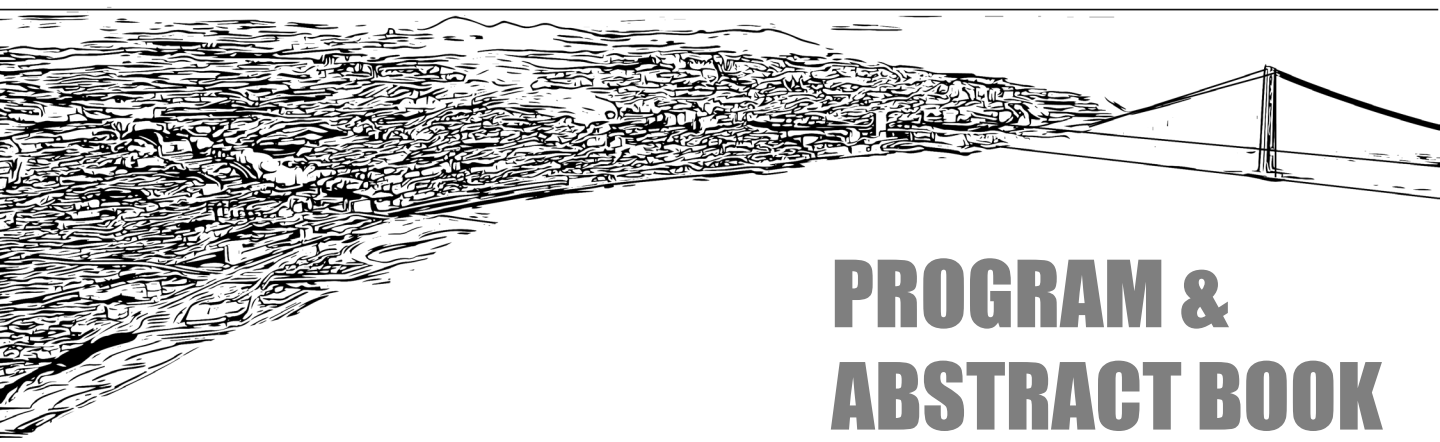
wnt 2022

40th anniversary of Wnt research

November 15-19, 2022 | Awaji, Japan



PROGRAM & ABSTRACT BOOK

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Wnt 2022

Program & Abstracts

November 15-19, 2022
Awaji, Japan

Organizers

Akira Kikuchi, Junichi Takagi, Shinji Takada, Tohru Ishitani, Yasuhiro MInami

Co-Organizers

Marian Bienz, Elizabeth Vincan, Xi He, Eek-hoon Jho, Yi Arial Zeng

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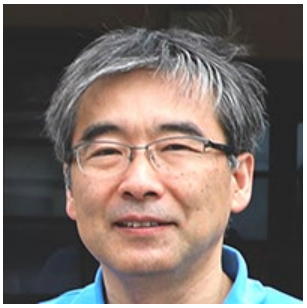
Akira Kikuchi



Junichi Takagi

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Tohru Ishitani

Yasuhiro Minami

International Committee Organizers

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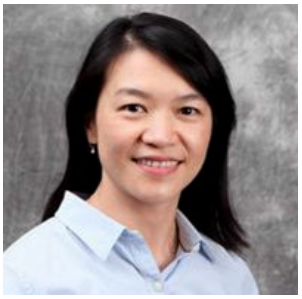


Yi Arial Zeng

Welcome Message

It is a great pleasure to welcome you at Wnt2022 of “40 years anniversary of Wnt research”. We sincerely hope that you will enjoy four days of meeting in Awaji Island located closely to Kobe, Osaka, Kyoto, and Nara, which are famous cities in the West part of Japan. According to the ancient Japanese literature, Awaji Island is said to be the first island that formed for Japan. You can enjoy excellent science and the beautiful nature, historic culture, and abundant cuisine in Awaji Island.

This meeting was originally planned to be held in 2020. Covid-19 has influenced our lives throughout the world since January 2020, and unfortunately Wnt 2020 was decided to be postponed. Our activities have been restricted for almost three years, both by governmental rules and regulations and by our appropriately cautious behaviour, to prevent the spread of Coronavirus. During the Covid-19 pandemic, the research communities have experienced many changes, including on-line education and meeting (e.g. WntTalks on-line). Even though the virtual communications can be used to overcome time and space, we still miss the good old way of having face-to-face discussion with our colleagues in the real cities with the different cultures as done in the past Wnt meeting.

The Organizing Committee in Japan thought deeply how we should have Wnt meeting this year, in person, fully remote, or hybrid format, because we cannot predict the situation in the next few months and it is changing in many different countries with the appearance of new variants of Covid-19. Finally, the Organizing Committee decided to have an in person meeting Wnt 2022 beyond all the organizing and administrative issues. It is the first time that the Japanese society of Wnt researchers hosts a Wnt meeting.

This meeting is organized to allow plenty of personal gathering, scientific discussion, and social interaction. Wnt signaling is an essential and highly conserved pathway. During the last 40 years Wnt signaling has been shown to play crucial roles in embryonic development, cell stemness, tissue regeneration, and human diseases. During oral sessions, evening poster sessions, excursion, breakfast, lunch, and dinner, we will have the opportunity to meet with colleagues from all over the world. We would very much appreciate the financial supports from European Biology Organization (EMBO), the Yamada Science Foundation, and all other sponsors. Without these supports, Wnt2022 could not be held.

We wish you all a successful and enjoyable meeting. Have a great time in Awaji.

On behalf of the Organizing Committee
Akira Kikuchi
Junichi Takagi
Shinji Takada
Tohru Ishitani
Yasuhiro Minami

Meeting Sponsors

We are extremely grateful to our sponsors for their generous contributions and support

General Information

Venue
Awaji Yumebutai International Conference Center
1Yumebutai, Awaji, Hyogo
T: +81-(0)799-74-1020
F: +81-(0)799-74-1021
Hotel : Grand Nikko Awaji
2Yumebutai, Awaji, Hyogo
T: +81-(0)799-74-1111
F: +81-(0)799-74-1100
*International Conference Center and Hotel are connected at 2F by corridor.

The registration desk will be open on:
Tuesday 15th November 15:00-18:00
Location: Front desk (Hotel 2F)

Wednesday 16th, Thursday 17th and Friday 18th November 08:30-18:00
Location: Registration counter (Conference Center 2F)

Cloakroom and luggage room
There is a cloakroom near the Front desk. The Luggage room, #201 and #202, can be accessed.

Security
Name badges must be worn at all times as these serve as the admission pass to scientific sessions and also for meals.

Internet Access
Internet can be accessed in each area with the following codes:
Main Hall Wi-Fi: apnn-main
Password: ybt21pma
Event Hall Wi-Fi: apnn-event
Password: ybt21pev
Reception Hall B Wi-Fi: apnn-receb
Password: ybt21prb
Lobby : “B1F”or”1F”or”2F-1”or”2F-2”

Group Photo Wednesday 16th November 12:20-

Refreshment breaks, lunch and dinner
Conference Center
B1F Event Hall: Welcome Dinner / Opening Mixer / Lunch (except 17th November) / Dinner
2F Main Hall Foyer: Coffee Break /

Hotel
1F Banquet Room STELLA : Farewell Dinner (18th November)
1F Banquet Room CIELO: Farewell Mixer (18th November)

Lecture room
Lectures will be held in the “Main Hall”.

Poster Presentations
Posters will be displayed in the “Reception Hall B” area. Please mount your posters on arrival.

Poster Session 1
Odd-numbered posters will be presented on the evening of Wednesday 16th November.

Poster Session 2
Even-numbered posters will be presented on the evening of Thursday 17th November.

Presenters should remove their posters prior to the dinner on the Friday 18th November.

Prizes will be presented at the evening on Friday 18th November.

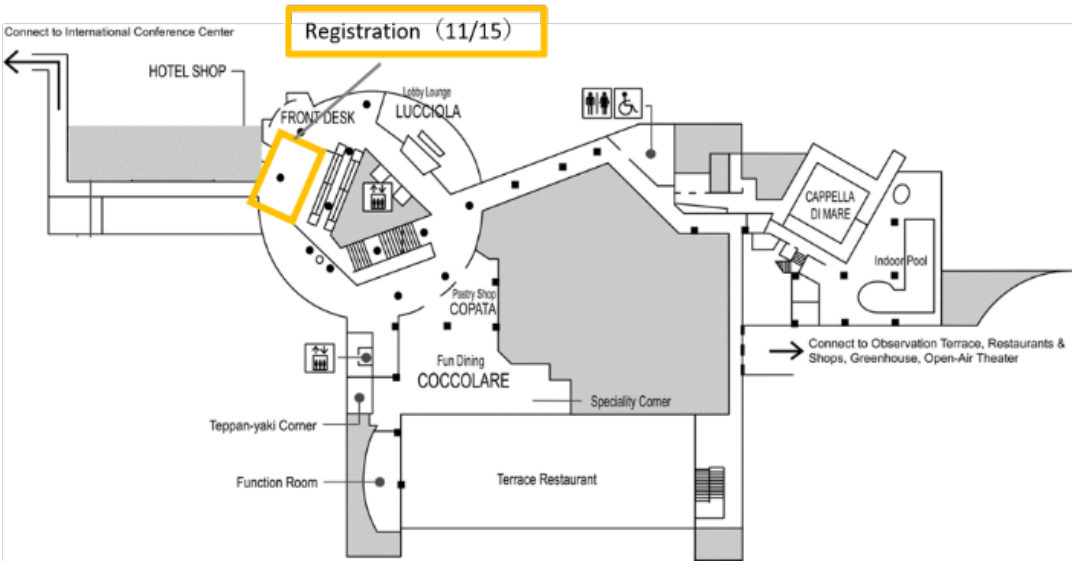
Excursion Thursday 17th November

Insurance
The organizers are unable to accept any responsibility for damage or loss of personal property during the conference and participants are advised to ensure that such items are adequately insured.

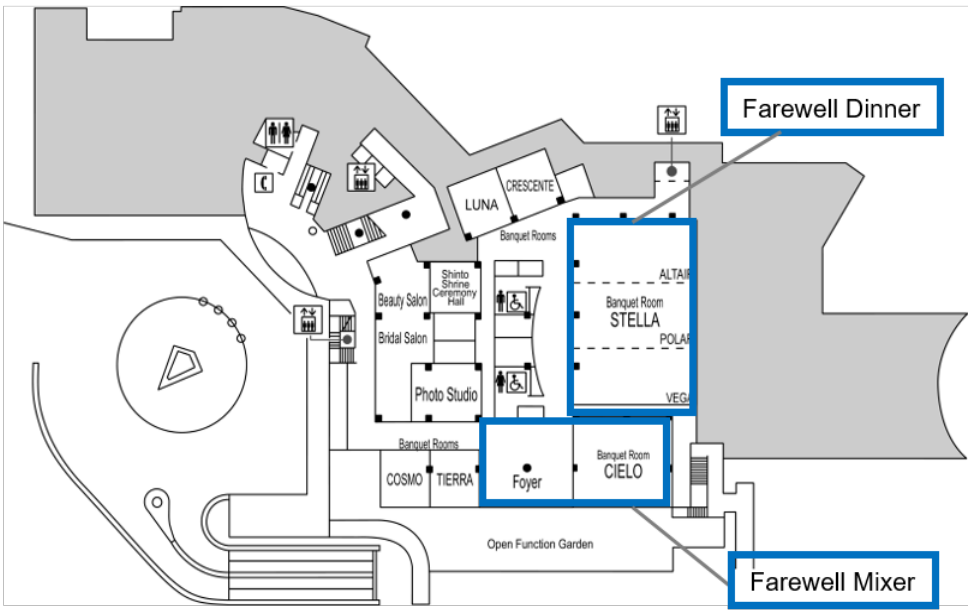
Hotel

Conference Center and Hotel are connected at 2F by Corridor

Grand Nikko Awaji
2F

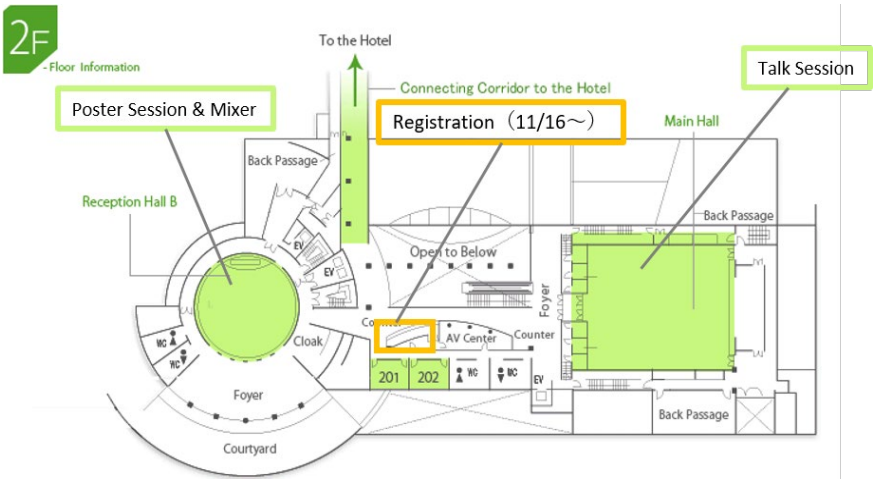


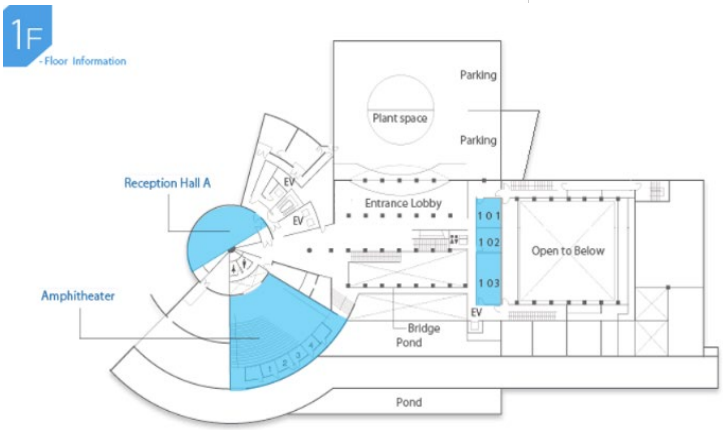
1F

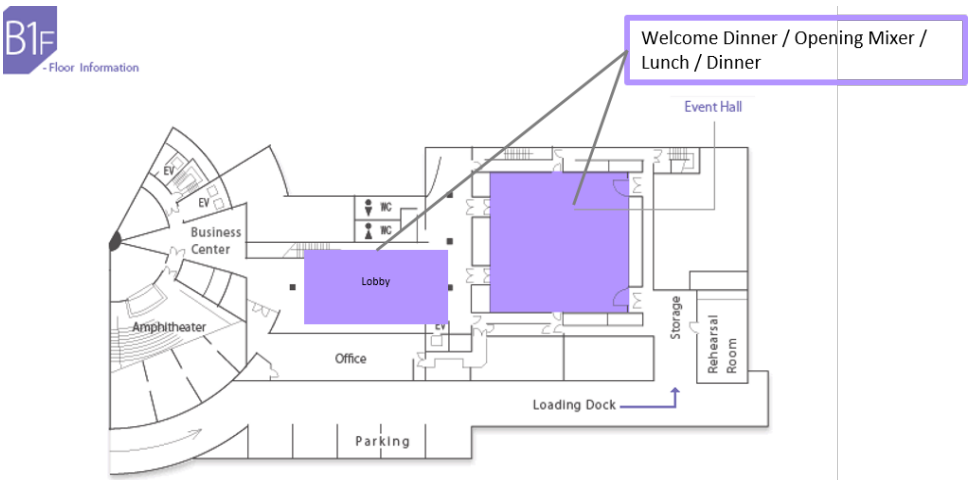


Conference Center

Conference Center and Hotel are connected at 2F by Corridor







Program

Day 1 – 15 November

15:00-18:00	Arrival/Registration
18:00-20:00	Welcome Dinner
20:00-20:10	Opening of the meeting and Greeting Ikuko Hara-Nishimura
20:10-20:40	Introduction Akira Kikuchi

Session-I: 40 years of Wnt research ~Plenary talk by Masatoshi Takeichi~

Chair: Shinji Takada

20:40-21:25	T-01 Surprising encounter between cadherin and Wnt systems Masatoshi Takeichi
21:25-22:30	Opening mixer

Day 2 – 16 November

07:00-09:00 Breakfast

Session-II: Wnt signal transduction mechanism-I

Chair: Marian Waterman

09:00-09:30 T-02 Aberrant cholesterol metabolism and Wnt/β-catenin signaling coalesce via Frizzled5 in supporting cancer growth
Xi He

09:30-09:45 T-03 Mutual regulations between Wnt11 and core PCP components establish planar cell polarity
Yusuke Mii

09:45-10:00 T-04 Molecular mechanisms of Frizzled-dependent planar polarity establishment in *Drosophila*
David Strutt

10:00-10:15 T-05 Cell competition ensures robust formation of morphogen gradient
Tohru Ishitani

10:15-10:30 T-06 β-Catenin plays dual functions in regulating cell competition to constitute tissue compartments
Xin Zhang

10:30-10:50 Coffee break

Session-III: Wnt signaling in development

Chair: Hee-Jung Choi

10:50-11:20 T-07 Intraflagellar transport complex A (IFT-A) and Kinesin 2 are required for nuclear translocation of β-catenin
Marek Mlodzik

11:20-11:50 T-08 PCP/Vangl2 mechanosensitive signaling in heart formation
Daniela Panakova

11:50-12:05 T-09 Crosstalk of Wnt and FGF signaling during anteroposterior embryonic development
Sergei Sokol

12:05-12:20 T-10 Canonical Wnt signalling in early vertebrate development: From direct Wnt/beta-catenin target genes to downstream Gene Regulatory Networks
Stefan Hoppler

12:20-12:40 Group Photo

12:40-13:40 Lunch

Session-IV: Wnt secretion from cells

Chair: Yvonne Jones

13:40-14:10 T-11 Towards a model of Wnt gradient formation

Jean-Paul Vincent

14:10-14:40 T-12 Regulation of the Wnt secretory machinery
Michael Boutros

14:40-15:10 T-13 Upstream Regulation of Wnt Signaling
David Virshup

15:10-15:25 T-14 Observing β-catenin dynamics through tandem fluorescent protein timers
Nicholas Tolwinski

15:25-15:40 T-15 Cytoneme-mediated transport of active Wnt5b/Ror2 complexes in zebrafish
Steffen Scholpp

15:40-16:00 Coffee break

Session-V: Assembling signalosomes in Wnt signaling
Chair: Karl Willert

16:00-16:30 T-16 Cilia are WNT ⊣ PP1 signalling organelles
Christof Niehrs

16:30-17:00 T-17 The function of the Dishevelled PDZ domain
Melissa Gammons

17:00-17:15 T-18 Quantitative analysis of real-time Wnt-FZD Interactions in live cells
Gary Davidson

17:15-17:30 T-19 Localized Wnt-sources instruct mitotic orientation: dissecting the Wnt-transduction mechanism coupled with NuMA machinery
Susanna Eli

17:30-17:45 Short break

17:45-18:45 Poster "Flash Talk (Odd number)" (Tohru Ishitani)

19:00-21:00 Dinner

21:00-22:30 Poster Session-I (Odd number)

Day 3 – 17 November

07:00-09:00 Breakfast

Session-VI: Structural studies of Wnt signaling

Chair: Xi He

09:00-09:30 T-20 Structural insights into the extracellular modulation of Wnt signalling
Yvonne Jones

09:30-10:00 T-21 PI(4,5)P2-stimulated positive feedback drives Dishevelled recruitment to Frizzled in Wnt/β-catenin signaling
Jacob Mahoney

10:00-10:15 T-22 Structural and functional studies of the interplay of TCF/LEF/β-catenin and FOXO/p53 signaling
Tobias Madl

10:15-10:30 T-23 Catalytic and Inhibitory Mechanisms of Porcupine-Mediated Wnt Acylation
Xiaochun Li

10:30-11:00 Coffee break

Session-VII: Wnt signal transduction mechanism-II

Chair: Elizabeth Vincan

11:00-11:30 T-24 Role of TFEB as a terminal mediator of Wnt signaling
Eek-hoon Jho

11:30-12:00 T-25 Functional and structural analysis of Frizzled receptor and its downstream transducer, β-arrestin
Hee-Jung Choi

12:00-12:15 T-26 A New CUT&RUN Low Volume-Urea (LoV-U) protocol uncovers Wnt/β-catenin tissue-specific target genes
Claudio Cantù

12:15-12:30 T-27 WNT signalling promotes genome stability in human pluripotent stem cells
Sergio P. Acebron

13:00-17:30 Excursion

18:00-19:00 Poster "Flash Talk (even number)" (Tohru Ishitani)

19:00-21:00 Dinner

21:00-22:30 Poster Session-II (even number)

Day 4 – 18 November

07:00-09:00 Breakfast

Session-VIII: 40 years of Wnt research ~Plenary talk by Roel Nusse~

Chair: Akira Kikuchi

09:00-09:45 T-28 Remarks on 40 years of Wnt
Roeland Nusse

09:45-10:00 Coffee break

Session-IX: Wnt signaling in cancer

Chair: Christof Niehrs

10:00-10:30 T-29 Identification of mouse pancreatic islet progenitors and its implication in islet regeneration
Yi Arial Zeng

10:30-11:00 T-30 Context dependent Wnt signalling in gastrointestinal epithelial stem cells and cancer
Elizabeth Vincan

11:00-11:30 T-31 Signaling Mechanisms and Tumor Contexts that link β-Catenin to Aggressive Colorectal Cancer
Marian Waterman

11:30-11:45 short break

11:45-12:00 T-32 Crosstalk between β-catenin and WT1 activity in acute myeloid leukaemia (AML)
Megan Payne

12:00-12:15 T-33 Anti-Usag-1 therapy by novel antibody drug for regeneration of missing teeth in patients with congenital tooth agenesis, a Rare Disease
Katsu Takahashi

12:15-12:30 T-34 GREB1 drives HNF4α-dependent oncogenic transcription and tumor growth in Wnt signal-activated hepatocellular carcinoma
Shinji Matsumoto

12:30-13:50 Lunch

Session-X: Wnt Signaling in stem cells and regeneration

Chair: Eek-hoon Jho

13:50-14:20 T-35 Manipulating stem cell fate using a selective Frizzled agonist
Stephane Angers

14:20-14:50 T-36 Characterizing and targeting the WNT receptor FZD7 in cancer
Karl Willert

14:50-15:05 T-37 Targeted lung regeneration with Fzd-specific Wnt-mimetics

Ahmad Nabhan

15:05-15:20 T-38 Epithelial regeneration, mucosal healing and reduction of inflammation by a Frizzled-specific Wnt mimetic
Wen-Chen Yeh

15:20-15:40 Coffee break

Session-XI: Wnt Signaling and organoid culture
Chair: Daniela Panakova

15:40-16:10 T-39 Reconstructing morphogen system to program multicellular patterning
Satoshi Toda

16:10-16:25 T-40 Inter-organ Wingless/Ror/Akt signaling regulates nutrient-dependent dendritic hyperarborization of somatosensory neurons
Tadashi Uemura

16:25-16:40 T-41 Wnt5a-Ror signaling regulates cell migration and contractility via RhoA-Myosin-Actin axis
Srisathya Srinivasan

16:40-17:10 T-42 Genotype-Phenotype mapping of patient-derived cancer organoids revealed divergent Wnt dependency during human GI carcinogenesis
Toshiro Sato

17:10-17:40 Poster prize (Tohru Ishitani)

17:40-17:55 Closing Remark (Junichi Takagi)

18:00-22:00 Farewell Dinner

20:00-22:00 Mixer & Discussion

Day 5 – 19 November

07:00-09:00	Breakfast
09:00	Departure

Abstracts-Oral Presentations

T-01

Surprising Encounter between Cadherin and Wnt Systems

Masatoshi Takeichi

RIKEN Center for Biosystems Dynamics Research, Kobe, Japan

Cadherin is a family of intercellular adhesion proteins, which is of central importance in formation of multicellular animals. Its adhesive function is accomplished by homophilic interaction between the extracellular domains. Importantly, however, their homophilic binding is not strong enough to produce firm cell-cell adhesion. For cadherin to be fully functional, it requires the cytoplasmic region to which β-catenin binds. This β-catenin further binds a-catenin forming a β/α-catenin complex. Removal of α-catenin causes dispersion of cells even when cadherin is active, indicating that this complex is indispensable for making characteristic cadherin-mediated intercellular bonds. It should also be noted that cadherin forms a superfamily comprising more than 100 members with diverse functions, and the 'classical cadherin' is the only target of β-catenin among the superfamily members,

Intriguingly, β-catenin also works as a key transcriptional regulator downstream of the canonical Wnt pathway. This discovery has prompted the fields to explore how cells manage β-catenin with a dual function. It seems, however, that this question remains largely unresolved. Another question is how a single protein has acquired multiple functions. Related to this problem, it is noteworthy that β- and α-catenin are detectable even in organisms that have no cadherin gene, such as cellular slime molds. Conversely, choanoflagellates, a unicellular species closest to metazoan, have cadherin superfamily genes, but none of their products are able to bind β-catenin. These pieces of information suggests that β- and α-catenin evolved independently of cadherin. Actually, we found that α-catenin exhibits functions not related to cell adhesin even in vertebrate cells. Thus, it should not be surprising that β-catenin also has cadherin-independent functions.

Based on these observations, we can infer that, during evolution, cadherin was accidentally chosen by β-catenin as its partner, resulting in generation of an adhesion machinery required for multicellular organization. Ancestral functions of β-catenin must have been retained at such events. Additionally, it is suggested that cadherin also cooperates with the PCP signaling pathways in which the non-canonical Wnt signals are known to participate. Thus, cadherin and Wnt are functionally intertwined in complex ways.

T-02

Aberrant cholesterol metabolism and Wnt/β-catenin signaling coalesce via Frizzled5 in supporting cancer growth

Shaoqin Zheng[1], Jiahui Lin[1], Zhongqiu Pang[1], Hui Zhang[1], Yinuo Wang[1], Lanjing Ma[1], Haijiao Zhang[1], Xi Zhang[2], Maorong Chen[3], Xinjun Zhang[4], Chao Zhao[5], Jun Qi[6], Liu Ca[7], Min Wang[8], Xi He[3], Ren Sheng[1, 3]

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3 F.M Kirby Neurobiology Center, Boston Children's Hospital, Department of Neurology, Harvard Medical School, Boston, MA 02115, USA
4 Key Laboratory of Molecular Biophysics of the Ministry of Education, National Engineering Research Center for Nanomedicine, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, P.R.China
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8 Department of Biliary-Pancreatic Surgery, Affiliated Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Ave, Wuhan 430030, P.R.China

Frizzled (Fzd) proteins are Wnt receptors and play essential roles in development, homeostasis and oncogenesis. How Wnt/Fzd signaling is coupled to physiological regulation remains unknown. Cholesterol is reported as a signaling molecule regulating morphogen such as Hedgehog signaling. it is well-established that pancreatic cancer specially requires abnormal cholesterol metabolism for growth. In this study, We unexpectedly found that among ten Fzds, Fzd5 has a unique capacity to bind cholesterol specifically through its conserved extracellular linker region. Cholesterol-binding enables Fzd5 palmitoylation, which is indispensable for the receptor maturation and trafficking to the plasma membrane. In Wnt-addicted pancreatic ductal adenocarcinoma (PDAC), cholesterol stimulates tumor growth via Fzd5-mediated Wnt/β-catenin signaling. A natural oxysterol, 25-hydroxylsterol competes with cholesterol and inhibits Fzd5 maturation and Wnt signaling, thereby alleviates PDAC growth. This cholesterol-receptor interaction and ensuing receptor lipidation uncover a novel mechanism by which Fzd5 acts as a cholesterol sensor and a pivotal nexus coupling lipid metabolism to morphogen signaling. These findings further suggests that cholesterol-targeting may provide new therapeutic opportunities for treating Wnt-dependent cancers.

T-03

Mutual regulations between Wnt11 and core PCP components establish planar cell polarity

Yusuke Mii [1], Minako Suzuki [1], Hiroshi Koyama [1], Ritsuko Takada [1], Makoto Matsuyama[2], Toshihiko Fujimori [1], Shinji Takada [1]

[1] *National Institute for Basic Biology, Okazaki, Japan*

[2] *Shigei Medical Research Institute, Okayama, Japan*

Planar cell polarity (PCP) is a kind of directional information within a tissue plane.
Recent studies show that a family of secreted signaling proteins Wnt can direct PCP. However, it is still controversial how Wnt regulates PCP, largely due to the lack of visualization of Wnt proteins. We have visualized endogenous Wnt8 (Mii et al., Nat. Commun. 2017) and Wnt11 (this study) in Xenopus embryos by generating antibodies. Interestingly, most of endogenous Wnt11 protein was arranged in a "parallel pattern" along the medio-lateral axis in the neural plate, implying involvement of core PCP components because extracellular distribution of a secreted protein requires a specific scaffold (Mii et al., eLife 2021). Indeed, knockdown of vangl2 reduced the medio-lateral distribution of Wnt11, suggesting involvement of core PCP components. Consistently, when PCP is established by ectopic Wnt11, GFP-Pk3 and Vangl2 (Chu & Sokol, eLife 2016), GFP-Pk3 and Vangl2 can accumulate Wnt11 on polarized cells. Vangl2 is considered to be phosphorylated upon Wnt stimulation. Super-resolution imaging revealed that phosphorylated Vangl2 was distributed on the opposite side of Pk3 at cell boundaries with Wnt11 accumulation, suggesting the formation of the Wnt11 scaffold involves phosphorylation of Vangl2.
Using phospho-deficient and phospho-mimetic mutants of Vangl2, we examined topological relationship among core PCP components including Pk3, phosphorylated and non-phosphorylated Vangl2, and Fz7 in the formation of the local scaffold of Wnt11.
Together with a mathematical model, the local scaffold formation of Wnt11 by core PCP components could explain asymmetric localization of core PCP components as well as the medio-lateral distribution of Wnt11.

T-04

Molecular mechanisms of Frizzled-dependent planar polarity establishment in Drosophila

David Strutt

University of Sheffield, Sheffield, United Kingdom

Planar polarity refers to the ability of structures in a tissue plane to adopt a common polarity and is a universal phenomenon in plant and animal development. The best- studied molecular system that defines planar polarity in animal tissues is the Frizzled- dependent ‘core’ planar polarity pathway. This pathway functions by forming asymmetric intercellular protein complexes between neighbouring cells, with the polarity of these complexes having a constant orientation relative to the plane of the tissue.

The establishment of core pathway planar polarity requires symmetry breaking at multiple levels. The first is establishing asymmetry within the intercellular complexes, the second is polarisation of complexes within individual cell junctions and cells, and the third is orienting polarity relative to the axes of the tissue. Ongoing work in the lab seeks to understand each of these steps and how they are integrated to produce a uniform pattern of planar polarity, using the Drosophila pupal wing as a model experimental system. Current projects examining mechanisms of molecular and cellular symmetry breaking and the influence of cell rearrangements will be discussed.

T-05

Cell competition ensures robust formation of morphogen gradient.

Tohru Ishitani

RIMD, Osaka University, Suita, Japan CiDER,
Osaka University, Suita, Japan

Morphogen signaling forms an activity gradient and instructs cell identities in a signaling strength-dependent manner to pattern developing tissues. However, developing tissues also undergo dynamic morphogenesis, which may produce cells with unfit morphogen signaling and consequent noisy morphogen gradients. Recently, we demonstrated that cell competition corrects noisy Wnt morphogen gradients in early embryos. Cell competition is a cell-cell interactive process in which cells with relatively higher fitness eliminate those with lower fitness. During early embryonic anterior-posterior patterning, unfit cells with abnormal Wnt signaling activity spontaneously appear and produce noise in the gradient but they are apoptotically eliminated after communicating with neighboring normal cells. However, it is still unknown whether cell competition mediates robust morphogen gradients formation just in early embryos or in various organogenesis processes patterned by other morphogens. Here we show that cell competition supports the robust morphogen gradients formation beyond morphogen types and tissue types. Zebrafish imaging analyses of the Shh morphogen gradient identify that unfit cells with abnormal Shh activity often arise and produce noise in the gradient, which is formed in neural tube and muscle primordia. Similar to the elimination of Wnt-unfit cells, Shh-unfit cells are also apoptotically eliminated via cadherin-mediated communication with neighboring normal cells and subsequent Smad signaling activation and reactive oxygen species production. These results indicate that cell competition-mediated correcting systems can function in various organogenesis to support diverse morphogen gradients' robustness. Moreover, as morphogen gradients also control adult tissue patterning, this system may be relevant for tissue homeostasis and preventing diseases.

T-06

β-catenin plays dual functions in regulating cell competition to constitute tissue compartments

Xin Zhang

Columbia University, New York, United States

Self-assortation of progenitor cells during development is essential for establishment of distinct tissue identity. This is exemplified in the eye, where the early optic cup is divided into the neural retina in the center and ciliary margin (CM) in the periphery. Previous studies have demonstrated that Wnt signaling is required for specification of the CM, but here we show that genetic ablation of Wnt signaling mediator β-catenin in the peripheral optic cup failed to prevent the formation of the CM-derived ciliary body and iris in adult animals. Mosaic analysis revealed that this was due to loss of adherens junctions among β-catenin deficient cells, which were preferentially excluded from the CM. Even in β- catenin mutant cells that can maintain adherens junction, their inability to mediate Wnt signaling resulted in a change from P-cadherin to N-cadherin expression. We showed that this cadherin switch was sufficient to segregate otherwise identical cells into separate clusters. As a result, the ciliary body and iris were still formed after inactivation of Wnt signaling in the peripheral retina. These results showed that the dual function of β-catenin in adherens junction and Wnt signaling is required for cell competition to constitute retinal compartments.

T-07

Intraflagellar transport complex A (IFT-A) and Kinesin 2 are required for nuclear translocation of β-catenin

Marek Mlodzik and Linh T. Vuong

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The nuclear translocation of β-catenin upon Wnt-pathway activation remains an unresolved puzzle of canonical Wnt-signaling, as β-catenin does not have a classical NLS. Primary cilia are dynamic organelles, which require a specialized protein network for proper biogenesis and function. Misregulation of ciliary function is linked to developmental pathway defects. Through a series of genome-wide genetic screens in non-ciliated epithelial tissues, imaginal discs, in *Drosophila* we have identified non-ciliary roles of ciliary proteins in several signaling pathways. We have thus identified cilia associated genes in both PCP and canonical Wnt-signaling in non-ciliated cells (all epithelial imaginal disc cells lack the primary cilium). In particular, these screens defined a function of the Intraflagellar transport complex A (IFT-A) in canonical Wnt-signaling. Follow up mechanistic studies on how IFT-A functions in Wnt/β-catenin signaling revealed that it works together with Kinesin 2 and is required for the nuclear translocation of β-catenin. We have demonstrated that Kinesin-2 and IFT-A proteins act as a complex during *Drosophila* Wg-signaling and canonical Wnt-signaling in general, binding to β -catenin (Armadillo/Arm in *Drosophila*). β-catenin/Arm, the mediator of nuclear Wnt/Wg-signaling, is upon its release from the destruction complex tethered on microtubules (MTs) via a Kinesin2/IFT-A/β-catenin complex. Following pathway activation, Kinesin-2/IFT-A mutant cells exhibit high cytoplasmic β-catenin levels, yet fail to activate Wg/Wnt-targets in both, *Drosophila* and mammalian cells, mouse/MEFs or HEK293, with nuclear localization of β-catenin/Arm markedly reduced or even not detectable. We demonstrate that this effect is mediated, in part, by protecting β-catenin from a conserved cytoplasmic retention process, and, importantly, nuclear translocation β-catenin/Arm is dependent on the motor-domain function of Kinesin2. We now show that Arm/b-catenin is transported along MTs towards the nucleus. Strikingly, upon Wnt-activation MTs are re-arranged with many "+"-ends localized near or even inside the nucleus. We detect MTs penetrating into the nucleus and we observe β-catenin/Arm moving along these into the nucleus. Again, this mechanism is conserved between *Drosophila* and mammalian cells (as seen in MEFs and HEK293 cells). Our work thus identifies a novel mechanism for Kinesin-2/IFT-A in Wg/Wnt-signaling that is independent of their ciliary role. Moreover, our studies provide first mechanistic insight into how β-catenin/Arm is translocated into the nucleus upon Wnt-signaling activation.

T-08

PCP/Vangl2 mechanosensitive signaling in heart formation

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Linear heart tube (LHT), a transient structure present in all vertebrates, undergoes major tissue deformations while forming the first functional organ. The tissue tension, torsion and anisotropic expansion, all contribute to the LHT remodeling and result in looped and chambered heart. How mechanical forces contribute to the heart formation and what developmental pathways and cellular processes regulate them is not fully explored. We have previously described PCP signaling, a morphogenetic pathway that organize cell adhesion and cytoskeletal dynamics through diverse downstream effectors, in generating polarized tissue tension within the forming LHT. Here, we examine the role of PCP, and specifically Vangl2, in the tissue-scale supracellular polarization of actomyosin within the myocardial epithelium. We demonstrate that the polarized distribution and activity of phosphorylated Myosin requires cardiac-specific Myosin Light Chain Kinase 3 (Mylk3) and Rho-associated Protein Kinase 2a (Rock2a). We find that like Rock2a, also Mylk3 is under genetic control of PCP signaling, thus identifying it as a novel tissue-specific effector of this pathway. Further, we show the opposing force-generating activities of Mylk3 and Rock2a regulate heart tube remodeling. We then examine, how the tissue tension forces affect nuclear compartment during LHT stage. We uncover the relationship between the changes in nuclear morphology and PCP signaling. Specifically, we discover a previously unidentified role for Vangl2 in regulating the nuclear mechanosensing that is coupled to transcriptional changes in gene programs regulating muscle differentiation and cardiac function. We propose PCP/Vangl2 axis as a key regulator of mechano-molecular signaling in heart development. Understanding how the mechanical inputs shapes tissues and organs in development will allow us to decipher the pathological states where the mechanical cues play a crucial role.

T-09

Crosstalk of Wnt and FGF signaling during anteroposterior embryonic development

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The formation of the vertebrate anteroposterior body axis is known to be regulated by Wnt and FGF signaling, however the molecular crosstalk between these pathways remains unstudied. During Xenopus gastrulation, canonical Wnt signaling results in the phosphorylation and inactivation of TCF3/TCF7L1, a transcription factor that associates with beta-catenin. The establishment of planar cell polarity in the neuroectoderm is another major event that takes place during gastrulation and has been also associated with Wnt signaling. Unexpectedly, we find that both processes are downstream of the FGF pathway. FGF signaling increased TCF3 phosphorylation and directed Vangl2 localization to the anterior side of each cell by the end of gastrulation. By contrast, inhibition of endogenous FGF receptor reduced TCF3 phosphorylation and disrupted PCP via distinct mechanisms. The observed effects on canonical Wnt signaling were due to transcriptional changes, whereas PCP was directly altered through FGF receptor enzymatic activity. Roles of R-spondins in the selection of the canonical and noncanonical Wnt signaling branches will be discussed.

T-10

Canonical Wnt signalling in early vertebrate development: From direct Wnt/beta-catenin target genes to downstream Gene Regulatory Networks.

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The experimentally accessible Xenopus embryo was instrumental in discovering many biochemical mechanisms of vertebrate Wnt signalling, but it also informed us about key roles of Wnt signalling in developmental patterning of the early vertebrate embryo:
1. Maternal Wnt/beta-catenin pathway components (maternal Wnt signalling) break the radial symmetry of the egg and promote dorsal development.
2. After the onset of zygotic gene expression, wnt8a/beta-catenin signalling (zygotic Wnt signalling) then promotes almost the opposite, i.e., ventral mesoderm.

We used this dramatic change in the biological response as an experimental model to identify stage- and tissue-specific direct Wnt/beta-catenin target genes. We combined RNA-seq and beta-catenin ChIP-seq experiments and defined direct Wnt target genes as having Wnt-regulated transcripts and beta-catenin association to the same gene loci at the relevant stages. We identified just over 100 target genes of maternal Wnt signalling, and just over 30 of zygotic wnt8 signalling. The apparently binary biological response to Wnt signalling (i.e., early: maternal->dorsal vs later: zygotic->ventral) suggested that we might also expect just those two corresponding classes of Wnt/beta-catenin target genes.

However, de-novo motif analysis of relevant beta-catenin-associated genomic sequences and validation experiments reveal more complexity, with a useful definition of about five classes: two classes of maternal target genes, both of which co-regulated by nodal signalling, but with one class (e.g., siamois) expressed before, and apparently co- regulating, the other (e.g. goosecoid); a third class of probably ubiquitous Wnt target genes regulated by maternal and zygotic Wnt signalling (e.g. axin2); and zygotic wnt8a/beta-catenin signalling additionally regulating at least two further classes of target genes; a fourth class co-regulated by BMP signalling (e.g., msx1) and a fifth class co- regulated by FGF signalling (e.g., cdx2).

With access to further laboratory experiments somewhat restricted during the COVID pandemic, we explored computational modelling to test our logic of an integrated Gene Regulatory Network response to maternal and zygotic Wnt signalling in this model vertebrate embryo, which now provides hypotheses to further investigate molecular mechanisms for regulation of these stage- and tissue-specific direct Wnt target genes, and for identification of equivalent Wnt target genes in early mammalian and human development.

T-11

Towards a model of Wnt gradient formation

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Wnts are secreted proteins that control a variety of processes including stem cell maintenance, cell fate decisions and growth. In some situations, Wnts act over many cell diameters, behaving as a long-range morphogen, while in others, Wnts are thought to act in a juxtacrine manner. The range of Wnts is likely to be controlled at multiple steps, during secretion, release, transport, and degradation. We aim to characterise how the rate of each of these steps is controlled, with the goal of building a theory of Wnt gradient formation. Previous genetic experiments and recent structural analysis suggest that, during progression through the secretory pathway, Wnts are tightly associated with Wls. Yet, this interaction must be relaxed for Wnt to be released from producing cells. To determine how this is controlled, we are using FRET, SNAP-tagging and high-resolution microscopy to track Wls and Wingless after they have reached the surface of secreting cell. Our preliminary results suggest that these two proteins dissociate soon after their co-internalisation, perhaps explaining why Wnt secretion requires endocytosis. We have previously shown (in collaboration with the group of EY Jones) that the glypican Dlp shields the lipid of Wnts. Recent analysis has shown that Wingless accumulates in aggregates at the basal surface of Dlp mutant cells, suggesting that Dlp is required to 'receive' Wingless upon its release. The ability of Dlp binding to Wingless relies on two features: a hydrophobic tunnel that binds the Wingless lipid and heparan-sulfate chains. We are mapping the HS-binding surface of Wingless with the aim of separating the two modes of interaction and testing the hypothesis that dual binding via low affinity sites is essential for Dlp to boost the spread of Wingless along the surface of the epithelium. The dynamics of gradient formation will be assessed with new optogenetic means of acutely triggering Wingless production and sensitive reporter of signalling.

T-12

Regulation of the Wnt secretory machinery

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Intercellular communication by Wnt proteins governs many processes during development, tissue homeostasis, and disease in all metazoan animals. Many context- dependent effects are initiated in Wnt-producing cells and depend on the export of lipidated Wnt proteins. Wnt proteins are lipidated by the acyl-transferase Porcupine which is required for interaction with many of its receptors. Previously, through *ex vivo* and *in vivo* screens, we identified factors that are required for the secretion of lipidated Wnt proteins. We identified the eight transmembrane protein Evi/Wls as the key cargo-receptor for lipidated Wnt proteins and demonstrated its role in the secretion of Wnt proteins *in vivo* in *Drosophila* and in vertebrates. Evi/Wls is highly conserved in metazoans and only one EVI/WLS gene with three transcript variants exists in the human genome, in contrast to 19 WNT or 10 FRIZZLED genes. Evi/Wls binds to Wnt proteins in the ER, shuttles from the Golgi to the plasma membrane and is recycled through the endocytic compartment back to the Golgi and the ER. To our current knowledge, Evi/Wls is the only carrier of lipidated Wnt proteins. However, whether and how Evi/Wls protein levels are adjusted to differentially expressed Wnt ligands with varying expression patterns has remained largely unclear. We observed that in colon adenocarcinoma samples, WNT3 expression is positively correlated with Evi/Wls protein abundance, suggesting a post-translational mechanism that adjusts Evi/Wls protein levels. Biochemical and genetic screeens were used to discover that Evi/Wls is an endogenous substrate of ER-associated degradation (ERAD), a molecular pathway that mediates the ubiquitination and degradation of ER-resident proteins. Evi/Wls is constantly produced in the Wnt secreting cells and immediately degraded in the absence of lipidated Wnts. We will discuss recent findings on underlying mechanisms, interacting proteins and regulatory factors that influence the capability of cells to secrete Wnt proteins.

T-13

Upstream Regulation of Wnt Signaling

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The delivery of Wnt signaling ligands to the right cells at the right time is essential for the development of multicellular animals and for the maintenance of stemness, proliferation and differentiation in adults. The downstream consequences of Wnt/β-catenin include both activation and repression of gene expression, and modulation of the MAPK signaling pathway. The intestinal crypt and intestinal organoids have been powerful system to understand Wnt signaling. We and others have recently demonstrated that in vivo, telocytes, an interstitial cell type named only in 2010, are the critical source of Wnts for the intestinal stem cell niche. Telocyte-mediated delivery of signaling molecules is likely to be important in many epithelial tissues, but we lack a clear understanding of how essential lipid-modified Wnts (and possibly many other signaling proteins) are transported from the producing telocytes to their stem cell targets. We used our organoid-telocyte co-culture system to visual at high resolution how Wnts move from telocytes to the intestinal stem cell niche. We find that Wnt delivery and distribution is a multistep process. At the resolution of electron microscopy, we find that telocytes use membrane extensions to establish synapse-like structures with target cells. Supporting the importance of these Wnt synapses, specific synapse- and membrane platform-associated proteins were needed for efficient Wnt presentation to target cells by regulating cell spreading and cytoneme initiation. Taken together, our findings link telocyte membrane platform associated proteins to Wnt secretion in cell-to cell contact resembling synaptic interactions.

T-14

Observing β-catenin dynamics through tandem fluorescent protein timers

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The Wnt pathway transduces its signal by preventing the degradation of the signaling protein β-catenin. But β-catenin serves a dual role in both transcription and adhesion. To study the dynamics of these processes in vivo we used tandem fluorescent protein timers inserted into the endogenous β-catenin locus, looking at the stability and turnover of β-catenin. We find that the turnover of the protein is too fast to observe during normal signaling conditions, but we identify the process of dorsal closure in embryonic development as a place with highly stabilized β-catenin. We examine the requirement for stable β-catenin at the leading edge of this wound healing-like morphogenetic process as well as the requirement for Wnt signaling and adhesion components using optogenetics. We propose a novel β-catenin function in stable junctions during force generating cell biological processes.

T-15

Cytoneme-mediated transport of active Wnt5b/Ror2 complexes in zebrafish.

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Chemical signalling is the primary means by which cells communicate in the embryo. The underlying principle refers to a group of ligand-producing cells and a group of cells that respond to this signal because they express the appropriate receptors. In the zebrafish embryo, Wnt5b binds to the receptor Ror2 to trigger the Wnt/Planar Cell Polarity (Wnt/PCP) signalling pathway to regulate tissue polarity and cell migration. However, it is still unclear how this lipophilic ligand is transported from the source cells through the aqueous extracellular space to the target tissue. Here we show that Wnt5b, together with Ror2, is loaded on long signalling filopodia, also known as cytonemes. The active Wnt5b/Ror2 complexes are formed in the producing cell and handed over from these cytonemes to the receiving cell. Then, the receiving cell activates Wnt/PCP signalling, regardless of whether the cell expresses functional receptors. On the tissue level, we further show that cytoneme-dependent spreading of active Wnt5b/Ror2 affects convergence and extension in the zebrafish gastrula. We suggest that cytoneme- mediated transfer of ligand-receptor complexes is a vital mechanism for paracrine signalling in a tissue and thus challenges the long-standing concept of characterising responsive and non-responsive tissues based solely on the expression of the receptors.

T-16

Cilia are WNT ┤ PP1 signalling organelles

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WNT ligands typically signal via receptor activation across the plasma membrane to induce β-catenin-dependent gene activation. In contrast, we find that in primary- and motile cilia, WNT receptors relay a WNT/GSK3 signal that β-catenin-independently promotes ciliogenesis and ciliary beating, respectively. LRP6 but not LRP5 contains a ciliary targeting sequence that locates the coreceptor to the ciliary membrane. Ciliary WNT signalling inhibits protein phosphatase 1 (PP1) activity, a negative regulator of ciliogenesis, by decommissioning GSK3-mediated phosphorylation of the PP1 regulatory inhibitor subunit PPP1R2. Deficiency of WNT/GSK3 signalling by depletion of cyclin Y and cyclin-Y-like protein 1 induces primary cilia defects in mouse and *Xenopus* embryos. We conclude that cilia are WNT signalling organelles, which transduce a WNT ┤ PP1 signal that is distinct from that transmitted through the plasma membrane.

T-17

The function of the Dishevelled PDZ domain.

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Dishevelled is a cytoplasmic hub that transduces Wnt signals to cytoplasmic effectors, broadly characterised as canonical (β-catenin-dependent) and noncanonical, to specify cell fates and behaviours during development. To transduce canonical Wnt signals, Dishevelled binds to the intracellular face of Frizzled through its DEP domain and polymerises through its DIX domain to assemble dynamic signalosomes. Dishevelled also contains a PDZ domain whose function remains controversial. Here, I will present our recent work uncovering the selective function of the PDZ domain of Dishevelled in non-canonical Wnt signalling processes in Drosophila and mammalian cells. We used a combination of proximity labelling assays, CRISPR-based genome editing, cell-based assays and structural biophysics to determine the molecular function of the Dishevelled PDZ domain. We discovered that the PDZ domain undergoes a structural rearrangement upon binding to the unique, highly conserved PDZ-binding motifs (PBMs) at the C-termini of Daple and Girdin. Furthermore, we show that the binding of Dishevelled to these PBMs is essential for its role in disassembling Wnt-dependent primary cilia. Thus, our work has uncovered Daple and Girdin as key physiological ligands of the Dishevelled PDZ domain.

T-18

Quantitative analysis of real-time Wnt-FZD Interactions in live cells

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Real-time quantification of ligand-receptor interactions in live cells aids our understanding of Wnt reception selectivity. We have used a combination of advanced fluorescent microscopy and bioluminescence resonance energy transfer (BRET) methods to quantify DKK binding to LRP6 as well as determine the selectivity of Wnt3a association to different Frizzleds (FZD's). Our initial BRET experiments were based on overexpression of FZD fusion proteins to establish and optimize the methodology. We are now generating data using stable cell lines that express lower levels of the Wnt receptors.
The combination of using NanoBiT/BRET together with reduced receptor expression levels allowes us to generate more accurate quantification of Wnt-FZD interactions. In our experiments FZD's are fused to HiBiT, an 11 aa peptide that can associate with a complementary LgBiT subunit to form a functional Nano-luciferase. LgBiT is membrane- impermeable so only BRET signals from the cell surface of HiBit-FZD expressing cells are detected after application of LgBiT together with a functional GFP-Wnt fusion protein. We are extending our analysis to different Wnt-FZD combinations to gain a more comprehensive overview of Wnt pathway ligand-receptor selectivity.

T-19

Localized Wnt-sources instruct mitotic orientation: dissecting the Wnt-transduction mechanism coupled with NuMA machinery

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In multicellular organisms, morphogenesis and homeostasis of epithelial tissues require oriented cell divisions, which rely on the proper positioning of mitotic spindle. Polarity and spindle orientation defects are often coupled with loss of tissue architecture and altered proliferation. During asymmetric cell division, the spindle orients to ensure the correct cell fate determinants segregation by exposing daughter cells to different molecular hints. In these processes, spindle positioning is defined by the activity of the molecular microtubule motors dynein/dynactin and the complex NuMA/LGN, coordinated with cortical polarity cues. In numerous stem cells niche, Wnt ligands have shown a restricted spatial localization that regulates mitotic axis but how cells sense Wnt signals to orient the metaphase plate is largely unknown.

Here, we present our finding on the transduction mechanism of localized Wnt3a signals during mitosis. Imaging experiments conducted in Hela cells dividing in contact with Wnt3a- beads revealed that canonical Wnt effectors including Lrp6, β-catenin, Dvl2 are implicated in the correct spindle orientation towards the Wnt source. Moreover, performing immunoprecipitation assays, we discovered unknown players of Wnt-dependent mitotic response such as the highly conserved core complex of NuMA/LGN, also essential for the right spindle positioning toward the Wnt3a- beads. In particular we show that, upon Wnt3a stimulation, NuMA forms macromolecular complexes with Wnt effectors including β-catenin, Axin1, APC, and Lrp6. In conclusion, our study reveals the existence of a cross-talk between mitotic spindle orientation and Wnt, and that this mechanism relies on the formation of NuMA complexes with Wnt effectors. Considering the spatial distribution of Wnt3-signals in niche microenvironment, we believe that this newly identified mechanism of mitotic Wnt might be essential for the correct execution of asymmetric stem cell divisions.

T-20

Structural insights into the extracellular modulation of Wnt signalling

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Wnt signalling is modulated in multiple ways by extracellular interactions involving both the Wnt ligands and their receptors. Within the Division of Structural Biology (Strubi) at Oxford we are using a combination of structural and biophysical analyses to reveal the mechanisms that govern this finely balanced signalling system and to identify potential tools for therapeutic intervention. Recent data and insights will be discussed.

T-21

PI(4,5)P2-stimulated positive feedback drives Dishevelled recruitment to Frizzled in Wnt/β-catenin signaling

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In the Wnt/β-catenin pathway, Wnt binding to Frizzled (Fzd) and LRP5 or LRP6 (LRP5/6) co-receptors recruits the cytosolic protein Dishevelled (Dvl) to the plasma membrane, ultimately inhibiting degradation of the transcriptional co-activator β-catenin. Fzd-bound Dvl recruits the β-catenin destruction complex, thereby promoting phosphorylation of LRP5/6 by the destruction complex–resident kinase GSK-3, a key step in inhibiting β-catenin degradation. LRP5/6 phosphorylation requires Wnt-stimulated production of the lipid phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), a process mediated by Dvl-associated lipid kinases. However, the mechanism of Wnt-stimulated Dvl recruitment and the role of PI(4,5)P2 in pathway activation have not been elucidated. Fzds are members of the G protein-coupled receptor superfamily, therefore it has been thought that Wnt binding allosterically enhances Fzd-Dvl affinity to promote Dvl recruitment. Using purified Fzd reconstituted in lipid nanodiscs, we found that extracellular ligands do not affect Fzd-Dvl affinity. However, PI(4,5)P2 strongly enhances Fzd-Dvl binding, independent of ligand binding. Our findings suggest a positive feedback loop in which Wnt-stimulated receptor clustering stimulates local PI(4,5)P2 production to enhance Dvl recruitment and support LRP5/6 phosphorylation.

T-22

Structural and functional studies of the interplay of TCF/LEF/β-catenin and FOXO/p53 signaling

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Here, I will present our recent published and unpublished work on the regulation and cross-talk of Wnt/β-catenin signaling with p53 and FOXO signaling.
Intrinsically disordered regions (IDRs) lack stable tertiary structure and instead rapidly interconvert between different conformations. This structural plasticity enables IDRs to act as key players in cellular signaling pathways, including Wnt, p53 and FOXO signaling. Transcription factors (TFs) are enriched in IDRs, many of which are stabilized by or acquire tertiary structure in the presence of DNA or other binding partners.
Examples for TFs with long IDRs are T-cell factor/lymphoid enhancer binding factor 1 (TCF/LEF), FOXOs and p53. In the recent years, increasing evidence points towards potential links between TCF/LEF, FOXO and p53 signaling. For example, elevated levels of p53 downregulate β-catenin and β-catenin overexpression seems to upregulate levels of p53 by inhibiting p53 proteolysis [1]. Related to FOXO signaling, binding of β-catenin to FOXO inversely correlates with Wnt/TCF/LEF-mediated transcription [2], and this shift of β-catenin from TCF/LEF to FOXO has been shown to play a role in colon cancer metastasis, osteoblast differentiation, liver metabolism, and kidney fibrosis. Despite the importance of the interplay between these two major signaling pathways at the level of TFs and β-catenin, the molecular mechanism as to β-catenin and TCF/LEF, FOXO and p53 TFs interact and how this may regulate TF function remains unknown.
I will present our recent results related to this major effort, and more specifically related to i) the interplay of FOXO4 and TCF/LEF/β-catenin [3], ii) structural and dynamic investigation of the TF DNA-binding domains (unpublished and [4]), iii) the interplay of p53 and FOXO4 [5, 6], and vi) the interplay of p53 and TCF/LEF/β-catenin (unpublished). In all of these links, disordered regions play a key role. For example, we unraveled the molecular regulatory mechanism of FOXO4 by β-catenin and found that the disordered FOXO4 C-terminal region, which contains its transactivation domain, binds β-catenin through two defined interaction sites, and that this is regulated by phosphorylation.
Binding of β-catenin competes with the auto-inhibitory interaction of the FOXO4 IDR with its DNA-binding Forkhead domain, and thereby enhances FOXO4 transcriptional activity. Furthermore, we show that binding of the β-catenin inhibitor protein ICAT is compatible with FOXO4 binding to β-catenin, suggesting that ICAT acts as a molecular switch between anti-proliferative FOXO and pro-proliferative Wnt/TCF/LEF signaling. Our recent data illustrate how the interplay of IDRs, DNA-binding domains, posttranslational modifications, and co-factor/β-binding binding contribute to transcription factor function.

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T-23

Catalytic and Inhibitory Mechanisms of Porcupine-Mediated Wnt Acylation

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Wnt signaling is essential to regulate embryonic development and adult tissue homeostasis. Aberrant Wnt signaling is frequently associated with cancers. Wnt proteins must undergo palmitoleoylation on their hairpin 2 by the endoplasmic reticulum (ER)- resident membrane-bound O-acyltransferase porcupine (PORCN) before acting as signaling molecules. This modification is indispensable for Wnt to bind its receptor Frizzled, thus triggering signaling. Here, we report four cryo-electron microscopy structures of human PORCN: 1) the complex with the palmitoleoyl-CoA substrate, 2) the complex with its inhibitor LGK974, an anti-cancer drug currently in clinical trials, 3) the complex with LGK974 and WNT3A hairpin 2 (denoted as WNT3Ap), and 4) the complex with a synthetic palmitoleoylated WNT3Ap analog. The structures reveal that the hairpin 2 of WNT3A, which is well conserved in all Wnt ligands, inserts into PORCN from the lumenal side and the palmitoleoyl-CoA accesses the enzyme from the cytosolic side. The cis double bond at C9 position of palmitoleoyl moiety of palmitoleoyl-CoA and palmitoleoylated WNT3Ap analog causes a kink of fatty acid chain to form a "C" shape in the catalytic core enzyme. The catalytic histidine triggers the transfer of unsaturated palmitoleoyl group to the target serine on the Wnt hairpin 2 owing to the proximity of the two substrates. The inhibitor-bound structure shows that LGK974 occupies the palmitoleoyl-CoA binding site to prevent the reaction. Thus, this work provides a mechanism of Wnt acylation and advances the development of PORCN inhibitors for cancer treatment.

T-24

Role of TFEB as a terminal mediator of Wnt signaling

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Transcription factor EB (TFEB) is a well-known master regulator of autophagy and lysosomal biogenesis processes. To activate the transcription of genes involved in lysosomal biogenesis when nutrients are scarce, TFEB is dephosphorylated and relocated to the nucleus. We discovered that Wnt signaling causes TFEB to localize to the nucleus, and that both TFEB-β-catenin-TCF/LEF1 and β-catenin-TCF/LEF1 complexes control the expression of Wnt target genes. Our biochemical studies showed that TFEB is a component of the β-catenin destruction complex, and that nuclear localization of TFEB results from the destabilization of the destruction complex by knockdown of either Axin or APC. Interestingly, RNA-sequencing analysis revealed that about 27% of Wnt3a-induced genes were TFEB dependent. The TFEB target genes involved in autophagy and lysosomal biogenesis were not present in these "TFEB mediated Wnt target genes," nevertheless. Mechanistically, we found that the TFEB is PARsylated by Tankyrase (TNKS) with Wnt ON condition, and the nuclear-localized PARsylated TFEB then forms a complex with β-catenin-TCF/LEF1 to induce the "TFEB mediated Wnt target genes". Finally, we discovered that in different cancer types, the levels of “TFEB-mediated Wnt target genes” show strong correlations with the level of Axin2, which represents the activity of Wnt signaling. Overall, our results indicate that Wnt signaling induces the expression of a subset of genes that are distinct from previously known genes regulated by the β-catenin-TCF/LEF1 complex or TFEB, by the formation of a transcription factor complex made up of PARsylated TFEB and β-catenin-TCF/LEF1. Throughout my talk, additional evidence in support of our conclusion will be provided.

T-25

Functional and structural analysis of Frizzled receptor and its downstream transducer, beta-arrestin

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Wnt signaling activation is initiated by ligand binding to the extracellular ligand binding domain, called the cysteine-rich domain (CRD), of Frizzleds (FZDs). Much of the molecular basis of ligand binding to the FZD CRD was revealed through the structural studies of the CRD–Wnt/Norrin complexes. However, how ligand binding at the extracellular region is transmitted to the cytoplasm through FZD has not been fully understood. Here, biochemical and biophysical data will be presented showing that a flexible linker domain, which connects the CRD to the transmembrane domain (TMD), plays an important role in high-affinity ligand binding and hence downstream signaling. In addition, we show that Wnt binding to FZD, a member of the class F G protein coupled receptor (GPCR) family, activates beta-arrestin-mediated signaling through Dishevelled. Biochemical and structural data of the interaction between beta-arrestin and Dishevelled will be presented and functional implication of beta-arresting-mediated signaling will be discussed.

T-26

A New CUT&RUN Low Volume-Urea (LoV-U) protocol uncovers Wnt/β-catenin tissue-specific target genes

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Upon WNT pathway activation, stabilized β-catenin travels to the nucleus where it associates with the TCF/LEF family of transcription factors, which constitutively bind to genomic Wnt Responsive Elements (WREs), to activate tran- scription of target genes. Discovering the binding profile of β-catenin is therefore required to unambiguously assign direct targets of WNT signaling. Cleavage Under Target and Release Using Nuclease (CUT&RUN) has recently emerged as a prime technique for mapping the binding profile of chromatin interacting proteins. In our attempts to profile different regulators of the WNT/β-catenin transcriptional complex, CUT&RUN performed reliably when targeting transcription factors such as TCF/LEF, but it failed to produce consistent binding patterns of the non-DNA-binding β-catenin. Here, we present a bio- chemical modification of the CUT&RUN protocol, which we refer to as LoV-U (Low Volume and Urea), that enables the generation of robust and reproducible β-catenin binding profiles. CUT&RUN-LoV-U uncovers direct WNT/β-catenin target genes in human cells, as well as in ex vivo cells isolated from developing mouse tissue. CUT&RUN-LoV-U can profile all classes of chromatin regulators tested and is well suited for simultaneous processing of several samples. We submit that the application of our protocol will allow the detection of the complex system of tissue-specific WNT/β-catenin target genes, together with other non-DNA-binding transcriptional regulators that act downstream of ontogenetically fundamental signaling cascades.

T-27

WNT signalling promotes genome stability in human pluripotent stem cells

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Human pluripotent stem cells hold a great promise for regenerative medicine and human development modelling. However, pluripotent stem cells cultured or differentiated in vitro often display chromosomal aberrations, which precludes their use in the clinic. The WNT signaling pathway is essential for embryonic patterning, stem cell maintenance and lineage specification. Here, we report that WNT signaling has a moonlighting role in genome maintenance in human pluripotent stem cells. We find that WNT inhibition triggers a domino effect through hitherto uncharacterised signalling cascades that results DNA damage and chromosome segregation defects in human pluripotent stem cells.
Furthermore, using single cell sequencing and multiplex chromosome fluorescent in situ hybridization, we show that WNT inhibition results in structural and numerical aneuploidy. Conversely, we demonstrate that WNT activity protects self-renewing human pluripotent stem cells from different genomic insults. Finally, differentiation experiments into the three embryonic germ layers reveal that WNT roles in genome stability are uncoupled of its functions in cell fate determination. In this unpublished work, we conclude that Wnt signals coordinate stem cell renewal and genome maintenance before lineage specification, which could be critical for the translational use of stem cells and to our understanding of how aneuploidy arises during early human development.

T-28

Remarks on 40 years of Wnt

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The 2022 Wnt meeting in Awaji, Japan coincides with the 40th anniversary of a publication that announced the discovery of a gene that proved to be the first mammalian Wnt gene (Nusse and Varmus, 1982). The Wnt community has taken that report as a somewhat arbitrary starting point of the field. The history of Wnt signaling as it evolved and expanded since 1982 has been told in several reviews (Nusse and Varmus, 2012; Niehrs 2022) and in my presentation, I will not provide another historical account. Rather, I would like to go back to 1982 and highlight several other discoveries reported in that year that collectively impacted our understanding of cancer in a dramatic way. I will also revisit the role of Wnt signaling in cancer, including posing some unresolved questions.

T-29

Identification of mouse pancreatic islet progenitors and its implication in islet regeneration

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It has generally proven challenging to produce functional β cells *in vitro*. Our previous study identifies Procr as a target of Wnt signaling. Our recent study uncovers a novel Procr cell population in adult mouse pancreatic islets. The cells do not express differentiation markers and feature epithelial-to-mesenchymal transition (EMT) characteristics. By genetic lineage tracing, Procr islet cells undergo clonal expansion and generate all four endocrine cell types during adult homeostasis. Sorted Procr cells, representing ~1% of islet cells, can robustly form islet-like organoids when cultured at clonal density. Exponential expansion can be maintained over long time periods by serial passaging, while differentiation can be induced at any time point in culture. β cells dominate in differentiated islet organoids, while α, δ and PP cells occur at lower frequencies. The organoids are glucose-responsive and insulin-secreting. Upon transplantation in diabetic mice, the organoids reverse disease. These findings demonstrate that the adult pancreatic islet contains a population of Procr progenitors. We will also describe the relevance of Procr progenitors in human islets.

T-30

Context dependent Wnt signalling in gastrointestinal epithelial stem cells and cancer

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Cancer initiation and progression are fuelled by cells with both stem cell and mesenchymal properties. The Wnt/β-catenin signal transduction pathway plays critical roles in these processes in colon cancer and in other Wnt-driven gastrointestinal cancers. Our aim is to understand how Wnt governs these properties and identify the molecular mechanisms of context dependent Wnt signalling.

Recently we demonstrated that Frizzled$_7$ functions as a Wnt receptor in intestinal (*Flanagan et al*., *Stem Cell Reports, 2015)* and gastric (*Flanagan et al., Disease Models and Mechanisms, 2017*) stem cells and that this need for Frizzled$_7$ function is carried through to cancers that arise in these tissues (*Vincan et. al., Differentiation, 2005; Schwab et al., Developmental Dynamics 2018; Flanagan et al., Cancer Research, 2019*). Blocking Frizzled$_7$-mediated Wnt signalling has potent anti-tumour effects (reviewed in *Phesse et al., Cancers 2016* and *Flanagan et al, Br J of Pharmacology, 2017*). These roles that we have identified for Frizzled$_7$ in Wnt-driven gastrointestinal cancers, make Frizzled$_7$ an attractive therapeutic target that has the potential to impact on cancer initiation, growth and progression and ultimately, patient survival.

As we interrogate Frizzled$_7$ signalling and function for therapeutic targeting in cancer, it is perhaps timely to revisit lower organisms to gain insight into the context dependent and dynamic nature of Wnt signalling for effective drug design. Much of what is known about the core components of the Wnt signalling pathways was derived from studying the function of Frizzled$_7$ orthologues in the development of lower organisms (reviewed in *Tran et al., Handbook of Experimental Pharmacology, 2021*).

T-31

Signaling Mechanisms and Tumor Contexts that link β-Catenin to Aggressive Colorectal Cancer

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The most common genetic mutations in colorectal cancer (CRC) are those that activate the Wnt signal transduction pathway with over 80% of CRCs harboring such mutations. And yet despite this common mutation pathway to cancer, there are multiple CRC subtypes that differ in their Wnt signature as well as their prognosis and response to therapies. These subtypes range from those with a strong Wnt signature ("Wnt-high") to those with a weak signature ("Wnt-low"). How differing subtypes arise, whether they co-exist in tumors, and importantly, how their individual signatures influence the tumor microenvironment and patient prognosis is unknown. To study the tumor phenotypes and mechanisms of Wnt-high and Wnt-low cancers, we isolated two subtypes from a well-studied CRC cell line that greatly differ in Wnt activity as measured by TOPflash (high vs. low). Using mouse modeling and single cell RNAseq approaches, we determined that the Wnt-high cancer cells create tumors that are inflammatory and less aggressive than Wnt-low tumors. In contrast, Wnt-low tumors are invasive and immune-suppressive and yet promote strong inflammatory effects on neighboring normal epithelia. Paradoxically, Wnt-high and Wnt-low signatures do not correlate with the level of beta-catenin protein which are similar in the two subtypes. Rather, we present mechanistic data that implicate specific signaling networks that influence beta-catenin differently, including the set of Wnt target genes that are regulated. These subtype-specific signaling networks reveal potential sensitivities to epigenetic and kinase inhibitors that might be new options for CRC therapies.

T-32

Crosstalk between β-catenin and WT1 activity in acute myeloid leukaemia (AML).

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Acute myeloid leukaemia (AML) is a clonal disorder of haematopoietic stem cells (HSCs), characterised by the expansion of abnormal myeloblasts. AML arises from leukaemia stem cells (LSCs) which clonally evolve to evade chemotherapy and mediate relapse.
Survival rates have improved over 50 years; however, the prognosis remains dismal for the elderly or those harbouring adverse cytogenetic events. There's an urgent unmet clinical need for targeted efficacious drugs in AML which reduce side-effects and induce robust remissions. One potential molecular drug target is β-catenin, the central mediator of Wnt signalling which is frequently dysregulated in myeloid leukaemia. β-Catenin is overexpressed, mislocalised, and overactive in AML, where it confers inferior patient survival and drives the emergence, maintenance, and drug resistance of LSC. The level, localisation and activity of β-catenin is governed heavily through protein interactions, and we recently characterised the first β-catenin interactome study in myeloid leukaemia cells. One novel interactor of β-catenin identified was Wilms Tumour 1 (WT1) protein which is frequently mutated and overexpressed in AML. In this study we have confirmed for the first time that β-catenin and WT1 interact in both AML cell lines and patient samples.
Functionally, WT1 knockdown significantly decreased β-catenin nuclear expression and TCF activity in KG-1 cells. Furthermore, induction of WT1 mutations (exons 8 and 9) increased β-catenin expression and augmented Wnt signalling output.
Reciprocally, we showed that β-catenin knockdown repressed WT1 expression and signalling which was at least party transcriptionally driven. Overall, this study reports the first physical and functional interaction between β-catenin and WT1 in AML and reveals a potential novel role for β-catenin in the regulation of post-transcriptional gene control, both of which could inform novel β-catenin targeting strategies in AML.

T-33

Anti-Usag-1 therapy by novel antibody drug for regeneration of missing teeth in patients with congenital tooth agenesis, a Rare Disease

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Congenital tooth agenesis appears from early childhood, which is the developmental stage of the jaw, it adversely affects nutritional security and growth. Many attempts have been made to regenerate teeth by tissue engineering techniques, but due to problems such as cost and safety, they have not yet reached clinical application. We found that the USAG-1 protein (BMP / WNT antagonist) gene-deficient mouse formed excess teeth (teeth existing above the normal number of teeth), and this finding shows that one protein affects increase of the number of teeth. We also found that the formation of missing teeth was restored by mating various congenital tooth agenesis model mice (Eda, Runx2 gene-deficient mice, etc.) with USAG-1 gene-deficient mice (excess tooth model mice). We selected 5 anti-USAG-1 neutralizing antibodies from 1037 candidate antibodies, considering the difference in activation mode for BMP and WNT signaling and the difference in an antigen recognition site of antibody by epitope binning. We have applied for patents for these antibodies. All five mouse anti-USAG-1 neutralizing antibodies were found to ameliorate tooth agenesis a with a single intraperitoneal administration in Eda-deficient mice, a model of congenital tooth agenesis. Three promising mouse anti-USAG-1 antibodies were humanized; preliminary toxicity studies were conducted in parallel with bioactivity equivalence confirmation of the three human anti-USAG-1 antibodies to confirm the final development candidate with the remainder as backup. In this application. The development of a human anti-USAG-1 antibody obtained in this project could be a curative treatment for regenerating one's own teeth by molecular targeted therapy which is different from a current treatment using general artificial teeth.

The human anti-USAG-1 antibody, which is a molecular target drug against USAG-1, could be administered before the phenotype of the child is clarified, using the mutation of the causative gene of the parents' congenital tooth agenesis as a biomarker.

T-34

GREB1 drives HNF4α-dependent oncogenic transcription and tumor growth in Wnt signal-activated hepatocellular carcinoma

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Among the molecular signaling pathways involved in hepatocellular carcinoma (HCC) carcinogenesis, the Wnt/β-catenin signaling pathway is one of the most frequently activated pathways. However, the molecular basis of HCC carcinogenesis by the β- catenin signaling pathway has not been clearly identified. Growth regulation by estrogen in breast cancer 1 (GREB1), previously identified as a nuclear cofactor for hormone- dependent transcription factors, has recently been shown to be induced by Wnt signaling in hepatoblastoma, a pediatric liver cancer. In contrast, GREB1 is not expressed in colorectal cancers, in which Wnt signaling is aberrantly activated at high frequency.
Here, we evaluated the expression and molecular function of GREB1 in adult HCC. Immunohistological analysis of over 400 specimens revealed a clear positive correlation between GREB1 and β-catenin expression in hepatocellular carcinoma. GREB1 expression was induced by a super-enhancer formed by β-catenin/TCF4 and two master transcription factors HNF4α and FOXA2 that maintain hepatocyte identity. GREB1 was enriched in the regulatory region of HNF4α target genes, including proliferation-regulated genes essential for HCC cell survival. GREB1 promotes cell proliferation by inducing an HCC-specific transcriptional signature that is independent of the native regulation of cell differentiation by HNF4α. TCGA cohort analysis confirmed the clinical relevance and significance of the β-catenin-GREB1-HNF4α axis target genes. GREB1 antisense oligonucleotide (ASO) suppressed the expression of GREB1 and the target genes of the GREB1-HNF4α axis and orthotopic HCC cell liver tumorigenesis. Thus, GREB1 expression was induced in a Wnt signaling-dependent manner by super-enhancers associated with HNF4α and FOXA2, which promoted HCC tumorigenesis by accelerating HNF4α-specific oncogenic transcription.

T-35

Manipulating stem cell fate using a selective Frizzled agonist

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Current ventral midbrain dopaminergic progenitor differentiation protocols utilize small molecule inhibitors targeting Glycogen synthase kinase-3 (GSK3) to activate Wnt signaling, a step required for the anterior-posterior patterning of the nervous system and acquisition of the midbrain fate. However, GSK3a/b are pleiotropic kinases involved in multiple signaling pathways and their inhibition is a known trigger of neurogenesis. We predicted that direct activation of specific Wnt receptors naturally involved during neural patterning will allow for a more precise spatiotemporal control of Wnt/β-catenin signaling and result in more homogenous differentiation outcomes and thereby increase the functionality of resulting cell population. Here, we characterized the expression of FZD receptors at the surface of neural progenitor cells with different regional identity. Our data shows that FZD5 expression is uniquely upregulated in forebrain progenitors and is governed by OTX2 and LDB1. Using a FZD5-selective Frizzled and LRP5/6 Agonist (FLAg) we show that midbrain patterning can be achieved with high efficiency and precision thereby decreasing cell heterogeneity obtained with conventional protocols from hPSC. Moreover, the FLAg-patterned progenitors give rise to functional dopaminergic neurons *in vitro* and efficiently rescue motor dysfunction when transplanted in a Parkinson Disease rodent model.

T-36

Characterizing and targeting the WNT receptor FZD7 in cancer

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Since their first discovery, Wnt proteins and associated signaling molecules have been inextricably linked to cancer. Wnt receptors, in particular the Frizzled (FZD) family of cell surface proteins, offer opportunities to selectively and specifically target the Wnt signaling pathway using immune-based strategies. Previously, we developed an antibody that exclusively binds FZD7, one of the 10 FZD family members, the expression of which is elevated in stem and cancer cell populations. In particular, using The Cancer Genome Atlas, we observed elevated FZD7 expression in multiple solid tumors, including breast invasive carcinoma, glioblastoma multiforme, lung squamous cell carcinoma, ovarian serous cystadenocarcinoma, prostate adenocarcinoma, and uterine carcinosarcoma. In a preclinical study, we showed that the FZD7 antibody conjugated with a potent antimitotic agent selectively killed FZD7-expressing cancer cells in vitro and in vivo. To advance these clinical studies and to better understand the role of FZD7 in tumorigenesis, we are using a mouse mammary tumor model, called MMTV-Wnt1. Tumors derived from these mice express elevated levels of Fzd7, and isolation of tumor cells expressing Fzd7 using fluorescence-based cell sorting followed by orthotopic transplantation demonstrates that tumor initiating potential is enriched in the Fzd7-high population. These findings support our hypothesis that Fzd7 marks tumor initiating cells and that targeting of FZD7-positive tumor cells represents a viable option for the treatment of cancer.

T-37

Targeted lung regeneration with Fzd-specific Wnt-mimetics.

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Background: Wnt is one of a handful of pathways that control the specification and activity of dozens, or perhaps hundreds, of stem cells across different species and organs. How Wnt is spatially controlled across different stem cell populations, often within the same organ, is not understood. Moreover, clinical efforts to modulate Wnt have failed due to off-target toxicity, highlighting the need to understand and mimic endogenous mechanisms of cell-type specific Wnt signaling. In the alveolus, Wnt is required for AT2 stem cell function and AT2 cells selectively activate Wnt during development and regeneration leading some to hypothesize that Wnt activation of AT2 cells would be beneficial in IPF to stimulate alveolar epithelial repair. Conversely, Wnt is highly active in pathologically expanding fibroblasts in IPF patients and inhibiting the pathway reduced extra-cllular matrix deposition in mouse models of the lung fibrosis leading others to propose Wnt inhibition to halt the fibroblast expansion in the same disease. Given that these cells are separated by the thinnest of barriers to enable gas exchange, how Wnt is selectively activated in AT2 cells or fibroblasts is unknown.

Results: Focusing on lung alveoli, wherein Wnt controls intermingled epithelial and stromal progenitors, we show Frizzled (Fzd) receptors, which have distinct affinities for Wnt ligands, are differentially expressed by epithelial (Fzd5/6), endothelial (Fzd4) and stromal (Fzd1) cells. Importantly, these patterns are conserved across species and disease states. We develop family-specific Fzd antagonistic antibodies and lung organoid gene-editing technology which reveal that Fzd5 is uniquely required for alveolar epithelial stem cell activity.
Furthermore, Wnt signaling can be specifically activated in mouse and human AT2 cells using our recently developed Wnt-mimetics which are engineered Fzd5-specific Fzd-Lrp agonist antibodies (FLAgs). Surprisingly, FLAgs can also be engineered to activate canonical Wnt in epithelial stem cells through the non-canonical Fzd6, even though endogenous Wnt ligands do not. FLAgs are superior to recombinant Wnt at stimulating AT2 stem cell activity. In vivo, Fzd5 and Fzd6-specific FLAgs stimulate supra- physiological alveolar epithelial stem cell activity during regeneration. Interestingly, agonizing the non-canonical Fzd6, but not Fzd5, expands the alveolar stem cell pool by promoting an alveolar fate in airway derived progenitors and increases protection in the bleomycin lung injury model.

Conclusion: Together, these studies identify the endogenous receptor for Wnt signaling in the alveolar stem cell, point to a new mechanism for cell type-specific Wnt signaling and provide proof-of-concept for new therapeutic avenues aiming to manipulate Wnt in specific cell types.

T-38

Epithelial regeneration, mucosal healing and reduction of inflammation by a Frizzled-specific Wnt mimetic

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Inflammatory bowel disease (IBD) affects millions globally. Disruption of the colon epithelial barrier is a defining characteristic of UC, allowing the exposure of luminal microbes to the intestinal stroma and the host immune system, creating a vicious cycle of inflammation and further damage to the epithelium. Histological remission is arising as a new target for UC treatment. However, agents that act directly to heal the epithelium are lacking, and the rates of mucosal healing and histological remission remain low.

Wnts are secreted, lipid-modified glycoproteins that function as key regulators of stem cells in many tissues. In the intestine, Wnt signaling is crucial in maintaining integrity of the epithelium under tissue homeostasis and during injury repair. Although exogenous R- spondin (RSPO), which amplifies Wnt signals by maintaining cell surface expression of FZD and LRP receptors, was reported to repair intestine epithelial damage, it also induced hyperplasia of normal epithelium. The ability of Wnt mimetics, recombinant antibody-based molecules mimicking endogenous Wnts, to repair intestine epithelial damage has not been explored.

We show here that Wnt mimetics repaired the injured colon epithelium in a Dextran sodium sulfate (DSS) mouse model of ulcerative colitis (UC), reducing the disease activity index (DAI) and histology damage scores with better efficacy than RSPO2 or the combination of RSPO2 and Wnt mimetics. Unlike RSPO2 or the combination treatment, Wnt mimetic treatment alone did not affect uninjured epithelium. Next, guided by the Fzd expression patterns in normal and disease conditions, we narrowed in on Fzd5 and showed that a subfamily Fzd5,8-specific Wnt mimetic, SZN-1326-p, was effective both in organoid culture and in vivo in intestine injury repair. Using scRNA-seq, we demonstrated that in the DSS model, SZN-1326-p activated Wnt signaling predominantly in colon epithelial cells, including multiple stem/progenitor cell populations, leading to transient proliferation followed by accelerated epithelial differentiation that ultimately promoted mucosal barrier healing with a concomitant reduction in inflammation. This effect was specific to the injured epithelium, without promoting proliferation of normal, uninjured intestinal tissue. Our work suggests that SZN-1326-p could be a new therapeutic option for the treatment of UC.

T-39

Reconstructing morphogen system to program multicellular patterning

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During animal development, cells communicate with each other using various molecules to control the behaviors of cell populations and assemble complex tissue structures. Secreted signaling molecules called morphogens diffuse inside tissues and form a concentration gradient, which will work as positional information for surrounding cells to guide their differentiation and generate tissue patterns. However, cell-cell interactions in vivo are extremely complicated: e.g., morphogens interact with many surrounding molecules in addition to receptors and cells dynamically change their own properties while exchanging signals. Therefore, it is still unclear how diffusing morphogens form patterns with distinct multicellular domains precisely. To explore what features are sufficient for tissue patterning, we asked whether arbitrary proteins (e.g. GFP) could be converted into synthetic morphogens. Synthetic morphogens expressed from a localized source formed a gradient of gene induction and can be used to program *de novo* tissue domain formation. In addition, our synthetic morphogen system is also useful to explore the regulatory mechanisms of natural morphogen diffusion such as Wnt. In this presentation, I will introduce our synthetic morphogen system and discuss the mechanisms of multicellular patterning and Wnt diffusion regulation.

T-40

Inter-organ Wingless/Ror/Akt signaling regulates nutrient-dependent dendritic hyperarborization of somatosensory neurons

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Nutrition in early life has profound effects on an organism, altering processes such as organogenesis. However, little is known about how nutritional status is conveyed to developing neurons, how those neurons regulate growth in response to such a signal, and whether the growth regulation at the cellular level is associated with behavioral changes. We are addressing these questions using Drosophila larval class IV dendritic arborization neurons (C4da neurons), which sense noxious light, thermal, and mechanical stimuli and provoke robust avoidance behaviors. We and others previously found that dendrites of C4da neurons become more complex when larvae are reared on a low-yeast diet compared to a high-yeast diet (hyperarborization phenotype under the low-nutritional condition; Watanabe et al., Genes to Cells 2017, Kanaoka et al., Genes to Cells 2019; Poe et al. eLife 2020).

Our systematic search for key nutrients of the phenotype revealed that C4da neurons increase their dendrite densities in response to a combined deficiency in vitamins, metal ions, and cholesterol. The deficiency of these nutrients upregulates wingless (wg) expression in closely located muscles. The muscle-derived Wg activates Akt-Tor signaling in C4da neurons through the receptor tyrosine kinase Ror, which results in the dendritic hyperarborization. Moreover, this muscle-neuron interorgan pathway is regulated partly by a systemic ligand Unpaired 2 (Upd2)-Stat92E pathway between the fat body and muscles. Additionally, the low-yeast diet blunts light responsiveness of the neurons and light avoidance behaviors, which may help larvae keep searching for high-nutrient foods under a potentially risky environment. Together, our studies illustrate how the availability of specific nutrients affects neuronal development through inter-organ signaling (Kanaoka et al., bioRxiv, 2022)

T-41

Wnt5a-Ror signaling regulates cell migration and contractility via RhoA-Myosin-Actin axis

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The Wnt5a-Ror pathway is a highly conserved developmental signaling pathway that regulates morphogenetic processes in organisms from cnidarians to vertebrates. The pathway functions independently of the canonical Wnt/β-catenin pathway to control cell migration and other cytoskeleton-dependent processes during embryonic development. Despite this, little is known about how Wnt5a-Ror signaling functions at the cellular level to exert its biological effects. Through a combination of pharmacological and genetic approaches, we experimentally elevated or diminished Wnt5a-Ror activity in immortalized mouse embryonic fibroblasts (iMEFs). Interestingly, we observed that in a 2D environment, elevated Wnt5a-Ror signaling suppresses migration, whereas, in a 3D environment, it promotes migration. Based on previous literature on the role of contractility in cell migration, we hypothesized that the contrasting effect of Wnt5a-Ror signaling in 2D and 3D migration might be explained by changes in cellular contractility. Indeed, we observed that increased Wnt5a-Ror activity not only increased cellular contractility in iMEFs, but also resulted in enhanced stress fiber formation and mature focal adhesions. Importantly, we further established that Wnt5a-Ror signaling induces changes in several key regulators of the cell contractility, including altered subcellular localization of RhoA, elevated activity of its downstream target Rho-associated protein kinase (ROCK), which subsequently increased phosphorylation of non-muscle myosin light chain (MLC) at Thr18/Ser19, a major molecular switch that controls contractility.
Collectively, these data suggest that a major cellular function of the Wnt5a-Ror pathway is to activate the “RhoA-Myosin-Actin” (RMA) axis, which in turn controls cell contractility and focal adhesion formation to modulate migratory cell behavior.

T-42

Genotype-Phenotype mapping of patient-derived cancer organoids revealed divergent Wnt dependency during human GI carcinogenesis

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In homeostatic adult tissues, niche factors regulate long-term self-renewal and multiple differentiation capacity of tissue stem cells. By reconstructing niche factors in vitro, tissue stem cells form a stereotypic organoid structure and self-renew for the long-term. We and others have identified tissue-specific niche factors, which enabled the propagation of organoids from a variety of adult tissues, including patient-derived tissues. Human tissue-derived organoids preserved genetic and epigenetic abnormalities of the original tissues and showed disease-relevant biological phenotypes in vitro and in vivo. Using these technologies, we have been working on the phenotypic profiling of patient-derived organoids and revealed molecular mechanisms of genotype-phenotype correlations underlying the disease pathogenesis. In this meeting, I would like to introduce our recent research on how to deepen the understanding of human disease biology and develop new therapies using organoid technology.

Abstracts-Poster Presentations

P-01

Conserved Map7/7D1/Ens proteins coordinate microtubule remodeling and Wnt/PCP signaling for cell polarity formation.

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Tissue morphogenesis requires the establishment and maintenance of cell polarity that involves microtubule (MT) remodeling. MT remodeling is often promoted by β-catenin- independent Wnt/PCP signaling. This signaling and microtubule remodeling are regulated interdependently, by an unknown mechanism. Here we show that the paralogous microtubule-associated proteins Map7 and Map7D1 (Map7/7D1) participate in a feedback loop between one of Wnt/PCP signaling pathways, Wnt5a signaling and MT remodeling through a direct interaction with Dishevelled (Dvl) in HeLa cells (here called MT-Wnt/PCP network). Map7/7D1 direct the cortical localization of Dvl, and facilitate the cortical targeting of MT plus-ends in response to Wnt5a signaling. Wnt5a signaling also promotes Map7/7D1 movement toward MT plus-ends, and depletion of the Kinesin-1 member Kif5b abolishes the Map7/7D1 dynamics and Dvl localization.
Furthermore, Dvl stabilizes Map7/7D1. Intriguingly, Map7/7D1 and its Drosophila ortholog, Ensconsin (Ens) show planar-polarized distribution in both mouse and fly epithelia, and Ens influences proper localization of Drosophila Dvl, Dsh in pupal wing cells. These results suggest that the role of Map7/7D1/Ens in Dvl/Dsh localization is evolutionarily conserved. Based on the above results, to understand the roles of MT- Wnt/PCP network regulators, Map7/7D1 for mammalian tissue morphogenesis, we are now analyzing expression patterns of Map7 and Map7D1 in various mouse tissues and phenotypes of Map7 or Map7D1 knock-out mice. The involvement of Map7 or Map7D1 in cell polarity formation during mammalian tissue morphogenesis will be discussed in this meeting.

P-02

Division orientation proteins are key for intestinal organoids development in a Wnt-dependent manner

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Colorectal cancer (CRC) is one of the deadliest human malignancies in which the high relapse rate is increased by the high frequency of cancer stem-like cells (CSC).
Aberrancies in stem cell proliferation and epithelial polarity in the intestinal epithelium are key events in the generation of CSCs. The Wnt signaling pathway is a critical regulator of intestinal stemness, orchestrated by Wnt3-secreting Paneth cells acting as niche.
Interestingly, recent studies identified a role for localized Wnt signals in oriented stem cell divisions, suggesting an implication in self-renewal in the crypts. However, the Wnt- related molecular mechanisms governing intestinal stem cell (ISCs) divisions and how they are deregulated in CRC remains largely unclear.
We report that ablation by lentiviral sh-RNA interference of Afadin and NuMA, two proteins involved in spindle orientation, leads to defective morphogenesis of intestinal organoids derived from wild-type mice. These defects are characterized by a significant decrease in the differentiation rate and reduced proliferation. Consistently, both Afadin- and NuMA-depleted organoids display alterations in the transcriptional profile compared to the wild-type, with differences in the regulation of specific pathways (such as apical junctions and mTOR pathway), suggesting that Afadin and NuMA contribute to the maintenance of epithelial integrity also by transcriptional activities. In addition, we observed impairment in the mitotic spindle orientation of dividing cells in the intestinal crypts, in agreement with the described role of both proteins in spindle positioning.
Interestingly, in spite of the misorientation defects, organoids retain a normal monolayered epithelia architecture, which we show can be ascribed to the presence of correction mechanisms that guide the reintegration of the misoriented daughter cells in the epithelia. Intriguingly, preliminary data show that depletion of Afadin in organoids derived from APC -/- mice, in which the Wnt pathways is constitutively active, results in an increased spheroids area (opposite effects compare to WT), hinting at the possibility that Afadin could be implicated in Wnt3-dependent differentiation of intestinal cells.
Our data suggest that Afadin and NuMA play a role in the molecular machinery orchestrating Wnt-dependent planar divisions and transcriptional programs in intestinal organoids. Importantly, our results provide molecular information on niche-regulated ISC divisions that drive epithelial morphogenesis under physiological conditions and are likely deregulated in neoplastic conditions. Our findings highlight an uncharacterized crosstalk between disruption of epithelial integrity by downregulation of cytoskeletal proteins in intestinal stem cells and lineage specification of intestinal cells in the crypts.

P-03

Investigating the role of β-catenin in post-transcriptional control of gene expression in acute myeloid leukaemia (AML)

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Acute myeloid leukaemia (AML) is a heterogeneous clonal disorder of the bone marrow with a poor prognosis. Current chemotherapies show limited efficacy and so novel therapies are required that target specific molecular aberrations. One such candidate is β-catenin, the central mediator of Wnt signalling, which is frequently overexpressed, mislocalised and overactive in AML where it sustains leukaemia stem cells (LSC). The stability, localisation and activity of β-catenin is dictated heavily by protein interactions which were previously poorly characterised in a haematopoietic context. We recently performed the first β-catenin interactome analysis in myeloid leukaemia cells and revealed a plethora of novel interactions for β-catenin including the significant enrichment of RNA binding proteins (RBPs). The RBPs were associated with GO search terms 'mRNA processing and transport' and 'rRNA processing and transport' and validated β-catenin:RBP interactions to date include LIN28B, WT1 and MSI2. β-Catenin has well-established roles in transcription however this data, coupled with previous reports of β-catenin binding mRNA and regulating alternative splicing, raises the intriguing possibility that β-catenin could also have a role in post-transcriptional gene control in AML. To identify transcripts with which β-catenin is associated we have performed RBP-immunoprecipitation (RIP) for β-catenin coupled to RNA sequencing (RIPseq) in AML cells. These analyses have identified and validated β-catenin association with several RNA targets involved in critical cellular processes including myeloid differentiation (CSF1, CEBPA, PML), autophagy (RPTOR, DAPK3, WDR6) and most abundantly the Wnt signalling pathway itself (AMER1, APC, BCL9L, LEF1, TCF7, AXIN2, RNF43). The existence of positive and negative feedback loops within Wnt signalling has been known for some time and are thought to be driven exclusively through TCF/LEF mediated transcriptional mechanisms since many are confirmed Wnt target genes. However, our new data raise the tantalising prospect that β-catenin may also govern Wnt signalling feedback loops post-transcriptionally, especially since many are not established transcriptional Wnt target genes (e.g., AMER1, APC, BCL9L). We are now investigating the functional impact of β-catenin on these bound RNAs, and the wider consequence of these interactions for the development of both normal haematopoietic stem cells (HSC) and AML LSC.

P-04

Mechanisms of asymmetric intercellular complex formation in planar polarity

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Planar cell polarity describes the polarisation of structures or cell behaviours within the plane of a tissue. One of the pathways which regulates this is the core planar polarity pathway, which utilises the Wnt signalling components Frizzled and Dishevelled. In the Drosophila pupal wing, core polarity proteins form intercellular complexes that link the polarity of neighbouring cells. Frizzled and Dishevelled localise to distal cell ends, and specify the distal localisation of a single trichome. Strabismus and Prickle localise proximally, while the atypical cadherin Flamingo localises both proximally and distally linking the two halves of the complex between neighbouring cells. The axis of asymmetry is thought to be guided by global tissue-specific cues, acting in conjunction with self-organising feedback interactions between the core proteins.
Attempts to understand the hierarchy of assembly of the asymmetric intercellular complexes have been complicated by the presence of feedback interactions, whereby individual complex components can have both stabilising and destabilising effects on other proteins in the complex. To address this, we are using Drosophila S2 cells to reconstruct asymmetric complex formation in the absence of feedback. We transfect Flamingo and other core components into different cell populations into Drosophila S2 cells, and then allow these cell populations to aggregate and form intercellular contacts. We have used this cell culture system to demonstrate that all complex components have a positive role in stabilising Flamingo at cell contacts. We then asked whether core planar polarity proteins alone are able to assemble into intrinsically asymmetric complexes. Modelling suggests that intrinsic asymmetry might be weak and even a mild bias would be amplified by feedback interactions to give strong asymmetry. Interestingly, our results suggest that in the absence of feedback there is a strong preference for the formation of asymmetric complexes over symmetric complexes. Furthermore, the presence of just Frizzled in one cell and Flamingo in both cells is sufficient to form an asymmetric complex without involvement of other proteins.
Current experiments are using both cell culture and pupal wings to determine the molecular basis for

P-05

The Tankyrase Inhibitor OM-153 Demonstrates Antitumor Efficacy and a Therapeutic Window in Mouse Models

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The catalytic enzymes tankyrase 1 and 2 (TNKS1/2) alter protein turnover by poly-ADP- ribosylating target proteins, which earmark them for degradation by the ubiquitin– proteasomal system. Prominent targets of the catalytic activity of TNKS1/2 include AXIN proteins, resulting in TNKS1/2 being attractive biotargets for addressing of oncogenic WNT/β-catenin signaling. Although several potent small molecules have been developed to inhibit TNKS1/2, there are currently no TNKS1/2 inhibitors available in clinical practice. The development of tankyrase inhibitors has mainly been disadvantaged by concerns over biotarget-dependent intestinal toxicity and a deficient therapeutic window. Here we show that the novel, potent, and selective 1,2,4-triazole–based TNKS1/2 inhibitor OM- 153 reduces WNT/β-catenin signaling and tumor progression in COLO 320DM colon carcinoma xenografts upon oral administration of 0.33–10 mg/kg twice daily. In addition, OM-153 potentiates anti–programmed cell death protein 1 (anti–PD-1) immune checkpoint inhibition and antitumor effect in a B16-F10 mouse melanoma model. A 28- day repeated dose mouse toxicity study documents body weight loss, intestinal damage, and tubular damage in the kidney after oral–twice daily administration of 100 mg/kg. In contrast, mice treated oral–twice daily with 10 mg/kg show an intact intestinal architecture and no atypical histopathologic changes in other organs. In addition, clinical biochemistry and hematologic analyses do not identify changes indicating substantial toxicity. The results demonstrate OM-153–mediated antitumor effects and a therapeutic window in a colon carcinoma mouse model ranging from 0.33 to at least 10 mg/kg, and provide a framework for using OM-153 for further preclinical evaluations.

P-06

Galphai2-induced conductin/axin2 condensates inhibit Wnt/beta-catenin signaling and suppress cancer growth

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Conductin/axin2 is a scaffold protein negatively regulating the pro-proliferative Wnt/beta- catenin signaling pathway. Accumulation of scaffold proteins in condensates frequently increases their activity, but whether condensation contributes to Wnt pathway inhibition by conductin remains unclear. Here, we show that the Galphai2 subunit of trimeric G- proteins induces conductin condensation by targeting a polymerization-inhibiting aggregon in its RGS domain, thereby promoting conductin-mediated beta-catenin degradation. Consistently, transient Galphai2 expression inhibited, whereas knockdown activated Wnt signaling via conductin. Colorectal cancers appear to evade Galphai2-induced Wnt pathway suppression by decreased Galphai2 expression and inactivating mutations, associated with shorter patient survival. Notably, the Galphai2-activating drug guanabenz inhibited Wnt signaling via conductin, consequently reducing colorectal cancer growth in vitro and in mouse models. In summary, we demonstrate Wnt pathway inhibition via Galphai2-triggered conductin condensation, suggesting a tumor suppressor function for Galphai2 in colorectal cancer, and pointing to the FDA-approved drug guanabenz for targeted cancer therapy.

P-07

Identification of response signatures for tankyrase inhibitor treatment in tumor cell lines

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Small-molecule tankyrase 1 and tankyrase 2 (TNKS1/2) inhibitors are effective antitumor agents in selected tumor cell lines and mouse models. Here, we characterized the response signatures and the in-depth mechanisms for the antiproliferative effect of tankyrase inhibition (TNKSi). The TNKS1/2-specific inhibitor G007-LK was used to screen 537 human tumor cell lines and a panel of particularly TNKSi-sensitive tumor cell lines was identified. Transcriptome, proteome, and bioinformatic analyses revealed the overall TNKSi-induced response signatures in the selected panel. TNKSi-mediated inhibition of wingless-type mammary tumor virus integration site/β-catenin, yes-associated protein 1 (YAP), and phosphatidylinositol-4,5-bisphosphate 3-kinase/AKT signaling was validated and correlated with lost expression of the key oncogene MYC and impaired cell growth. Moreover, we show that TNKSi induces accumulation of TNKS1/2-containing β-catenin degradasomes functioning as core complexes interacting with YAP and angiomotin proteins during attenuation of YAP signaling. These findings provide a contextual and mechanistic framework for using TNKSi in anticancer treatment that warrants further comprehensive preclinical and clinical evaluations.

P-08

Regulation of Neuronal Function by Wnt Signaling

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Wnt signaling seems to play an important role in the mature central nervous system (CNS) as evidenced by the association of genes regulating Wnt signaling pathways with several neuropathologies that exhibit a post developmental onset, including schizophrenia (Emamian et al., 2004; Mao et al., 2009), bipolar disorder (Klein and Melton, 1996; Zandi et al., 2008), and Alzheimer's disease, where deregulation of Wnt signaling has been proposed as an etiological cause (Caricasole et al., 2003). A major obstacle to understanding how Wnt signaling may contribute to these pathologies is poor understanding of the Wnt signaling pathways present in the postnatal brain. We have described a neuronal Wnt signaling cascade that upregulates trafficking of NMDA-type glutamate receptors consequently facilitating synaptic plasticity, a widely accepted cellular model of learning and memory necessary for proper circuit development and brain function. This pathway is initiated by Wnt5a and requires RoR2 to increase dendritic intracellular Ca2+, activate PKC and JNK kinases in dendrites, and increase trafficking of NMDARs to synapses (Cerpa et al. 2011, 2015; McQuate et al. 2017). Our data also indicate that Wnt5a, in a RoR2 and PLC-dependent manner, depolarizes the resting membrane potential of neurons enhancing their excitability. This effect seems to be mediated by regulation of the metabolism of membrane phospholipids (de la Cruz et al. 2022) that control plasma membrane conductances responsible for setting the resting membrane potential and intrinsic properties of neurons.

We now show that Wnt5a induces homodimerization of RoR2 and that this homodimerization is sufficient to trigger the cascade of events leading to increased trafficking of NMDARs toward synapses. Chemically induced homodimerization of RoR2 produces RoR2 autophosphorylation, activates PKC and JNK kinases, increases cytosolic Ca2+, and increases trafficking of NMDARs. The potential role of potential RoR2-Frizzled receptors heterodimers in regulating intrinsic properties of neurons will also be discussed.

This novel neuronal Wnt5a/RoR2 signaling cascade regulates the synaptic properties of neurons as well as their intrinsic properties, identifying Wnt signaling as an important factor in the regulation of neuronal functioning in the mature CNS.

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P-09

A panel of in-house pyridine-pyrazole derivatives as potential antibacterial and antituberculosis agents

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Compounds 6a, 6b, and 6f are novel pyridine-pyrazole derivatives, which were evaluated for antibacterial, antifungal, and antitubercular activities in vitro. Streptococcus pyogenes ATCC 14289, Staphylococcus aureus ATCC 9144, Pseudomonas aeruginosa ATCC 9027, Escherichia coli ATCC 25922, and Candida albicans ATCC 10231, were all susceptible to Compound 6b (2-[5-(4-chloro-phenyl)-4-cyano-2-(pyridine-4-carbonyl)-2H- pyrazol-3-ylamino]-N-phenyl acetamide). Finally, the current article presents clinically relevant pyridine-pyrazole compounds for further lead optimization research.

P-10

MAPKs and Wnt signaling in whole body regeneration of Hydra

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The freshwater polyp Hydra (Cnidaria) possesses an extraordinary whole body regeneration capacity. Genome, transcriptome and (phospho-) proteome approaches have revealed that most of signaling pathways and transcription factors are already present in this clade, which is the sister group of Bilateria. These pathways become activated not only during embryogenesis but also by injury signals that initiate regeneration. We found that Wnt signaling has a dual function in the regeneration process. In a first phase Wnt genes are activated generically as part of the generic injury response, in which mitogen-activated protein kinases (MAPKs) are initially activated via calcium and reactive oxygen species (ROS). The MAPKs, p38, c-Jun N-terminal kinases (JNKs) and extracellular signal-regulated kinases (ERK) are essential for Wnt activation in Hydra head and foot regenerates. The antagonism between the ERK signaling pathway and stress-induced MAPKs results in a balanced induction of apoptosis and mitosis. However, Wnt genes are activated by MAPK signaling rather than apoptosis. In a second phase, Wnt genes are activated in a position specific manner in order to pattern the missing body parts. Our data show that the early Wnt gene activity is differentially integrated with a stable, β-Catenin-based gradient along the primary body axis maintaining axial polarity and activating further Wnts in the regenerating head. We hypothesize that this mechanism is also present in vertebrates, but may be activated only to different degree at the level of early Wnt gene integration.

Petersen et al. https://doi.org:10.1093/molbev/msv079 Tursch et al. https://doi.org:10.1073/pnas.2204122119

P-11

Structural Characterization of Ternary Complex of Cachd1 with Frizzled-5 and LRP6

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CACHD1 (Cache Domain Containing 1) is an α2δ-Like Protein, that is required for neurons to acquire left-right asymmetric character. CACHD1 binding partners screen identified Frizzled-7, and further biochemical assays discovered Frizzled-5 and Frizzled-8 binding to CACHD1 at better affinity. Additionally, Wnt co-receptor LRP6 binds to CACHD1 at even higher affinity. We co-crystallised and determined the ternary complex structure of the soluble extra cellular domains of CACHD1:FZD5:LRP6(P3P4). CACHD1 ECD shows overall structural similarity to the α2δ1 auxiliary subunits of the voltage-gated Ca2+ channel Cav1.1. which contain four Cache domains and a Von Willebrand factor type A (VWA) domain. However, towards the C-terminal region of the ECD, a unique domain interacts with FZD5_CRD. The CACHD1 N-terminal two α helices interact with the LRP6_P3 propeller. Thus, CACHD1 serves as a crosslinking component in the ternary complex, independently binding to FZD5CRD and LRP6_P3P4. Structural superpositions show that the Cachd1 binding site on FZDCRD overlaps with the "thumb" and palmitoleic acid (PAM) lipid binding site, required for the receptor-ligand interaction with Wnt. The LRP6_P3 binding interface is overlapping DKK1-C binding surface. This suggests that Cachd1 may also compete with Wnt3a for binding to the LRP6P3 propeller. Taken together our biophysical and structural analyses showed that CACHD1 is a novel binder to both members of the FZD family of Wnt receptors and the LRP6 co-receptors.

P-12

The role of deubiquitination in regulating Wnt/PCP signaling

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Wnt/Planar cell polarity (Wnt/PCP) signaling is an evolutionarily conserved critical mechanism that controls the coordinated alignment of cell polarity across the tissue plane. Disruption of Wnt/PCP signaling underlies a variety of developmental defects, but its aberrant activation has been implicated in cancer malignancy. Vangl1 and Vangl2 are core components of Wnt/PCP signaling pathway. Their activities are tightly controlled by the posttranslational modification, such as phosphorylation and ubiquitination. Previously, we have identified the E3 ligase CUL3-KBTBD7 that can regulate Vangl through the endoplasmic reticulum-associated degradation pathway. Here, by screening deubiquitinating enzymes (DUBs) that have significant impacts on Vangl protein abundance, we identified two ubiquitin-specific peptidases, USP6 and USP32, which share high similarity in amino acid sequence. The ectopic expression of USP6/32 increased Vangl protein level, whereas the depletion of USP6/32 promoted Vangl degradation. Loss of USP32 in mice also led to a decrease of Vangl expression and thus an increase of the penetrance of loop tail phenotype in Vangl mutant mice. Interestingly, we found that Vangl1 and USP32 are both highly expressed in human pancreatic ductal adenocarcinoma (PDAC) tissues and their expression levels are strongly correlated among various PDAC cell lines. Moreover, USP32 depletion significantly inhibited the metastasis of PDAC cells to the lung by promoting Vangl degradation. Overall, our data reveal a new regulatory mechanism of Wnt/PCP signaling through USP6 and USP32- mediated Vangl deubiquitination, which play important roles in both development and disease.

P-13

Function and Regulation of Vangl2 phosphorylation at the C-terminal PDZ-binding motif

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Wnt/planar cell polarity (Wnt/PCP) signaling regulates polarized cell behaviors in a diverse array of developmental and physiological processes and is controlled by a set of highly conserved core PCP proteins, including Vangl2. Dysregulation of Wnt/PCP signaling underlies a variety of human diseases, such as neural tube defects, skeletal dysplasias, and cancers. Proper protein expression, subcellular localization, and protein- protein interactions are critically important for the functions of core PCP proteins in transducing Wnt/PCP signaling or establishing PCP asymmetry. Post-translational modifications of core PCP proteins are emerging as key regulatory mechanisms. Our previous studies showed that Vangl activities are controlled by ubiquitination and N- terminal phosphorylation in a Wnt-responsive manner. Here, we first generated and analyzed a specific Vangl2 knock-in mouse model that lacks the C-terminal PDZ-binding motif (PBM) and found that the PBM is essential for Vangl2 protein function during development. Interestingly, the PBM of Vangl2 is also phosphorylated at two conserved serine residues, which fine-tune the function of PBM by defining the interactome of Vangl2. We found that the PBM phosphorylation of Vangl2 is highly enriched in the brain, the loss of which leads to defects in synapse formation and fear-associated memory. In summary, our findings validate the functional significance of Vangl2 PBM in mammals and unravel a new tissue-specific post-translational regulatory mechanism in Wnt/PCP signaling.

P-14

Intercellular tension ensures robust morphogen gradient formation in zebrafish embryo

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Activity gradients of morphogen signaling, such as Wnt/β-catenin signaling, instructs cell identities to pattern developing tissues. Recently, our group found that, although cells with unfit Wnt/β-catenin activity frequently appear and generate noisy Wnt morphogen gradients, these unfit cells were apoptotically eliminated through communication with neighboring fit cells in zebrafish embryos. The Wnt/β-catenin activity gradients along the embryonic anterior–posterior axis generates quantitative gradient of membrane E- cadherin, and the unfit Wnt/β-catenin activity is converted into the unfit E-cadherin levels, which were sensed by neighboring fit cells and consequently triggers the unfit cell apoptosis to repair Wnt/β-catenin gradients (Akieda et al. 2019). However, how the unfit E-cadherin levels initiate the unfit cell apoptosis is still unclear.
Here, we show that the local change of intercellular tension through unfit E-cadherin levels to trigger the Wnt morphogen-collecting apoptosis in zebrafish embryo. Imaging analyses of cortical actomyosin and the RhoA activity revealed that Wnt/β-catenin gradients generate intercellular tension gradients by forming E-cadherin gradients throughout the embryonic tissue. In unfit Wnt/β-catenin cells, the activities of local tension were changed depending on Wnt/β-catenin activity and E-cadherin levels.
Apoptosis of Wnt/β-catenin-unfit cells was suppressed by modifying the intercellular tension. In addition, cells overexpressing the constitutive active α-catenin, a mechanotransducer connecting E-cadherin and actin cytoskeleton, efficiently underwent apoptosis in the anterior region where the Wnt/β-catenin activity and intercellular tension was low. In conclusion, our results suggest that Wnt/β-catenin activity gradients are converted into the gradients of intercellular tension via E-cadherin and actomyosin, and neighboring fit cells would sense this change of mechanical force and initiate cell competition. This mechanism of sensing unfit cells via the difference of intercellular tension is expected to be used ubiquitously in other tissues to maintain tissue robustness.

P-15

Uncovering the context-dependent roles of WNT signalling governing the maintenance and differentiation of human naive embryonic stem cells

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Authentic human naïve embryonic stem (HNES) cells cultured routinely in defined conditions feature a transcriptomic signature that closely recapitulates the preimplantation epiblast of the early human blastocyst. These cells are routinely cultured in a cocktail containing PD0325901 (a potent FGF-MEK-ERK inhibitor), XAV939 (an inhibitor of TANKYRASE activity), Go6983 (a pan-PKC signalling inhibitor), and Leukaemia inhibitory factor or LIF, collectively termed PXGL). In comparison to mouse embryonic stem cells, HNES cells are inherently unstable and readily differentiate into both trophoblast- and hypoblast-like cells upon induction with specific signalling cues.
One significant difference between mouse and human naïve ES cells is their requirement and responsiveness to WNT signalling. HNES cells cannot be stably propagated in traditional 2i+LIF culture conditions used for mouse ES cells.
To uncover the context-dependent WNT signalling mechanisms in human naïve ES cells, we carried out a multiparametric, high-throughput cell signalling immunofluorescence screen to modulate seven different signalling cascades. We reveal that active WNT/β- Catenin signalling is the critically deterministic factor in differentiating human naïve ES cells towards hypoblast-like cells. This is facilitated through active NODAL and FGF signalling and blocking either of these pathways perturbs differentiation. Interestingly, when WNT signalling is blocked using either DKK1 or IWP-L6, no other signalling cascade can effectively compensate. However, when WNT/β-Catenin is activated alongside the simultaneous blockade of both FGF-MEK-ERK and NODAL activity, differentiation is swiftly diverted and HNES cells adopt a trophoblast-like signature.
Moreover, switching from the activation to inhibition of WNT signalling does not adversely affect trophoblast-like cell differentiation.
We found that HNES cells can withstand elevated and sustained levels of WNT/β- Catenin signalling in the presence of potent FGF receptor (FGFR) but not MEK-ERK inhibitors, exhibiting significantly higher expression of key naïve pluripotent transcription factors. This suggests that the human naïve pluripotent transcription factor network has intrinsic mechanisms to control the levels of WNT signalling. Finally, we used immunofluorescent microscopy to examine the expression of all four TCF/LEF transcription factors in human pluripotent, trophoblast- and hypoblast-like cell populations.

P-16

Identifying specificity of Wnt-RTK-binding

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Wnt proteins play a crucial role in the development and cell homeostasis by activating essential signaling pathways like the beta-catenin-dependent gene transcription pathway. Wnts bind to the cysteine-rich domain of Frizzleds and LRP5/6 co-receptors, bringing them together to initiate a cascade of events resulting in beta-catenin translocation to the nucleus. Wnts are also thought to bind and recruit other co-receptors such as Ryk, Ror1, Ror2, and Ptk7 to the FZD/Wnt complex to activate less well-understood signaling pathways. These co-receptors belong to the receptor-tyrosine-kinase (RTK) family, but are characterized by having inactive intracellular kinase domains (pseudokinases). Despite their lack of catalytic activity, these RTKs are associated with various developmental defects and diseases in the skeletal, cardiovascular and respiratory systems. Overexpression of these receptors is also frequently correlated with tumor progression and metastasis. Nonetheless, these Wnt-binding RTKs are understudied, and their specific functions are enigmatic. For example, it is not known which of the 19 human Wnts can bind and recruit a particular RTK-co-receptor (although Wnt5 is frequently implicated) or which of the ten FZDs can participate in formation of Wnt/FZD/co-receptor complexes. Based on preliminary data from our lab and published results, we hypothesize that a small region within Wnts, called the Wnt-linker, is responsible for co-receptor binding. We are using in vitro surface-plasmon-resonance (SPR) binding studies to investigate which Wnt-linkers binds to each co-receptor. In addition, we are using cellular assays with co-receptor/LRP5 chimeras to analyze how the trimeric complex is formed between specific Wnts, FZDs, and co-receptors. We hope to show that specific binding sites on Wnts are responsible for binding to co-receptors and identify which Wnt proteins bind to individual co-receptors. In addition, we identify the specific FZDs involved in Wnt/FZD/co-receptor complexes in cellular assays. This work will add crucial information to the Wnt field by identifying the Wnt/co-receptor binding sites and adding a new framework of biochemically possible Wnt/FZD/co-receptor interactions.

P-17

Retracted

P-18

WNT10A variant in Japanese nonsyndromic oligodontia case

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The number of missing teeth classifies congenital tooth agenesis (TA). Hypodontia is defined as five or fewer missing teeth excluding the wisdom teeth, and oligodontia as six or more teeth. Congenital TA is the most common anomaly in humans, with a frequency of 6.8% (95% confidence interval: 6.1–7.7%) for hypodontia, and 0.1% (95% confidence interval: 0.04–0.3%) for oligodontia in Japanese. We have previously demonstrated that the sibling recurrence risk ratio of oligodontia is 43.8%, and thus suggested that oligodontia would inherit with dominant style in most cases. Robust tooth development requires a variety of growth factors produced by the oral ectodermal epithelium, such as FGFs, BMP, and WNTs. WNT10A is reported as the most frequent cause of human non-syndromic TA in Japan.
Genetic causes are still unknown in more than 50% of TA cases. Therefore, we have performed whole exome analysis of Japanese TA patients to identify causative gene of congenital TA.
The patient was a 21-year-old female with nine missing teeth, without systemic abnormality, including the crown morphology of the extant tooth and the jawbone. Then, we performed whole exome analysis to explore the genetic cause of the case. A novel heterozygous WNT10A gene variant was identified in the patient. To investigate the biological activity of the p.Lys364* variant of WNT10A, we performed TOP-Flush assay with a stable transfectant of PC3 cells expressing a luciferase reporter gene with a TCF- LEF response element, and following expression vectors; human LRP6 and human FRIZZLED4.
In this study, we identified a novel heterozygous WNT10A gene variant, c.1090A>T in the patient, but not in other members of the family. a novel non-sense variant of WNT10A (c.1090A>T) as a cause of tooth agenesis, which produces a C-terminal truncated gene product, p.Lys364*. The C-terminal truncated p.Lys364* of WNT10A lost the WNT/beta- catenin signaling activity. Besides the assay, we detected the degraded immunopositive bands of the variant, suggesting that the truncated WNT10A would be more instable than wild-type in PC3 cells.

P-19

Exploring the Regulatory Mechanisms of the Wnt Diffusion with a Synthetic Notch-Based System

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Wnt is one of the secreted proteins called morphogen, providing a concentration gradient which works as positional information and playing essential roles in embryogenesis and homeostasis. However, it is still unclear that how the distribution and dynamics of Wnt are precisely controlled in intercellular spaces. Biochemical analyses have revealed that Wnt-binding factors such as heparan sulfate proteoglycans (HSPGs) and secreted frizzled-related protein (sFRP) families can regulate the Wnt diffusion and its activity. Wnt lipidation, serum proteins and extracellular matrix have been also considered to be involved in the regulation of Wnt diffusion. Despite the understanding of molecular players in the Wnt regulation, it is difficult to separately analyze the roles of these factors in the Wnt diffusion one by one in vivo.
Here, we established an in vitro model system to analyze Wnt localization using a synthetic Notch-based protein detection system. Synthetic Notch (synNotch) system, an engineered Notch receptor, can recognize a user-defined ligand and output the expression of user-defined target genes. Importantly, this system can recognize a ligand binding to cell surface, but not a soluble ligand, allowing us to distinguish between cell surface-binding form and soluble form of ligands. We prepared GFP-tagged Wnt3a (GFP-Wnt)-secreting cells and its receiver cells that express anti-GFP synNotch system to detect cell surface-binding GFP-Wnt. Using these cells, we explored what kind of factors can bind Wnt on cell surface and analyze the Wnt diffusion on receiver cells in the presence of Wnt-binding factors. In this poster, I will introduce our findings on the Wnt diffusion process using our model system.

P-20

Super-resolution microscopy localizes endogenous Dvl2 to Wnt signaling-responsive biomolecular condensates

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During organismal development, homeostasis, and disease, Dishevelled (Dvl) proteins act as key signaling factors in beta-catenin-dependent and -independent Wnt pathways. While the importance of Dvl for Wnt signaling transmission has been demonstrated in many organisms, our mechanistic understanding is still limited. Previous studies using overexpressed proteins showed Dvl localization to large, punctate-like cytoplasmic structures that are dependent on its DIX domain.

To analyze Dvl's role in Wnt signaling, we genome-engineered an endogenously expressed Dvl2 protein tagged with an mEos3.2 fluorescent protein for super-resolution imaging. In our recently published study, we demonstrate the functionality and specificity of the fusion protein in beta-catenin-dependent and -independent signaling using multiple independent assays. We performed live-cell imaging of Dvl2 to analyze the dynamic formation of the supra-molecular cytoplasmic Dvl2_mEos3.2 condensates. While overexpression of Dvl2_mEos3.2 mimics the previously reported formation of abundant large "puncta", supra-molecular condensate formation at physiological protein levels is only observed in a subset of cells with approx. one per cell. We show that in these condensates, Dvl2 co-localizes with other Wnt pathway components, such as APC and Axin1, at gamma-tubulin and CEP164-positive centrosomal structures. We demonstrate that the localization of Dvl2 to these condensates is Wnt-dependent.
Single-molecule localization microscopy using PALM of mEos3.2 in combination with DNA-PAINT reveals the distinct organization and repetitive patterns of these condensates at the centrosome in a cell cycle-dependent manner. Our results indicate that the localization of Dvl2 in supra-molecular condensates is coordinated dynamically and dependent on cell state and Wnt signaling levels.

To identify general mechanisms of condensate formation, we performed a high-content small molecule screen investigating Dvl2 condensate regulation. This high-throughput assay identified several kinase inhibitors modulating Dvl2 condensate formation. Further experiments are ongoing to specify the interplay of Wnt activity, the temporal-spatial response to Wnt signals, and the co-condensation of various other signaling components.

Taken together, our studies highlight the importance and regulation of biomolecular condensates in the Wnt pathways and their role for proper mitotic progression at single- molecule resolution.

P-21

SZN-413, a FZD4-specific WNT Agonist, as a Potential Novel Therapeutic for the Treatment of Diabetic Retinopathy

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WNT proteins play crucial roles in the maintenance and self-renewal of stem cells in a variety of tissues. While the WNT pathway is critical to injury- and disease-induced tissue repair, technical challenges inherent to WNTs, such as lipid modification and lack of receptor selectivity, are major obstacles to developing recombinant WNTs as therapeutics. Here, we overcame these challenges and developed a FZD4-specific agonist and explored its potential use in the treatment of diabetic retinopathy.

VEGF is an important factor in the development of wet age-related macular degeneration (wAMD), proliferative diabetic retinopathy (DR) and diabetic macular edema (DME), inducing pathological neovascularization and altering retinal capillary permeability. Anti- VEGF agents control ocular neovascularization, leakage and intraocular inflammation and are currently the standard of care in the treatment of DME and wAMD. However, anti-VEGF therapy does not induce reperfusion of ischemic retinal areas. Therefore, there remains a high unmet need for therapies with new mechanisms of action to achieve clinically meaningful retinal reperfusion of ischemic areas in DR, and reduce the ensuing production of factors contributing to vascular leakage and pathological angiogenesis.

Human mutations in genes encoding either receptors (FZD4, LRP5, TSPAN12) or the ligand Norrin (NDP), involved in Wnt signaling, result in a variety of inherited vitreoretinopathies. Given the key function of FZD4-mediated Wnt signaling in vascular development, we generated a novel FZD4-specific agonist, SZN-413, and observed that it inhibited pathological neovascularization in a mouse model of oxygen-induced retinopathy (OIR). Furthermore, SZN-413 induced proper retinal vessel regrowth, reduced avascular area, suggesting the potential to re-perfuse the ischemic retina in DR. In a separate model, the FZD4-specific agonist also prevented the retinal leakage induced by VEGF in rabbit eyes. The current findings suggest that activation of FZD4 signaling can simultaneously address the retinal non-perfusion and leakage characteristics of DR pathology. We believe that this novel mechanism of action of our Norrin mimetic opens new possibilities to treat diabetic and potentially other retinopathies. Furthermore, because SZN-413 regenerates normal retinal vasculature in ischemic areas of the retina, it is possible that the durability of this mechanism alone or in combination with anti-VEGF may be extended.

P-22

Wnt-Ror2 signaling in mesenchymal progenitor cells contributes to maintenance and adipogenic degeneration of the skeletal muscles

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It has recently been shown that mesenchymal progenitor cells (MPCs), one of the skeletal muscle tissue-specific stem cells, play critical roles in regulating maintenance of the skeletal muscles. On the other hand, it is well known that MPCs are source of aberrant intramuscular adipose tissue (IMAT), one of the pathological characteristics of sarcopenia and muscular dystrophy. Although MPCs contribute to both maintenance and pathogenesis of skeletal muscles, the molecular mechanism regulating functions of MPCs remains unclear.
The Ror-family receptors, consisting of Ror1 and Ror2, play critical roles in developmental morphogenesis, tissue regeneration and cancer progression by acting as a receptor(s) for Wnt ligands, including Wnt5a. We have previously shown that Ror1 is expressed selectively in satellite cells (SCs), another type of skeletal muscle tissue- specific stem cells, and plays crucial roles in regulating proliferation of SCs during regeneration of the skeletal muscles. However, the role of Ror2 in skeletal muscle is largely unknown.
Here, we show that Ror2 is expressed selectively in MPCs, and regulates proliferation, adipogenic differentiation and cellular senescence of MPCs. Indeed, MPCs-specific Ror2 conditional knockout (Ror2 cKO) mice exhibit atrophy of the skeletal muscles, and regenerative ability and IMAT of the skeletal muscles after injection with glycerol, which can induce IMAT along with injury, were suppressed in Ror2 cKO mice. We also found that expression of Wnt5b (the closest relative of Wnt5a), but not Wnt5a, was increased in the tibialis anterior muscles after glycerol injection. Wnt5b-Ror2 signaling has been shown to promote proliferation of MPCs through activation of p38. Since it has been reported that drastic increase of MPCs induces IMAT, it can be envisaged that Wnt5b- Ror2 signaling leads to IMAT by promoting aberrant proliferation of MPCs. Interestingly, Wnt11, another possible ligand of Ror2, is also involved in the regulation of MPCs. It was found that Wnt11-Ror2 signaling might play crucial roles in proliferation, adipogenic differentiation and cellular senescence of MPCs. Collectively, these findings indicate that Wnt11- and/or Wnt5b-Ror2 signaling in MPCs are critical determinants for maintenance and/or IMAT in the skeletal muscles.

P-23

Myocardin-related transcription factors regulate vertebrate gastrulation via interaction with Wnt signaling

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Myocardin-related transcription factors (Mrtfs), also known as Mkl or MAL, associate with serum response factor (Srf) to modulate transcription in response to actin dynamics; however, the functions of Mrtfs in early development remain to be clarified. In Xenopus embryos, both activation and inhibition of Mrtf/Srf-dependent transcription interfered with gastrulation and neurulation, indicating an essential role of Mrtf/Srf signaling in morphogenesis. RNA sequencing revealed candidate Srf-dependent target genes encoding actin and other cytoskeletal proteins that may be responsible for the observed defects in Mrtf-expressing early embryos. In addition, Wnt3a, Wnt5a, Wnt7a and Wnt11 were identified as potential Mrtf target genes. Functional interactions of Wnt signaling with Mrtf were assessed using Mrtf/SRF luciferase reporter and GFP-Mrtf that localizes to the nucleus upon activation. Both canonical and non-canonical Wnts modestly upregulated Mrtf/SRF reporter and promoted Mrtf nuclear localization, while dominant negative Wnt11 strongly inhibited the reporter and nuclear translocation of Mrtf. The results suggest that Mrtf and Wnts functionally interact to control vertebrate gastrulation.

P-24

Novel small molecule agonist of GFRA1, JW0061 facilitates hair regeneration by activation of WNT signaling in hair follicular stem cells.

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The WNT signaling is playing a critical role in the skin development and hair follicle formation during embryogenesis, and is required for the differentiation of skin stem cells into hair follicles. In particular, Dermal Papilla (DP) cells as a dermal organizing center are well-known for providing inductive signals to promote growth and differentiation of surrounding cells during hair growth, which mainly relies on an intracellular WNT signalling. Therefore, we have screened own compound library to identify and optimize small molecules which can activate WNT signalling in hair stem cells, expecting them to be a novel therapeutic agent for androgenic alopecia. Here, we demonstrate the JW0061, small-molecule WNT activator that increases amount of nuclear β-catenin level, stimulating WNT signaling in various cell types including DP cells. Using a nematic protein organization technique, we identified that JW0061 is directly binds to GFRA1 from DP cell extracts, and validated the specific binding via surface plasmon resonance assay (Kd: 2.5uM). Furthermore, we found that JW0061 acts as an agonist on GFRA1 to activate GFRA1-RET signaling cascades, followed by WNT activation through β-catenin stabilization. JW0061 eventually promotes DP cell proliferation via increased VEGFR2 expression mediated by WNT signaling. We also demonstrated that JW0061 induces anagen phase in hair cycle and hair growth that are highly correlated with WNT activation in various efficacy models. In conclusion, JW0061 has been validated in various efficacy and safety models (not shown), and is currently under GLP toxicology studies.
Considering limited therapeutic options for androgenic alopecia, we propose the JW0061 as a novel therapeutic option that is complimentary and alternative to the standard of care.

P-25

Mining the Wnt signalling responsive surfaceome for novel drug targets in acute myeloid Leukaemia (AML)

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Acute myeloid leukemia (AML) is an aggressive malignancy of haematopoietic stem/progenitor cells with a poor prognosis. New targeted therapies are urgently required to induce long term remissions and improve survival. One such molecular target is the Wnt/β-catenin signalling pathway, long known to be frequently deregulated in AML leading to the emergence, maintenance, and drug resistance of leukaemia stem cells (LSC) in AML. Attempts to pharmacologically disrupt this pathway internally through small molecule inhibitors has achieved limited success to date, and instead targeting Wnt signalling from the cell exterior may hold promise for novel biomarker and immunotherapy design. Several Wnt target genes are known to be membrane proteins, however, a detailed characterisation of the Wnt signalling responsive surfaceome has not been performed in any cell type. To identify novel Wnt signalling regulated cell surface antigen in AML cells, we performed cell surface capture (CSC) from two AML cells (NB4 and KG1) harbouring β-catenin knockdown (using lentivirally delivered shRNA) coupled to tandem mass tag (TMT) quantitative mass spectrometry (MS). Differential expression analysis of MS data identified 265 significantly altered proteins (minimum of 2 unique peptides, > 1.3-fold change; $P < 0.05$), shown by gene ontology (GO) analyses as membrane localised proteins and functionally associated with cancer hallmark events. Validation of putative differentially regulated surface proteins by western blot analyses confirmed downregulation of CD300A in response to β-catenin repression indicating it may be positively regulated through Wnt signalling. Furthermore, stabilisation of β- catenin using the GSK3β inhibitor CHIR99021 increased CD300A expression in KG1 and OCI-AML3 cell lines. CD300A is known to be overexpressed in AML, confers inferior patient survival and has been associated with suppressing immune responses. in summary, this study represents the first attempt to characterise the Wnt signalling responsive surfaceome in myeloid cell line and has identified CD300A as a potential target of β-catenin. Work is now underway to understand how CD300A is expressed in normal myelopoiesis, how it contributes to leukaemogenecity and if it has any reciprocal relationship with Wnt signalling.

P-26

A conserved distal chromatin hub controls Wnt4 gene expression in the mammary gland

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WNT signaling is crucial for morphogenesis and maintenance of multiple tissues in our body. Different Wnt genes are expressed in well-defined, tissue-specific domains throughout embryonic development as well as postnatally. Yet, how Wnt gene expression itself is controlled, remains virtually unknown. Here, we use the unique features of the Wnt4 gene to study tissue specific Wnt gene regulation. In the mammary gland, Wnt4 expression is gradually induced during postnatal development and robustly maintained in distinct mammary cell types in the adult. During pregnancy, Wnt4 expression, regulated by progesterone, is essential for ductal side branching. Based on predictions from Hi-C data, Wnt4 is the only protein coding gene in its topologically associating domain (TAD). We constructed a comprehensive map of the regulatory DNA landscape for the murine and human Wnt4 gene. Spanning ~370 kb, we have identified 17 putative cis-acting regulatory sequences (CRSs) for mouse Wnt4. Using luciferase and dCas9-VPR assays we have demonstrated that a subset of these CRSs is functionally active and able to activate Wnt4 expression in mammary cells. Additionally, we show that a subset of these elements is progesterone responsive. One of our identified CRSs, CRS6, is likely to play an important role in regulating Wnt4 expression, as we have shown that it can interact and form a (CTCF mediated) loop with the promotor of Wnt4 as well as with other CRSs. Moreover, CRS6 harbors the transcription start site of an annotated LncRNA, gm13003, the expression of which correlates with Wnt4 expression. We have shown that deleting (parts of) CRS6 led to significant reduction of Wnt4 and gm13003 expression in mouse mammary cells. In a parallel approach, we have identified 9 CRSs in human breast cells, which are partly conserved and partly non-conserved between mouse and human. In human breast cells, the (CTCF mediated) loop between CRS6 and the Wnt4 promotor is conserved. However, the human CRS6 lacks an annotated LncRNA. Furthermore, we have identified the progesterone receptor and grainy head like 2 as transcription factors associated with Wnt4 expression. Progesterone stimulation, GRHL2 overexpression and knockdown, and combinations these experiments revealed that PR and GRHL2 are important regulators of Wnt4 expression in both mouse and human cells, further indicating a conserved regulatory mechanism of Wnt4. We propose a model where CRS6 acts as a conserved distal chromatin hub that serves to bring other CRSs as well as the Wnt4 promotor in close physical proximity. In this hub, transcription factors such as PR and GRHL2, attached to (multiple) different CRSs, come together to control tissue specific Wnt4 expression.

P-27

Novel Wnt signal modulators and their applications in research and therapeutic development

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WNTs are highly conserved, secreted lipoglycoproteins that regulate stem/progenitor cell function and play a crucial role in tissue development, homeostasis, injury repair and regeneration. Nineteen mammalian WNTs, 10 frizzled receptors (FZD1-10), and two co- receptors, low-density lipoprotein receptor-related proteins (LRP5 and LRP6) have been identified. Elucidating the functions of individual WNTs and FZDs has been hampered by difficulties in expressing these proteins and lack of receptor selectivity. R-spondins (RSPO1-4) are a family of ligands that stabilizes WNT receptors and amplifies Wnt signaling through binding to the two E3 ligases [zinc and ring finger 3 (ZNRF3) and ring finger protein 43 (RNF43)] and the co-receptors leucine-rich repeat-containing G-protein coupled receptors 4-6 (LGR4-6). One major challenge to exploring RSPO for tissue repair and regeneration is limiting RSPO effects to the specific tissue(s) of interest, as LGR4-6 and ZNRF3/RNF43 are widely expressed in various tissues.

We have solved the challenges associated with WNTs and RSPOs through the development of three novel classes of targeted antibodies to replicate WNT and RSPO functions. Our WNT mimetic platform is a tetravalent, bispecific, full antibody-based platform that can target a specific FZD and LRP pair. This set of tool molecules allows the interrogation of individual FZDs and LRPs in a particular tissue in vitro and in vivo during homeostasis and disease. Our RSPO mimetic platform can direct RSPO function to a specific cell type of interest, allowing the study of different RSPO mimetics in a cell/tissue specific manner. And lastly, we have also developed a novel platform called “superagonist” that equals the combination of WNT and RSPO. This set of tool molecules allows the study of specific receptors, cell types, and different signaling strengths during homeostasis and injury repair of tissues of interest in vitro and in vivo.

These novel WNT modulating platforms further allow us to develop novel therapeutics to regenerate healthy tissue, improve organ function in indications with high unmet need.
Phase 1 clinical studies have been initiated in healthy volunteers for our lead candidates, SZN-043 and SZN-1326, in development for AH and IBD. The impact of other molecules in lung and various ocular indications will also be presented.

P-28

The ubiquitin ligase HUWE1 potentiates WNT/beta-catenin signaling through a mechanism mediated by APC, AXIN1 and GSK3A/B that is independent from the control of CTNNB1 stability

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HUWE1 is a HECT domain-containing ubiquitin ligase that regulates dozens of cellular processes. Through comparative genetic suppressor screens in haploid human cells, we previously identified HUWE1 as a potent positive regulator of the WNT/beta-catenin pathway. Surprisingly, the contribution of HUWE1 to signaling was only evident when the pathway was hyperactivated by loss of the beta-catenin (CTNNB1) destruction complex (DC) kinase casein kinase 1alpha (CSNK1A1), but not by loss of the DC scaffold adenomatous polyposis coli (APC). This result was striking because CSNK1A1 and APC have a shared function in the DC controlling CTNNB1 stability, and therefore loss of either protein would be expected to have the same effect on WNT signaling. However, the differential contribution of HUWE1 to WNT/beta-catenin signaling in cells lacking CSNK1A1 or APC suggested additional, unique functions for CSNK1A1 or APC. Genetic interaction analyses and a novel 'suppressor of suppressor' genetic screen revealed the reason for these paradoxical results: a subset of CTNNB1 DC components, including AXIN1, APC and glycogen synthase kinase 3alpha or beta (GSK3A/B), but excluding CSNK1A1 and AXIN2, are required for HUWE1 to potentiate WNT/beta-catenin signaling. Furthermore, HUWE1 promotes WNT/beta-catenin signaling through at least two distinct mechanisms, one that increases CTNNB1 protein abundance, and another that is independent from the control of CTNNB1 stability. These results reveal a new role for AXIN1, APC and GSK3A/B in mediating WNT/beta-catenin signaling through HUWE1, distinct from their established activity controlling CTNNB1 stability as part of the DC.

P-29

The oncogenic transcription factor FOXQ1 is differential regulator of Wnt target genes

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The forkhead box transcription factor FOXQ1 is aberrantly induced in various cancers and particularly contributes to colorectal cancer progression and metastasis. It has been suggested that FOXQ1 exacerbates cancer in part by activating the oncogenic Wnt/β-catenin signalling pathway. However, the mode of action of FOXQ1 in the Wnt pathway remains largely enigmatic. Here, we report that FOXQ1 acts primarily via the transcriptional regulation of Wnt target gene expression both in a β-catenin-TCF/LEF dependent and independent manner. On the one hand, results from functional assays indicate that FOXQ1 promotes β-catenin activity downstream of the destruction complex to increase TCF/LEF-dependent gene expression. Our results show that FOXQ1 can physically associate with and boost the activity of the Wnt transcriptional complex. On the other hand, we use gene-edited cell lines, proximity proteomics, and chromatin immunoprecipitation to show that FOXQ1 differentially controls the expression of major Wnt target genes, most likely by directly binding to their promoters and recruiting many of the same co-factors as TCF/LEFs. In addition, RNA-sequencing results in intestinal cancer cell lines confirmed that FOXQ1 regulates the transcription of major Wnt genes and differentially shapes their expression in synergy with an active Wnt pathway.
Altogether, these results suggest that FOXQ1 specifies the Wnt transcriptional response through two converging modes of action, which may be of particular importance for Wnt- driven colorectal cancer.

P-30

Exosome-mediated secretion of Wnt3a is promoted by Wnt-binding protein sFRP2

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During the development of multicellular organisms, cell-to-cell communication mediated by cell signaling molecules controls cell proliferation and differentiation. Wnt, a diffusible ligands, regulates these processes by activating signal transduction in receiving cells through its binding to Frizzled receptors. Many evidences have been reported that Wnt proteins are loaded on exosomes to travel in the extracellular environment. However, it still remains unclear how Wnt could be secreted and incorporated on exosomes to activate signaling pathway in long distance.
Here, we show that the amount of secreted Wnt3a loaded on exosomes is significantly inceased, specifically in the present of sFRP2. Co-culture of Wnt3a-expressing cells with sFRP2-expressing cells or treatment of several different cells with the conditioned meidum of these cells significantly enhanced exosome-mediated secretion of Wnt3a. In contrast, sFRP3, another member of the sFRP family, did not cause such an increase. Futhermore, sFRP2 specifically inceases Wnt3a attachment to cells when cells are treated with Wnt3a conditioned medium.

To understand why the effect on Wnt3a is specific to sFRP2, we next analyzed binding of Wnt3a to different sFRPs in conditioned media. Analysis with analytical ultracentrifugation with flouresecent detection system showed that secreted Wnt3a specifically forms a complex only with sFRP2 but not other sFRP members, such as sFRP3, sFRP4, suggesting that bining of Wnt3a to sFPR2 is specific and required for the secretion of Wnt3a-containing exosomes
Finally, to understand the mechanism of sFRP2-mediated enhancement of loading of Wnt3a on exosomes, we are examing requirement of recepotr and co-receptor of Wn3a. Until now, we found that, in LRP5/6 knockout MDCK cells, the level of exosome-loaded Wnt3a is not effected, despite inhibition in Wnt-β-catenin signaling.

These results suggest that sFRP2 acts as an effector to promote binding of Wnt3a ligand to cell membrane proteins, probably by forming a complex with Wnt3a, resulting in an increase in Wnt3a endocytosis and re-secretion by exosomes for further long-range transmission.

P-31

Wnt5a-Ror2 signaling protects astrocytes from hemin-induced cytotoxicity through activating p62-Nrf2 axis in the injured brains.

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The Ror-family proteins, Ror1 and Ror2, have been shown to act as receptors or co- receptors for Wnt5a, and play essential roles in regulating cell polarity, migration, proliferation and differentiation during developmental morphogenesis, tissue-/organo- genesis and regeneration of adult tissues following injury. We have shown that expression levels of Ror2 are up-regulated markedly in some populations of astrocytes in the adult brains following injuries, including traumatic brain injury, ischemic stroke, and intracerebral hemorrhage. Astrocytes, a type of glial cells in the central nervous system (CNS), are highly responsive cells that change their characteristics in response to CNS injuries. These astrocytes, termed reactive astrocytes, play critical roles in promoting recovery of the CNS from their pathological conditions. However, it is unclear how expression of Ror2 is up-regulated in the reactive astrocytes in the injured brains, and whether or not its function in reactive astrocytes is associated with tissue repair.

We found that bFGF, IL-1β and TNF-α, whose expressions are up-regulated at the lesion sites within 1 day post injury, act synergistically to up-regulate expression of Ror2 in cultured astrocytes. RNA-Seq analysis revealed that expression of heme oxygenase-1 (HO-1) is also up-regulated drastically in astrocytes stimulated with bFGF, IL-1β and TNF-α in a manner dependent on increased expression of Ror2. It has been well known that expression of HO-1 is induced by the transcription factor Nrf2, which can be activated by oxidative stress. In fact, nuclear accumulation of Nrf2 was increased gradually in astrocytes following stimulation with bFGF, IL-1β and TNF-α, while reactive oxygen species (ROS) production was decreased in these cells compared with untreated cells. These results suggest that Ror2 might mediate activation of Nrf2 in a manner independent of oxidative stress. Furthermore, we provide evidence indicating that Wnt5a-Ror2 signaling increases expression level of p62 protein that can prevent ubiquitination of Nrf2 through the interaction with Keap1, thereby promoting nuclear accumulation of Nrf2. p62 forms liquid droplets that serve as a platform for autophagosome formation as well as Nrf2 activation. Therefore, autophagic degradation of the p62-liquid droplets results in a reduction of Nrf2 activity. Analyses using the lysosome inhibitor Bafilomycin A1 revealed that autophagy is activated strongly in astrocytes stimulated with bFGF, IL-1β and TNF-α and that Wnt5a-Ror2 signaling might promote the formation of p62-liquid droplets, with delaying their autophagic degradation irrespective of the autophagy flux.

In the injured brains, disruption of blood-brain barrier (BBB) results in secondary brain damages partly due to the toxic effects of hemin, a breakdown product of hemoglobin. It has been shown that Nrf2 acts to resist against hemin-induced cytotoxicity by inducing expression of its target genes, including HO-1. Indeed, we found that the hemin cytotoxicity is relieved in cultured astrocytes stimulated with bFGF, IL-1β and TNF-α, compared to unstimulated cells, and that suppressed expression of Ror2 or treatment with recombinant Wnt5a results in increased or decreased hemin cytotoxicity in these astrocytes, respectively. Our findings indicate that Wnt5a-Ror2 signaling might play a critical role in protecting reactive astrocytes from hemin-induced cytotoxicity by activating p62-Nrf2 axis in the injured brains. Therefore, Wnt5a-Ror2 signaling might be a potential therapeutic target to prevent secondary brain damages associated with BBB breakdown following various brain injuries.

P-32

Evolution of TCF/LEF diversity in expression and function in development

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The molecular mechanisms of the canonical Wnt/β-catenin pathway are remarkably conserved among invertebrate and vertebrate animals, including nuclear β-catenin binding to TCF/LEF DNA-binding proteins to regulate gene expression. However, in vertebrates, there is much more variety of TCF/LEF genes and isoforms. We are investigating this variety at the core of vertebrate Wnt signalling mechanisms by studying the evolution, expression, and function of vertebrate TCF/LEF genes and isoforms.

To explore the evolution of vertebrate TCF/LEF genes we compared the genomes of chordates. The usually four TCF/LEF genes in vertebrates (TCF7, LEF1, TCF7L1 and TCF7L2) may derive from two rounds of whole genome duplications from a single TCF gene conserved in invertebrates. In vertebrates, some TCF/LEF genes can generate a variety of isoforms by using alternative promoters and splicing sites, which results in the production of protein isoforms lacking functional domains, such as the β-catenin binding domain or the auxiliary DNA-binding domain (C-clamp). Our analysis suggests that the ancestral vertebrate may have possessed a TCF gene that was most similar to TCF7L2 and that the acquisition of complexity, including alternative transcription start sites and C-clamp-lacking variants, may be an early vertebrate-specific evolutionary innovation.

Vertebrate TCF/LEF genes are known to be differentially expressed in development, however the expression and function of multiple isoforms in development are yet to be characterized. Using Xenopus tropicalis as vertebrate model system, we are investigating vertebrate-specific TCF/LEF isoform function during embryonic development. Expression analyses revealed that in most cases there is no stage-specific isoform attribution, suggesting that their different activity may be more tissue-specific. To understand the molecular activity of these isoforms we also started functional experiments. We have successfully knocked out TCF7 in F0 embryos using CRISPR/Cas9 and we are now establishing a mutant line. We are also testing Morpholino oligonucleotides to interfere with the production of TCF/LEF specific isoforms. These specific knockdown, CRISPR knockout together with isoform-specific rescue experiments will provide experimental samples for planned transcriptomics analysis to explore whether different TCF/LEF isoforms differentially regulate Wnt target genes and ultimately provide novel insights into the role of Wnt signalling in vertebrate development and evolution.

P-33

Mitotic NuMA/β-catenin containing complexes instruct division orientation towards localised Wnt3a sources.

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Wnt signalling governs developmental programs and tissue homeostasis by regulating stem cells functions. Its primary role in cell proliferation has been long believed to be circumscribed to β-catenin transcriptional activation, however increasing evidence uncovered important Wnt functions also in mitosis. In the healthy intestine, oriented cell divisions fine tune the differentiation of intestinal stem cells (ISCs) from the crypt bottom to villi, maintaining the correct crypt architecture. Secretion of Wnt3a by the niche Paneth cells residing at the crypt bottom regulates crypt homeostasis by instructing planar cell divisions. Consistently, dysregulation of the Wnt-pathway and mutations in its components are frequently observed in colorectal cancer (CRC). Recent evidence revealed a key role of localised Wnt3a signals in regulating oriented stem cell divisions and self-renewal. The Nuclear Mitotic Apparatus protein (NuMA) is a master regulator of mitotic progression that orchestrates mitotic spindle formation and placement. How the mitotic spindle responds to localised Wnt3 stimuli instructing ISC divisions and proliferation in the crypts, and whether NuMA is part of this response is unknown. I will present evidence that in mitosis the dynein activating-adaptor NuMA interacts with Wnt pathway components including Axin1, APC, GSK3β, CK1α and β-catenin in a Wnt3a- dependent manner. Importantly, these interactions can be reconstituted with purified proteins expressed recombinantly, with organisational principles that are under study.
Our data revealed a novel physical connection between localised Wnt3a sources and division orientation mechanisms, and identified the macromolecular complexes executing Wnt3-instructed oriented mitosis, which consist of components of the mitotic spindle orientation machinery and the Wnt destruction complex. Our findings shed light on the mechanistic whereby Wnt3a drives the oriented cell division, likely contributing to intestinal crypt homeostasis.

P-34

The effects of matrix stiffness and cell adhesion on CTNNB1 distribution and WNT/CTNNB1 signaling activity

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CTNNB1 (b-catenin) is a central component of the WNT/CTNNB1 pathway, which is crucial in animal development and adulthood. It also plays a key role in cell-cell junctions, maintaining cell adhesion. CTNNB1 thus plays a dual role in the cell. This raises the question whether cells merely balance the distribution of CTNNB1 between its structural role in cell adhesion and its transcriptional role in WNT/CTNNB1 signaling or if functional interplay occurs. If so, CTNNB1 could form a link between mechanical information and transcriptional output. While interplay has been indicated upon artificial modulation of cadherin expression levels and during epithelial–mesenchymal transition, it is unclear to what extent CTNNB1 exchange occurs under physiological conditions and in response to WNT stimulation (as reviewed in van der Wal & van Amerongen, 2020). Here we use a quantitative real time imaging approach use to study the dynamics and distribution of CTNNB1 at (near) endogenous levels in the different cellular compartments (i.e. membrane, cytoplasm and nucleus). Because CTNNB1 containing adherens junctions have been shown to remodel upon tension, we subtly modulate cell adhesion by adjusting the matrix stiffness to induce a mechanoresponse. I will present our results on CTNNB1 distribution in response to changes in matrix stiffness and WNT modulation. To determine if redistribution of CTNNB1 also leads to a transcriptional response, we combine this with unbiased RNA-seq analysis.

P-35

Cell competition supports robust morphogen gradients formation during organogenesis.

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Morphogen signaling forms an activity gradient and instructs cell identities in a signaling strength-dependent manner to pattern developing tissues. However, developing tissues also undergo dynamic morphogenesis, which may produce cells with unfit morphogen signaling and consequent noisy morphogen gradients. Recently, we demonstrated that cell competition corrects noisy Wnt morphogen gradients in early embryos. Cell competition is a cell-cell interactive process in which cells with relatively higher fitness eliminate those with lower fitness. During early embryonic anterior-posterior patterning, unfit cells with abnormal Wnt signaling activity spontaneously appear and produce noise in the gradient but they are apoptotically eliminated after communicating with neighboring normal cells. However, it is still unknown whether cell competition mediates robust morphogen gradients formation just in early embryos or in various organogenesis processes patterned by other morphogens. Here we show that cell competition supports the robust morphogen gradients formation beyond morphogen types and tissue types.
Zebrafish imaging analyses of the Shh morphogen gradient identify that unfit cells with abnormal Shh signaling activity often arise and produce noise in the gradient, which is formed in neural tube and muscle primordia. Similar to the elimination of Wnt-unfit cells, Shh-unfit cells are also apoptotically eliminated via cadherin-mediated communication with neighboring normal cells and subsequent Smad signaling activation and reactive oxygen species production. These results indicate that cell competition-mediated correcting systems can function in various organogenesis to support diverse morphogen gradients' robustness. Moreover, as morphogen gradients also control adult tissue patterning, this system may be relevant for tissue homeostasis and preventing diseases.

P-36

Novel amino acid substitution in LRP6 as the cause of human congenital tooth agenesis

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Non-syndromic tooth agenesis (NSTA) is a common human developmental anomaly that causes masticatory, speech, aesthetic, and psychological problems. Since gene mutations are frequently detected in TA cases, the WNT/β-catenin signaling pathway is considered crucial for regulating human teeth number. LRP6 is a single transmembrane type co-receptor for the WNT ligands, which plays a pivotal role in tooth development and is the causative gene for selective tooth agenesis-7 (STHAG7; Phenotype MIM number; 616724).

Two Japanese cases were diagnosed as TA by radiological examinations. The two pairs of parents had no variations in the teeth number, and all the members, including the probands, had no systemic abnormalities. The proband in family 1 was a 20-year-old female with oligodontia, congenitally 12-missing permanent teeth except for the third molars. In family 2, the proband was a 40-year-old female with hypodontia, congenitally missing five permanent teeth except for the third molar. Her fourth child was also affected by NSTA.

Saliva samples were collected from the patient and the family members who gave consent for participation in the research. We performed whole-exome sequencing and found two missense nucleotide substitutions in the LRP6 gene (NM_002336.3). Then, the dual-luciferase reporter assay was performed with these variant LRP6 expression vectors. Though wild-type LRP6 enhanced WNT signaling dose-dependently, one variant did not affect the WNT signal. However, the other variant showed significant enhancement.

Both amino acid substitutions were located at the extracellular domain of LRP6, where WNT ligands can interact. According to the 3D models of LRP6 domains (Protein Crystal Structure Database; File ID 3S94 and 4A0P) visualized with PyMOL software (www.pymol.org), a sizeable steric hindrance in one variant was observed because of a bulky functional group. In contrast, another variant shows almost no steric hindrance by the replacement, suggesting why one variant retained WNT/β-catenin signaling activity in vitro, while the other did not.

Taken together, we conclude that the variant in the family 2 is the cause of TA, however, the contribution of the other variant on tooth malformation is still unknown. Further future analysis is needed to clarify the pathogenicity of the variant.

P-37

β-catenin in mouse thymic epithelial cells fine-tunes postnatal T-cell production

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In the thymus, the thymic epithelium provides a microenvironment essential for the development of functionally competent and self-tolerant T cells. Previous findings showed that modulation of Wnt/β-catenin signaling in mouse thymic epithelial cells (TECs) disrupts embryonic thymus organogenesis. However, the role of β-catenin in TECs for postnatal T-cell development remains to be elucidated due to the difficulty of performing an in vivo analysis without generating extrathymic side effects. Here, we analyzed gain-of-function (GOF) and loss-of-function (LOF) of β-catenin highly specific in mouse TECs using β5t-Cre line, which enables highly efficient and specific genetic manipulation in TECs. We found that GOF of β-catenin in TECs results in severe thymic dysplasia and T-cell deficiency beginning from the embryonic period. By contrast, LOF of β-catenin in TECs reduces the number of cortical TECs and thymocytes modestly and only postnatally. Nonetheless, LOF of β-catenin did not cause an arrest in the development of the thymus, including the corticomedullary architecture, and the subsequent generation of T cells. These results indicate that the β-catenin signaling in TECs is dispensable for TEC development and T-cell development, but fine-tuning of β-catenin expression within a permissive range is required for TECs to generate an optimal microenvironment to support postnatal T-cell development.

P-38

Receptor-feedback robustly and quickly shapes Wnt gradient in heart development

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Morphogens govern tissue patterning in a concentration-dependent manner. However, it is still unclear how reproducible patterning can be achieved with diffusing molecules, especially when that patterning concerns differentiation of thin tissues. Wnt6 patterns cardiogenic mesoderm to induce differentiation of a thin tissue, the pericardium, in Xenopus. In this study, we revealed that a Wnt receptor, frizzled-7, is expressed in a Wnt-dependent manner. With a combination of experiments and mathematical modeling, this receptor-feedback appears essential to shape a steep gradient of Wnt signaling. In addition, computer simulation revealed that this feedback imparts robustness against variations of Wnt ligand production and allows the system to reach a steady state quickly. We also found that a Wnt antagonist sFRP1, which is expressed on the opposite side of the Wnt source, accumulates on N-acetyl-rich heparan sulfate (HS). N-acetyl-rich HS concentration is high between the sources of Wnt and sFRP1, achieving local inhibition of Wnt signaling via restriction of sFRP1 spreading. These integrated regulatory systems restrict the Wnt signaling range and ensure reproducible patterning of the thin pericardium (Yamamoto et al., eLife 2022).
Antagonists such as sFRP1 expand Wnt ligand distribution. However, they do not always broaden the signal activation range. We are extending the present simulation to analyze the mechanisms behind the differences in ligand distribution and signal activation ranges.

P-39

Discovering the effect of Bmps: the main opponents of Wnt signaling in the mammalian intestine

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Wnt signaling plays a crucial role in the intestinal epithelial stem cells maintenance and thus it is essential for the intestinal homeostasis. Besides the stem cells, differentiated epithelial cells, which play absorptive and protective roles, are also required for the proper functioning of the mammalian intestine. Differentiation of intestinal epithelial cells seems to be preferentially driven by Bmp signaling. Bmp signaling is presumed to counteract stem cell programs induced by Wnt signaling. However, if Bmp pathway just represses Wnt signaling or actively triggers certain differentiation programs remains puzzling. Therefore, we probed the role of individual Bmp ligands as counter actors of Wnt signaling on the intestinal epithelial cells. We performed short-term cultivation of freshly isolated murine intestinal crypts treated with individual Bmp ligands followed by deep expression profiling, cell type characterization and proliferation analysis. This setup in combination with in vivo Bmp receptor type I inhibition revealed distinct functions of individual Bmp ligands. Our data indicate that individual Bmp ligands uniquely influence the differentiation fate of intestinal epithelial cells. Our results also imply that Bmp signalling does not just simply counteract Wnt signaling, but it actively induces differentiation programs towards mucin producing goblet cells and various subtypes of enterocytes. Bmp ligands strikingly differ in the ability to influence the intestinal epithelial cells in terms of regulating differentiation, proliferation rate and the regulation of gene expression. Furthermore, our results suggest that the places of action of individual Bmps are spatially separated along crypt-villus axis.

P-40

Exploring the biological role of β-catenin-dependent and - independent gene regulation in embryonic stem cells

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Wnt/β-catenin is a key signaling pathway in the regulation and maintenance of epithelial stem cells. The stem cell activity is coordinated by the microenvironment, regulating the Wnt/β-catenin signaling. The signaling cascade involves many redundant receptors and downstream components (e.g., 12 Frizzled receptors and 4 TCF/LEF's), with the exception of β-catenin. Thus, β-catenin represents the «bottleneck» of this signaling cascade; yet it is not required for the regulation of all Wnt target genes. Although β- catenin plays a crucial role in Wnt/β-catenin signaling, the role of β-catenin-dependent and -independent gene regulation, particularly in a stem cell context, remains to be explored. In a non-stem cell context, we previously identified a subset of β-catenin transcriptional targets, so called GHOST genes, that were not regulated by TCF/LEF factors. Here we extend this work into a stem cell context. To address this, we performed unbiased whole transcriptome sequencing analysis in wild-type and β-catenin knock-out mouse embryonic stem cells to identify genes regulated dependently and independently of β-catenin. We identified genes which are up- or downregulated independently of β- catenin. We are studying the biological role of these genes to gain deeper understanding of the Wnt/β-catenin signaling pathway in governing the properties and differentiation of stem cells.

P-41

ATXN10 was Identified as a Wnt5a downstream target, which regulates planar cell polarity establishment during early development in mouse

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Wnt5a is a secreted molecule, which is expressed in the presomitic mesoderm (PSM) and has important roles for the anterior-posterior (A-P) axis elongation and the left-right (L-R) axis formation during early mouse development. For the A-P axis elongation, Wnt5a regulates directional migration of mesodermal cells after their ingression from the primitive streak into the PSM. For the L-R axis formation, Wnt5a instructs planar cell polarity (PCP) establishment in the node, which regulates the cilia position in each node cell. Even though Wnt5a is shown to involve in those important events, the underlying molecular mechanisms are poorly understood. To tackle this problem, we tried to identify downstream targets of Wnt5a signaling by conducting Mass-spec analysis of the complexes that interact with two core PCP regulators, Dvl2 and Pk2, in WT and Wnt5a- KO ES cells, which were differentiated into PSM-like cells. We isolated a cilia component, ATXN10, as a protein which interacted with both Dvl2 and Pk2. Interestingly, ATXN10 interacted with Dvl2 only in Wnt5a-KO ES cells, and interacted with Pk2 only in WT ES cells. These data suggest that ATXN10 changes the interacting partners upon Wnt5a stimulation. The conditional KO of Atxn10 as well as over-expression (OE) of Atxn10 revealed that ATXN10 is essential for the establishment of PCP in the node. We are currently examining the molecular mechanisms how the cilia component regulates the PCP establishment.

P-42

Modulating the Wnt Pathway through Lipid-dependent Interactions

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Coordinated cell fate determination during the early stages of life is dependent on several key signals. One such class of signals are Wnt proteins, a family of lipid-modified morphogens that play important roles in embryonic development and adult tissue homeostasis. Wnt signalling relies on the palmitoleoylation of a serine residue that is contained within a highly conserved region named the 'thumb'. This event promotes the engagement of Wnts with the essential proteins that drive their secretion, their transport through the extracellular space and, ultimately, binding to the receptor. Importantly, misregulation of Wnt signalling has been associated with several cancers, developmental disorders and neurodegenerative diseases. However, effective therapeutic strategies aimed at Wnt disorders remain sparse. This work is aiming to identify novel Wnt interactors through an unbiased interdisciplinary approach by combining in vivo genetic manipulations in Drosophila with a chemical strategy of covalently trapping palmitoleate binding proteins in human tissue culture. Firstly, by expressing a Wnt-biotin ligase (TurboID) fusion protein either from the endogenous locus of Wingless (main Drosophila Wnt) or by overexpression, this study identified important regulators of Wingless, such as Dally-like protein, as well as several novel Wg interactors which have been characterised in this study, albeit not extensively. In parallel, this study designed and synthesised lipidated and non-lipidated photoactivatable peptidomimetics of the Wnt 'thumb'. These probes can colocalise with GPC6 – a known Wnt interactor, however they fail to efficiently crosslink into nearby proteins. Engagement of Wnt peptidomimetics with GPC6 has highlighted a new avenue for its potential inhibition, which could have an indirect effect on Wnt signalling. Towards that, this study has developed a biophysical, as well as a cell-based assays to determine the binding of novel cyclic peptides to GPC6 and their ability to inhibit GPC6 and Wnt signalling. Together with the potential new targets identified from the Drosophila studies, the cyclic peptide inhibitors could become important tools for modulating the Wnt pathway which will not only deepen our understanding of Wnt signalling but can also potentially deliver new therapeutic strategies for the treatment of Wnt-driven medical conditions, as well as providing insight for other similar signalling pathways.

P-43

Human interleukin-1 receptor-associated kinase 1, a component of TLR/IL-1R pathways, induces the activation of β-catenin via its human-specific motif.

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Whereas inflammatory responses are crucial for the recovery from tissue damage and the protection from pathogens, the chronic inflammation in the lesion microenvironment occasionally accelerates tumorigenesis and metastasis and the mechanisms underlying its tumorigenic actions are poorly understood. Interleukin 1 receptor-associated kinase family (IRAK) consisting of four members, a shared component of Toll-like receptor/IL-1 receptor pathways, has been known to regulate the inflammation responses via downstream transcription factors such as NFκB and AP-1. Here we show that human IRAK1 (hIRAK1), one of IRAK family proteins, was required for the IL-1-mediated activation of β-catenin and that overexpression of hIRAK1 led to the activation of not only NFκB but also β-catenin in human cancer cells. The activity of hIRAK1 for β-catenin depended on its unique phosphorylation motif, which does not exist in other hIRAK family and mouse IRAK1. This motif-specific role of hIRAK1 was further supported by the observations that either other hIRAK family or mouse IRAK1 failed to activate β-catenin and that the addition of this motif by two amino acids substitution in mouse IRAK1 resulted in the acquisition of the activity for β-catenin. Besides, Kaplan-Meier plotter analysis revealed that hIRAK1 expression was, among hIRAK family, particularly associated with poor prognosis in lung, liver and breast cancer, implying that hIRAK1 has unique role in cancer malignancy. Next, we looked into somatic mutations of hIARK1 in cancers, which were reported in previous studies or Catalogue Of Somatic Mutation In Cancer (COSMIC) and found that multiple hIARK1 point-mutants in its kinase domain exhibited stronger activation of β-catenin than wild type of hIRAK1 did. Our results raise the possibility that the hIRAK1-specific role in β-catenin signaling may involve chronic inflammation-triggered carcinoma in human.

P-44

Time-resolved analysis of nuclear Wnt-signaling reveals β-catenin temporal genomic repositioning and cell type-specific plastic or elastic chromatin responses

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The current model entails that the association of β-catenin to TCF/LEF-bound genomic regions is followed by the upregulation of target gene transcription. However, whether beta-catenin targeted genomic regions are bound simultaneously or in a temporal fashion, and how this impacts the chromatin dynamics after Wnt activation, have not been investigated. To address these questions, we first assessed the genome- wide binding activity of β-catenin over time, in two Wnt-responsive human cell types. Indeed, while TCF/LEF and other components of the Wnt transcriptional complex are constitutively associated with the chromatin, it is beta-catenin's arrival, upon Wnt induction, that launches target genes transcription. Therefore, discovering the dynamics of the genome-wide β-catenin binding pattern is required to unambiguously define the direct targets of Wnt signaling. We found that β-catenin physically associates with different loci at specific time-points after stimulation, implying that the definition of Wnt targets is fundamentally temporal. Moreover, of these targets, only a small fraction are shared between cell types, pinpointing the extent to which β-catenin activity is context-dependent. Second, we asked whether the time- and cell-specific activities are reflected in differential chromatin dynamics. We discovered that Wnt/beta-catenin progressively shapes the chromatin profile of human embryonic stem cells consistent with their acquisition of a mesodermal identity: we refer to this genomic response as plastic. In contrast, in human embryonic kidney cells, Wnt/β-catenin drives a transient opening of relevant chromatin regions, followed by a re-establishment of the pre-stimulation chromatin state: we define this response as elastic. Finally, we discover that the Wnt-induced transient chromatin opening at target genes depends on the presence of β-catenin and associated chromatin remodeling activities carried out by CBP/p300 and HDACs, unearthing a previously unappreciated pioneering role of β-catenin. We submit that the plastic and elastic chromatin behaviors constitute part of the mechanism explaining how the Wnt/β-catenin pathway drives divergent cell-fate decisions during development and homeostasis.

P-45

Structure and function of the ROR2 cysteine-rich domain in vertebrate noncanonical WNT5A signaling

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The receptor tyrosine kinase ROR2 mediates noncanonical WNT5A signaling to orchestrate tissue morphogenetic processes, and dysfunction of the pathway causes Robinow syndrome, Brachydactyly B and metastatic diseases. The domain(s) and mechanisms required for ROR2 function, however, remain unclear. We solved the crystal structure of the extracellular cysteine-rich (CRD) and Kringle (Kr) domains of ROR2 and found that, unlike other CRDs, the ROR2 CRD lacks the signature hydrophobic pocket that binds lipids/lipid-modified proteins, such as WNTs, suggesting a novel mechanism of receptor action. Functionally, we showed that the ROR2 CRD, but not other domains, is required and minimally sufficient to promote WNT5A signaling, and Robinow mutations in the CRD and the adjacent Kr alter ROR2 function. Moreover, we demonstrated that WNT5A binds the CRD of Frizzleds, but not that of ROR2, and synthetic antibodies that dimerize the Frizzled CRD are sufficient to initiate signaling. Thus, we propose a model in which ROR2 acts via its CRD to potentiate the function of a receptor supercomplex that includes Frizzleds to transduce WNT5A signals.

P-46

O-GlcNAcylated LRP6-Merlin interaction acts as a nutrient sensor for the regulation of Hippo signaling mediated cell proliferation

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The Wnt signaling is critical for regulating cell proliferation and the Hippo signaling is a well-known cellular pathway that negatively regulates cell proliferation. Various crosstalk between these pathways have been reported, however, it is not yet clear whether the Wnt signaling component can modulate Hippo signaling depending on extracellular nutrient availability. We recently found that the LDL-related receptor protein 6 (LRP6) is O-GlcNAcylated, a post-translational modification whose level is linked to nutritional availability. In the nutrient-rich conditions, O-GlcNAcylated LRP6 negatively regulates the Hippo pathway. Mechanistically, O-GlcNAcylation of LRP6 in nutrient-rich conditions not only reduces lysosome-mediated LRP6 degradation but also induces the interaction of LRP6 with Merlin. Sequestration of Merlin by O-GlcNAcylated LRP6 leads to block LATS1 activation, which induces nuclear translocation of YAP for the expression of genes involved in promoting cell proliferation and anti-apoptosis etc. However, in starvation condition, reduction of O-GlcNAcylation on LRP6 leads to release of Merlin, which can activate LATS1 and block nuclear localization of YAP. Our findings suggested an important role for LRP6 O-GlcNAcylation as a nutrient sensor for the regulation of Hippo pathway. We speculate that O-GlcNAc-mediated LRP6 stability and Hippo signaling control may provide new mechanistic insights into why abnormal O-GlcNAcylation levels, which may boost Wnt signaling while reducing Hippo signaling, are found in numerous tumors, metabolic disorders, and Alzheimer's disease.

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P-47

PARsylated transcription factor EB (TFEB) regulates the expression of Wnt target genes by forming a complex with TCF/LEF1-β-catenin

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Wnt signaling pathway is a conserved signaling pathway which plays crucial roles in embryonic development and adult homeostasis. It is generally known that when Wnt binds to its receptors, stabilized β-catenin forms a complex with TCF/LEF transcription factor and stimulates target gene expression. However, we found that Wnt signaling induces the nuclear localization of TFEB, a well-known master regulator of autophagy and lysosomal biogenesis processes, and the expression of Wnt target genes is regulated by TFEB-TCF/LEF1-β-catenin as well as β-catenin-TCF/LEF1 complexes. Our biochemical data revealed that TFEB is a part of the β-catenin destruction complex, and destabilization of the destruction complex causes nuclear localization of TFEB. Interestingly, RNA-sequencing analysis revealed that about 27% of Wnt3a-induced genes were TFEB dependent. These "TFEB mediated Wnt target genes" were different from well-known TFEB target genes involved in autophagy and lysosomal biogenesis processes. Mechanistically, we found that Tankyrase (TNKS) PARsylates TFEB with Wnt ON signaling, and the nuclear localized PARsylated TFEB forms a complex with TCF/LEF1 to induce the "TFEB mediated Wnt target genes". Overall, our data suggest that Wnt signaling induces the expression of a subset of genes that are distinct from previously known genes regulated by the β-catenin-TCF/LEF1 complex or TFEB, by forming a transcription factor complex consisting of PARsylated TFEB and TCF/LEF1.
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P-48

Structural and functional characterization of Frizzled 7-specific monoclonal antibody

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The WNT signaling is crucial for diverse biological processes such as early embryonic development and stem cell proliferation in adult tissues. Deregulated WNT signaling is implicated in many pathological conditions, especially various cancers1. Frizzled 7 (FZD7), one of receptor subtypes for WNT ligand, has been reported to be highly expressed in many cancer types compared to normal adult tissues, and is expected to be a promising therapeutic target in cancer. To date, various strategies have been implemented to develop small molecules, peptides, and antibodies that specifically block FZD7-mediated WNT signaling2. Here, we report the structural and functional characterization of a newly generated FZD7-specific monoclonal antibody. To understand the molecular basis of the FZD7-specific binding of this antibody, we solved the cryo- electron microscopy (cryo-EM) structure of the FZD7-antibody complex and compared its structure with that of apo-state human FZD7, which we recently determined at 3.1 Å resolution by cryo-EM. We further analyzed this antibody with cell-based functional assays.

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P-49

Elucidation of the recognition mechanism of LRP4 by Muscle-specific kinase (MuSK) critical for the establishment of the neuromuscular junction

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LRP4, a type of LDL receptor protein, plays a crucial role in the formation and maintenance of the vertebrate neuromuscular synapses by bridging muscle-specific receptor tyrosine kinase (MuSK) and Agrin, leading to MuSK activation. Formation of Agrin-LRP4-MuSK complex in muscle cells results in MuSK activation, which in turn clusters acetylcholine receptors(AChRs) and establishes a special synaptic junction called neuromuscular junction (NMJ). As MuSK-LRP4 interaction plays a central role in the organization of proper structure of NMJ and defect of this signaling pathway leads to various muscular diseases, elucidation of the molecular organization of this signaling complex is eagerly awaited. However, there are no structural information about the physical interaction between MuSK and LRP4 available.

By means of the RaPID (Random non-standard Peptides Integrated Discovery) system, we had performed a library screening against human MuSK (hMuSK) and obtained a 27- residues linear peptide called L1 which binds to hMuSK with a high affinity (KD= 1.6 nM). Intriguingly, comparison of the amino acid sequence of L1 with that of LRP4 readily identified four peptide segments in LRP4, located at the boundary between the EGF module and the YWTD □-propeller domain, that were homologous to L1, suggesting a possibility that these four segments may represent binding site(s) for MuSK.

To test this idea, we tested if these peptides are capable of binding MuSK by using peptide-Fc fusion and MuSK ectodomain proteins, and in fact confirmed that one of the peptide segments bound to MuSK as strong as L1. When these peptide segments were mutated in the context of full-length LRP4, some mutants indeed showed reduced binding toward full-length MuSK expressed on the same cells, suggesting their involvement in the LRP4-MuSK binding.

P-50

A role of RNF43 other than Wnt receptor regulation in oncogenesis

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Wnt signaling pathways are tightly regulated by ubiquitination of their signal transducers and dysregulation of these pathways promotes the tumorigenesis. It has been reported that a Wnt target ubiquitin ligase RNF43 plays important role in the Fzd-dependent regulation of Wnt signaling pathways by forming a negative feedback circuit. We recently reported that a phospho-switch of RNF43 reversibly controls the function of RNF43 as a tumor suppressor for degrading Wnt receptors and suppressing Wnt signaling. Some of RNF43 mutations invert RNF43 tumor suppressor to oncogene by turning the phospho- switch off and forming positive feedback. Our findings about RNF43 function along Wnt receptor regulation was consistent largely with results from other groups and a consensus of the community. On the other hand, it is still not known well for the alternative role of RNF43 than Wnt receptor regulation in tumorigenesis. We reported in the past that RNF43 suppresses p53 pathway and apoptosis without the change of p53 protein level. And the mechanism of p53 suppression by RNF43 is independent to the function in regulating Wnt receptors. These facts suggest that RNF43 regulates two of the three key signaling pathways in multi-step tumorigenesis and these two oncogenic signals are crosstalking via RNF43. Excess of RNF43 expression induced by aberrant Wnt signaling possibly reduces tumor-suppressive activity of p53 pathway further. However, the molecular mechanism of the p53 suppression by RNF43 remains unclear. We recently found that cytoplasmic region of RNF43 interacts with p53 and a molecular complex that is known to modify p53 protein and the function. Therefore, we are investigating the detail how RNF43 inhibits p53 at the downstream of Wnt signaling and whether it could be a potential target for cancer therapy.

P-51

In vivo CRISPR screens reveal multiple routes to Wnt independence in RNF43-mutant/RSPO-fusion cancers

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Genetic alteration-caused hyperactivated Wnt signaling is a potent driver of tumorigenesis. Inactivating mutations in RNF43 or gene fusions involving RSPO2/3, detected in multiple cancer types, increase the cell-surface abundance of Wnt receptors and make these cancer cells hypersensitive to and dependent on ligand-activated Wnt signaling. This generates an opportunity of treating RNF43-mutant/RSPO-fusion cancers by pharmacologically targeting the biogenesis of Wnt ligands, e.g., with PORCN inhibitors that are in clinical trials now. However, resistance to PORCN inhibition has been observed within this cohort. To identify the underlying drug resistance mechanisms, we performed in vivo CRISPR screens during PORCN inhibitor therapy in mice bearing RNF43-mutant pancreatic cancer xenografts. This identified several categories of genes whose knockout conferred drug resistance. First, as a proof of concept, knockout of known negative regulators of the Wnt/β-catenin signaling cascade, including APC, AXIN1, and CTNNBIP1, activated β-catenin signaling downstream of the Wnt ligand and thereby caused resistance to upstream Wnt pathway inhibition. Second, inactivation of FBXW7, the substrate recognition component of the SCF E3 ubiquitin ligase complex, stabilized multiple oncoproteins including MYC and Cyclin E and mediated Wnt/β- catenin-independent tumor growth. Third, in RNF43-mutant pancreatic cancers, silencing of the p300/GATA6 axis led to a phenotypic transition from the classical subtype to the dedifferentiated basal-like/squamous subtype. This bypassed the antidifferentiation activity of Wnt signaling, causing Wnt independence. In summary, this study revealed diverse mechanisms leading to Wnt ligand independence in RNF43-mutant/RSPO-fusion cancers and identified potential biomarkers for patient stratification for anti-Wnt therapies.

P-52

USAG-1-mediated activation of Wnt signaling can regenerate missing teeth in a mouse model of congenital tooth agenesis

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Investigations of single-gene knockout mice have demonstrated that loss of function of USAG-1, also referred to as Sclerostin domain containing 1 (Sostdc1), ectodin, or Wnt modulator in surface ectoderm Wise), result in the production of supernumerary teeth. USAG-1 is a bifunctional protein that antagonizes BMP and Wnt, the two signaling molecules essential for tooth development. It has been suggested that BMP signaling is essential for morphogenesis of extra teeth, while Wnt signaling is important for supernumerary tooth formation. We previously reported that tooth development arrested in Runx2−/− and Ectodysplasin A (Eda) mice, a mouse model for congenital tooth agenesis, was rescued in double mutant mice, a supernumerary mouse model. A clear link between USAG-1 and rescue of congenital tooth agenesis has been established.

In this study, we examined the relationship between Wnt10a which are known to be causative genes of human congenital agenesis, and USAG-1 in tooth formation using mutant mice.
We interbred heterozygous USAG-1 and Wnt10a mice and analyzed the F2 generation. To eliminate the influence of the mouse background, only F2 progeny USAG- 1−/−/Wnt10a−/− mice were analyzed. Embryos were obtained by timed mating; day E0 started from midnight, before finding a vaginal plug. Offspring were analyzed at 8 weeks of age. After removing the skin, dissected maxillae, and mandibles from the heads of the offspring were soaked in 0.02% proteinase K prepared in PBS at 37°C for 4 days and cleaned with 5% H2O2 at 15° to 25°C for 5 min. Last, they were rinsed in H2O and air- dried.

88 males and 80 females were studied. In USAG-1-/-/Wnt10a-/- mice, the jawbone was small, formation of supernumerary teeth and fused teeth were observed. In USAG-1+/-/Wnt10a-/- mice, the jawbone was small, but supernumerary teeth were formed, and no fused teeth were observed. Compared to USAG-1-/-/Wnt10a+/+ mice, USAG-1-/-/Wnt10a+/- mice had an increased number of supernumerary and fused teeth in the mandible. Furthermore, the incidence of mandibular supernumerary teeth increased to 100% and their thickness increased. Wnt signaling was suggested to promote supernumerary tooth formation in USAG-1 heterozygous mice. Not only BMP but also Wnt signaling is important in controlling tooth number.

P-53

Wnt signaling cross talks with mechanical stimulation to establish Hydra axial patterning

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Hydra is a freshwater Cnidarian with the shape of a tube and organized along a single oral/aboral axis. The animal's proverbial regeneration potential is consequent to the ability of Hydra tissue to establish de novo Wnt signaling organizer centers. These Wnt organizers mark the oral end of the main axis, and coordinate a chain of events that ultimately leads to the characteristic structure of the animal head with its tentacles. The emergence of Wnt organizers depends on mechanical stimulation of the cells by stretching. Without mechanical stretching, that is caused by osmotically driven inflation and deflation cycles of spherical Hydra fragments, regeneration fails. The strength of HyWnt3 expression reflects quantitatively the level of tissue stretching and overexpression of HyWnt3 renders the mechanical stimulation obsolete for successful establishment of an animal axis. Moreover, Wnt signaling seems to alter the mechanical properties of the cells, enabling further stretching. This positive feedback of mechanochemical nature can be the core of a mechanism for the spontaneous emergence of organizers, and thus the patterning of the Hydra along the main axis.

P-54

Glycolytic flux-control of Wnt signaling in regulation of developmental timing

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Central carbon metabolism plays a role beyond bioenergetic functions, impacting gene expression and signal transduction. Previously, we have shown that glycolytic flux inhibits Wnt signaling through a sentinel glycolytic metabolite fructose 1,6-bisphosphate (Miyazawa et al., 2021, doi.org/10.1101/2021.12.20.473441). In this meeting, we will present the significance of such a functional link in controlling mouse embryo segmentation timing. Vertebrate embryos undergo periodic segmentation of presomitic mesoderm (PSM), and a molecular clock called segmentation clock dictates the timing of segmentation. We first observed that higher glycolytic flux led to a slower pace of the segmentation clock in wild-type embryos. Slowing down of the clock was more prominent in transgenic embryos overexpressing dominant active, cytoplasmic Pfkfb3 (cytoPfkfb3), with enhanced glycolytic flux. To probe the possibility that Wnt signaling suppression underlies the clock phenotype, we aimed to restore the pace of clock by activating Wnt signaling. Of great interest, deleting a single allele of a Wnt antagonist, Dickkoppf-1, was able to restore the clock tempo partially, without affecting glycolytic flux, in cytoPfkfb3 embryos, highlighting that glycolysis functions at the upstream of the Wnt signaling pathway. Combined, these findings demonstrate that glycolytic flux controls the timing of embryo segmentation via Wnt signaling. Metabolic pathway is highly conserved across species, and hence our work demonstrates the potential that cellular metabolism is a general regulator of developmental timing and potentially embryonic patterning.

P-55

A Wnt repressed cholesterol biosynthetic enzyme regulates RTK/MAPK signaling and cellular senescence.

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A widely held assumption about Wnt signaling is that activated Wnt signaling generally activates the transcription of target genes via β-catenin. We recently reported that Wnt signaling also represses the expression of a large subset of genes (Harmston et al., 2020; Madan et al., 2018). These results shift the paradigm from where Wnt is viewed primarily as an activator of transcription to a more nuanced view where Wnt signaling drives widespread gene repression and activation. Many Wnt repressed genes have ETS family transcription factor binding motifs in their promoter regions that function downstream of the MAPK pathway. Our analysis found that high Wnt signaling repressed MAPK activity and prevented Ras-mediated senescence, thereby permitting unrestricted proliferation of cancer cells. Given the well-established pro-oncogenic roles of both Wnt and MAPK signaling, we postulate that Wnt signaling adjusts the intensity of MAPK signaling to be "just right" to drive cancer proliferation without inducing senescence (colloquially known as the "Goldilocks" model). Seeking to understand how Wnts regulate MAPK signaling, our studies have identified a novel Wnt repressed cholesterol biosynthetic enzyme (Madan et al., 2021) as this pathway's key and unexpected regulator. This enzyme regulates the RTK-MAPK axis, thereby sculpting dynamic changes in the transcriptional landscape and a differentiation/senescence response. This supports a novel paradigm that multiple cancers reprogram the flux of the cholesterol pathway to regulate signaling pathways and cell fate.

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P-56

Canonical and Non-canonical Wnt signaling are simultaneously activated by Wnts in colon cancer cells

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The Wnt signaling pathway is a crucial regulator of the intestinal epithelium homeostasis and is altered in most colon cancers. While the role of aberrant canonical, β-catenin- dependent Wnt signaling has been well established in colon cancer promotion, much less is known about the role played by noncanonical, β-catenin-independent Wnt signaling in this type of cancer. This work aimed to characterize the noncanonical signal transduction pathway in colon cancer cells. To this end, we used the prototype noncanonical ligand, Wnt5a, in comparison with Wnt3a, the prototype of a canonical β-catenin activating ligand. The analysis of the expression profile of Wnt receptors in colon cancer cell lines showed a clear increase in both level expression and variety of Frizzled receptor types expressed in colon cancer cells compared with non-malignant cells. We found that Wnt5a activates a typical Wnt/Ca++ - noncanonical signaling pathway in colon malignant cells, inducing the hyperphosphorylation of Dvl1, Dvl2 and Dvl3, promoting Ca++ mobilization as a result of phospholipase C (PLC) activation via pertussis toxin-sensitive G-protein, and inducing PLC-dependent cell migration. We also found that while the co-receptor Ror2 tyrosine kinase activity is not required for Ca++ mobilization-induced by Wnt5a, it is required for the inhibitory effects of Wnt5a on the β-catenin-dependent transcriptional activity. Unexpectedly, we found that although the prototype canonical Wnt3a ligand was unique in stimulating the β-catenin-dependent transcriptional activity, it also simultaneously activated PLC, promoted Ca++ mobilization, and induced Rho kinase and PLC-dependent cell migration. Our data indicate, therefore, that a Wnt ligand can activate at the same time the so-called Wnt canonical and noncanonical pathways inducing the formation of complex signaling networks to integrate both pathways in colon cancer cells.

P-57

Wnt-dependent regulation of cell-proliferation by SP1:β-catenin complex in colorectal cancer

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Wnt/β-catenin signaling is a highly conserved pathway in multicellular organisms. It is involved in the regulation of basic cellular properties including, cell proliferation, differentiation, migration, and self-renewal of stem cells. It has been implicated in multiple processes, such as cell fate determination and axis formation during development.
Dysregulation of Wnt signaling causes diseases, most commonly colorectal cancer (CRC). Previously we showed that the generic transcription factor SP1 is an integral part of the Wnt/β-catenin pathway and is regulated by the same in CRC. In the absence of Wnt signaling, SP1 interacts with the destruction complex, leading to its degradation.
This degradation is mediated by phosphorylation via GSK3β and ubiquitination via βTrCP. Upon Wnt activation, SP1 is stabilized due to loss of its interaction with βTrCP. Additionally, the interaction of SP1 and β-catenin is required for their mutual stabilization. Furthermore, Wnt-induced stabilization of β-catenin is inhibited under SP1 knockdown conditions. To gain insights into the global implications of this complex in the context of development and disease, we performed ChIP-seq analysis for both of these proteins individually. Next, to validate the occupancy of these proteins as a complex, we performed a sequential ChIP-seq analysis. Strikingly, the set of genes co-regulated by the SP1:β-catenin complex were found to be enriched for cell cycle and DNA replication, both of which are essential to maintain normal cell proliferation which can otherwise promote cancer. We also observed dysregulation of the same set of target genes in CRC patient datasets which exhibited high levels of SP1 and β-catenin, corroborating our previous findings. Xenograft studies in mice treated with a combination of Wnt and Sp1 inhibitors led to a substantial decrease in tumor burden than when compared to any single treatment. Further, to establish the significance of the SP1:β-catenin complex during vertebrate development, we ectopically overexpressed them in zebrafish. We observed that overexpression of SP1 and β-catenin resulted in developmental defects in the embryos. Interestingly, we observed that this effect is synergistically higher in embryos injected with both SP1 and β-catenin. Moreover, myca, which is a marker of cell proliferation, is upregulated in these embryos. Collectively, we show that the function of SP1:β-catenin complex is conserved and is required for cell proliferation during development as well as intestinal homeostasis in adults.

P-58

Daam plays opposing roles in the canonical and non-canonical Wnt signaling pathways regulating intestinal stem cells

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The small intestine is among the fastest self-renewing tissues in adult mammals. This rapid turnover is fueled by intestinal stem cells residing in the intestinal crypt, capable of generating all the differentiated cells that populate the intestinal epithelium to maintain homeostatic function. Among the several signaling pathways governing the balance between stem cell renewal and differentiation, Wnt signaling plays a pivotal role, and dysregulation of this pathway leads to cancer formation. Several feedback mechanisms have evolved to tightly control Wnt signaling, especially at the cell surface as exemplified by the transmembrane E3 ligases Rnf43/Znrf3. Using biochemical and molecular analysis on cell cultures we show that the diaphanous-related formin (DRF) Daam1 interacts with Rnf43, and this complex is required to attenuate Wnt/beta-catenin signaling at the receptor level. Accordingly, Daam1/2 knock-out allows R-spondin-independent growth in mouse intestinal organoids, similar to Rnf43/Znrf3 knock-out. Furthermore, genetic analysis in vivo suggest that Daam is also required for secretory lineage specification through the non-canonical Wnt, also known as Planar Cell Polarity (PCP) pathway. In conclusion, we show that Daam1/2 play opposing roles on Wnt/beta-catenin and PCP pathways via Rnf43, a function required for homeostatic maintenance of the murine small intestine.

P-59

Quantitative analysis of diffusing population of endogenous Wnt8 protein in zebrafish

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Wnt is a secreted protein involved in multiple processes of embryogenesis and tissue homeostasis, including cell fate specification, cell polarity formation and maintenance of progenitor cell population. Wnt protein is known to carry a palmitoleate moiety that is essential for signaling activity. In several contexts, secreted Wnt protein is considered to move long distance from source cells and to form the concentration gradient, but mechanisms regulating dispersal of such hydrophobic ligands in the extracellular space are not fully understood yet.
In zebrafish development, Wnt8 is expressed at the ventro-lateral marginal zone during gastrulation and thought to function as a morphogen, which generates multiple neural subdomains along the A-P axis in a manner dependent on signaling dosage. A previous study proposed that cytonemes-mediated transfer is involved in long-range actions of Wnt8 signal in neural patterning of zebrafish early gastrula. However, since this model was based on the results obtained by ectopic-expression of fluorescent tagged Wnt protein, behaviors of endogenous Wnt8 protein still remained unclear.
To examine behavior of endogenous Wnt protein, a coding sequence of Achilles, a variant of YFP with fast maturation, was introduced into the 3' region of wnt8.1 locus of zebrafish genome by CRISPR/Cas9 system. By quantitative analysis using FCS (Fluorescence Correlation Spectroscopy), freely diffusing Wnt8-Achilles protein was detected not only in the extracellular space around Wnt8 secreting cells but also in the animal pole. Wnt8-Achilles protein was also detected in the ventral marginal zone and animal pole by transplantation assay of "morphotrap"-expressing cells, in which a membrane-tethered form of anti-GFP nanobody is expressed. In contrast, transplanted cells expressing membrane-tethered Frizzled8 ECD could capture Wnt8-Achilles protein in the ventral marginal zone but not in animal pole. These suggest that while freely diffusing population of Wnt8-Achilles protein are distributed across the entire embryo, they lose ability for receptor binding in the place far from secreting cells.

P-60

Different modification states of heparan sulfate proteoglycans are differently involved in spatial distribution of Wnt11 and in feedback regulation of planar cell polarity

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Planar cell polarity (PCP), directional information of cells within a tissue plane, is established with asymmetric localizations of core PCP components. In *Xenopus* neural plate, Wnt11 is known to regulate PCP, supposedly forming a concentration gradient.
However, we found that Wnt11 was distributed uniformly but not in a graded manner in the neural plate. In a higher resolution, Wnt11 was localized on cell boundaries along a mediolateral direction in the neural plate similar to core PCP components. This result implies a mutual regulation between Wnt11 and core PCP components. To understand the molecular bases of the characteristic local accumulation of Wnt11, we focused on heparan sulfate (HS) proteoglycans (HSPGs), which are involved in the distribution and signaling of Wnt8 (Mii et al., *Nat. Commun*. 2017). Modification states of HS chains are important for the activities. Here, we focused on *N*-deacetylation and *N*-sulfation of HS, both of which are catalyzed by NDST1. Knockdown of *ndst1* reduced Vangl2, which is a core PCP component, and caused neural tube closure defects, a commonly observed phenotype in loss-of-function of core PCP components and Wnt11. Both of these HS chains were distributed in a polarized manner similar to Wnt11 and core PCP components in the neural plate. Furthermore, we found that deacetyl HS exhibited polarized distribution when exogenous Wnt11 directs PCP, similar to core PCP components and Wnt11, although it is uniformly distributed without exogenous Wnt11. These findings suggest that HSPGs form a feedback loop with Wnt11 and core PCP components to regulate PCP. Furthermore, deacetyl HS and *N*-sulfo HS exhibited different subcellular localizations in comparison with Wnt11 or core PCP components. These data suggest that those modification states of HS have different roles in the regulation of PCP. To understand the role of each modification state of HS chains, we generated mutants of NDST1 to manipulate the HS modification. We would like to discuss the specificity of modification states of HS chains.

P-61

Using super-resolution microscopy to study Wnt secretion.

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Wnts are secreted proteins that control a variety of processes including stem cell maintenance, cell fate decisions and growth. Misregulation of Wnt signalling is associated with several diseases, including cancer and Alzheimer's. Understanding how Wnt signalling is modulated is therefore of considerable importance. A shared characteristic of most Wnts is that they are lipidated in the ER. The lipid is shielded from the aqueous environment of the secretory pathway's lumen by Wntless (Wls/Evi), which escorts Wnts to the plasma membrane. However, it remains to be determined how Wnts are then released from Wls and how the lipid moiety of Wnt is shielded once it has disengaged from Wls/Evi. Here, we describe our efforts towards answering these questions. Using super-resolution microscopy, we track SNAP-tagged, endogenously expressed Wingless (Wg), as it is secreted and transported in the developing Drosophila wing imaginal disc. Thus, by following the dynamics of Wg secretion at high spatial and temporal resolution, we find that Wingless is endocytosed by producing cell and then enriched at the limiting membrane of Rab4 positive vesicles and Rab7 positive endosomes. Since Wls/Evi is not enriched in these subcellular compartments, we suggest that Wg separates from Wls/Evi earlier, possibly in early endosomes, or at the early stages of clathrin-mediated endocytosis. We are developing FRET assays to further map the location where Wingless and Wls/Evi dissociate in the hope that this information will suggest the underlying molecular mechanism.

P-62

Wnt5a regulates cellular state of specific subtype of fibroblast and accelerates tumor progression.

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Wnt signaling is divided into β-catenin dependent (canonical) and independent (non- canonical) pathway. Although contribution of canonical pathway to the progression of cancer is well-understood, the involvement of non-canonical pathway in cancer remains controversial. This might be because the signal cascade is transmitted across tumor microenvironment which makes it difficult to appreciate it in tumor cell line model.
Although Wnt5a, one of the ligand that activates non-canonical signal, is reported to be expressed in a subset of stroma cell, it is unknown how these cells act in stroma. To make these questions clear we applied single-cell RNA sequencing (scRNA-seq) to murine cancer model (AOM/DSS model) and tried to decode the intra- and inter- cellular signal communication.
Gene expression profile of all the cells in tumor tissue revealed that Wnt5a was dominantly expressed in fibroblast and the receptors of Wnt5a were also found in fibroblast specifically. Next we isolated fibroblasts from the tumor tissue and integrated three distinct scRNA-seq data from different environments; normal region of healthy control mice, inflammatory and tumor region. Wnt5a expressing fibroblast in healthy control mice switched to Wnt5a high expressing state during inflammatory and tumorigenesis stage due to TGF-β signaling. CAFs only detected in tumor region was subdivided into two groups, mCAF and iCAF. Wnt5a expressing fibroblasts associated with mCAF particularly as inferred from the scRNA-seq data.
To reveal the involvement of Wnt5a in cancer development, Wnt5a conditional knockout mice were used in the AOM/DSS model. Tumors in knockout mice were significantly fewer than the control mice suggesting the pro-tumor function of Wnt5a, and knockout mice harbored reduced population of mCAF cells. Although Wnt5a had no direct effect on proliferation of colon cancer organoid, it seems that Wnt5a drives tumorigenesis by increasing the abundance of mCAF cells. Fibroblast primary culture model revealed that Wnt5a maintains the cell identity of mCAF through regulation of specific transcription factor.
Together, in vivo analysis of signal transduction clarified the novel pathway of Wnt5a through cell-cell communication.

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DKK3 Driven Wnt Pathway in Radiation-induced Inflammatory and Fibrosing Skin Injury

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Radiation induced fibrosis is common side effect of radiation treatment in cancer patients. Reactive oxygen species (ROS) massively produced in radiation damage and present study found that DKK3 is upregulated as a result of ROS exposure in keratinocytes. DKK3 is regulator (positive or negative) of canonical Wnt signaling in cellular context dependent manner. It is found that a global or keratinocyte specific Dkk3 knockout results in lessening of fibrotic phenotype in keratinocytes as well as mice with a reduced overall Wnt reporter activity and a pro-inflammatory phenotype on immune spectrum. DKK3 mediated Wnt activity might serve as drug target for treatment of radiation based fibrosis.

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Recycling endosomes restrain Wnt signalling induced intestinal stem cell proliferation

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Defects in Wnt signalling underlies the majority of Colorectal cancer cases. While we have a good understanding of how this cascade is regulated by the destruction complex, we know less about the sub-cellular trafficking pathways and organelles involved in Wnt signalling activity, especially in disease conditions. To close this gap, we performed a genetic modifier screen of trafficking regulators in the Drosophila intestine that constitutively activated Wnt signalling in intestinal stem cells (ISCs) as a result of inducible APC knockdown. We identified recycling endosomes as key players in regulating Wnt signalling after APC loss. Overexpression of a specific Rab GTPase (associated with the recycling pathway) or a constitutively activate mutant rescued ISC proliferation and Wnt activation after APC loss, while knockdown or a dominant negative mutant exacerbated these phenotypes. Using genetic experiments, we place the recycling pathway downstream of APC but upstream of Arm (β-catenin). Interestingly, we show that the recycling pathway is involved in regulating the levels of membrane Arm and this correlated with Wnt activity. We are currently investigating how recycling endosomes controls Wnt signalling in ISCs and translating our findings in mammalian intestinal organoid models.

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The Frizzled-Dvl interaction is not allosterically regulated by ligand binding or receptor dimerization

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Wnt/β-catenin signaling is initiated upon Wnt binding to and heterodimerization of Frizzled (Fzd) and Low-density lipoprotein receptor-related protein 5/6 (LRP5/6). Ligand binding to Fzd has been proposed to allosterically enhance the binding affinity between Frizzled and the Dishevelled (Dvl) DEP domain, leading to the recruitment and inhibition of the β-catenin destruction complex. However, we have directly measured the binding affinity of purified DEP for nanodisc-embedded Fzd4 and Fzd5 using biolayer interferometry, and have not observed any enhancement of affinity in the presence of the ligands xWnt8 or Norrin. We then asked whether DEP recruitment to Fzd is allosterically enhanced by the presence of LRP5/6. In order to directly investigate this biochemically, we developed a technique to obtain purified homogeneous receptor dimers in nanodiscs using a split GFP (sGFP) tether. In this method, complementary sGFP tags associate to tether the co-expressed dimers and control both stoichiometry and orientation within the nanodiscs. We did not observe any change in DEP affinity for Fzd4-LRP6 heterodimers relative to Fzd4 alone, nor did we see any Norrin-induced change in affinity. Given that homodimerization of Fzd has been proposed to contribute to signaling, we additionally purified Fzd4 homodimers in nanodiscs, and again saw no effect of dimerization on DEP recruitment, regardless of the presence of Norrin. Given these results, we are able to rule out the model of ligand- or dimerization-mediated allosteric recruitment of Dvl as the mechanism of Wnt/β-catenin signal initiation. However, our method to purify stoichiometric, parallel receptor dimers will enable further biochemical and structural studies of Fzd, LRP5/6, and other co-receptors involved in Wnt/β-catenin signaling.

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A conserved Wnt/Sp5 signaling cassette specifies and patterns germ layer gene regulatory networks along the anterior-posterior axis in sea urchin embryos

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The establishement and patterning of the primary body axis (termed the anterior- posterior [AP] axis in most bilaterians) is one of the first fundemental events in an embryo's development. Data from metazoan embryos indicates that Wnt signaling is an ancient molecular mechanism required for this developmental process. In deuterostome sea urchin embryos an integrated network of three different Wnt signaling pathways (Wnt/β-catenin, Wnt/JNK, and Wnt/PKC) governs primary germ layer gene regulatory network (GRN) specification and patterning along the AP axis. Here, we analyzed the transcription factor Sp5's role as a critical effector downstream of two AP Wnt signaling network pathways. Our data indicate posterior Wnt/β-catenin signaling, which initiates endomesoderm specification, activates Sp5 which is subsequently integrated into an evolutionarily-conserved gene regulatory 'kernal' critical for endomesoderm specification in echinoderm species that diverged ~550 million years ago (sea urchins and sea stars). In the anterior half of the embryo, we found that the non-canonical Wnt1/Wnt8-Fzl5/8- JNK signaling pathway, which postions the anterior neuroectoderm (ANE) GRN around the anterior pole, also activates Sp5 and that it forms feedback loops with Wnt8 and itself that are essential for ANE GRN postioning. Comparisons of expression and functional data from several animal embryo, including vertebrates, show that Sp5 is often activated by Wnt signaling to specify posterior endoderm and/or mesodermal territories as well as position early ANE GRNs along the AP axis. Our data support the idea that Wnt-Sp5 interactions represent an evolutionary-conserved signaling cassette that is essential for remarkably similar developmental processes in several metazoan embryos.

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Wnt8a is disseminated via cytonemes in zebrafish gastrulation

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Wnt molecules must be distributed from source to receiving cells to set up a morphogen gradient. Cell-cell communication is essential in multicellular organisms to regulate responses such as cell proliferation and differentiation. During zebrafish development, Wnt8a intercellular transport is via signaling filopodia- cytonemes - and are crucial during antero-posterior neural plate patterning (1). How cytonemes precisely control Wnt8a delivery is still being studied.
In this project, we address the question of how dissemination can regulate Wnt gradient formation. We hypothesize that the length and number of Wnt8a cytonemes would impact on Wnt gradient and consequently anteroposterior patterning. Furthermore, we propose that the number of Wnt8a transported by cytonemes could also affect the morphogenetic gradient.
To test our first hypothesis, we investigate the function of Vangl2. We show that Vangl2 can induce long, branching cytonemes to deliver Wnt8a (2). Consistently, Vangl2 inhibition reduces cytoneme length and number, reducing Wnt activation. Altering Vangl2 levels and therefore cytoneme number and length shifts anteroposterior neural plate boundaries in the zebrafish and therefore, is important for regulating cytoneme behaviour.
To test our second hypothesis, we have started to quantify Wnt8a molecules on cytonemes using super-resolution microscopy. DNA qPAINT is a quantitative single- molecule detection technique which allows us to visualise and quantify signalling molecules. Using a microscope with super-resolution capabilities, we record the 'blinking' single-molecule events caused by complementary DNA binding/unbinding kinetics of a DNA imager strand to a DNA docking strand which labels the protein-of-interest.
Firstly, we optimized our microscope for super-resolution, using a known protein - the nuclear pore complex (NPC). We used NPC-GFP stable cells and GFP nanobodies modified for DNA PAINT to visualise the 8 protein cluster NPC ring. Once optimized, we utilised the GFP nanobody to visualise Wnt8a-GFP in zebrafish fibroblasts. We could detect Wnt8a-GFP clusters along and on cytoneme tips. We then used qPAINT to analyse number and size of Wnt8a clusters and quantified Wnt8a molecule numbers on cytonemes. We will next quantify Wnt8a on an endogenous level. By advancing this research area, we will forward our understanding of the critical quantitative switch needed to initiate a Wnt response.

1. Stanganello et al., 2015, Nat Comms
2. Brunt et al., Nat Comms, 2021

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The contribution of non-canonical WNT11 to colorectal cancer

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WNT signaling plays a crucial role during development, homeostasis and regeneration. Mutations in the WNT pathway are frequently found in various tumor types, both in sporadic and hereditary cancers. Due to the critical role of WNT signaling in tumorigenesis, we hypothese that not only mutations in WNT pathway components but also expression of specific WNT ligands contributed to tumorigenesis. Initial assessment of the expression of family of 19 human WNT ligands in colorectal cancer entities using the TCGA database showed distinct upregulation of WNT5A and WNT11. Since the contribution of WNT5A and WNT11 to tumorigenesis in colorectal cancer is elusive, we studied the role of WNT5A and WNT11 in this cancer entity using various in vitro models including cancer cell lines, mouse and human-derived colon organoid lines. We find that in concordance with the TCGA database, WNT11 is specifically overexpressed in APC truncated tumors and tumor models. Furthermore, knock-down of WNT11 reduces proliferation in colorectal cancer cell lines. Ongoing research is conducted to identify the molecular contributions of WNT11 to colorectal tumorigenesis.

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