REVIEW

Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2

Simona M. Wagner*, ShuJun Zhu*, Adrian C. Nicolescu, Lois M. Mulligan

Division of Cancer Biology and Genetics, Cancer Research Institute and Department of Pathology & Molecular Medicine, Queen's University, Kingston, ON, Canada. *contributed equally to the study.

Multiple endocrine neoplasia type 2 is an inherited cancer syndrome characterized by tumors of thyroid and adrenal tissues. Germline mutations of the *REarranged during Transfection* (RET) proto-oncogene, leading to its unregulated activation, are the underlying cause of this disease. Multiple endocrine neoplasia type 2 has been a model in clinical cancer genetics, demonstrating how knowledge of the genetic basis can shape the diagnosis and treatment of the disease. Here, we discuss the nature and effects of the most common recurrent mutations of *RET* found in multiple endocrine neoplasia type 2. Current understanding of the molecular mechanisms of *RET* mutations and how they alter the structure and function of the RET protein leading to its aberrant activation, and the effects on *RET* localization and signaling are described.

KEYWORDS: RET; Multiple Endocrine Neoplasia Type 2; Genotype–Phenotype.

Wagner SM, Zhu SJ, Nicolescu AC, Mulligan LM. Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2. Clinics. 2012;67(S1):77-84.

E-mail: mulligal@queensu.ca

Tel.: 1 613 533 6000 ext.77475

INTRODUCTION

Multiple endocrine neoplasia type 2 (MEN 2) is an inherited cancer syndrome, characterized by medullary thyroid carcinoma (MTC). The disease has three clinically defined subtypes, as described elsewhere in this volume. Briefly, familial MTC (FMTC), considered the least aggressive form of MEN 2, exhibits MTC without additional tumors or phenotypes, and frequently shows later onset than other disease subtypes. MEN 2A is characterized by MTC with pheochromocytoma, which occurs in approximately 50% of cases, and parathyroid hyperplasia or adenoma in 10–35%. Finally, MEN 2B is also characterized by MTC and pheochromocytoma, but parathyroid hyperplasia is rare. This is the most aggressive subtype, with earliest onset of disease and metastasis, and poorest prognosis. In MEN 2B, MTC has been documented in patients as young as 2 months (1). In addition, patients with MEN 2B frequently present with other non-tumor features including ganglioneuromatosis of the mouth and gut, corneal nerve thickening, delayed puberty, and a marfanoid habitus (2,3).

MEN 2 is dominantly inherited, and its genetic cause, mutations of the *REarranged during Transfection* (*RET*) proto-oncogene, was first recognized nearly 20 years ago (4–6). Since then, the range of mutations identified, their potential for predicting clinical course, and the underlying functional effects have been explored. Detection of *RET* mutations in MEN 2 represents a paradigm for genetically guided patient management, and genotype–phenotype correlations in this disease now inform recommended interventions, patient and family screening, and long-term follow-up (7,8). Functional characterization of these mutations also has the potential to define optimal therapeutic regimens, and may identify additional phenotypic implications that have not been broadly recognized. Here, we discuss our current understanding of the molecular mechanisms for the more common *RET* mutations and their potential significance.

RET RECEPTOR

The *RET* proto-oncogene encodes a receptor tyrosine kinase that is required for the development of neural-crest-derived cells, the urogenital system, and the central and peripheral nervous systems, notably the enteric nervous system (9,10). The RET protein has a large extracellular domain containing a cysteine-rich region and a series of cadherin homology domains, a transmembrane domain, and an intracellular tyrosine kinase domain, required for RET phosphorylation and downstream signaling (Figure 1A) (11,12). The RET kinase is structurally similar to other tyrosine kinases, sharing many conserved functional motifs and regulatory residues that have been shown to have importance for kinase enzyme function (13). RET is activated by binding of a multi-protein ligand complex. RET binds a soluble ligand of the glial cell-line-derived neurotrophic factor (GDNF) family but also requires a co-receptor of the GDNF family receptors α (GFRα), which is tethered to the cell membrane via glycosylphosphatidylinositol linkage

No potential conflict of interest was reported.

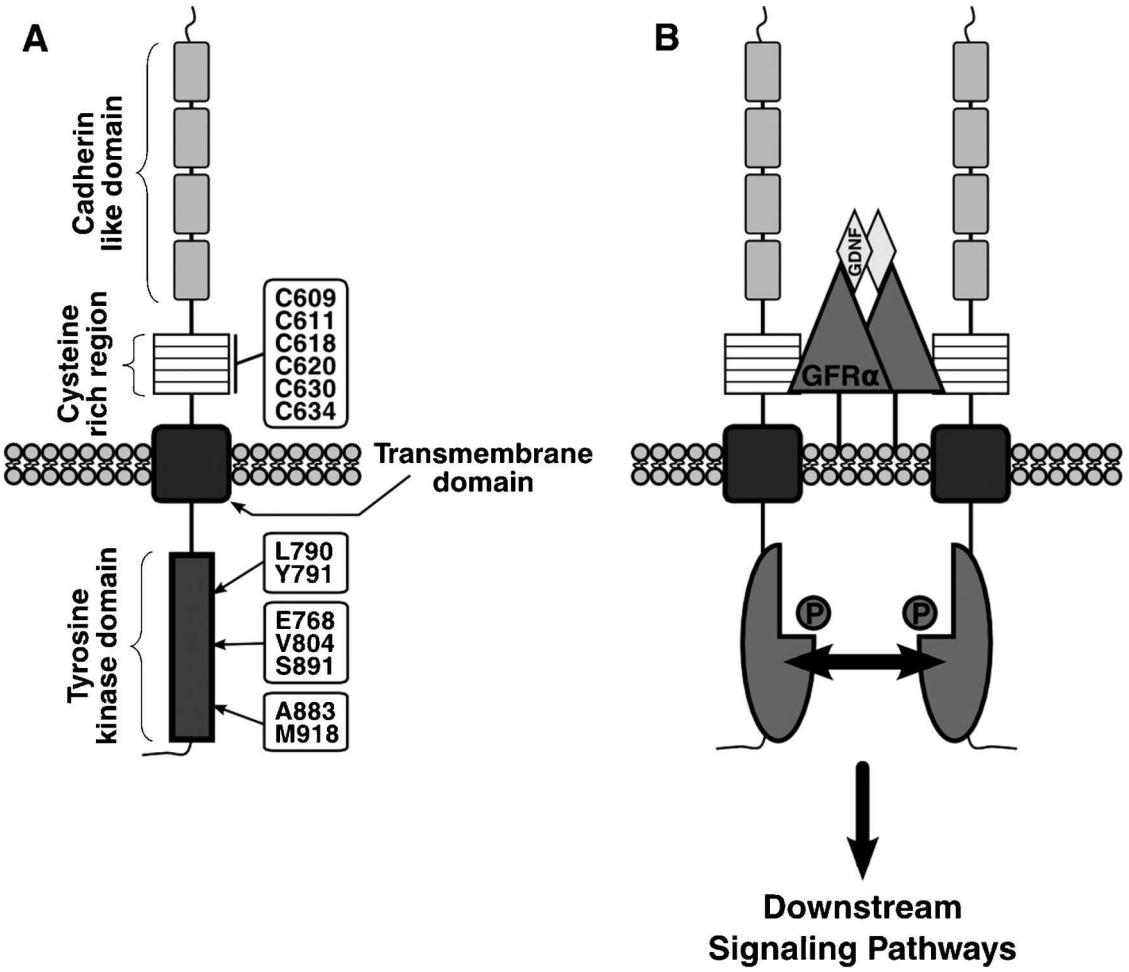

Figure 1 - The RET receptor: structure, activation, and oncogenic mutations. (A) Schematic diagram depicting RET tyrosine kinase receptor domains and the location of recurrent oncogenic mutations. The RET protein has a large extracellular domain containing a cysteine-rich region and a series of cadherin homology domains, a transmembrane domain, and an intracellular tyrosine kinase domain. The positions of the most common mutations found in patients with multiple endocrine neoplasia type 2 (MEN 2) are shown. (B) Mechanism of RET activation. Wild-type RET activation requires the dimerization of RET, mediated through formation of a multicomponent complex. RET is activated by binding both a soluble ligand (glial cell-line-derived neurotrophic factor; GDNF) and a non-signaling extracellular co-receptor (GDNF family receptor; GFRα). Upon activation of RET, phosphorylation of multiple intracellular tyrosines leads to stimulation of downstream signaling pathways.

(Figure 1B) (14,15). Initially, GDNF binds to GFRα, and these complexes are then able to recruit RET to form heterohexamers that are concentrated in regions of the cell membrane called lipid rafts (14,16). These are membrane domains enriched in glycosylphosphatidylinositol-linked proteins and signaling molecules that provide a platform not only for enhanced cell signaling, but also for regulation of receptor kinase activity and downregulation (17). Activation of RET leads to stimulation of multiple downstream pathways, including mitogen-activated protein kinase and extracellular signal-regulated kinase, phosphoinositide 3-kinase and protein kinase B, signal transducer and activator of transcription 3, proto-oncogene tyrosine-protein kinase Src1, and focal adhesion kinase (18,19), that promote cell growth, proliferation, survival, and/or differentiation.

THE *RET* PROTO-ONCOGENE IN MEN 2

MEN 2 is associated with point mutations of RET, predictably leading to its activation in the absence of ligands and co-receptors. Mutations are primarily amino acid substitutions affecting a very small number of *RET* codons in either the extracellular domain or within the kinase domain (Table 1; Figure 1A). Mutations are dominant, requiring only a single mutant allele to confer the disease phenotype. Summaries of MEN 2 *RET* mutation occurrence are well reviewed elsewhere (20–23) or are available online (http://www.arup.utah.edu/database/MEN2/MEN2_welcome.php). Together, these data suggest strong overall themes as to functional effects of these mutations, but also as to their clinical significance.

Strong associations of disease subtype, and also specific disease phenotypes, with individual RET mutations have made it possible to stratify risk of MEN 2 by genotype (7,8). The management guidelines of the American Thyroid Association (8) base the recommendations for initial diagnosis, therapeutic intervention, and long-term follow-up on patient genotype and the current understanding of the natural history of the disease associated with each *RET* mutation. Mutations of cysteine residues (primarily

Table 1 - Molecular effects of RET mutations in multiple endocrine neoplasia 2.

Mutation location	Affected RET Codons	Putative function of the wild-type residue	Predicted mutation effects	Phenotype	Recommended intervention (8)
Extracellular-cysteine rich domain	C609 C611 C618 C620 C630	Contributes to tertiary structure of RET through the formation of intramolecular disulfide bonds	Weakly activating. Alteration in protein folding and maturation. Formation of mutant RET dimers that are constitutively active in the absence of ligands	MEN 2A and FMTC	Prophylactic thyroid surgery before the age of 5. Under some conditions may delay beyond 5 years
	C634	Role in formation of intramolecular disulfide bonds	Strongly activating. Ligand-independent dimerization of receptor molecules, enhanced phosphorylation of intracellular substrates.	MEN 2A	Surgery before age 5
Intracellular tyrosine kinase domain	L790, Y791	In the N-terminal lobe of the RET kinase	Moderately activating. Affects ATP binding and inter-lobe flexibility.	MEN 2A and FMTC	May delay surgery beyond 5 years
	E768	In close proximity with the ATP binding site	Alters interactions within the region and facilitates the transition to an active conformation	FMTC	
	V804	A gatekeeper residue which regulates access to the ATP binding site	Alters hinge flexibility and positioning of RET helices for catalysis	FMTC	
	S891	C-terminal lobe of the kinase, adjacent to the activation loop of the kinase	Alters activation loop conformation and promotes monomeric RET activation	MEN 2A and FMTC	
	A883	Situated next to activation loop	Strongly activating. Local conformational change which destabilizes the inactive form of the protein and promotes its activation	MEN 2B	As early as possible (within first year of life)
	M918	Lies in the substrate-binding pocket of the kinase and plays a role in stabilizing the receptor–ATP complex	Strongly activating. Alters protein conformation and substrate specificity. The mutant can dimerize and become phosphorylated in the absence of ligand stimulation	MEN 2B	

FMTC, familial medullary thyroid carcinoma; MEN 2, multiple endocrine neoplasia 2; RET, REarranged during Transfection.

cysteines 609, 611, 618, 620, 630, and 634) in the RET extracellular domain account for the majority of MEN 2A cases, and are also common in patients with FMTC. Intracellular kinase domain mutations are mainly associated with FMTC and MEN 2B. Mutations in the intracellular codons 768, 790, 791, 804, and 891 underlie FMTC, and occur less commonly in patients with MEN 2A (20,24), while specific mutations of codon 918 (M918T) or 883 (A883F) account for the vast majority of MEN 2B cases, and are exclusive to the subtype (3,25). In addition to association with disease subtype, significant correlations of specific mutations with disease features are reported. For example, RET codon 634 mutations carry a greater patient risk for pheochromocytoma and parathyroid hyperplasia (4,26–28), and are associated with a higher frequency of detection of MTC at the time of early thyroidectomy (29). Variation in clinical presentation has even been observed with different codon 634 substitutions. The specific substitution of an arginine at codon 634 (C634R) is strongly associated with increased risk of parathyroid hyperplasia (4,26–28), increased frequency of distant metastases, earlier onset of both lymph node and distant metastases, and bilaterality of pheochromocytoma (30,31).

MOLECULAR MECHANISMS OF RET MUTATIONS

Evidence-based assessment of MEN 2 genotypic data demonstrate that not all RET mutations have equivalent clinical significance, although all reported mutations are thought to lead to ligand-independent constitutive activation of the RET receptor, autophosphorylation of RET, and aberrant stimulation of downstream signaling pathways. It follows that the molecular mechanisms of mutations associated with these different phenotypes may also be distinct and that these mechanisms may provide clues to disease origin and, potentially, treatment for patients with these mutations. Here, we discuss some of the current understanding of the mechanisms of RET dysfunction seen in MEN 2, and explore the potential implications of these mechanisms.

RET Extracellular Domain Cysteine Residues

The most frequently identified *RET* mutations in MEN 2 affect cysteines in the extracellular cysteine-rich region (primarily residues between Cys515 and Cys634) (Figure 1A; Table 1). In the normal protein, intramolecular cysteine–cysteine disulfide bonds contribute to the tertiary structure of the RET extracellular domain. Correct positioning of residues in this region is critical to interactions with GDNF–GFRα ligand complexes (14,15,32). Amino acid substitutions, resulting in replacement of a normal cysteine with any amino acid, lead to loss of intramolecular bonds and to an unpaired cysteine that is available for intermolecular interactions with other mutant RET proteins (Figure 2A) (33–35). These mutant RET dimers are constitutively active in the absence of ligands. Furthermore, mutant dimers are not recruited to lipid rafts through GFRα interactions, and may be activated in other membrane compartments, which can affect the nature and intensity of the resultant downstream signals (36,37). Downstream signaling regulation, via

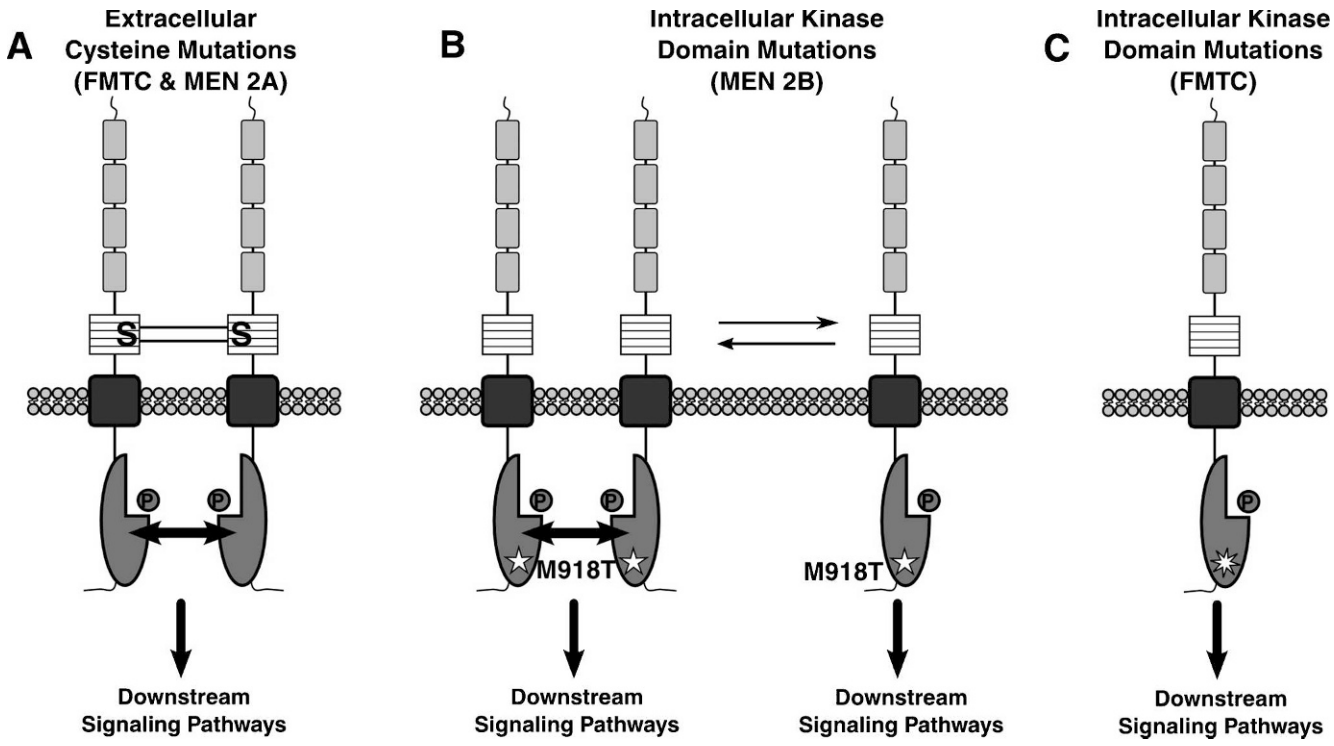

Figure 2 - Molecular mechanisms of pathogenic RET activation. Schematic diagrams showing mechanisms of RET activation in the presence of various multiple endocrine neoplasia type 2 (MEN 2) mutations. (A) Substitutions of extracellular cysteines lead to formation of intermolecular disulfide bonds and to constitutive RET dimerization and activation. (B) The MEN 2B mutation, M918T (star) in the kinase domain, leads to a conformational change with multiple effects including an increase in RET kinase activity and activation of receptors in either dimeric or monomeric form. (C) Intracellular kinase domain mutations asterisk implicated in familial medullary thyroid carcinoma (FMTC) (e.g. residues 791 and 891), permit activation of monomeric RET, allowing for a partially active conformation.

interactions with ubiquitin ligases such as CasitasB-lineage lymphoma proto-oncogene (CBL) (38), or with cellular phosphatases such as SHP1 and SHP2 (39,40) that are involved in limiting or terminating signals, differ from that of the raft-associated wild-type receptor, enhancing the effect of the oncogenic mutation.

Although the molecular mechanisms of activation are similar, cysteine RET mutations also vary in impact. In general, mutations located closer to the RET transmembrane domain have greater transforming ability and are linked to increased risks of more aggressive MEN 2 disease (41) (Table 1). Codon 634 mutations confer the greatest degree of RET activation, with higher levels of autophosphorylation and transforming ability than the other cysteine mutations, and are linked to broader phenotypes and more severe disease, as described above. Interestingly, mutations of other cysteine residues are believed to affect the efficiency of RET protein folding and maturation, and to impair transport to the cell membrane, resulting in decreased levels of cell surface protein and weaker signaling capability (33–35,42,43). In fact, a subset of RET cysteine mutations, sometimes referred to as Janus mutations, can lead to a partial loss-of-function phenotype, as well as to oncogenic effects. These mutations, generally affecting codons 609, 611, 618 or 620, are thought to confer cell-type-specific decreases in functional protein on the cell surface. Inactivating mutations of RET can lead to the congenital abnormality Hirschsprung disease, which is characterized by the absence of the enteric neurons from the distal colon (44). Janus mutations have been linked to an insufficiency of mature RET protein in the gut, resulting in the Hirschsprung phenotype, yet at the same time, risks remain high for MEN 2 phenotypes, as sufficient mature protein is expressed in the thyroid for development of MTC (45–47).

RET Intracellular Domain Mutations

Intracellular RET kinase mutations fall into two groups: high-penetrance mutations causing MEN 2B, and less aggressive mutations that lead to FMTC or, more rarely, MEN 2A (Table 1). These RET mutations fall within the N-terminal and C-terminal lobes of the kinase (Figure 3). Although the mutations are spread out along the linear protein sequence (Figure 1A), they appear to cluster on either the ATP-binding face or substrate-binding/autoinhibitory face of the protein tertiary structure, suggesting some common themes in their functional effects (Figure 3; Table 1). The precise mechanisms by which these intracellular mutations activate RET are various, but it is suggested that they all do so through destabilizing the inactive form of RET, and shifting the equilibrium of RET receptors towards the active state (48).

MEN 2B Mutations

Over 95% of MEN 2B cases are associated with the same methionine to threonine change at codon 918 (M918T) in the RET kinase domain (Figure 3). Structurally, this residue lies in the substrate-binding pocket of the kinase, and the M918T mutation appears to increase RET–ATP binding affinity and the stability of the active ATP-bound form, effectively

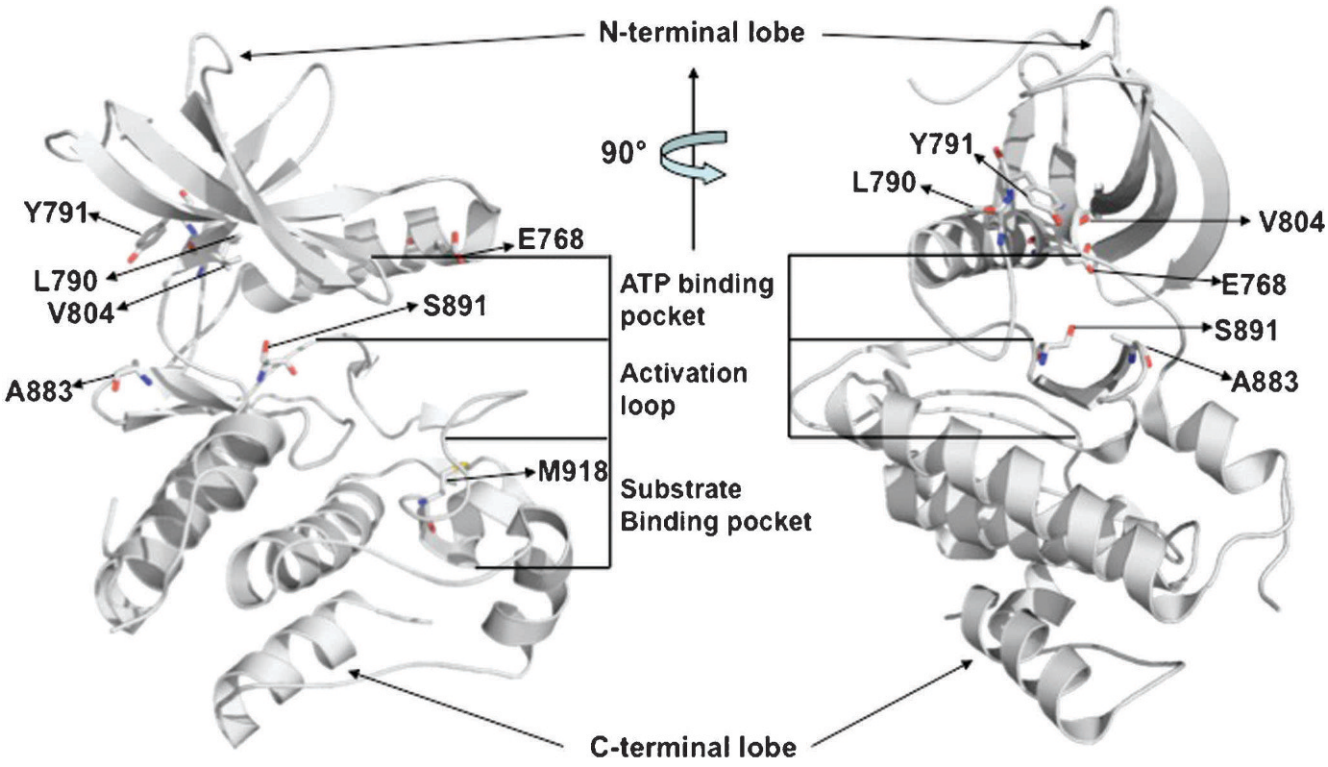

Figure 3 - Structure of the RET tyrosine kinase domain. Ribbon diagrams of the intracellular regions of activated RET, in two orientations, showing the positions of key functional features of the kinase: the ATP binding pocket; the activation or autoinhibitory loop; and the substrate binding pocket. Two orientations of the model, displaying the autoinhibitory/substrate binding face (left) and the ATP-binding face (right), are shown. Amino acid residues that are mutated in patients with multiple endocrine neoplasia type 2 (MEN 2) are represented in the stick form. The three-dimensional representation was based on the crystal structure of the phosphorylated (activated) RET tyrosine kinase domain (residues 709–990).

making RET more active, more of the time (48–51). The M918T mutation appears to increase the stability of monomeric active forms of RET, but activation of these mutants can also be further enhanced by binding of GDNF–GFRα complexes, suggesting that these mutant RET forms may induce signal transduction from both within and outside the lipid rafts, perhaps via distinct signaling complexes (Figure 2B). As a result, RET downstream signals are enhanced, and activation of targets is increased, notably including upregulation of gene transcripts that contribute to cell proliferation or to metastasis-promoting cell behaviors (52,53). Although it has been postulated that the M918T mutation alters the preferred substrates of the mutant RET protein with respect to both autophosphorylation of RET tyrosine residues, and phosphorylation of downstream signaling molecules (54,55), novel downstream targets that cannot also be stimulated at lower levels by other less active RET mutants or by ligand activation of wild-type RET have not been broadly identified (48–51,53).

An intriguing finding has been that activation of M918T RET begins before the receptor arrives at the cell surface, stimulating signaling pathways from the endoplasmic reticulum before the receptor reaches its fully glycosylated mature form (56), which has not been observed for other mutants. RET signaling from intracellular compartments may differ (in intensity or otherwise) from that at the plasma membrane, which has been shown to be the case when wild-type RET is internalized into endosomes following ligand stimulation (57) and for cytosolic RET mutants found in papillary thyroid carcinoma (37).

An alanine to phenylalanine substitution at codon 883 (A883F) is the only other recurring MEN 2B mutation (58,59). Structurally, this residue lies between the activation and catalytic loops of the kinase (Figure 3), and would be predicted to increase the flexibility of these domains, so destabilizing the inactive form of the protein and promoting its activation. Although generally considered a high-risk mutation, some studies suggest that it may have a lesser effect than the M918T mutation (60).

A handful of instances of double mutations in MEN 2B have also been reported: V804M/E805K (51), V804M/Y806C (61), and V804M/S904C (62). It appears that the combination of two mild intracellular mutations can cooperate to produce a more severe mutant. Each mutation alone (V804M, E805K, Y806C, S904C) has low or no transforming ability, consistent with the observation that V804M generally leads to FMTC (discussed below), but when coupled together, they exert a synergistic effect on the transforming ability of mutated RET (51,63).

Lower Risk Intracellular Domain Mutations

Recurrent mutations in the intracellular codons 768, 790, 791, 804, and 891 are found in patients with FMTC and, less commonly, MEN 2A (20,64). This group of mutations is the most diverse in functional effects, phenotypic variability, and long-term clinical implications.

Mutations of glutamic acid 768 occur almost exclusively in patients with FMTC, whereas leucine 790 mutations have been recognized in both FMTC and MEN 2A families (20–23). These are considered lower penetrance mutations, associated with later-onset disease, as reflected by evidence-based clinical management recommendations suggesting that delayed prophylactic surgery may be acceptable (Table 1) (8). The E768 and L790 residues lie close to the ATP binding site (Figure 3) and may alter interactions in this region, and/or increase flexibility of domains, making the transition to an active conformation relatively easy.

Mutation of serine 891 to an alanine was initially recognized as an FMTC mutation, but more recently has been linked to MEN 2A features (65). Codon 891 lies in a conserved region of the RET protein, and its mutation appears to alter protein autoinhibition and ATP binding, favoring an active conformation. Interestingly, S891A and Y791F mutations are functionally unique in that they do not require RET dimerization for full activation, and so RET autophosphorylation and downstream signaling are not further enhanced by ligand binding (Figure 2C) (66). As for other RET mutants, this means that RET is not recruited into lipid rafts by GFRα, and hence it is likely that the nature, intensity, and duration of signaling is altered for these mutants (36–40).

The most common of these lower-risk mutations is substitution of valine 804 (67). This residue lies in the sequence linking the N-terminal and C-terminal lobes of the kinase domain, in a conserved region critical for RET–ATP binding, which is required for activation of the kinase. Substitution of valine 804 for a leucine (V804L) or methionine (V804M) changes the conformation of the ATP binding pocket, making it more permissive for binding ATP, and thus enhancing RET activation (51,68,69). Residue 804 represents a classical gatekeeper residue (51), positioned so as to regulate access to the ATP-binding site. Competitive binding to this region is the mechanism of action of multikinase inhibitors such as vandetanib, which has recently been approved for treatment of advanced MTC (70). Although vandetanib effectively inhibits wild-type and other mutant RET forms, the V804L or V804M mutations confer resistance to the drug (48,68). As other kinase inhibitors (such as sorafenib, which is currently under review for managing advanced thyroid carcinoma (71,72)) are not affected by codon 804 mutations (68), *RET* mutation status can have profound clinical importance for optimizing treatment regimens.

The molecular effects of substitution of phenylalanine for tyrosine at codon 791 (Y791F) of RET are not clearly defined. This residue is not a known site of tyrosine phosphorylation (73), so direct protein interactions of RET with other molecules are unlikely to be altered by this mutation. The position of the residue, close to the ATP binding pocket, may enhance ATP access, or may again alter protein flexibility, favoring the active conformation. *In vitro*, Y791F mutations have been shown to enhance signal transducer and activator of transcription 3 (STAT3) signaling (74). Like S891A mutants, the Y791F form of RET appears to exist as an active monomer as it does not require dimerization to be activated, and ligand binding does not further enhance autophosphorylation or downstream signaling (66). The significance of the Y791F mutation remains somewhat controversial. Reports have identified this mutation, alone or in combination with other mutations, in MEN 2A and FMTC, and in sporadic MTC and pheochromocytoma tumors (24,75). Co-occurrence of Y791F and codon 634 mutations has been shown to increase the risk of pheochromocytoma in some families (76), whereas other studies have concluded that this mutation is not pathogenic (77). Interestingly, a clue is perhaps provided by studies identifying Y791F and Y791N mutations in patients with Hirschsprung disease (75,78,79), possibly suggesting that mutations of tyrosine 791 may act as modifiers of multiple phenotypes.

CONCLUSIONS AND PERSPECTIVES

The landscape of MEN 2 disease management has been transformed by the identification and cataloguing of its underlying genetic causes. Mutation genotype has guided the evidence-based diagnosis, prediction, and management of MEN 2 as for few other diseases. However, we are only beginning to reap the benefits of functional characterization of *RET* mutations. The new crop of anti-RET therapeutics being developed has implications not just for MEN 2, but for thyroid cancer in general, and for other diseases that have recently been linked to RET activity including pancreatic and breast cancers (80–82). Conversely, mutations or altered expression of RET that result in decreased receptor function have been linked to developmental defects, such as Hirschsprung disease (44) and kidney anomalies (83,84), and current research also links GDNF and survival of dopaminergic neurons in Parkinson disease (85). Together, these studies clearly indicate that understanding of the normal functions and physiological role of RET are essential in assessing the short-term and long-term benefits and potential harms of novel RET-targeted therapeutics.

AUTHOR CONTRIBUTIONS

Wagner SM prepared the figures and was also responsible for the manuscript writing. Zhu S contributed to the manuscript writing. Nicolescu A prepared the models and figures. Mulligan LM is the senior author who prepared the final manuscript version. Wagner SM and Zhu S contributed equally to the study.

REFERENCES

1. Leboulleux S, Travagli JP, Caillou B, Laplanche A, Bidart JM, Schlumberger M, et al. Medullary thyroid carcinoma as part of a multiple endocrine neoplasia type 2B syndrome: influence of the stage on the clinical course. Cancer. 2002;94(1):44-50, http://dx.doi.org/10.1002/cncr.10205.
2. Brauckhoff M, Gimm O, Weiss CL, Ukkat J, Sekulla C, Brauckhoff K, et al. Multiple endocrine neoplasia 2B syndrome due to codon 918 mutation: clinical manifestation and course in early and late onset disease. World J Surg. 2004;28(12):1305-11, http://dx.doi.org/10.1007/s00268-004-7637-4.
3. Brauckhoff M, Machens A, Hess S, Lorenz K, Gimm O, Brauckhoff K, et al. Premonitory symptoms preceding metastatic medullary thyroid cancer in MEN 2B: An exploratory analysis. Surgery. 2008;144(6):1044-50; discussion 50-3, http://dx.doi.org/10.1016/j.surg.2008.08.028.
4. Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JBJ, Gardner E, et al. Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. Nature Genet. 1994;6:70-4, http://dx.doi.org/10.1038/ng0194-70.
5. Mulligan LM, Kwok JBJ, Healey CS, Elsdon MJ, Eng C, Gardner E, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature. 1993;363:458-60, http://dx.doi.org/10.1038/363458a0.
6. Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, et al. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet. 1993;2(7):851-6, http://dx.doi.org/10.1093/hmg/2.7.851.
7. Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab. 2001;86(12):5658-71, http://dx.doi.org/10.1210/jc.86.12.5658.

8. Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid. 2009;19(6):565-612, http://dx.doi.org/10.1089/thy.2008.0403.
9. Pachnis V, Mankoo B, Costantini F. Expression of the c-ret proto-oncogene during mouse embryogenesis. Development. 1993;119:1005-17.
10. Attie-Bitach T, Abitbol M, Gerard M, Delezoide AL, Auge J, Pelet A, et al. Expression of the RET proto-oncogene in human embryos. Am J Med Genet. 1998;80(5):481-6, http://dx.doi.org/10.1002/(SICI)1096-8628(19981228)80:5<481::AID-AJMG8>3.0.CO;2-6.
11. Takahashi M, Cooper G. ret transforming gene encodes a fusion protein homologous to tyrosine kinases. Mol Cell Biol. 1987;7:1378-85.
12. Takahashi M, Buma Y, Iwamoto T, Inaguma Y, Ikeda H, Hiai H. Cloning and expression of the ret proto-oncogene encoding a tyrosine kinase with two potential transmembrane domains. Oncogene. 1988;3:571-8.
13. Hanks SK, Quinn AM, Hunter T. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science. 1988;241(4861):42-52, http://dx.doi.org/10.1126/science.3291115.
14. Parkash V, Leppanen VM, Virtanen H, Jurvansuu JM, Bespalov MM, Sidorova YA, et al. The structure of the glial cell line-derived neurotrophic factor-coreceptor complex: insights into RET signaling and heparin binding. J Biol Chem. 2008;283(50):35164-72, http://dx.doi.org/10.1074/jbc.M802543200.
15. Schlee S, Carmillo P, Whitty A. Quantitative analysis of the activation mechanism of the multicomponent growth-factor receptor Ret. Nat Chem Biol. 2006;2(11):636-44, http://dx.doi.org/10.1038/nchembio823.
16. Paratcha G, Ledda F, Baars L, Coulpier M, Besset V, Anders J, et al. Released GFRalpha1 potentiates downstream signaling, neuronal survival, and differentiation via a novel mechanism of recruitment of c-Ret to lipid rafts. Neuron. 2001;29(1):171-84, http://dx.doi.org/10.1016/S0896-6273(01)00188-X.
17. Staubach S, Hanisch FG. Lipid rafts: signaling and sorting platforms of cells and their roles in cancer. Expert Rev Proteomics. 2011;8(2):263-77, http://dx.doi.org/10.1586/epr.11.2.
18. Arighi E, Borrello MG, Sariola H. RET tyrosine kinase signaling in development and cancer. Cytokine and Growth Factor Reviews. 2005;16:441-67, http://dx.doi.org/10.1016/j.cytogfr.2005.05.010.
19. Plaza-Menacho I, Morandi A, Mologni L, Boender P, Gambacorti-Passerini C, Magee AI, et al. Focal adhesion kinase (FAK) binds RET kinase via its FERM domain, priming a direct and reciprocal RET-FAK transactivation mechanism. J Biol Chem. 2011;286(19):17292-302, http://dx.doi.org/10.1074/jbc.M110.168500.
20. Margraf RL, Crockett DK, Krautscheid PM, Seamons R, Calderon FR, Wittwer CT, et al. Multiple endocrine neoplasia type 2 RET proto-oncogene database: repository of MEN2-associated RET sequence variation and reference for genotype/phenotype correlations. Hum Mutat. 2009;30(4):548-56, http://dx.doi.org/10.1002/humu.20928.
21. Machens A, Dralle H. Familial prevalence and age of RET germline mutations: implications for screening. Clin Endocrinol (Oxf). 2008;69(1):81-7, http://dx.doi.org/10.1111/j.1365-2265.2007.03153.x.
22. Raue F, Frank-Raue K. Update multiple endocrine neoplasia type 2. Fam Cancer. 2010;9(3):449-57, http://dx.doi.org/10.1007/s10689-010-9320-2.
23. Richards ML. Thyroid cancer genetics: multiple endocrine neoplasia type 2, non-medullary familial thyroid cancer, and familial syndromes associated with thyroid cancer. Surg Oncol Clin N Am. 2009;18(1):39-52, viii, http://dx.doi.org/10.1016/j.soc.2008.08.002.
24. Berndt I, Reuter M, Saller B, Frank-Raue K, Groth P, Grussendorf M, et al. A new hot spot for mutations in the ret protooncogene causing familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2A. J Clin Endocrinol Metab. 1998;83(3):770-4, http://dx.doi.org/10.1210/jc.83.3.770.
25. Machens A, Dralle H. Pheochromocytoma penetrance varies by RET mutation in MEN 2A. Surgery. 2008;143(5):696.
26. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2: International RET Mutation Consortium. JAMA. 1996;276:1575-9, http://dx.doi.org/10.1001/jama.1996.03540190047028.
27. Yip L, Cote GJ, Shapiro SE, Ayers GD, Herzog CE, Sellin RV, et al. Multiple endocrine neoplasia type 2: evaluation of the genotype-phenotype relationship. Arch Surg. 2003;138(4):409-16.
28. Quayle FJ, Fialkowski EA, Benveniste R, Moley JF. Pheochromocytoma penetrance varies by RET mutation in MEN 2A. Surgery. 2007;142(6):800-5; discussion 5 e1, http://dx.doi.org/10.1016/j.surg.2007.09.013.
29. Szinnai G, Meier C, Komminoth P, Zumsteg UW. Review of multiple endocrine neoplasia type 2A in children: therapeutic results of early thyroidectomy and prognostic value of codon analysis. Pediatrics. 2003;111(2):E132-9, http://dx.doi.org/10.1542/peds.111.2.e132.
30. Punales MK, Graf H, Gross JL, Maia AL. RET codon 634 mutations in multiple endocrine neoplasia type 2: variable clinical features and clinical outcome. J Clin Endocrinol Metab. 2003;88(6):2644-9, http://dx.doi.org/10.1210/jc.2002-021422.
31. Rodriguez JM, Balsalobre M, Ponce JL, Rios A, Torregrosa NM, Tebar J, et al. Pheochromocytoma in MEN 2A syndrome. Study of 54 patients. World J Surg. 2008;32(11):2520-6, http://dx.doi.org/10.1007/s00268-008-9734-2.
32. Amoresano A, Incoronato M, Monti G, Pucci P, de Franciscis V, Cerchia L. Direct interactions among Ret, GDNF and GFRalpha1 molecules reveal new insights into the assembly of a functional three-protein complex. Cell Signal. 2005;17(6):717-27, http://dx.doi.org/10.1016/j.cellsig.2004.10.012.
33. Carlomagno F, Salvatore G, Cirafici AM, De Vita G, Melillo RM, de Franciscis V, et al. The different RET-activating capability of mutations of cysteine 620 or cysteine 634 correlates with the multiple endocrine neoplasia type 2 disease phenotype. Cancer Res. 1997;57:391-5.
34. Chappuis-Flament S, Pasini A, De Vita G, Segouffin-Cariou C, Fusco A, Attie T, et al. Dual effect on the RET receptor of MEN 2 mutations affecting specific extracytoplasmic cysteines. Oncogene. 1998;17:2851-61, http://dx.doi.org/10.1038/sj.onc.1202202.
35. Ito S, Iwashita T, Asai N, Murakami H, Iwata Y, Sobue G, et al. Biological properties of Ret with cysteine mutations correlate with multiple endocrine neoplasia type 2A, familial medullary thyroid carcinoma, and Hirschsprung's disease phenotype. Cancer Res. 1997;57:2870-2.
36. Freche B, Guillaumot P, Charmetant J, Pelletier L, Luquain C, Christiansen D, et al. Inducible dimerization of RET reveals a specific AKT deregulation in oncogenic signalling. J Biol Chem. 2005;280(44):36584-91, http://dx.doi.org/10.1074/jbc.M505707200.
37. Richardson DS, Gujral TS, Peng S, Asa SL, Mulligan LM. Transcript level modulates the inherent oncogenicity of RET/PTC oncoproteins. Cancer Res. 2009;69(11):4861-9, http://dx.doi.org/10.1158/0008-5472.CAN-08-4425.
38. Scott RP, Eketjall S, Aineskog H, Ibanez CF. Distinct turnover of alternatively-spliced isoforms of the RET kinase receptor mediated by differential recruitment of the Cbl ubiquitin ligase. J Biol Chem. 2005;280(14):13442-9, http://dx.doi.org/10.1074/jbc.M500507200.
39. Incoronato M, D'Alessio A, Paladino S, Zurzolo C, Carlomagno MS, Cerchia L, et al. The Shp-1 and Shp-2, tyrosine phosphatases, are recruited on cell membrane in two distinct molecular complexes including Ret oncogenes. Cell Signal. 2004;16(7):847-56, http://dx.doi.org/10.1016/j.cellsig.2004.01.002.
40. Perrinjaquet M, Vilar M, Ibanez CF. Protein-tyrosine phosphatase SHP2 contributes to GDNF neurotrophic activity through direct binding to phospho-Tyr687 in the RET receptor tyrosine kinase. J Biol Chem. 2010;285(41):31867-75, http://dx.doi.org/10.1074/jbc.M110.144923.
41. Machens A, Hauptmann S, Dralle H. Modification of multiple endocrine neoplasia 2A phenotype by cell membrane proximity of RET mutations in exon 10. Endocr Relat Cancer. 2009;16(1):171-7, http://dx.doi.org/10.1677/ERC7-08-0096.
42. Mograbi B, Bocciardi R, Bourget I, Juhel T, Farahi-Far D, Romeo G, et al. The sensitivity of activated Cys Ret mutants to glial cell line-derived neurotrophic factor is mandatory to rescue neuroectodermic cells from apoptosis. Mol Cell Biol. 2001;21(20):6719-30, http://dx.doi.org/10.1128/MCB.21.20.6719-6730.2001.
43. Kjaer S, Hanrahan S, Totty N, McDonald NQ. Mammal-restricted elements predispose human RET to folding impairment by HSCR mutations. Nat Struct Mol Biol. 2010;17(6):726-31, http://dx.doi.org/10.1038/nsmb.1808.
44. Amiel J, Sproat-Emison E, Garcia-Barcelo M, Lantieri F, Burzynski G, Borrego S, et al. Hirschsprung disease, associated syndromes and genetics: a review. J Med Genet. 2008;45(1):1-14, http://dx.doi.org/10.1136/jmg.2007.053959.
45. Arighi E, Popsueva A, Degl'Innocenti D, Borrello MG, Carniti C, Perala NM, et al. Biological effects of the dual phenotypic Janus mutation of ret cosegregating with both multiple endocrine neoplasia type 2 and Hirschsprung's disease. Mol Endocrinol. 2004;18(4):1004-17, http://dx.doi.org/10.1210/me.2003-0173.
46. Mulligan LM, Eng C, Attié T, Lyonnet S, Marsh DJ, Hyland VJ, et al. Diverse phenotypes associated with exon 10 mutations of the RET proto-oncogene. Hum Mol Genet. 1994;3:2163-7, http://dx.doi.org/10.1093/hmg/3.12.2163.
47. Moore SW, Zaahl M. Familial associations in medullary thyroid carcinoma with Hirschsprung disease: the role of the RET-C620 ''Janus'' genetic variation. J Pediatr Surg. 2010; 45(2):393-6, http://dx.doi.org/10.1016/j.jpedsurg.2009.10.080.
48. Dixit A, Torkamani A, Schork NJ, Verkhivker G. Computational modeling of structurally conserved cancer mutations in the RET and MET kinases: the impact on protein structure, dynamics, and stability. Biophys J. 2009;96(3):858-74, http://dx.doi.org/10.1016/j.bpj.2008.10.041.
49. Gujral TS, Singh VK, Jia Z, Mulligan LM. Molecular Mechanisms of RET Receptor-Mediated Oncogenesis in Multiple Endocrine Neoplasia 2B. Cancer Res. 2006;66(22):10741-9, http://dx.doi.org/10.1158/0008-5472.CAN-06-3329.
50. Knowles PP, Murray-Rust J, Kjaer S, Scott RP, Hanrahan S, Santoro M, et al. Structure and chemical inhibition of the RET tyrosine kinase domain. J Biol Chem. 2006;281(44):33577-87, http://dx.doi.org/10.1074/jbc.M605604200.
51. Cranston AN, Carniti C, Oakhill K, Radzio-Andzelm E, Stone EA, McCallion AS, et al. RET is constitutively activated by novel tandem

mutations that alter the active site resulting in multiple endocrine neoplasia type 2B. Cancer Res. 2006;66(20):10179-87, http://dx.doi.org/10.1158/0008-5472.CAN-06-0884.

52. Jain S, Watson MA, DeBenedetti MK, Hiraki Y, Moley JF, Milbrandt J. Expression profiles provide insights into early malignant potential and skeletal abnormalities in multiple endocrine neoplasia type 2B syndrome tumors. Cancer Res. 2004;64(11):3907-13, http://dx.doi.org/10.1158/0008-5472.CAN-03-3801.
53. Hickey JG, Myers SM, Tian X, Zhu SJ, JL VS, Andrew SD, et al. RET-mediated gene expression pattern is affected by isoform but not oncogenic mutation. Genes Chromosomes Cancer. 2009;48(5):429-40, http://dx.doi.org/10.1002/gcc.20653.
54. Salvatore D, Melillo RM, Monaco C, Visconti R, Fenzi G, Vecchio G, et al. Increased in vivo phosphorylation of ret tyrosine 1062 is a potential pathogenetic mechanism of multiple endocrine neoplasia type 2B. Cancer Res. 2001;61(4):1426-31.
55. Songyang Z, Carraway KL, Eck MJ, Harrison SC, Feldman RA, Mohammadi M, et al. Catalytic specificity of protein-tyrosine kinases is critical for selective signalling. Nature. 1995;373:536-9, http://dx.doi.org/10.1038/373536a0.
56. Runeberg-Roos P, Saarma M. Neurotrophic factor receptor RET: structure, cell biology, and inherited diseases. Ann Med. 2007;39(8):572-80, http://dx.doi.org/10.1080/07853890701646256.
57. Richardson DS, Lai AZ, Mulligan LM. RET ligand-induced internalization and its consequences for downstream signaling. Oncogene. 2006;25(22):3206-11, http://dx.doi.org/10.1038/sj.onc.1209349.
58. Gimm O, Marsh DJ, Andrew SD, Frilling A, Dahia PLM, Mulligan LM, et al. Germline dinucleotide mutation in codon 883 of the RET proto-oncogene in multiple endocrine neoplasia type 2B without codon 918 mutation. J Clin Endocrinol Metab. 1997;82:3902-4, http://dx.doi.org/10.1210/jc.82.11.3902.
59. Smith DP, Houghton C, Ponder BAJ. Germline mutation of RET codon 883 in two cases of de novo MEN 2B. Oncogene. 1997;15:1213-7, http://dx.doi.org/10.1038/sj.onc.1201481.
60. Jasim S, Ying AK, Waguespack SG, Rich TA, Grubbs EG, Jimenez C, et al. Multiple Endocrine Neoplasia Type 2B with a RET Proto-Oncogene A883F Mutation Displays a More Indolent Form of Medullary Thyroid Carcinoma Compared with a RET M918T Mutation. Thyroid. 2011;21(2):189-92, http://dx.doi.org/10.1089/thy.2010.0328.
61. Miyauchi A, Futami H, Hai N, Yokozawa T, Kuma K, Aoki N, et al. Two germline missense mutations at codons 804 and 806 of the RET proto-oncogene in the same allele in a patient with multiple endocrine neoplasia type 2B without codon 918 mutation. Jpn J Cancer Res. 1999;90(1):1-5.
62. Menko FH, van der Luijt RB, de Valk IA, Toorians AW, Sepers JM, van Diest PJ, et al. Atypical MEN type 2B associated with two germline RET mutations on the same allele not involving codon 918. J Clin Endocrinol Metab. 2002;87(1):393-7, http://dx.doi.org/10.1210/jc.87.1.393.
63. Iwashita T, Murakami H, Kurokawa K, Kawai K, Miyauchi A, Futami H, et al. A two-hit model for development of multiple endocrine neoplasia type 2B by RET mutations. Biochem Biophys Res Commun. 2000;268(3):804-8, http://dx.doi.org/10.1006/bbrc.2000.2227.
64. Romei C, Mariotti S, Fugazzola L, Taccaliti A, Pacini F, Opocher G, et al. Multiple endocrine neoplasia type 2 syndromes (MEN 2): results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes. Eur J Endocrinol. 2010;163(2):301-8, http://dx.doi.org/10.1530/EJE-10-0333.
65. Schulte KM, Machens A, Fugazzola L, McGregor A, Diaz-Cano S, Izatt L, et al. The clinical spectrum of multiple endocrine neoplasia type 2a caused by the rare intracellular RET mutation S891A. J Clin Endocrinol Metab. 2010;95(9):E92-7, http://dx.doi.org/10.1210/jc.2010-0375.
66. Plaza Menacho I, Koster R, van der Sloot AM, Quax WJ, Osinga J, van der Sluis T, et al. RET-familial medullary thyroid carcinoma mutants Y791F and S891A activate a Src/JAK/STAT3 pathway, independent of glial cell line-derived neurotrophic factor. Cancer Res. 2005;65(5):1729-37, http://dx.doi.org/10.1158/0008-5472.CAN-04-2363.
67. Mukherjee S, Zakalik D. RET codon 804 mutations in multiple endocrine neoplasia 2: genotype-phenotype correlations and implications in clinical management. Clin Genet. 2011;79(1):1-16, http://dx.doi.org/10.1111/j.1399-0004.2010.01453.x.
68. Carlomagno F, Guida T, Anaganti S, Vecchio G, Fusco A, Ryan AJ, et al. Disease associated mutations at valine 804 in the RET receptor tyrosine kinase confer resistance to selective kinase inhibitors. Oncogene. 2004;23(36):6056-63, http://dx.doi.org/10.1038/sj.onc.1207810.
69. Carlomagno F, Guida T, Anaganti S, Provitera L, Kjaer S, McDonald NQ, et al. Identification of tyrosine 806 as a molecular determinant of RET kinase sensitivity to ZD6474. Endocr Relat Cancer. 2009;16(1):233-41, http://dx.doi.org/10.1677/ERC-08-0213.
70. Wells SA, Jr., Gosnell JE, Gagel RF, Moley J, Pfister D, Sosa JA, et al. Vandetanib for the treatment of patients with locally advanced or metastatic hereditary medullary thyroid cancer. J Clin Oncol. 2010;28(5):767-72, http://dx.doi.org/10.1200/JCO.2009.23.6604.
71. Gupta-Abramson V, Troxel AB, Nellore A, Puttaswamy K, Redlinger M, Ransone K, et al. Phase II trial of sorafenib in advanced thyroid cancer. J Clin Oncol. 2008;26(29):4714-9, http://dx.doi.org/10.1200/JCO.2008.16.3279.
72. Kloos RT, Ringel MD, Knopp MV, Hall NC, King M, Stevens R, et al. Phase II trial of sorafenib in metastatic thyroid cancer. J Clin Oncol. 2009;27(10):1675-84. Epub 2009/03/04, http://dx.doi.org/10.1200/JCO.2008.18.2717.
73. Kawamoto Y, Takeda K, Okuno Y, Yamakawa Y, Ito Y, Taguchi R, et al. Identification of RET autophosphorylation sites by mass spectrometry. J Biol Chem. 2004;279:14213-24, http://dx.doi.org/10.1074/jbc.M312600200.
74. Plaza-Menacho I, van der Sluis T, Hollema H, Gimm O, Buys CH, Magee AI, et al. Ras/ERK1/2-mediated STAT3 Serine 727 phosphorylation by familiar medullary thyroid carcinoma-associated RET mutants induces full activation of STAT3 and is required for c-fos promoter activation, cell mitogenicity and transformation. J Biol Chem. 2007;282(9):6415-24, http://dx.doi.org/10.1074/jbc.M608952200.
75. Vaclavikova E, Dvorakova S, Sykorova V, Bilek R, Dvorakova K, Vlcek P, et al. RET mutation Tyr791Phe: the genetic cause of different diseases derived from neural crest. Endocrine. 2009;36(3):419-24, http://dx.doi.org/10.1007/s12020-009-9242-7.
76. Toledo RA, Wagner SM, Coutinho FL, Lourenco DM, Jr., Azevedo JA, Longuini VC, et al. High penetrance of pheochromocytoma associated with the novel C634Y/Y791F double germline mutation in the RET protooncogene. J Clin Endocrinol Metab. 2010;95(3):1318-27, http://dx.doi.org/10.1210/jc.2009-1355.
77. Erlic Z, Hoffmann MM, Sullivan M, Franke G, Peczkowska M, Harsch I, et al. Pathogenicity of DNA variants and double mutations in multiple endocrine neoplasia type 2 and von Hippel-Lindau syndrome. J Clin Endocrinol Metab. 2010;95(1):308-13, http://dx.doi.org/10.1210/jc.2009-1728.
78. Seri M, Yin L, Barone V, Bolino A, Celli I, Bocciardi R, et al. Frequency of RET mutations in long- and short-segment Hirschsprung disease. Hum Mutat. 1997;9(3):243-9, http://dx.doi.org/10.1002/(SICI)1098-1004(1997)9:3<243::AID-HUMU5>3.0.CO;2-8.
79. Fitze G, Paditz E, Schlafke M, Kuhlisch E, Roesner D, Schackert HK. Association of germline mutations and polymorphisms of the RET proto-oncogene with idiopathic congenital central hypoventilation syndrome in 33 patients. J Med Genet. 2003;40(2):E10, http://dx.doi.org/10.1136/jmg.40.2.e10.
80. Funahashi H, Okada Y, Sawai H, Takahashi H, Matsuo Y, Takeyama H, et al. The role of glial cell line-derived neurotrophic factor (GDNF) and integrins for invasion and metastasis in human pancreatic cancer cells. J Surg Oncol. 2005;91(1):77-83, http://dx.doi.org/10.1002/jso.20277.
81. Esseghir S, Todd SK, Hunt T, Poulsom R, Plaza-Menacho I, Reis-Filho JS, et al. A role for glial cell derived neurotrophic factor induced expression by inflammatory cytokines and RET/GFR alpha 1 receptor up-regulation in breast cancer. Cancer Res. 2007;67(24):11732-41, http://dx.doi.org/10.1158/0008-5472.CAN-07-2343.
82. Gil Z, Cavel O, Kelly K, Brader P, Rein A, Gao SP, et al. Paracrine regulation of pancreatic cancer cell invasion by peripheral nerves. J Natl Cancer Inst. 2010;102(2):107-18, http://dx.doi.org/10.1093/jnci/djp456.
83. Skinner MA, Safford SD, Reeves JG, Jackson ME, Freemerman AJ. Renal aplasia in humans is associated with RET mutations. Am J Hum Genet. 2008;82(2):344-51, http://dx.doi.org/10.1016/j.ajhg.2007.10.008.
84. Zhang Z, Quinlan J, Hoy W, Hughson MD, Lemire M, Hudson T, et al. A common RET variant is associated with reduced newborn kidney size and function. J Am Soc Nephrol. 2008;19(10):2027-34, http://dx.doi.org/10.1681/ASN.2007101098.
85. Hoffer BJ, Harvey BK. Parkinson disease: Is GDNF beneficial in Parkinson disease? Nat Rev Neurol. 2011;7(11):600-2, http://dx.doi.org/10.1038/nrneurol.2011.149.