Defining the Relationship between RET Receptor Tyrosine Kinase and Cancer Biomarkers

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Abstract

RET (Re-arranged during Transfection) is a transmembrane receptor tyrosine kinase (RTK) that is involved in several cellular activities. The dysregulation of these transmembrane RTKs is closely linked with cancer development and is of interest as a potential therapeutic target. Mutations in RET have been associated with cancers of the thyroid, lung, breast, and pancreas, as well as melanomas. Abnormal cytosolic and nuclear translocations of cell to cell adhesion proteins, such as β catenin, have been reported in RET related cancers. These proteins may have potential to act as cancer biomarkers for RET point mutant and/or fused cancers. My project aims to identify the biomarkers associated with cell adhesion regulation for cancers expressing RET mutations and determine the mechanisms of these biomarkers interacting with mutant RET. In addition, to find potential mechanisms of action of RET kinase inhibitors that show change in expression and distribution on the cell adhesion biomarkers in relation to the RET mutations.

Introduction

RET (Rearranged during transfection) is a receptor tyrosine kinase (RTK) which is associated with cell proliferation, differentiation, motility, apoptosis, and survival. It contains a cadherin-related domain and a cysteine rich domain in the extracellular region, as well as a kinase domain and a carboxyl terminal domain in the intracellular region.

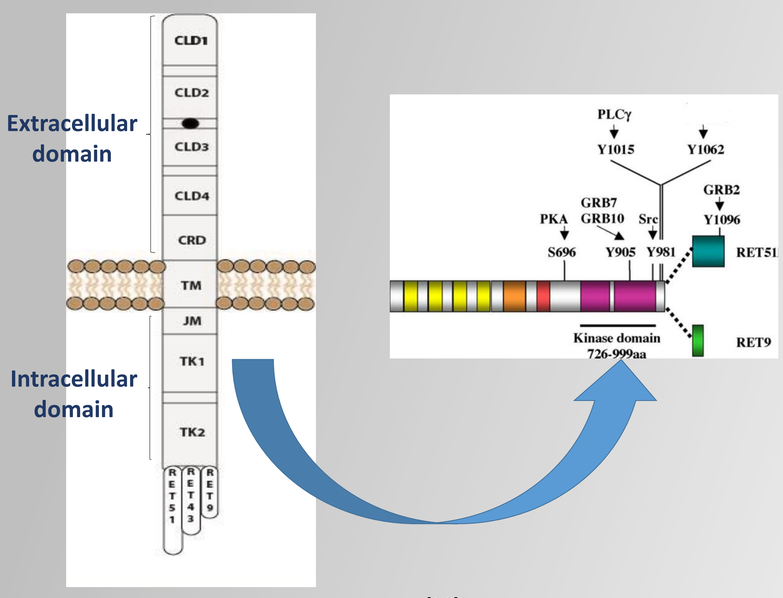


Figure 1: Structure and docking site of RET[1-2]. RET contains extracellular domain, transmembrane domain and intracellular domain. In addition, in intracellular domain, several docking sites for downstream signalling pathway are included.

RET mutations, fusions and overexpression are associated with several cancers.

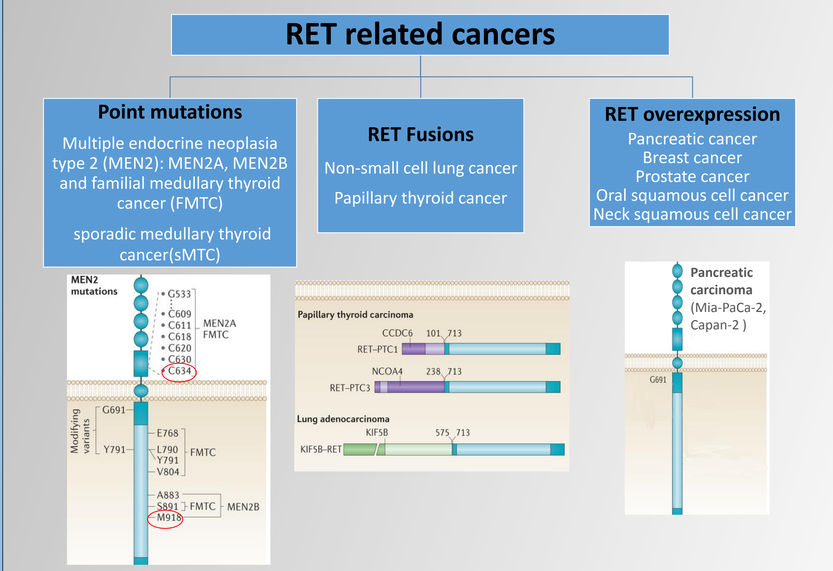
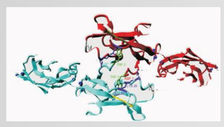


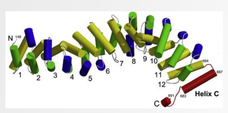
Figure 2: RET related cancers. RET point mutations, RET fusions and RET overexpression are found in several cancers, causing the constitutive activation of RET protein.

Cancer biomarkers have been utilized in multiple clinical fields, including cancer diagnosis, monitoring the progression in cell metastasis, and determining the effects of therapy. Cell-adhesion proteins, such as β-catenin, E-cadherin, vimentin and laminin, which also regulate cell invasion, differentiation and metastasis, may function as a potential cancer biomarkers.

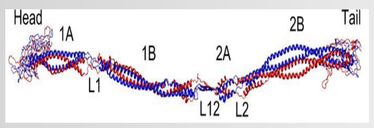
E-cadherin
(Breast cancer)



β-catenin
(Sporadic colorectal cancer)



Vimentin
(Gastric cancer)



Laminin
(Upper gastrointestinal cancer)

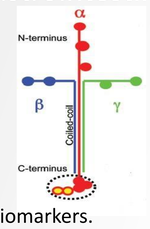


Figure 3: Several cell adhesion molecules used for cancer biomarkers.

Recently, several cell to cell adhesion proteins have been found to interact with RET or to be overexpressed in RET related cancer cells. β-catenin, a cell-adhesion protein and also a regulator for transcription of Wnt target genes, was found to have an abnormal nuclear translocation in the TPC1 cells (expressing RET/PTC1)[3]. Vimentin, an intermediate filament family protein, was found to be overexpressed in papillary thyroid carcinoma cells and expressed more truncated species with RET fusion cells[4-5].

Table 1. IC_{50} values of several RET receptor tyrosine kinase inhibitors.

Name	Vandetanib (ZD6474)	Cabozantinib (XL-184)	Sunitinib (SU11248)	Sorafenib (BAY 43-9006)	Ponatinib (AP24534)	Alectinib (CH5424802)	Quizartinib (AC220)
IC_{50}(nM)*	34	5.2	12	13	25.8	4.8	8
Main targets	RET, VEGFR, EGFR	VEGFR, MET, RET	VEGFR, PDGFR, c-KIT, RET	VEGFR, PDGFR, RET, FGFR, c-KIT, BRAF	BCR-ABL, VEGFR, PDGFR, KIT, FGFR, FLT3	ALK, RET	Flt3, PDGFRα, PDGFRβ, KIT, RET

* Based on Biochemical Data

There are several RET kinase inhibitors on the market or in the clinical trials. Although all these kinase inhibitors display a low IC_{50} values, they also inhibit off-target kinases, causing serious toxicity. For example, both Vandetinib and Cabozantinib are inhibitors of kinase insert domain receptor (KDR), so an improved RET selective kinase inhibitor is required. Recently, 2-substituted phenol quinazolines and Alectinib have been discovered to be more selective to RET with weak or no effect on KDR.

Aims

Aim 1: To identify biomarkers associated with cell adhesion regulation for cancers expressing RET mutations and determine the mechanisms of these biomarkers interacting with mutant RET.

Aim 2: To find potential mechanisms of action of RET kinase inhibitors that show changes in expression and distribution on the cell adhesion biomarkers in relation to the RET mutations.

Methods

Human normal embryonic kidney cell (HEK293), papillary thyroid cancer cell line (TPC1) and pancreatic cancer cell line (MIA PaCa-2) were utilized in the experiments. Confocal immunofluorescence microscopy was used to determine the distribution of the biomarkers and western blot analysis was conducted to determine the expression level of these biomarkers. RET inhibitors will used to determine the change of expression level and distribution of these adhesion proteins, using western blot and confocal immunofluorescence microscopy. In addition, the images were quantified by using Columbus™ Image Data Storage and Analysis System.

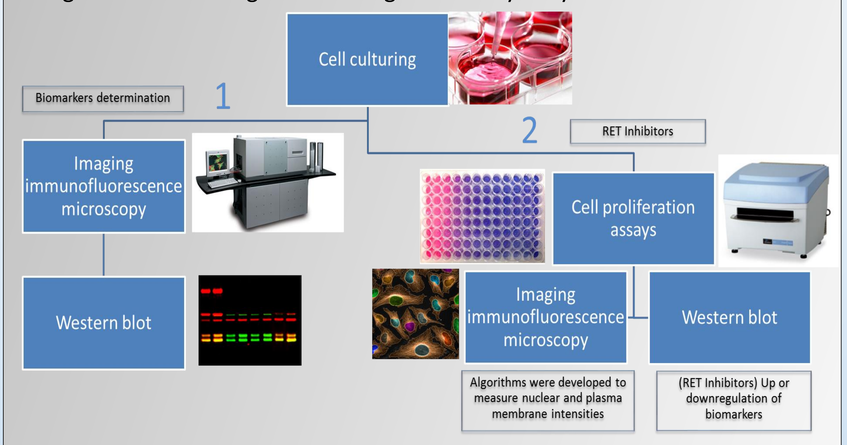


Figure 4: Flowchart for the experimental work. Branch 1 focus on biomarkers determination and branch 2 focus on RET inhibitors.

Results

Aberrant expression of β-catenin, γ-tubulin and Talin in RET related cancer cells

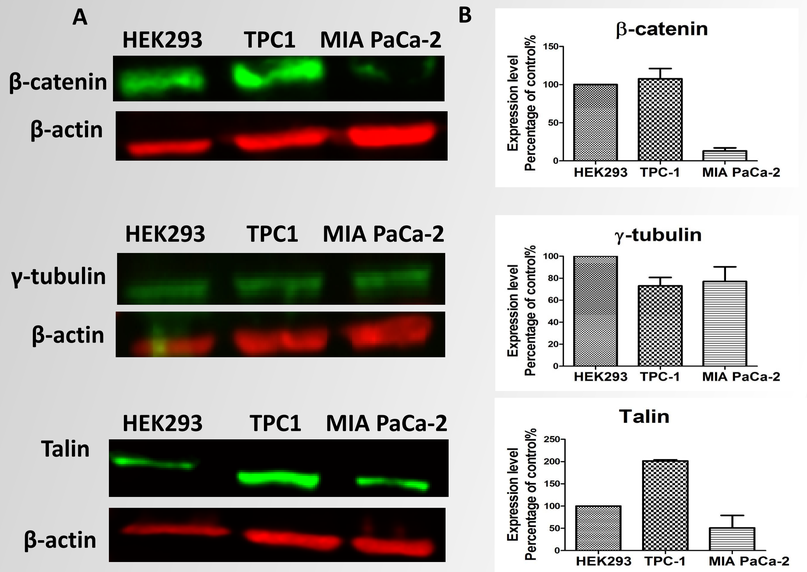


Figure 5: Western blot analysis for detection of β-catenin, γ-tubulin and talin. Experiments have been conducted twice. A. Infrared western blot images. B. Quantification of β-catenin, γ-tubulin and talin expression levels. All data was normalized against β-Actin and compared to control (HEK293 cell line).

β-catenin expression levels are reduced in MIA PaCa-2 cells, and talin is overexpressed in TPC-1 cells. In addition, γ-tubulin also shows slightly lower expression levels both in TPC-1 and MIA PaCa-2 cells.

Distribution of β-catenin and E-cadherin in RET related cancer cells

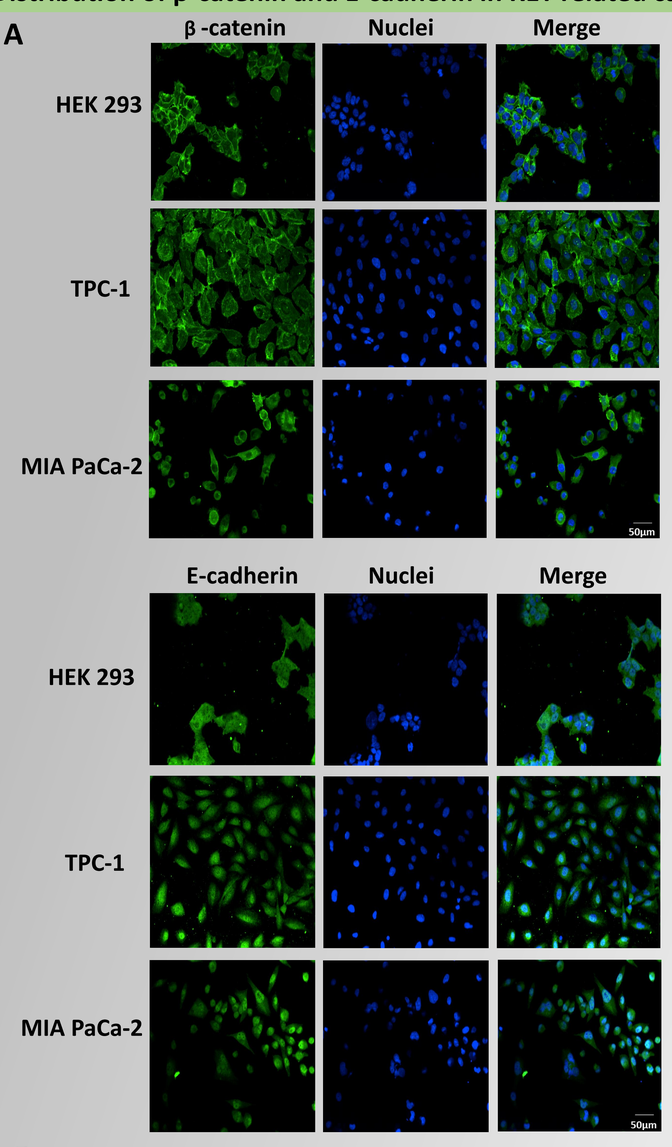


Figure 6: Imaging assay. Cells were imaged using confocal microscopy (20X objective, scale bar 50μm). A. Confocal immunofluorescence images. B. Quantification of biomarkers intensity in nuclei, plasma membrane and whole cells.

Images illustrate that E-cadherin experience nuclear translocation, meanwhile β –catenin intensity displays a remarkable decrease in TPC-1 and MIA PaCa-2 cells. The abnormal translocation and distribution of E-Cadherin and β –catenin could be associated with RET mutations.

Conclusion

1. TPC-1 cells which are RET fused model show upregulation of talin, whilst MIA PaCa-2 cells with a RET point mutation appear to have downregulated β-catenin expression. RET mutations may also contribute to the downregulation of γ-tubulin.
2. RET point mutations and RET fusions appear to be associated with the nuclear translocation of E-cadherin.

Future direction

1. RET inhibitors will be used to determine the effect of cell proliferation on these RET mutant or fused cells.
2. The expression level and distribution of these adhesion proteins will be tested with treatment of RET inhibitors, using western blot and imaging immunofluorescence assays.

Acknowledge

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References

1. Knowles, P.P., et al., Structure and chemical inhibition of the RET tyrosine kinase domain. J Biol Chem, 2006. 281(44): p. 33577-87.
2. Arighi, E., M.G. Borrello, and H. Sariola, RET tyrosine kinase signaling in development and cancer. Cytokine Growth Factor Rev, 2005. 16(4-5): p. 441-67.
3. Tartari, C.J., et al., Dissection of the RET/beta-catenin interaction in the TPC1 thyroid cancer cell line. Am J Cancer Res, 2011. 1(6): p. 716-25.
4. Yamamoto, Y., K. Izumi, and H. Otsuka, An immunohistochemical study of epithelial membrane antigen, cytokeratin, and vimentin in papillary thyroid carcinoma. Recognition of lethal and favorable prognostic types. Cancer, 1992. 70(9): p. 2326-2333.
5. Zeindl-Eberhart, E., et al., Influence of RET/PTC1 and RET/PTC3 oncoproteins in radiation-induced papillary thyroid carcinomas on amounts of cytoskeletal protein species. Amino Acids, 2011. 41(2): p. 415-25.