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The scope of our interests encompasses underlying mechanisms for the development of the vertebrate central nervous system (CNS) and various functions of the mature brain, including body fluid homeostasis, blood pressure control, food intake control, learning and memory.

## I. Mechanisms for neural circuit formation

Adenomatous polyposis coli 2 (APC2) is preferentially expressed in the nervous system from early developmental stages through to adulthood. APC2 is distributed along actin fibers as well as microtubules in neurons. Our investigation suggests that APC2 is involved in the signaling pathway from membrane receptors for extracellular guidance factors to the intracellular migration machinery.

Sotos syndrome (OMIM #117550) is characterized by intellectual disability and a combination of typical facial features and large head circumference. Sotos syndrome has been known to be caused by haploinsufficiency in the *NSD1* gene. Our knockdown experiments revealed that the expression of APC2 in the nervous system was under the control of *NSD1*. Moreover, *Apc2*-knockout (KO) mice also showed Sotos syndrome-like abnormalities. We are now investigating the relationship between *NSD1* and APC2 in more detail by examining *Nsd1*-KO mice.

## II. Physiological roles of receptor-like protein tyrosine phosphatases

Protein tyrosine phosphorylation plays crucial roles in various biological events such as cellular proliferation, differentiation, survival, migration, and metabolism. Cellular tyrosine phosphorylation levels are governed by the opposing activities of protein tyrosine kinases (PTKs) and protein

tyrosine phosphatases (PTPs). The physiological functions and regulatory mechanisms of receptor-like PTPs (RPTPs) are not fully elucidated. We have been making efforts to reveal the functional roles of RPTPs, especially of the R3 and R5 subfamilies.

### 2-1 R3 RPTP subfamily

The R3 RPTP subfamily, which is comprised of PTPRB, PTPRH, PTPRJ, and PTPRO, play pivotal roles in the development of several tissues including the vascular and nervous systems. Eph receptor tyrosine kinases are known to play indispensable roles in the topographic central projection of retinal ganglion cell (RGC) axons. Using the chick retinotectal projection system, we previously showed that PTPRO controls the sensitivity of retinal axons to ephrins and thereby has a crucial role in the establishment of topographic projections. We also demonstrated that R3 RPTPs dephosphorylate Eph receptors. Among R3 RPTPs, PTPRJ and PTPRO were expressed in developing mouse RGCs. We are now investigating the relationship between roles of PTPRJ and PTPRO in the central projection of the retinal axons by using gene knockout mice.

Leptin, an adipocyte-derived hormone, is a critical factor controlling food intake: it strongly inhibits food intake through the regulation of neuronal activities in the hypothalamic arcuate nucleus (ARC). Most obese individuals have an increased food intake despite high circulating leptin levels, which is referred to as leptin resistance. However, the exact mechanisms underlying leptin resistance in obese patients have yet to be elucidated. We recently demonstrated that PTPRJ is a physiological enzyme attenuating insulin signaling *in vivo*. In *Ptprj*-KO mice, the activation of insulin receptor (IR) kinase and Akt (or protein kinase B) was enhanced, and glucose and insulin tolerance was improved. Furthermore, *Ptprj*-KO mice exhibited lower weight gain associated with lesser food intake compared with wild-type mice.

We have now found that PTPRJ is involved in the regulation of leptin signaling in the ARC through the dephosphorylation of JAK2, the primary tyrosine kinase in leptin signaling. In line with these results, leptin signaling is enhanced in *Ptprj*-KO mice. Diet-induced obesity up-regulated PTPRJ expression in the hypothalamus, and induction of leptin resistance was strongly attenuated in *Ptprj*-KO mice. Upon leptin administration, *Ptprj*-KO mice fed a high-fat/high-sucrose

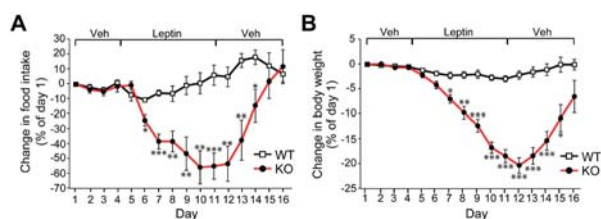


Figure 1. *Ptprj*-KO mice are protected from the development of diet-induced leptin resistance. (A) Daily change in food intake by and (B) Daily change in body weights of wild-type (WT) and *Ptprj*-KO mice fed high-fat/high-sucrose diet (HF/HSD) for 14 weeks upon daily administration of leptin. Leptin (500 ng) or vehicle was i.c.v. injected during the indicated period. ANOVA was used to detect significant differences at the same time point between the two groups ( $n = 8$  each; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

diet showed significant reductions in food intake and body weight as compared with corresponding WT mice (Figure 1). Moreover, the overexpression of PTPRJ in the ARC in non-obese mice with a viral vector induced significant leptin resistance. Overall, our results indicated that PTPRJ plays critical roles in the development of leptin resistance *in vivo*.

## 2-2 R5 RPTP subfamily

PTPRZ is the most abundant RPTP in oligodendrocyte precursor cells (OPCs), which are the principal source of myelinating oligodendrocytes. Three PTPRZ isoforms are generated by alternative splicing from a single gene: two transmembrane isoforms, PTPRZ-A and PTPRZ-B, and one secretory isoform, PTPRZ-S (or phosphacan). All isoforms are heavily modified with chondroitin sulfate (CS) chains, and identified as chondroitin sulfate proteoglycans (CSPGs) in the CNS. The CS moiety on their extracellular domain of PTPRZ is essential for achieving high-affinity binding sites for the endogenous ligands such as pleiotrophin (PTN). We have proposed that PTPRZ is a new molecular target for drug development in glioblastoma and demyelinating diseases such as multiple sclerosis (MS).

CSPGs enriched in demyelinating plaques are known to impair remyelination by inhibiting the migration and differentiation of OPCs in MS patients. This year, we revealed that protamine (PRM) effectively neutralizes the inhibitory activities of CSPGs, thereby enhancing OPC differentiation and (re)myelination in mice. Cell-based assays revealed that PRM exerted masking effects on extracellular CSPGs, and also acted as a ligand mimetic for PTPRZ-A/B, thereby improving oligodendrocyte differentiation even under unfavorable differentiation conditions with CSPGs (Figure 2). Intranasal administration of PRM accelerated myelination in the developing mouse brain, and its intracerebroventricular administration stimulated remyelination after cuprizone-induced demyelination.

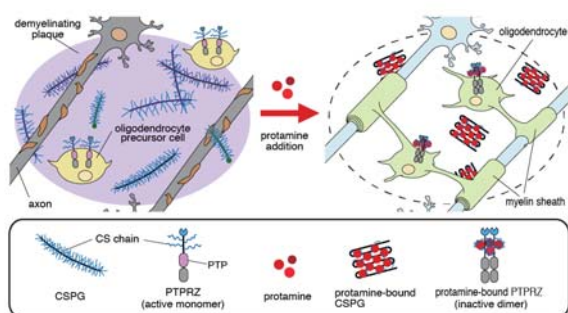


Figure 2. A possible regeneration model: Protamine may neutralize CSPG-mediated inhibition of migration of OPCs into demyelinating plaques and differentiation of OPCs to oligodendrocytes (see text).

A recent study on primary human glioblastomas suggested a close association between PTPRZ1 (human PTPRZ) expression and cancer stemness. However, the functional roles of PTPRZ in glioma stem cells have remained unclear. As was expected, sphere-forming cells from separated rat C6 and human U251 glioblastoma cell lines showed high expression of PTPRZ-B, the short receptor isoform of PTPRZ. Stable PTPRZ knockdown altered the expression levels of stem cell

transcription factors such as SOX2, OLIG2, and POU3F2, along with decreased sphere-forming abilities of these cells.

We identified an allosteric PTPRZ inhibitor, NAZ2329 (Figure 3A) for the first time. This compound efficiently reduced the expression of SOX2 in C6 and U251 cells, and abrogated the sphere-forming abilities of these cells. Furthermore, tumor growth in the C6 xenograft mouse model was significantly slower with the co-treatment of NAZ2329 with temozolomide, an alkylating agent, than with the individual treatments (Figure 3B). These results indicate that PTPRZ is a promising molecular target for differentiation-inducing therapy of malignant gliomas.

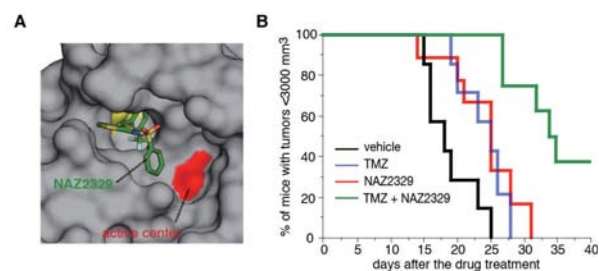


Figure 3. Identification of a new allosteric inhibitor, NAZ2329. **A**, X-ray structure of PTPRZ1-D1 complexed with NAZ2329. Cys1933 at the active site and Val-1911 at the bottom of the allosteric pocket are indicated in red and yellow, respectively. **B**, Antitumor effect of NAZ2329. Nude mice subcutaneously implanted with C6 glioblastoma cells were treated with DMSO, NAZ2329, temozolomide (TMZ), or the combination of NAZ2329 with TMZ until the humane endpoint ( $>3,000\text{ mm}^3$  tumor size or 40 days after the treatment). Kaplan-Meier analysis of the four treatment groups.

## III. Brain systems for body-fluid homeostasis

We have shown that  $\text{Na}_x$ , which structurally resembles voltage-gated sodium channels ( $\text{Na}_v1.1-1.9$ ), is the brain  $[\text{Na}^+]$  sensor to detect increases in  $[\text{Na}^+]$  in body fluids.  $\text{Na}_x$  is preferentially expressed in specific glial cells of sensory circumventricular organs (sCVOs) including the subfornical organ (SFO) and organum vasculosum lamina terminalis (OVLT). We have reported that  $\text{Na}_x$  signals in these brain regions deficient in a blood-brain barrier are involved in the control of water and salt intake.

### 3-1 Thirst control by $\text{Na}_x$ and TRPV4

We previously demonstrated that  $\text{Na}_x$  signals in  $\text{Na}_x$ -positive glial cells leads to the activation of TRPV4-positive neurons by using epoxyeicosatrienoic acids as gliotransmitters in sCVOs to stimulate water intake. Also, we suggested that another  $[\text{Na}^+]$ -dependent signal is needed besides the  $\text{Na}_x$ -TRPV4 signal to explain the whole amount of water intake. In order to identify the sensor molecule which generates the unknown  $[\text{Na}^+]$ -dependent signal, we performed RNA-seq analysis of sCVOs and identified several candidates. We are now examining the functional roles of these candidates in water intake.

### 3-2 Thirst and salt-appetite control by angiotensin II, $[\text{Na}^+]$ , and cholecystokinin

Angiotensin II (Ang II) is known to drive both thirst and salt appetite, and Ang II levels in blood are increased under both water- and salt-depleted conditions. However, we selec-

tively feel a thirst or salt appetite dependent on body fluid conditions. The brain mechanisms by which we properly take in water or salt have not been fully elucidated. Our local deletion experiments of *AT1a*, an Ang II receptor gene, indicated that AT1a signals in the SFO are involved in both water and salt intakes, whereas those in the OVLT are only involved in water intake. AT1a-positive neurons in the SFO project to several nuclei, including the OVLT and ventral part of the bed nucleus of the stria terminalis (vBNST). By using optogenetics, we demonstrated that thirst and salt appetite are driven by distinct groups of AT1a-positive neurons in the SFO: Neurons projecting to the OVLT control water intake, while those projecting to the vBNST control salt intake. We named these two different driver neurons ‘water neurons’ and ‘salt neurons’, respectively (Figure 4).

‘Water neurons’ were suppressed under Na-depleted conditions through cholecystokinin (CCK)-mediated activation of GABAergic neurons. As was expected, CCK levels in the SFO were increased under Na-depleted (water-satiated) conditions. The control mechanism of CCK secretion in the SFO remains to be elucidated. We had reported that *Na<sub>x</sub>*-KO mice do not stop salt intake even under water-depleted conditions. We herein revealed that ‘salt neurons’ were suppressed under the water-depleted conditions through activation of another population of GABAergic neurons by *Na<sub>x</sub>* signals. These studies thus provided new insights into the central mechanisms by which body fluid conditions control thirst and salt appetite. On the other hand, it is also known that aldosterone enhances salt appetite besides Ang II. Our next goal is integration mechanisms of salt-appetite signals from ‘salt neurons’ and ‘aldosterone-sensitive neurons’ in the vBNST.

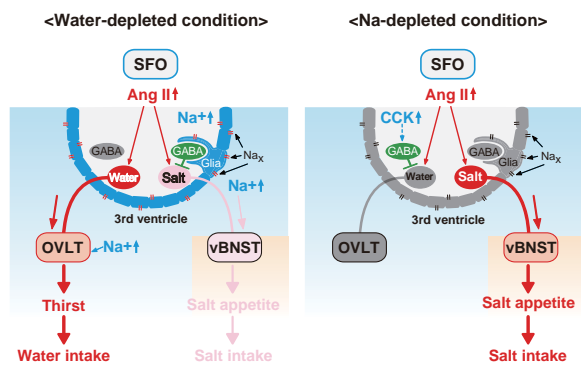


Figure 4. A schematic overview of control mechanisms for thirst and salt appetite in the SFO. Under the water-depleted condition (left), [Ang II] and [Na<sup>+</sup>], but not [CCK], increase in the SFO, and “water neurons” innervating the OVLT are selectively activated. Under the Na-depleted condition (right), [Ang II] and [CCK], but not [Na<sup>+</sup>], increase, and “salt neurons” innervating the vBNST are selectively activated. These two inhibitory systems by distinct GABAergic neurons are responsible for the specific activation of “water neurons” or “salt neurons” dependent on the body-fluid conditions. On the other hand, both water and Na intakes are simultaneously stimulated under the water- and Na-depleted condition, because the both neurons are active by Ang II.

## Publication List:

### [Original papers]

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- Kuboyama, K., Tanga, N., Suzuki, R., Fujikawa, A., and Noda, M. (2017). Protamine neutralizes chondroitin sulfate proteoglycan-mediated inhibition of oligodendrocyte differentiation. *PLoS ONE* *12*, e0189164.
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- Utsunomiya, A.N., Okada, S., Hiyama, T.Y., Noda, M., and Kobayashi, M. (2017). Clinical characteristic features of adipsic hypernatremia patients with subfornical organ-targeting antibody. *Clin. Pediatr. Endocrinol.* *26*, 197-205.