A *RET::GRB2* fusion in pheochromocytoma defies the classic paradigm of *RET* oncogenic fusions

Hector Gonzalez-Cantu[1], Cynthia M. Estrada-Zuniga[1], Zi-Ming Cheng[1], Purushoth Ethiraj[1], Qianjin Guo[1], Elaina Adderley[2], Hector Lopez[1], Bethany N. Landry[1], Abir Zainal[2], Neil Aronin[2], Yanli DIng[3], Xiaojing Wang[4,5], Ricardo C.T. Aguiar [1, 6, 7], Patricia LM Dahia[1, 6]

[1]Division of Hematology and Medical Oncology, Dept Medicine, UTHSCSA, [2]Division of Endocrinology, University of Massachusetts Worcester, [3]Department of Pathology, UTHSCSA, [4]Department of Population Sciences, UTHSCSA, [5]Greehey Children's Cancer Research, UTHSCSA, [6]Mays Cancer Center, UTHSCSA, [7]South Texas Veterans Health Care System, Audie Murphy VA Hospital, San Antonio

Introduction

RET is a receptor tyrosine kinase with restricted tissue expression and relevance to cancer[3]. Genetic aberrations in *RET*, including missense mutations and gene fusions lead to constitutive activation of RET and its effectors, endowing target cells with oncogenic phenotypes[3]. Pheochromocytomas and paragangliomas are highly heritable neural-crest derived tumors that are components of VHL disease. The great majority of these tumors are sporadic, with their main molecular drivers remaining poorly elucidated. Here we report the characterization and experimental validation of a novel driver oncogenic *RET* fusion with *GRB2* exhibiting an uncommon fusion configuration (RET as the 5' partner) and show its potential actionability with clinical-grade RET selective inhibitors.

Results

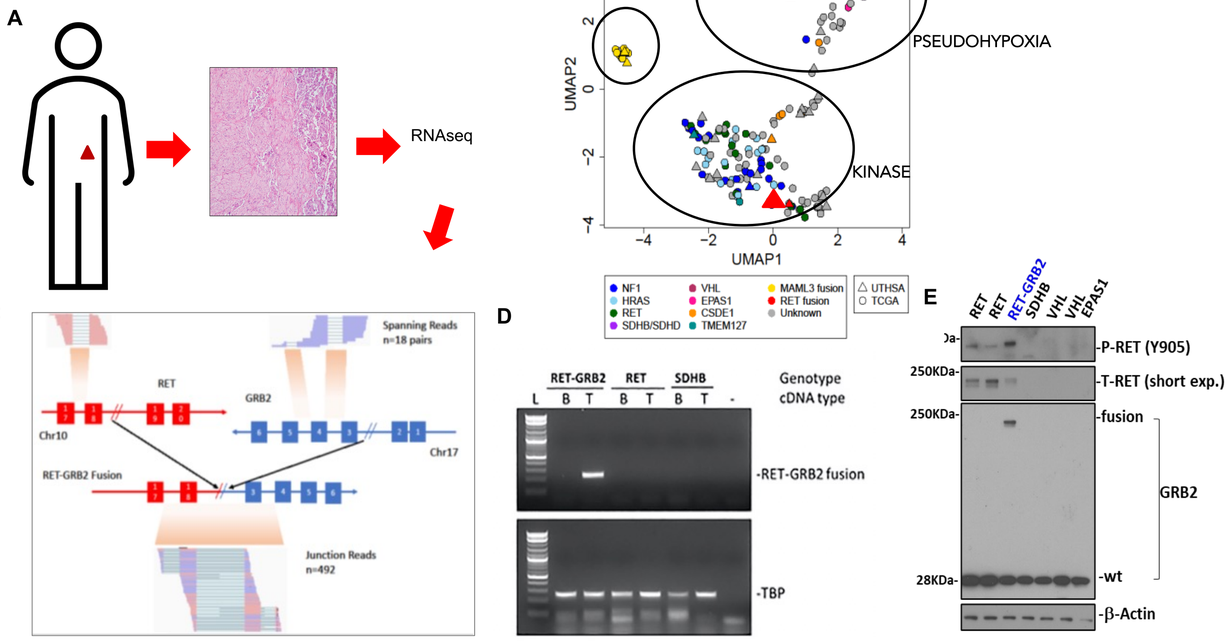

Fig.1 *RET::GRB2* fusion is detected as a somatic event in a pheochromocytoma. A. A sporadic pheochromocytoma was analyzed via RNAseq for its molecular origin. B. Uniform manifold approximation and projection (UMAP) plot of RNA-seq data from PPGL of our cohort (n=30, UTHSCSA) and TCGA (n=178), color-coded by genotype; gray symbols are tumors with unknown mutations, *RET::GRB2* fusion in red. C. Representation of the region spanning the RET exon 18 and GRB2 exon 3 in a pheochromocytoma predicted to carry the *RET::GRB2* fusion. D. Agarose gel of PCR products spanning the *RET::GRB2* fusion transcript exclusively in tumor but not matched leukocyte (blood) cDNA. Other *RET* and *SDHB*-mutant tumors do not show product. E. Immunoblot analysis of protein lysates from PPGLs harboring different mutations. An antibody targeting the C-terminal region of GRB2 detected GRB2 at the molecular size consistent with the predicted size of the RET::GRB2 fusion protein. Other PPGLs did not show this. Sample with the RET::GRB2 fusion is shown in blue.

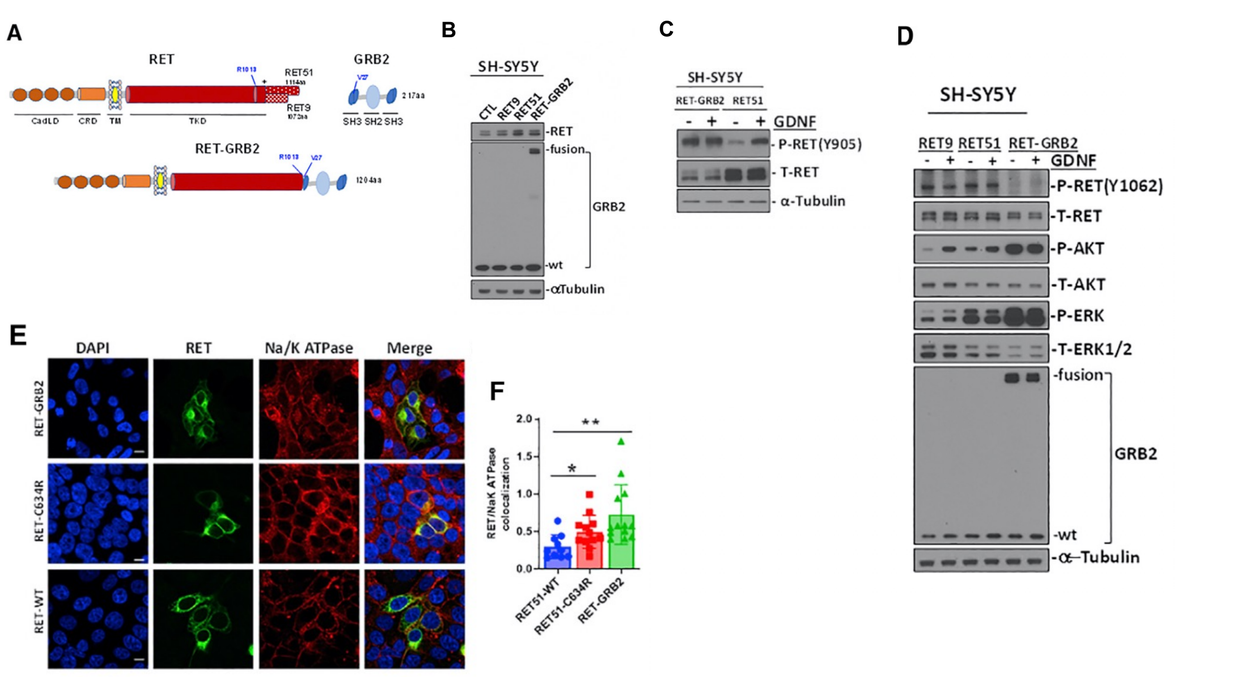

Fig. 2 Validation of RET::GRB2 fusion protein *in vitro*. A. Diagram of wild-type RET displaying relevant domains. RET51 and RET9 are the two main RET isoforms, diverging at amino acid 1,063. *RET* and *GRB2* breakpoints in RET::GRB2 fusions are indicated. B. Immunoblot analysis of SH-SY5Y cells stably expressing RET9, RET51, RET::GRB2 or control vector. C. Immunoblot analysis of SH-SY5Y cells expressing RET::GRB2 or RET51, exposed to 100ng/mL GDNF (+) or vehicle (-) for 10 minutes following 3 hours of serum starvation. D. Immunoblot analysis of SH-SY5Y cells expressing RET9, RET51, and RET::GRB2 treated with GDNF as in (C). E. Confocal microscopy of HEK293T cells expressing WT, mutant (C634R) RET, or RET::GRB2 fusion, labeled with a tag antibody in green (MYC for WT and C634R or hemagglutinin [HA] for RET::GRB2 fusion) and a membrane marker, Na/K ATPase (red). Nuclei are stained with DAPI (blue). F. Quantification of the colocalized signals between RET and Na/K ATPase using ImageJ.

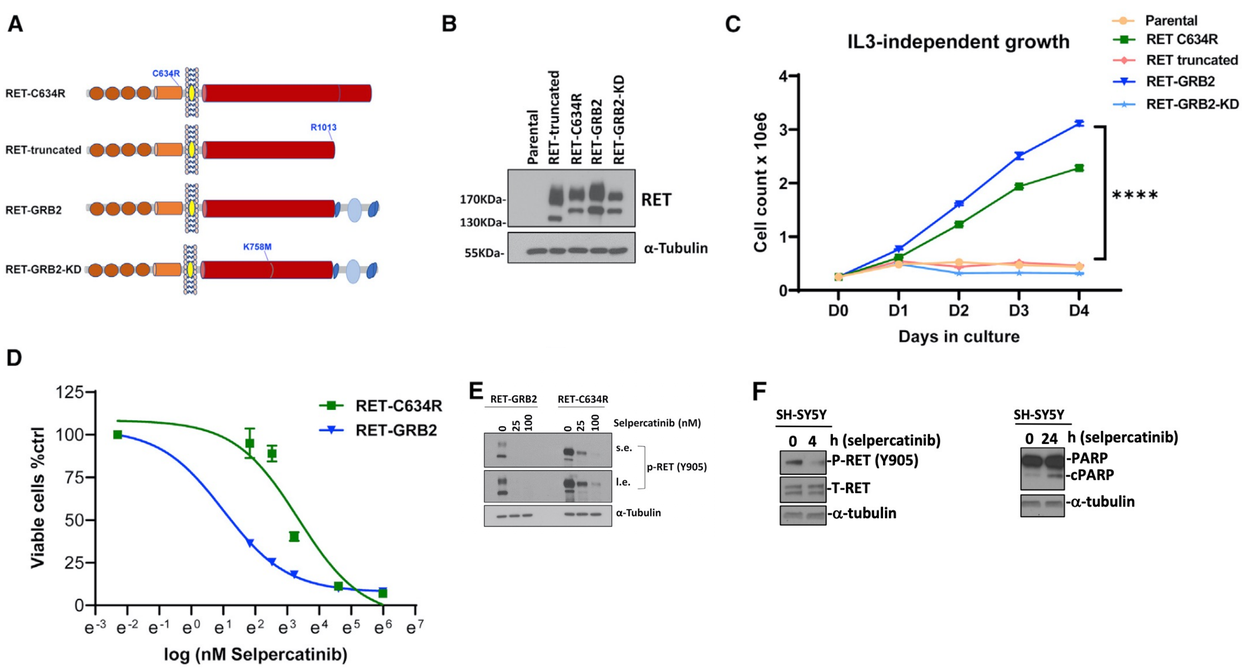

Fig. 3 *RET::GRB2* fusion is oncogenic and sensitive to clinical grade RET inhibitors. A. Constructs used to evaluate transforming activity in Ba/F3 cells: RET-C634R, pathogenic RET mutant, RET-truncated, RET component of RET::GRB2; full length RET::GRB2 fusion and RET::GRB2-KD, kinase dead version of fusion. B. Immunoblot analysis of Ba/F3 lysates stably expression the constructs in (A). C. Growth rate of Ba/F3 cells stably expressing constructs in (A) and (B), and parental cells in the absence of interleukin 3 (IL-3). D. IC50 concentration-response curves to selpercatinib at 0, 6.25, 12.5, 25, 50, 100, and 400nM for 72h measuring inhibition of growth of Ba/F3 cells expressing RET::GRB2 and RET-C634R. E. Immunoblot analysis of Ba/F3 cells expressing RET::GRB2 or RET-C634R treated with 25 or 100nM selpercatinib for 4 hours. F. Immunoblot analysis of lysates from SH-SY5Y cells expressing RET-GRB2 and treated with 50nM selpercatinib.

Conclusions

The *RET::GRB2* fusion found in a pheochromocytoma is shown to constitutively activate RET downstream signaling events and posses transforming capabilities, supporting its role as the molecular basis of this tumor's development. Mechanistically, the RET::GRB2 fusion is dependent on the kinase activity of the RET partner and the presence of GRB2 to confer its oncogenic properties. Translationally, cells harboring the *RET::GRB2* fusion are shown to be sensitive to clinical-grade RET selective inhibitors.

A distinct RET fusion with RET as the upstream partner(RET::SEPTIN2) was detected in a metastatic pheochromocytoma and the patient responded to selpercatinib, supporting our findings[2]. Future studies should determine if this fusion configuration is exclusive to PPGLs or if it can also be detected in other neural-crest derived tumors, or more broadly, different cancers; such tumors may also be amenable to RET selective inhibition

References

1. Estrada-Zuniga, C., Cheng, Z.-M., Guo, Q., Ethiraj, P., Adderley, E., Aronin, N., Ding, Y., Wang, X., Aguiar, R. C. T., & Dahia, P. L. M. (2022). A Novel RET-GRB2 Fusion in Pheochromocytoma Defies the Classic Paradigm of RET Oncogenic Fusions. Cell Reports Medicine. https://doi.org/10.2139/ssrn.4005584

2. Mweempwa, A., Xu, H., Vissers, J. H. A., Tothill, R. W., Pattison, A. D., Fellowes, A. P., Thomas, D. M., Richardson, G., Hicks, R. J., Grimmond, S. M., Fox, S. B., Luen, S. J., Desai, J., & Solomon, B. J. (2021). Novel RET Fusion RET-SEPTIN9 Predicts Response to Selective RET Inhibition With Selpercatinib in Malignant Pheochromocytoma . JCO Precision Oncology, 5, 1160–1165. https://doi.org/10.1200/po.21.00127

3. Mulligan LM. (2014). RET revisited: expanding the oncogenic portfolio. Nat Rev Cancer. 4(3):173-86.

Acknowledgements

P.LM.D. is a recipient of funds from the NIH (GM114102 and CA264248) and the Neuroendocrine Tumor Research Foundation and is the holder of the Robert Tucker Hayes Distinguished Chair in Oncology. This work was supported by funds from UT System Star Awards (to P.L.M.D.). R.C.T.A. acknowledges funding support from NIH R01ES031522, NIH R01GM140456, I01BX001882 (Veterans Administration Merit Award), and RP190043 (CPRIT).