Exploiting RET isoforms in managing medullary and papillary thyroid cancer

Eric Y Lian¹, Serisha Moodley¹ & Lois M Mulligan^{*,1}

¹Division of Cancer Biology & Genetics, Cancer Research Institute, & Department of Pathology & Molecular Medicine, Queen's University, Kingston, ON K7L 3N6, Canada

*Author for correspondence: Tel.: +1 613 533 6000; Fax: +1 613 533 6830; mulligal@queensu.ca

“The recent use of high throughput screening techniques has identified novel RET rearrangements in other tumors, suggesting roles for RET in the development and progression of multiple human cancers.”

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The RET receptor mediates cell proliferation, survival and migration in embryogenesis, and is implicated in transformation and tumor progression in multiple cancers. RET is frequently mutated and constitutively activated in familial and sporadic thyroid carcinomas. RET is expressed as two isoforms, RET9 and RET51, that differ in C-terminal amino acids. These isoforms interact with distinct downstream signaling and regulatory complexes, but their functional differences are not well defined, and the effects of RET isoforms on disease prognosis and treatment remain poorly understood. In this editorial, we review the current understanding of differences between the RET isoforms and how they might affect disease course and clinical outcomes and serve as potential therapeutic targets in medullary or papillary thyroid carcinoma, and other RET-associated tumors.

Thyroid cancer affects over 3 million people worldwide. Papillary thyroid carcinoma (PTC) is the most commonly diagnosed thyroid malignancy, accounting for approximately 85% of all these cases, and is generally treatable, with a 5-year survival rate of 98% [1]. Medullary thyroid carcinoma (MTC) is a less common subtype that makes up some 3–5% of all thyroid cancers, displays more aggressive phenotypes, and can be more difficult to manage [1]. Surgical removal of the thyroid, followed by postoperative thyroid hormone replacement therapy, contributes to successful disease management in the majority of both PTC and MTC cases [2]. However, a subset of patients experience recurrence and/or distant metastases [2,3]. To date, additional treatment strategies, such as external beam radiation or chemotherapy, have had limited efficacy and are associated with adverse effects [2,3] posing an urgent need for predictive markers to distinguish treatment-resistant cases, as well as new treatment options for these persistent thyroid cancers. The advent of methodologies to target common oncogenic proteins or mutants that contribute to thyroid cancers is providing new therapeutic opportunities to address these challenging cases.

The rearranged during transfection (RET) receptor tyrosine kinase is a transmembrane tyrosine kinase expressed in many neuroendocrine tissues, nerves and the kidney [4]. RET is activated by binding of GDNF family ligands and GFR α family co-receptors (Figure 1) to mediate numerous cellular processes including cell survival, proliferation, migration, and differentiation [4]. However, *RET* is also an oncogene associated with growth and spread of cancer in multiple human tissues, including thyroid, where it promotes both MTC and PTC [5]. Activating point mutants of RET are associated with MEN2, a cancer syndrome characterized by early onset MTC, and similar mutations are also linked to poor clinical outcome in 40–65% of sporadic MTC [4]. These mutations result in ligand independent receptor dimerization or reduced autoinhibition and increased ATP-binding, leading to constitutive RET activity [4].

In PTC tumors, chromosomal rearrangement of the *RET* gene can generate RET chimeric proteins consisting of the RET kinase-domain fused to N-terminal sequences of a different protein that contains homodimerization domains. These constitutively activated proteins are aberrantly localized to the cytosol [4,5]. The recent use of high throughput screening techniques has identified novel RET rearrangements in other tumors, most notably in lung

Figure 1. RET receptor tyrosine kinase structure and activation. (A) Activation of RET requires the formation of a multicomponent complex which involves a GDNF family ligand (GDNF, NRTN, ARTN or PSPN) and its respective co-receptor (GFR α 1–4). (B) RET is a transmembrane tyrosine kinase receptor with three main regions. The extracellular domain consists of four cadherin-like domains and a cysteine-rich region, linked to an intracellular tyrosine kinase domain and isoform-specific C-terminal tails. (C) RET has two main isoforms with unique nine or 51 amino acid (RET9 and RET51, respectively) C-terminal sequences. RET9 and RET51 possess different phospho-tyrosine and protein-binding motifs and display differential abilities to bind downstream adaptor and signaling molecules.

adenocarcinomas and chronic myelomonocytic leukemia, as well as in spitzoid tumors [4,6], suggesting roles for RET in the development and progression of multiple human cancers.

Differential functions of RET isoforms

Our understanding of RET's roles in tumorigenesis, and its effects on disease progression, is complicated by the expression of two RET protein isoforms, generated by alternative splicing of 3' *RET* gene sequences. These proteins, termed RET9 and RET51, are identical, save for the unique nine or 51 amino acids in their respective C-terminal tails [7] (Figure 1). Despite the minor structural differences between these isoforms, a large body of evidence now suggests that RET9 and RET51 have very different contributions to RET-mediated cellular processes. Although RET9 and RET51 are coexpressed, the relative amounts of each isoform are temporally and spatially regulated, suggesting differences in their biological roles. The RET isoforms are known to mediate distinct processes during embryogenesis, with RET9, but not RET51, being critical for normal kidney morphogenesis and enteric nervous system development [8]. RET9 is highly expressed during early development, but RET51 expression remains low until later developmental stages, suggesting involvement in differentiation related events [9,10]. RET9 is also expressed ubiquitously throughout the olfactory nervous system, while RET51 expression is confined to regions innervated by sensory neurons [11], consistent with a more specialized role in directing cell migration, axon growth and guidance [12,13].

More recently, we have begun to understand the molecular and biological mechanisms that contribute to these RET isoform-specific roles. RET isoforms recruit distinct signaling complexes, leading to different patterns of target gene expression [14,15]. Although both RET9 and RET51 promote downstream signaling through multiple pathways, including PI3K/Akt/mTOR, and ERK/MAPK, distinct protein interactions with RET isoform unique C-terminal sequences affect the timing, duration and subcellular localization of these events [4,14,16,17]. Most notably, RET51 interacts with adaptor proteins containing SH2 domains, such as GRB2, through a unique phosphorylated tyrosine residue (pY1096) in its C-terminal tail [16,18]. In contrast, RET9 has distinct binding capabilities at pY1062, and also contains a unique C-terminal binding motif (FTRF₁₀₇₂), which allows it to interact with PDZ domain proteins such as SHANK family members [4,16] (Figure 1). RET9 and RET51 have been found to differ in their membrane localization, and in their internalization into the cell and intracellular trafficking upon activation [16,17,19]. RET51 matures more efficiently than RET9, and is present in relatively greater quantities at the cell membrane, while RET9 matures slowly and accumulates as immature protein [17,20]. Upon activation, RET51 is more rapidly internalized from the cell surface, but can be recycled from endosomal compartments to the cell membrane, enhancing or prolonging its signals [17,19], while RET9 is targeted for degradation. Differential ubiquitination, mediated through unique C-terminal interactions, has recently been shown to contribute to these differences in RET isoform processing [16].

RET51 expression is an indicator of more aggressive disease

Despite the abundance of evidence for distinct roles of the RET isoforms, the implications of RET isoform expression in cancer, and its potential as a marker of disease course, have not been fully explored. In recent studies, we have shown that RET51 modulates multiple critical processes that promote tumor growth and invasive spread in thyroid carcinomas [21]. In both MTC and PTC cell types, RET51 is significantly more effective than RET9 in enhancing cell proliferation, migration and anchorage-independent growth, suggesting that it may be the primary isoform contributing to tumor aggressiveness and disease progression [21]. Consistent with this, RET51 expression is correlated with increased malignancy in human thyroid tumors [21]. RET51 is also more highly expressed relative to RET9 in some MEN2-associated tumors [22] and in breast carcinomas, where it may grant a growth advantage [23]. RET51 is also upregulated in some pancreatic tumors, where it may contribute to invasive potential [24]. Together, these data suggest that RET51 may be the primary oncogenic form of RET in all these tumors. In addition, the relatively higher expression of the RET51 isoform may be an important indicator of RET-mediated tumor invasiveness, overall tumor aggressiveness and poorer prognosis that could identify tumors requiring additional or more extensive treatment interventions. Further, the relative increase in RET51 in these tumors may also make it an attractive target for cancer therapy.

RET as a therapeutic target

There are currently no approved RET-specific targeted agents, however, several tyrosine kinase inhibitors (TKIs) with activity against multiple kinases have also shown efficacy against RET-expressing cancers. Vandetanib and cabozantinib, multi-kinase TKIs originally developed against VEGFR2, are also effective at inhibiting RET and have been approved for use in patients with advanced MTC or treatment-resistant PTC [4]. In clinical trials,

TKIs sorafenib, sunitinib and lenvatinib also demonstrate inhibitory activity against both wildtype and mutant RET, and have been identified as options for the treatment of patients with differentiated thyroid carcinomas and MTC [25,26]. More recent innovations in drug design have generated RET-selective TKIs like LOXO-292 (Loxo Oncology), RXDX-105 (Ignyta) and BLU-667 (Blueprint Medicines) that potently inhibit RET over other RTKs, and may provide a valuable new therapeutic tool for use in RET-dependent cancers while avoiding adverse effects associated with broad-spectrum kinase inhibitors [2,26].

Despite the promise of targeted therapy, long-term inhibition of RET signaling may have widespread effects on neuronal maintenance and regeneration or survival, particularly in aged or damaged neurons [27]. Reduced levels of RET activity result in degeneration of dopaminergic neurons and abolishes GDNF-mediated neuroprotective effects in cell and animal models of Parkinson's disease [28], while dysregulation of RET signaling results in nociceptor dysfunction, possibly leading to increases in inflammation-induced pain, as experienced by cancer patients [4]. Importantly, although RET inhibition reduces or stabilizes thyroid tumor size in some patients, tumors quickly develop resistance to these inhibitors and resume growth [29], suggesting the management or maintenance of disease rather than a curative strategy. Despite these drawbacks, the potential of RET-targeted treatments to extend patient life cannot be underestimated and suggests that strategies to further refine the effects of RET inhibition, such as focusing on the primary oncogenic isoform, RET51, could be valuable.

The majority of currently available TKIs recognize the highly conserved sequences of the ATP-binding site of RTKs, allowing them to bind and inhibit multiple kinases. Allosteric inhibitors, which block specific protein interactions or signals, may function as more selective alternatives to existing inhibitors [30]. Targeting of individual RET isoforms or RET isoform-dependent interactions and signaling pathways, using antibodies, peptidomimetics and/or small molecule inhibitors, may offer novel strategies to improve RET selectivity and obviate the adverse events associated with multi-targeting TKIs. Several antibodies that specifically target the RET51 C-terminal tail have been generated and may be effective steric inhibitors to block RET dimerization, autophosphorylation and/or interactions with signaling and adaptor molecules [31]. Peptidomimetics, small peptides that mimic natural substrates, also have the potential for development into potent and selective inhibitors against signaling molecules that bind unique protein domains and motifs [32]. For example, peptides mimicking the sequence and/or structure of the RET C-terminal tails may be useful as 'molecular sponges' to bind RET isoform-specific interactors, such as RET51-binding adaptor GRB2 [4,16,18], thereby inhibiting RET51-specific downstream signals. Alternatively, direct targeting of RET isoforms could be complemented by blocking established signaling pathways activated by RET including ERK/MAPK, PI3K and mTOR [4]. Small molecule inhibitors of these pathways have already been assessed in mechanistic studies and cancer clinical trials for the treatment of thyroid cancer. For example, studies have suggested targeting of the RET-associated PI3K/Akt/mTOR pathway, which is implicated in MTC tumorigenesis and progression and is specifically associated with sustained RET51 activity [17,33]. These novel strategies may provide additional therapeutic options to specifically inhibit RET isoform-dependent functions for the treatment of MTC and PTC or other RET-associated aggressive cancers.

Although work in the field of isoform-specific inhibitors remains nascent, recent studies have identified RET51 as a promising therapeutic target for the treatment of cancers [21]. Ongoing development of therapeutics with greater specificity and the ability to preferentially inhibit this more oncogenic isoform of RET may open new treatment options while avoiding adverse effects associated with current inhibitors.

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