Review

RET receptor signaling: Function in development, metabolic disease, and cancer

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Abstract: The *RET* proto-oncogene encodes a receptor tyrosine kinase whose alterations are responsible for various human cancers and developmental disorders, including thyroid cancer, non-small cell lung cancer, multiple endocrine neoplasia type 2, and Hirschsprung's disease. RET receptors are physiologically activated by glial cell line-derived neurotrophic factor (GDNF) family ligands that bind to the coreceptor GDNF family receptor α (GFRα). Signaling via the GDNF/GFRα1/RET ternary complex plays crucial roles in the development of the enteric nervous system, kidneys, and urinary tract, as well as in the self-renewal of spermatogonial stem cells. In addition, another ligand, growth differentiation factor-15 (GDF15), has been shown to bind to GFRα-like and activate RET, regulating body weight. GDF15 is a stress response cytokine, and its elevated serum levels affect metabolism and anorexia-cachexia syndrome. Moreover, recent development of RET-specific kinase inhibitors contributed significantly to progress in the treatment of patients with *RET*-altered cancer. This review focuses on the broad roles of RET in development, metabolic diseases, and cancer.

Keywords: *RET* protooncogene, GDNF family ligands, cancer, Hirschsprung's disease, kidney development, body weight control

1. Introduction

We identified *RET* (*R*earranged during *T*ransfection) as an oncogene activated by DNA rearrangement in 1985.[1] *RET* encodes a transmembrane tyrosine kinase with a unique extracellular domain that consists of four cadherin-like domains and a cysteine-rich region with 16 cysteine residues in a stretch of 120 amino acids (Fig. 1).[2)–4)] Alternative splicing in the 3′ region produces three different isoforms (1072, 1106, and 1114 amino acids) with short (9 amino acids, RET9), intermediate (43 amino acids, RET43), and long (51 amino acids, RET51) carboxyl-terminal tails, respectively,[5] and the expression level of RET43 was reported to be low compared with RET9 and RET51. As observed for cadherin, Ca^{2+} ions bind to the cadherin-like domains, and these are required for RET activation by GDNF and neurturin (NRTN).[4),6)]

Following RET activation by GDNF family ligands (GFLs), specific tyrosine residues in its intracellular domain are autophosphorylated. At least 14 of the 18 tyrosine residues in the intracellular

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Non-standard abbreviation list: AP: area postrema; ARTN: artemin; BRA: bilateral renal agenesis; CAKUT: congenital anomalies of the kidney and urinary tract; ENCDC: enteric neural crest-derived cell; ENS: enteric nervous system; FMTC: familial medullary thyroid carcinoma; GDF15: growth differentiation factor-15; GDNF: glial cell line-derived neurotrophic factor; GFL: GDNF family ligand; GFRα: GDNF family receptor α; GFRAL: GDNF family receptor-α like; HSCR: Hirschsprung's disease; MEN2: multiple endocrine neoplasia type 2; MM: metanephric mesenchyme; MTC: medullary thyroid carcinoma; MTKI: multiple tyrosine kinase inhibitors; NRTN: neurturin; NSCLC: non-small cell lung carcinoma; NST: nucleus of the solitary tract; ORR: objective response ratio; PFS: progression-free survival; PLCγ: phospholipase C γ; PSPN: persephin; PTC: papillary thyroid carcinoma; RET: *R*earranged during *T*ransfection; TGF-β: transforming growth factor-β; UB: ureteric bud; URA: unilateral renal agenesis; WD: Wolffian duct.

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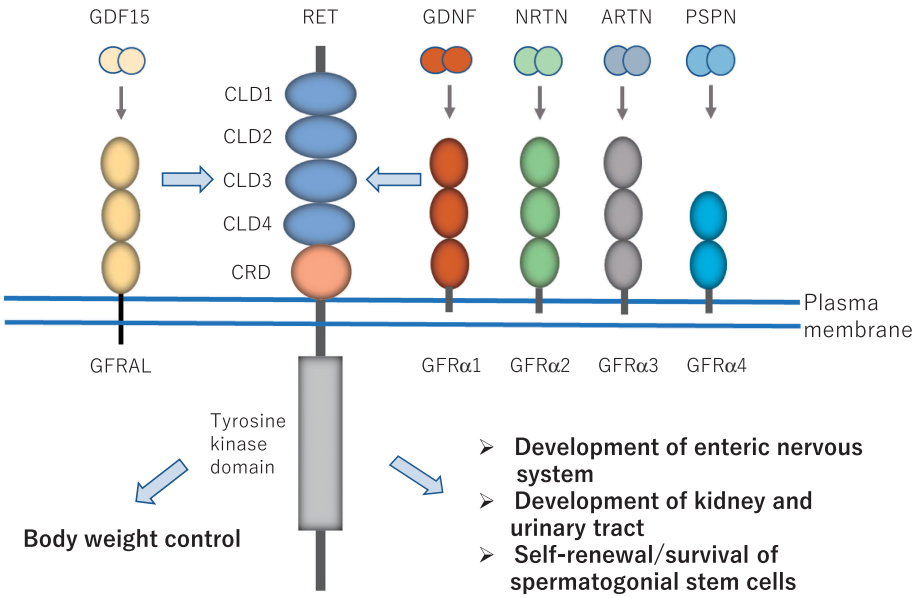

Fig. 1. RET activation by GDNF family ligands (GFLs) that bind to coreceptor GDNF family receptor α (GFRα). GDNF, NRTN, ARTN and PSPN preferentially bind to GFRα1, GFRα2, GFRα3 and GFRα4, respectively. GDNF/GFRα1/RET signaling complex is essential for the development of the enteric nervous system, kidney and urinary tract, and self-renewal/survival of spermatogonial stem cells. GDF15/GFRAL/RET complex plays a crucial role in body weight control. GDNF, glial cell line-derived neurotrophic factor; NRTN, neurturin; ARTN, artemin; PSPN, persephin; GDF15, growth differentiation factor-15; GFRAL, GDNF family receptor α-like; CLD, cadherin-like domain; CRD, cysteine-rich domain.

domain can be phosphorylated, and some of them represent docking sites for key adaptor proteins, leading to the activation of important signaling pathways (Fig. 2).[4),7),8)] For example, phosphorylated tyrosine 1062 mediates binding of the adaptor proteins SHC and FRS2, which are responsible for activation of the RAS/MAPK and/or PI3K/AKT signaling pathways.[9)–11)] Phosphorylated tyrosine 1015 mediates binding of phospholipase C γ (PLCγ), resulting in the activation of protein kinase C.[12)] These pathways play important roles in cell migration, proliferation, survival, and differentiation.

RET has been shown to be a causative gene for a variety of human diseases.[5)] *RET* activating point mutations are responsible for the development of the hereditary cancer syndrome multiple endocrine neoplasia type 2 (MEN2), which develops into medullary thyroid carcinoma (MTC) and pheochromocytoma.[13)–16)] *RET* activation by gene rearrangement is found in papillary thyroid carcinoma (PTC), non-small cell lung carcinoma (NSCLC), salivary gland intraductal carcinoma, and other cancers.[17)–24)] In addition, *RET*-inactivating point mutations or deletions lead to the development of Hirschsprung's disease (HSCR),[25),26)] which is a congenital malformation characterized by aganglionosis of variable length of the distal gastrointestinal tract. To date, the molecular mechanisms through which *RET* mutations lead to disease development have been extensively studied.

2. RET activation by GDNF family ligands

In 1993, GDNF was purified and cloned as a neurotrophic factor that enhances the survival of midbrain dopaminergic neurons.[27)] GDNF is a distant member of the transforming growth factor-β (TGF-β) superfamily, and three other proteins of GFLs, including NRTN, artemin (ARTN), and persephin (PSPN) were identified.[28)] These four family ligands show approximately 40% amino acid identity with each other and can activate RET kinase. However, GFLs cannot bind to RET directly, but GPI-anchored coreceptors named GFRα1–4 are necessary for their binding.[28)–32)] GDNF, NRTN, ARTN, and PSPN bind preferentially to GFRα1, GFRα2, GFRα3, and GFRα4, respectively (Fig. 1), although crosstalk occurs to a certain extent between the ligand and coreceptor pairs.[8)] Formation of the GFL-GFRα-RET 2:2:2 ternary complex results in activation of intracellular signaling, which supports the survival and differentiation of various neurons,

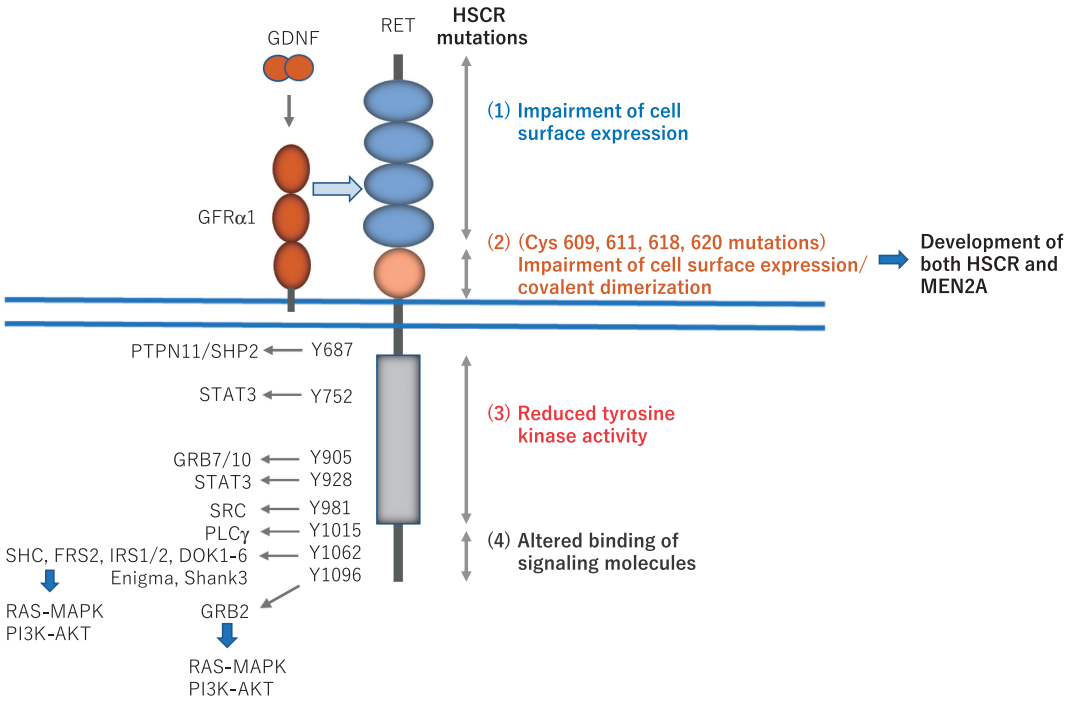

Fig. 2. Intracellular signaling pathways activated by RET via phosphotyrosines and *RET* mutations in Hirschsprung's disease (HSCR). Phosphorylated tyrosines in the intracellular domain of RET interact with a wide range of adaptor proteins, which leads to the activation of downstream signaling pathways, including the RAS/MAPK and PI3K-AKT pathways. For example, phosphotyrosine 1062 represents a multifunctional docking site for SHC, FRS2, and DOK family proteins. Missense mutations identified in HSCR patients are distributed along the whole coding sequence of the *RET* gene. Based on functional analyses of mutant RET, the HSCR mutations are classified as follows. (1) Most mutations in the RET extracellular domain impair its cell surface expression, most likely due to misfolding of the RET protein. (2) Mutations of Cys609, 611, 618, and 620 can result in the development of both HSCR (loss-of-function) and multiple endocrine neoplasia type 2A (MEN2A) (gain-of-function) phenotypes that are caused by impaired cell surface expression of RET in ENCDCs and covalent dimerization in thyroid C cells, respectively. (3) Mutations in the tyrosine kinase domain almost completely or partially disturb the RET kinase activity, resulting in the impairment of RAS/MAPK, PI3K/AKT, and/or PLCγ signaling pathways. (4) Mutations in the carboxyl-terminal tail alter the binding of adaptor proteins such as SHC.

including peripheral sensory and autonomic neurons as well as central motor and dopaminergic neurons.[28] Recent cryo-EM analysis demonstrated that the extracellular region of RET is folded and packed in a 'C-clamp' shape, which is stabilized by extensive inter-domain interactions. Because of this unique C-clamp shape, two RET molecules are recruited onto dimeric GFL-GFRα complexes, and the two cysteine-rich domains of RET are brought into close proximity, thereby promoting dimer formation and activation of the activity of the intracellular tyrosine kinase domain.[33)–35)]

More recently, another member of the TGF-β superfamily, GDF15 (also known as MIC-1) was found to bind to GFRα-like (GFRAL) with high affinity and then activate RET (Fig. 1).[36] The significance of this signaling complex is described in detail in a separate section.

3. Role of GDNF/GFRα1/RET signaling in the development of the enteric nervous system and Hirschsprung's disease

Gdnf-, *Gfr*α-, and *Ret*-deficient mice share phenotypes characterized by a lack of enteric neurons in the whole gastrointestinal tract and kidney agenesis or dysgenesis.[37)–42)] This finding clearly revealed the importance of signaling via GDNF/GFRα1/RET multicomponent receptors in development (Fig. 1). We and others found that phosphorylated tyrosine 1062 in the RET carboxyl-terminal tail represents a docking site for several adaptor proteins such as SHC and FRS2 and is important for activation of the RAS/MAPK and PI3K-AKT pathways (Fig. 2).[5] *Ret* mutant mice in which tyrosine 1062 was replaced with phenylalanine exhibited severe defects of enteric neurons in the

intestine and small kidneys, indicating a crucial role of signaling via tyrosine 1062 in organogenesis.[43),44)]

The enteric nervous system (ENS) originates from the neural crest, mostly at the vagal level. Neural crest-derived cells invade the foregut and begin their long rostrocaudal migration toward the end of the colon.[45)] In addition to extensive migration, establishment of the ENS requires controlled cell proliferation, differentiation and network formation by differentiated enteric neurons. During embryogenesis, migrating enteric neural crest-derived cells (ENCDCs) express RET and GFRα1. *Gdnf* mRNA is expressed in the mesenchyme of the gut and is abundant in the stomach on embryonic day 9.5 and extends to the cecum on embryonic day 10.5, suggesting a role of GDNF as a chemoattractant for ENCDC migration.

RET is a major causative gene for HSCR (prevalence: one in 5000 live births),[25),26)] which is a congenital malformation of the ENS that lacks enteric neurons mainly in the distal gastrointestinal tract. Based on the length of the aganglionic segment, HSCR is classified into two groups: short-segment HSCR (patients with aganglionosis as far as the rectosigmoidal junction) and long-segment HSCR (patients with aganglionosis beyond the rectosigmoid junction). *RET* mutations were found in approximately 50% of patients with familial HSCR and 10–20% of sporadic cases.[45)] Notably, there is a clear association between *RET* mutations and long-segment HSCR, total colonic aganglionosis, and total intestinal aganglionosis. A variety of missense, nonsense, and frameshift mutations have been identified along the entire coding sequence of *RET*, but meta-analysis data showed that *RET* mutations in HSCR are more commonly found in exon 10 (7.55%), 13 (11.32%), and 15 (7.55%).[46)] These mutations are inactivating and abrogate RET signaling, which is responsible for the migration and proliferation of ENCDCs during embryogenesis. Biochemical and cell biological analyses have elucidated various mechanisms through which *RET* missense mutations cause HSCR (Fig. 2).[47)] (1) Mutations in the RET extracellular domain impair its cell surface expression, most likely due to misfolding of the RET protein.[48)–50)] (2) Mutations of Cys609, 611, 618, and 620 can result in the development of both HSCR (loss-of-function) and multiple endocrine neoplasia type 2A (MEN2A) (gain-of-function) that are caused by impaired cell surface expression of RET in ENCDCs and covalent dimerization in thyroid C cells, respectively (described below in the section on *RET* mutations in cancer).[5)] (3) Mutations in the tyrosine kinase domain almost completely or partially disturb RET kinase activity, resulting in the impairment of RAS/MAPK, PI3K/AKT, and/or PLCγ signaling pathways.[51)–53)] (4) Mutations in the carboxyl-terminal tail alter the binding of adaptor proteins such as SHC.[54),55)]

In addition to the coding sequence, non-coding regions of the *RET* gene play a pivotal role in the development of HSCR. A common intronic enhancer polymorphism (RET +3, or rs2435357) was identified, which is a risk factor for HSCR and impairs *RET* expression.[56)] This common polymorphism might also interact with other genetic alterations, modulating the HSCR phenotype and may explain the failure to identify coding sequence mutations in the majority of HSCR cases, even in patients from families showing linkage to *RET*.

Nrtn- and *Gfrα2*-deficient mice showed a reduced number of myenteric neurons in the small intestine and a drastic reduction in cholinergic innervation in the salivary and lacrimal glands. *GDNF* and *NRTN* are considered rare susceptibility HSCR genes, sometimes in conjugation with *RET* mutations.[57),58)]

4. Role of GDNF/GFRα1/RET signaling in the development of kidney and renal anomaly

RET-mediated GDNF signaling has been shown to be essential for kidney development.[59)] During early development, RET and GFRα1 are expressed along the Wolffian duct (WD), whereas GDNF is expressed in the metanephric mesenchyme (MM) adjacent to the caudal portion of the WD (Fig. 3A). The ureteric bud (UB) emerges from the caudal portion of the WD, invaginates into the MM and begins to branch repeatedly. Of note, RET is highly expressed in a particular region of the WD where UB formation occurs (Fig. 3B) and subsequently at the tips of branching UB in the MM (Fig. 3C). During the whole process of kidney development, the UB tips appear to be formed entirely by RET-expressing cells that respond to GDNF in the MM, whereas the UB trunk largely consists of Ret-negative cells (Fig. 3D),[60),61)] indicating that RET expression is fine-tuned during UB branching. In the absence of *Gdnf*, *Ret*, or *Gfrα1* gene, the most frequent consequence is a failure of UB formation, resulting in renal agenesis or dysgenesis and ureter defects (no ureters, small ureters, abnormally connected ureters, *etc.*).[37)–42)] Several *Ret*-mutant mice showed decreased UB branching by affecting the RAS/MAPK,

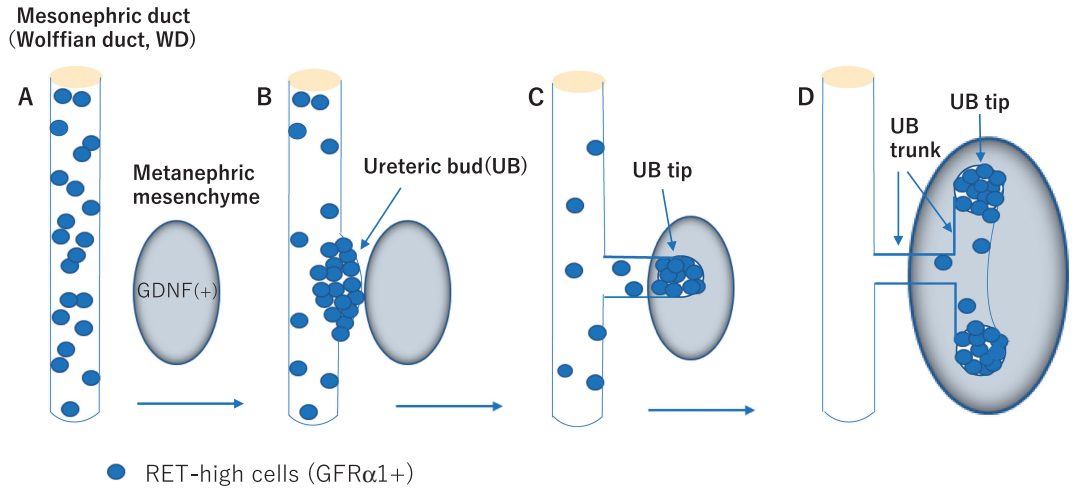

Fig. 3. RET-dependent cell movement during ureteric bud (UB) formation and branching. RET-positive cells (blue) are initially dispersed along the mesonephric duct (Wolffian duct, WD) (A). RET-positive cells start to move to form the primary UB and the ventral mesonephric duct is depleted of RET-positive cells (B). As the UB grows out, RET-positive cells form the UB tips while RET-negative cells follow and form the UB trunk (C, D).

PI3K/AKT, and/or PLCγ pathways.[8),43),62)] In addition, the transcription factors ETV4 and ETV5 were identified as key components of a gene network downstream of RET that promotes branching morphogenesis.[63),64)]

A previous report showed that heterozygous *RET* mutations were found in approximately 30% of a series of 29 human fetuses with bilateral or unilateral renal agenesis (BRA or URA) and one heterozygous *GDNF* mutation in a fetus with URA.[65)] However, another analysis of a large series of 105 cases, including 90 fetuses with either BRA or URA and contralateral renal hypodysplasia or multicystic dysplastic kidney reported only seven potential mutations in the *RET* coding sequence (6.6%) and no mutation in *GDNF*.[66)]

Congenital anomalies of the kidney and urinary tract (CAKUT) account for 40–50% of chronic renal diseases in children. CAKUT covers a wide range of structural malformations that result from a defect in the morphogenesis of the kidney and/or urinary tract. One study of 122 living patients, encompassing various CAKUT, found *RET* or *GDNF* variations in approximately 5% of patients.[67)] Moreover, in a large cohort of 749 individuals from 650 families with CAKUT, the coding exons of the 17 known dominant CAKUT-causing genes were analyzed. Among them, 37 different heterozygous mutations in 12 out of 17 genes examined were detected in 47 patients from 41 of the 650 families (6.3%), in which only three mutations in *RET* (0.5%) were included.[68)] These findings indicated that *RET* mutations are less commonly associated with CAKUT than expected.

5. Role of GDF15/GFRAL/RET signaling in body weight control

GDF15 is a member of the TGF-β family and is associated with body weight regulation.[36)] The administration of GDF15 to obese mice reduced food intake and body weight, demonstrating its anti-obesity effects.[69)] GDF15 is a stress-induced hormone and its plasma levels increase with age, intense exercise, obesity, smoking, and pregnancy. Higher levels are also observed in various human diseases, including cardiovascular disease, chronic kidney disease, diabetes, many advanced cancers, and serious infections (Fig. 4).[70)–72)]

In 2017, the receptor for GDF15 was identified by four pharmaceutical company research laboratories as GFRAL, the expression of which is detected in neurons of the hindbrain area postrema (AP) and nucleus of the solitary tract (NTS), leading to a decrease in food intake and body weight in mice.[73)–76)] More notably, GDF15-GFRAL requires RET tyrosine kinase as a signaling receptor for body weight regulation. GDF15 binding to GFRAL initiates RET phosphorylation, resulting in the activation of ERK1/2, AKT, PLCγ, and FOS (Fig. 4). The co-expression of RET and GFRAL in AP and NTS was confirmed by *in situ* hybridization analysis.

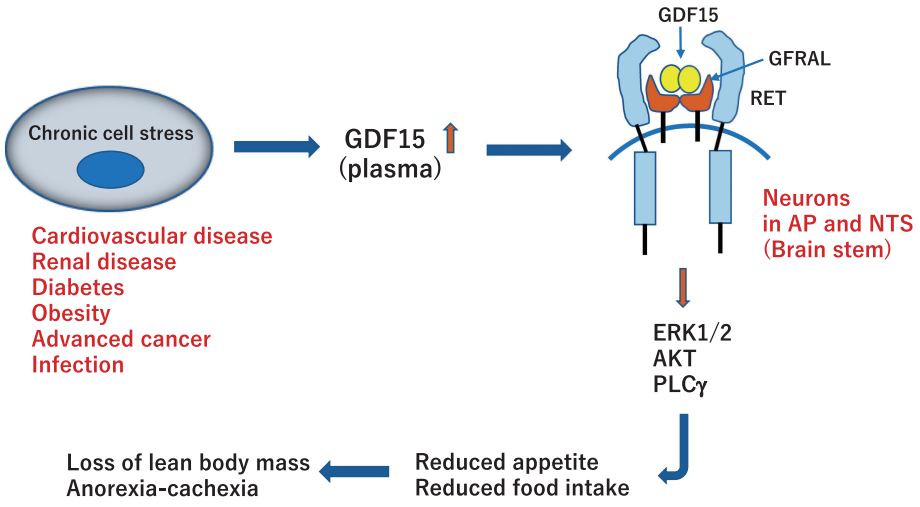

Fig. 4. Action of GDF15 under conditions of chronic stress. Circulating GDF15 increases in response to various cellular stresses and crosses the blood-brain barrier. GDF15 binds to GFRAL and activates RET in neurons in the hindbrain area postrema (AP) and nucleus of the solitary tract (NTS). This central pathway leads to reduced food intake, causing loss of lean body mass and anorexia-cachexia syndrome.

In homeostatic conditions, animals use hypothalamic neural circuits to maintain body weight by integrating metabolic and hormonal signals from the periphery. However, under stress conditions, they use an alternative neuronal pathway for metabolic changes.[36),77)] Elevated GDF15 plasma levels are observed in various chronic human diseases and are associated with body weight loss. In advanced cancers, the circulating levels of GDF15 increase by up to 10–100-fold and induce anorexia-cachexia syndrome (Fig. 4). Cachexia is defined as a metabolic syndrome associated with extreme involuntary wasting of lean body mass with or without loss of fat mass. GDF15 is now considered the main actor in cachexia observed in patients with cancer. However, there are no approved drugs for this condition. Recently, a monoclonal antibody that targets GFRAL and inhibits RET signaling has been developed. The antibody prevented the GDF15-driven interaction of RET with GFRAL and cancer-related cachexia by reversing excessive lipid oxidation in tumor-bearing mice.[78)] Further clinical trials of drugs targeting the GDF15-GFRAL pathway will shed light on the treatment of patients with cancer-related cachexia.

6. *RET* rearrangements in cancer

Since *RET* was discovered as an oncogene in 1985,[1)] a variety of *RET* rearrangements and point mutations have been identified in human cancers.[79)] Somatic *RET* rearrangements involve the 3′ sequence of *RET*, which contains the tyrosine kinase domain, and the 5′ sequence of various partner genes that contain dimerization domains such as the coiled-coil domain. *RET* breakpoints often occur within intron 11 and less frequently within introns 7 and 10 (Fig. 5A). To date, more than 35 genes have been reported to form fusion genes with *RET*.[79)]

RET fusion has been detected in 5–35% of adult PTCs, in which rearrangement with the *CCDC6* gene has most frequently been observed (named *RET/PTC1*) (Fig. 5B).[17)–19)] The other 5′ partner genes for *RET* fusion in PTC include *PRKAR1A*, *NCOA4*, *GOLGA5*, *TRIM24*, *TRIM33*, *KTN1*, and *RFG9*. The prevalence of *RET* rearrangements is much higher (50–80%) in radiation-induced PTCs following the Chernobyl radioactive fallout or the atomic bomb in Japan.[80)–83)] The highest frequency of rearrangement was found in post-Chernobyl children. A significant predominance of *RET* fusion with the *NCOA4* gene (named *RET/PTC3*) (Fig. 5B) over *RET/PTC1* was observed in PTCs of the first post-Chernobyl decade. *RET*, *CCDC6*, and *NCOA4* are located on the long arm of chromosome 10; thus, both *RET/PTC1* and *RET/PTC3* are induced by paracentric inversion. It is notable that *RET* rearrangements and *BRAF* mutations are largely mutually exclusive in PTCs. In addition to PTC, *RET* rearrangements were detected at a much lower prevalence in other types of thyroid cancer, such as follicular thyroid, anaplastic thyroid, and medullary thyroid carcinomas.[79)]

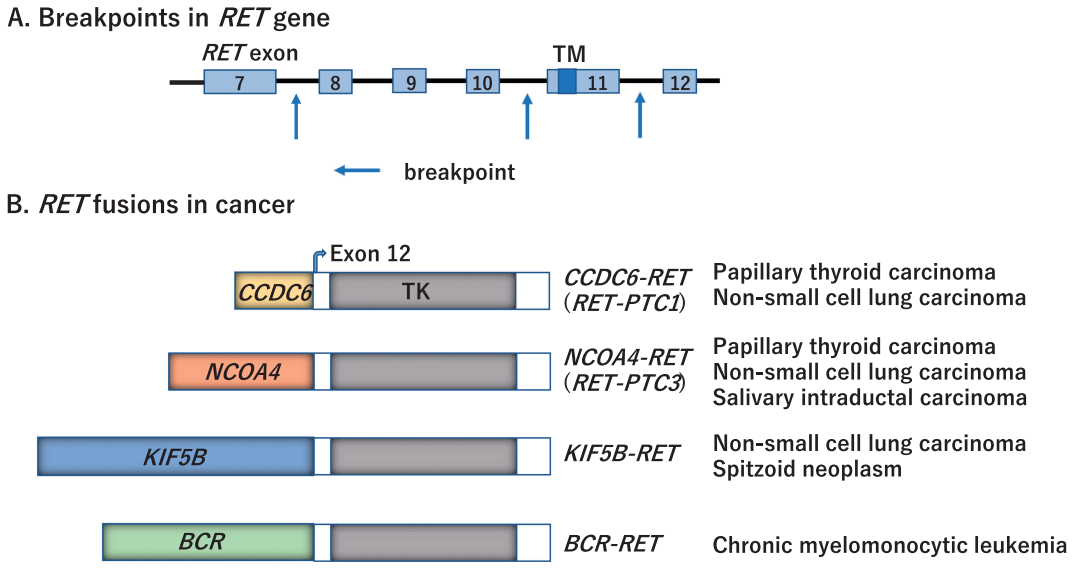

Fig. 5. *RET* rearrangement in human cancer. **A.** Breakpoints in the *RET* gene. The breakpoints of *RET* often occur within intron 11 and less frequently within introns 7 and 10 (indicated by arrows). **B.** Representative *RET* rearrangement identified in human cancers. PTC, papillary thyroid carcinoma; TM, transmembrane domain; TK, tyrosine kinase domain.

RET rearrangements are found in a portion (1–2%) of NSCLC cases with *KIF5B-RET* fusion being the most commonly identified (Fig. 5B).[20)–23)] Because *KIF5B* is located on the short arm of chromosome 10, the *KIF5B-RET* fusion is created by pericentric inversion. *CCDC6*, *NCOA4*, *TRIM33*, and *CUX1* are also partner 5′ genes for *RET* fusion in NSCLCs.[84)] Patients with *RET* fusion-positive NSCLCs have shown unique clinicopathological characteristics; they are young (<60 years old), female, and non-smoking patients.

Next-generation DNA and/or RNA sequencing approaches are used to identify less frequent *RET* rearrangements in a wide variety of cancer types. These include colorectal,[85)] breast,[86)] ovarian,[87)] chronic myelomonocytic leukemia[88)] and spitzoid tumors (Fig. 5B).[89)] Large-scale analyses have revealed that *RET* fusion can be detected in 0.2% of colorectal cancers (6/3117 cases)[85)] and 0.1% of breast cancers (8/9693 cases).[86)] Recently, a high frequency of *RET* rearrangements (>40%) has been detected in salivary intraductal carcinomas, including *NCOA4-RET* (Fig. 5B) and *TRIM27-RET*,[24),90)] suggesting that its detection is useful for diagnosing a particular type of salivary carcinoma.

7. *RET* mutations in cancer

Germline *RET*-activating mutations give rise to a hereditary cancer syndrome, MEN2.[13)–16)] Based on the clinical phenotypes, MEN2 is classified into three subtypes: MEN2A, MEN2B, and familial medullary thyroid carcinoma (FMTC). MEN2A is the most common subtype, which is characterized by MTC in all patients combined with the development of pheochromocytoma and parathyroid hyperplasia/adenoma (hyperparathyroidism) in ~50% and ~20% of the patients, respectively. Lichen amyloidosis is occasionally observed in patients with MEN2A. MEN2B is a more aggressive subtype with early onset of MTC. In addition to the development of MTC and pheochromocytoma, MEN2B patients display mucosal neuroma, hyperganglionosis of the intestine, thickening of the corneal nerve, and marfanoid habitus, but not hyperparathyroidism. FMTC is the most indolent subtype of MEN2 and usually develops MTC in the later stages of life. FMTC is now considered a variant of MEN2A.[91)]

The majority of *MEN2A* mutations (>95%) have been identified in one of six cysteine residues (codons 609, 611, 618, and 620 in exon 10 and codons 630 and 634 in exon 11) in the cysteine-rich region of the RET extracellular domain (Fig. 6A). Among these, Cys634 mutations have been found in ~85% of the patients.[92)] The same cysteine mutations in the RET extracellular domain also cause the FMTC phenotype with a high frequency of ~60% for Cys609, 611, 618, 620, or 630 substitutions and a

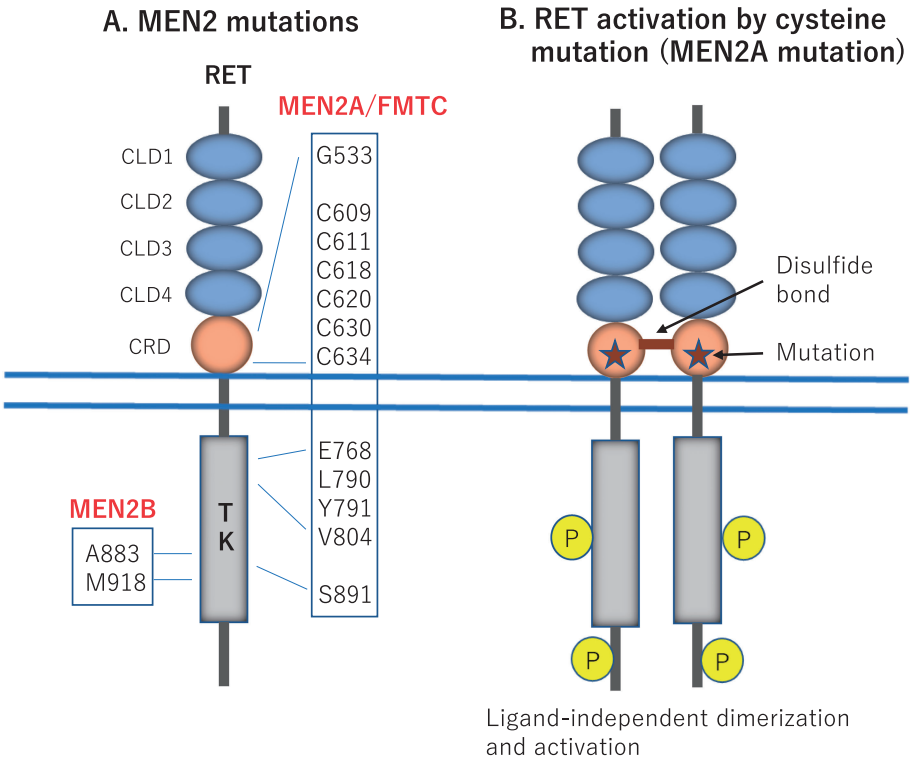

Fig. 6. Germline *RET* mutations in MEN2. **A.** The majority of MEN2A mutations (>95%) are identified in one of six cysteine residues (codons 609, 611, 618, and 620 in exon 10 and codons 630 and 634 in exon 11) in the cysteine-rich domain (CRD) of the RET extracellular region. In addition to cysteine substitutions, FMTC mutations are frequently found at noncysteine residues in both the extracellular and intracellular regions. The M918 mutation is detected in >95% of MEN2B patients. **B.** Mechanism of RET activation by cysteine mutations. When a cysteine residue is replaced with another amino acid in MEN2A/FMTC (indicated by stars), mutant RET proteins form ligand-independent covalent dimerization, resulting in constitutive activation.

lower frequency of ~30% for Cys634 substitutions.[93] We and Santoro *et al.* demonstrated that cysteine substitutions result in ligand-independent constitutive activation of RET through the formation of an aberrant intermolecular disulfide bond between two mutant RET (Fig. 6B).[94),95] In addition, the transforming activity of RET with Cys609, 611, 618, or 620 mutations is considerably lower than that of RET with Cys634 mutation, due to the impaired cell surface expression of the former four mutants.[96] This may explain why the Cys609, 611, 618, or 620 mutations predispose to the development of indolent FMTC rather than MEN2A. Of notes, RET cysteine mutations affecting Cys609, 611, 618, and 620 can develop both MEN2A/FMTC (gain-of-function) and HSCR (loss-of-function) phenotypes, which are caused by RET covalent dimerization in thyroid C cells and impaired cell surface expression in ENCDCs, respectively (Fig. 2).[97]

Moreover, Gly533Cys (G533C) (exon 8 in the extracellular domain),[98] Glu768Asp (E768D), Leu790Phe (L790F), Tyr791Phe (Y791F), Val804Met/Leu (V804M/L), and Ser891Ala(S891A) substitutions (exons 13–15 in the kinase domain) have been reported in some families with FMTC and/or MEN2A (Fig. 6A).[47),91]

Two specific mutations, Met918Thr (M918T) and Ala883Phe (A883F), are associated with the development of MEN2B (Fig. 6A).[15),16),99] The M918T mutation is found in >95% of patients and the A883F mutation in less than 4% of patients. These substitutions may induce conformational changes in the kinase domain that increase kinase activity and ATP binding and may alter substrate specificity.[100)–103] However, the mechanisms by which different *RET* mutations in MEN2A and MEN2B induce distinct clinical phenotypes remain elusive.

According to data published in a public database in 2015 (Catalog of Somatic Mutations in Cancer), somatic *RET* mutations have been identified with a high frequency (>40%) in sporadic MTC

patients. The M918T mutation is most frequent in these patients, and other less common somatic mutations are also observed at residues C634, C630, A883, and others.[79] A recent study using next-generation sequencing uncovered the presence of *RET* mutations in a variety of cancers at a low frequency, including colorectal carcinoma,[85),104] breast carcinoma,[86] endometrial and ovarian carcinoma, skin melanoma, Merkel cell carcinoma, and paraganglioma.[87]

8. Therapeutic application of RET kinase inhibitors to *RET* mutation-positive tumors

A variety of multiple tyrosine kinase inhibitors (MTKIs) have been used in clinical trials to treat *RET*-mutation-positive tumors. These MTKIs include vandetanib, cabozantinib, sorafenib, sunitinib, ponatinib, lenvatinib, alectinib, and RXDX-105, which target several tyrosine kinases.[105] Each molecule has distinct inhibitory activities against various targets. The clinical efficacy of MTKIs in *RET*-altered cancers is limited as shown by the lower overall objective response ratios (ORRs: 16–53% in *RET*-rearranged NSCLCs) and the shorter progression-free survival (PFS: 2.3–7.3 months in *RET*-rearranged NSCLCs). The limited efficacy of MTKIs is at least partially attributed to the off-target activity of these molecules.[79),105] In addition, while cabozantinib and vandetanib can effectively inhibit the activity of the RET M918T mutant, they fail to block the activity of the gatekeeper mutants RET V804M and V804L.[106),107]

Thus, more selective RET inhibitors are expected to achieve higher potency and lower toxicity. Recently, two RET-specific inhibitors, pralsetinib (BLU-667) and selpercatinib (LOXO-292), have been developed to inhibit wild-type RET and a broad spectrum of RET mutants, including those carrying the M918T mutation, the gatekeeper mutations V804L and V804M, and the *CCDC6-RET* and *KIF5B-RET* rearrangements.[79),108)–110] The use of these inhibitors improved ORR and the median duration of response, and revealed greater benefit for *RET*-altered MTC and NSCLC. For example, in the patients with *RET*-fusion-positive NSCLC who had previously received platinum-based chemotherapy, selpercatinib showed an ORR of 64% and a median duration of response of 17.5 months. Sixty-three percent of the responses were ongoing at a median follow-up of 12.1 months.[110] Notably, both inhibitors also exhibited anti-tumor activity in patients with brain metastasis.[111),112] Selpercatinib has been approved (September, 2021) and implemented (December 2021) for *RET*-rearranged NSCLC in Japan.

9. Conclusions

Over the past 30 years, a wide range of RET kinase functions have been studied, including roles in development, neurological disorders, and cancer. Recently, RET activation by GDF15 has been shown to play a pivotal role in the regulation of appetite and body weight during stress conditions, opening up new horizons in RET research. Further studies on GDF15/GFRAL-RET signaling will promote our understanding of cachexia metabolic signatures in patients with cancer and the development of therapeutic interventions to improve their outcomes. In addition, although not discussed in this review, the roles of RET in self-renewal and/or survival of spermatogonial stem cells[113)–115] and hematopoietic stem cells,[116] in intestinal immunity,[117),118] and in neuropathic pain[119] are also being actively studied. Research on RET kinases continues to have a profound impact on a wide range of fields of life science.

The development of RET kinase inhibitors has been actively pursued, and many clinical studies are in progress. Selective RET inhibitors, selpercatinib and pralsetinib, have demonstrated remarkable clinical efficacy and safety, and are beneficial to patients with advanced *RET*-altered cancers. RET is also expressed in different neuronal cells, including dopaminergic neurons, motor neurons, sympathetic neurons, and parasympathetic neurons. Thus, it is possible that RET-dependent signaling plays a role in neurodegenerative diseases, such as Parkinson's disease and amyotrophic lateral sclerosis. RET agonists and their appropriate delivery to the nervous system will contribute significantly to the development of new therapeutic strategies for such diseases.

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Profile

Masahide Takahashi was born in Gifu Prefecture, Japan, in 1954 and graduated from Nagoya University School of Medicine in 1979. He majored in Pathology at Nagoya University Graduate School of Medicine and received his PhD degree in 1983. He subsequently studied as a research fellow at Dana-Farber Cancer Institute and Harvard Medical School in Boston between 1983 and 1985. After returning to Japan, he worked as a researcher at Aichi Cancer Center Research Institute in Nagoya between 1985 and 1990, and he moved to Nagoya University as an Assistant Professor. He became Professor of Pathology at Nagoya University in 1996. He was appointed as Dean of Nagoya University School of Medicine between 2012 and 2017, and Trustee and Vice President of Nagoya University between 2017 and 2020. He became Director and Professor of the International Center for Cell and Gene Therapy at Fujita Health University in 2020. His pioneering work included the discovery of the *RET* protooncogene, mutations in which are responsible for various human cancers and developmental diseases. His research group has elucidated the mechanisms of disease development caused by *RET* mutations. For his accomplishments, he has received The Japan Pathology Award (2001), The Chunichi Cultural Award (2010), Medical Award of The Japan Medical Association (2019), Princess Takamatsu Cancer Research Fund Prize (2020), and Medal with Purple Ribbon from the Government of Japan (2020).