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We have been studying the molecular and cellular mechanisms underlying the development of the vertebrate central nervous system (CNS), mainly using mice. This research covers many developmental events including the patterning of the retina, neuronal terminal differentiation, axonal navigation, branching and targeting, synapse formation, refinement and plasticity. The scope of our interests also encompasses the mechanisms for various functions of the mature brain, including body-fluid regulation, behavior 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, reportedly plays pivotal roles in the development of several tissues including the vascular and nervous systems. We demonstrated, for the first time, that PTPRJ is a physiological enzyme attenuating insulin signaling *in vivo*. *Ptprj*-KO mice show lower weight gain associated with lesser food intake compared with wild-type mice. Thus we are now investigating a role of PTPRJ in the regulation of energy homeostasis.

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 *PTPRZ* gene: two transmembrane isoforms, PTPRZ-A and PTPRZ-B, and one secretory isoform, PTPRZ-S, all of which expressed in the CNS are heavily modified with chondroitin sulfate (CS) chains. The CS moiety on their extracellular domain of PTPRZ is essential for achieving high-affinity binding sites for the endogenous ligands; pleiotrophin (PTN), midkine, and interleukin-34. However, its functional significance and molecular mechanism in regulating PTPRZ activity remains obscure.

We have shown that PTPRZ receptors have functions to maintain the immature status of OPCs by dephosphorylating various substrate molecules including p190 RhoGAP. This year, we revealed that protein expression of CS-modified PTPRZ-A began earlier, peaking at postnatal days 5–10 (P5–P10), and then that of PTN peaked at P10 at the developmental stage corresponding to myelination onset in the mouse brain. *Ptn*-KO mice consistently showed a later onset of the expression of myelin basic protein, a major component of the myelin sheath, than wild-type mice. Upon ligand application, PTPRZ-A/B in cultured oligodendrocyte precursor cells exhibited punctate localization on the cell surface instead of diffuse distribution, causing the inactivation of PTPRZ and oligodendrocyte differentiation. Notably, the same effect was observed with the removal of CS chains with chondroitinase ABC (chABC), but not polyclonal antibodies against the extracellular domain of PTPRZ (Figure 1). These data indicate that PTN-PTPRZ-A signaling controls the timing of oligodendrocyte precursor cell differentiation *in vivo*, in which the negatively charged CS moiety maintains PTPRZ in a monomeric active state, and that the positively-charged PTN induces receptor clustering, potentially by neutralizing electrostatic repulsion between CS chain.

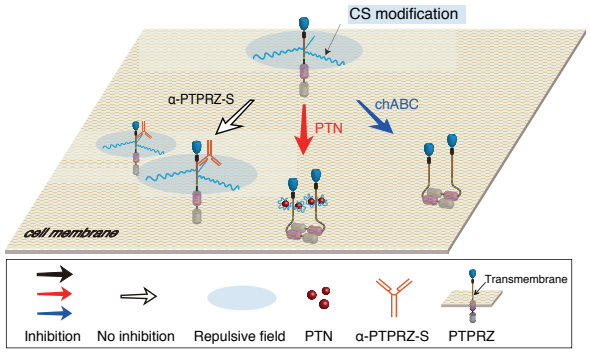

Figure 1. Schematic representation of the role of the CS chains in regulating PTPRZ activity. The CS modification in PTPRZ was essential for maintaining the monomeric active form, possibly by electrostatic repulsion, and, at the same time, provided ligand binding sites. The binding of PTN appears to neutralize the negative charges of the CS chains, causing the dimerization and inactivation of PTPase (see text).

Emerging data have indicated that PTPRZ is aberrantly over-expressed in glioblastoma and it is a causative factor for its malignancy. However, small molecules that selectively inhibit the catalytic activity of PTPRZ have not been developed so far. We identified SCB4380 as the first potent inhibitor for PTPRZ by *in vitro* screening of a chemical library. We determined the crystal structure of the catalytic domain of PTPRZ. Furthermore, the structural basis of the binding of SCB4380 elucidated by a molecular docking method was validated by site-directed mutagenesis studies (Figure 2A). The intracellular delivery of SCB4380 by liposome carriers inhibited PTPRZ activity in C6 glioblastoma cells, and thereby suppressed their migration and proliferation *in vitro* and tumor growth in a rat allograft model (Figure 2B). Therefore, selective inhibition of PTPRZ represents a promising approach for glioma therapy.

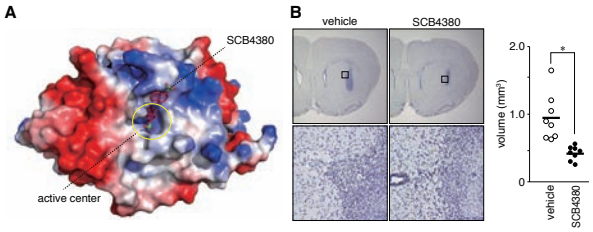

Figure 2. Development of SCB4380 and its application to a rat glioblastoma model. **A**, whole view of the best docking pose of SCB4380 shown by stick representation. **B**, rat C6 glioblastoma cells transplanted into rat brains were allowed to grow for 7 days. One day after surgery, SCB4380/liposome, or vehicle was icv injected daily for 5 days. Representative sections are shown together with an enlargement of the boxed area. The graph shows tumor volume. The horizontal bars indicate the average of each group. $*P<0.05$, by the Student's *t*-test.

III. Brain systems for body-fluid homeostasis

We have previously shown that Na_x, which structurally resembles voltage-gated sodium channels ($Na_v1.1$–1.9), is a Na^+-concentration ($[Na^+]$)-sensitive Na channel with a gating threshold of ~150 mM for extracellular $[Na^+]$ ($[Na^+]_o$) *in vitro*. Na_x is preferentially expressed in the glial cells of sensory circumventricular organs (sCVOs) including the subfornical organ (SFO) and organum vasculosum lamina terminalis (OVLT), and is involved in $[Na^+]$ sensing for the control of salt-appetite.

3-1 Thirst control in the brain by Na_x and TRPV4

We found that Na_x is also involved in thirst control in dehydrated animals (Figure 3). We investigated voluntary water intake immediately induced after an intracerebroventricular (icv) administration of a hypertonic NaCl solution in mice. Because the transient receptor potential vanilloid (TRPV) channels, TRPV1 and TRPV4, have been proposed to function as osmosensors, we examined *TRPV1*-, *TRPV4*-, *Na_x*-, and their double-gene KO mice. The induction of water intake by *TRPV1*-KO mice was normal, whereas that by *TRPV4*-KO and *Na_x*-KO mice was significantly less than that by WT mice. Water intake by *Na_x*/*TRPV4*-double KO mice was similar to that by the respective single KO mice. When TRPV4 activity was blocked with a specific antagonist (HC-067047), water intake by WT mice was significantly reduced, whereas that by *TRPV4*-KO and *Na_x*-KO mice was not. The same results were obtained by the administration of miconazole, which inhibits the biosynthesis of epoxyeicosatrienoic acids (EETs), endogenous agonists for TRPV4, from arachidonic acid (AA). Moreover, icv injection of hypertonic NaCl with AA or 5,6-EET restored water intake by *Na_x*-KO mice to the WT level, but not that by *TRPV4*-KO mice. These results suggest that the Na^+ signal generated in Na_x-positive glial cells leads to the activation of TRPV4-positive neurons in sCVOs in order to stimulate water intake by using EETs as gliotransmitters.

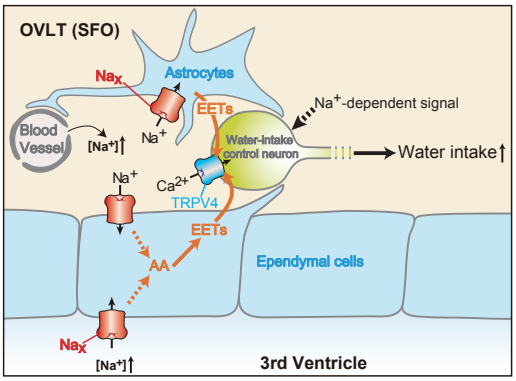

Figure 3. A possible signaling pathway in sCVOs underlying the induction of water intake. When $[Na^+]_o$ in plasma and cerebrospinal fluid increases, Na_x channels (red) in glial cells (astrocytes and ependymal cells) in the SFO or OVLT are activated, leading to the synthesis of EETs. EETs released from glial cells activate TRPV4 channels (blue) in the neighboring neurons, which conceivably control water-intake behavior. The additional activation signal of an unknown Na^+-dependent mechanism is required for the Na_x/TRPV4-dependent induction of water intake.

3-2 Thirst and salt-appetite control in the brain by angiotensin II, $[Na^+]$, and cholecystokinin

Angiotensin II (Ang II) is known to drive both thirst and salt appetite; however, the neural mechanisms underlying selective water- and/or salt-intake behaviors remain unknown. Using optogenetics, we showed that thirst and salt appetite are driven by distinct groups of angiotensin

II receptor type 1a-positive excitatory neurons in the SFO (Figure 4). Neurons projecting to the OVLT control water intake, while those projecting to the ventral part of the bed nucleus of the stria terminalis (vBNST) control salt intake. Thirst-driving neurons are suppressed under sodium-depleted conditions through cholecystokinin (CCK)-mediated activation of GABAergic neurons. In contrast, the salt-appetite-driving neurons were suppressed under dehydrated conditions through activation of another population of GABAergic neurons by Na_x signals. These distinct mechanisms in the SFO may underlie the selective intakes of water and/or salt and may contribute to body fluid homeostasis.

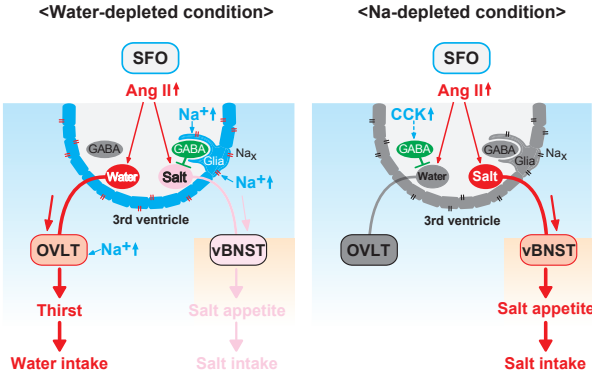

Figure 4. A schematic overview of controls for thirst and salt appetite from SFO. Under the water-depleted condition (left), both Ang II and [Na^+] increase in SFO. "Water neurons" innervating OVLT are selectively activated under the water-depleted condition. Under the Na-depleted condition (right), both Ang II and CCK increase in SFO, and "salt neurons" which innervate vBNST are selectively activated. Under the water- and Na-depleted condition, both water and Na intakes are stimulated, because neither the Na_x signal nor CCK signal is induced.

3-3 Adipsic hypernatremia accompanied by autoantibodies to the SFO

Adipsic (or essential) hypernatremia is a rare hypernatremia caused by a deficiency in thirst regulation and vasopressin release. In 2010, we reported a case in which autoantibodies targeting Na_x caused adipsic hypernatremia without hypothalamic structural lesions demonstrable by magnetic resonance imaging (MRI). This year, we reported three newly identified patients with similar symptoms. Immunohistochemical analyses using the sera of these patients revealed that antibodies specifically reactive to the mouse SFO were present in the sera of all cases. Passive transfer to mice of the patient immunoglobulin revealed that autoimmune destruction of the SFO may be the cause of the adipsic hypernatremia. This study provides a possible explanation for the pathogenesis of adipsic hypernatremia without demonstrable hypothalamus-pituitary lesions.

Publication List:

〔Original papers〕

- Doura, T., Kamiya, M., Obata, F., Yamaguchi, Y., Hiyama, T.Y., Matsuda, T., Fukamizu, A., Noda, M., Miura, M., and Urano, Y. (2016). Detection of *lacZ*-positive cells in living tissue with single-cell resolution. Angew. Chem. Int. Ed. Engl. *55*, 9620–9624.
- Fujikawa, A., Nagahira, A., Sugawara, H., Ishii, K., Imajo, S., Matsumoto, M., Kuboyama, K., Suzuki, R., Tanga, N., Noda, M., Uchiyama, S., Tomoo, T., Ogata, A., Masumura, M., and Noda, M. (2016). Small-molecule inhibition of PTPRZ reduces tumor growth in a rat model of Glioblastoma. Sci. Rep. *6*, 20473.
- Kuboyama, K., Fujikawa, A., Suzuki, R., Tanga, N., and Noda, M. (2016). Role of chondroitin sulfate (CS) modification in the regulation of protein tyrosine phosphatase receptor type Z (PTPRZ) activity: Pleiotrophin-PTPRZ-A signaling is involved in oligodendrocyte differentiation. J. Biol. Chem. *291*, 18117-18128.
- Sakuta, H., Nishihara, E., Hiyama, T.Y., Lin, C.-H., and Noda, M. (2016). Na_x signaling evoked by an increase in [Na^+] in CSF induces water intake via EET-mediated TRPV4 activation. Am. J. Physiol. Regul. Integr. Comp. Physiol. *311*, R299-R306.

〔Original papers (E-publication ahead of print)〕

- Hiyama, T.Y., Utsunomiya, A.N., Matsumoto, M., Fujikawa, A., Lin, C.-H., Hara, K., Kagawa, R., Okada, S., Kobayashi, M., Ishikawa, M., Anzo, M., Cho, H., Takayasu, S., Nigawara, T., Daimon, M., Sato, T., Terui, K., Ito, E., and Noda, M. Adipsic hypernatremia without hypothalamic lesions accompanied by autoantibodies to subfornical organ. Brain Pathol. 2016 Aug 2.
- Matsuda, T., Hiyama, T.Y., Niimura, F., Matsusaka, T., Fukamizu, A., Kobayashi, K., Kobayashi, K., and Noda, M. Distinct neural mechanisms for the control of thirst and salt appetite in the subfornical organ. Nat. Neurosci. 2016 Dec 19.

〔Review articles〕

- Fujikawa, A., and Noda, M. (2016). Role of pleiotrophin-protein tyrosine phosphatase receptor type Z signaling in myelination. Neural Regen. Res. *11*, 549-551.
- Hiyama, T.Y., and Noda, M. (2016). Sodium sensing in the subfornical organ and body-fluid homeostasis. Neurosci. Res. *113*, 1-11.