TMEM127 may downregulate receptor tyrosine kinases to prevent tumorigenesis

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Introduction

TMEM127 is a ubiquitously expressed, transmembrane encoding gene of poorly known function that is mutated in pheochromocytomas and paragangliomas. TMEM127 is located in the endomembrane and plasma membrane and may be involved in protein trafficking. TMEM127 mutant tumors cluster with pheochromocytoma with kinase activation (cluster 2). To gather insights into TMEM127 function in vivo, we generated a recombinant C57/BL6 mouse lacking *Tmem127* under a generic promoter (*Tmem127* CMV-KO), and here analyzed the abundance of RTKs in adrenal gland, thyroid, spleen and liver. *Tmem127* KO mice do not develop spontaneous tumors. However, they have higher insulin sensitivity and activation of PI3K/AKT signaling in liver and muscle, suggesting that this mouse strain should be a useful model for analyzing preneoplastic signaling.

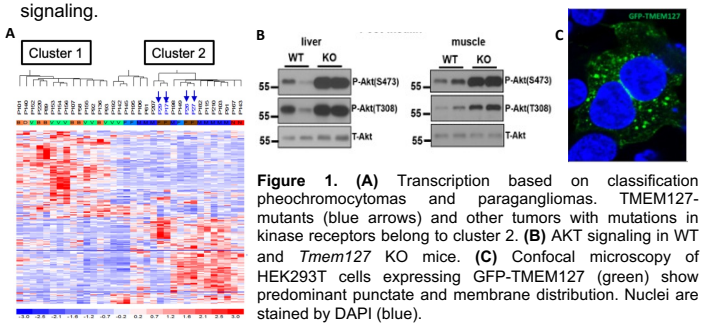

Figure 1. (A) Transcription based on classification pheochromocytomas and paragangliomas. TMEM127-mutants (blue arrows) and other tumors with mutations in kinase receptors belong to cluster 2. **(B)** AKT signaling in WT and *Tmem127* KO mice. **(C)** Confocal microscopy of HEK293T cells expressing GFP-TMEM127 (green) show predominant punctate and membrane distribution. Nuclei are stained by DAPI (blue).

Hypothesis

TMEM127 might function as a tumor suppressor through antagonizing cell surface proteins including receptor tyrosine kinase receptors.

Sample Collection

Tmem127 was knocked out under a CMV promoter in C57/BL6 mice. Standard dissection techniques were used to remove the liver (n=6), adrenal glands (n=14), spleen (n=6), and thyroids (n=13) from adult male and female WT and *Tmem127* KO mice.

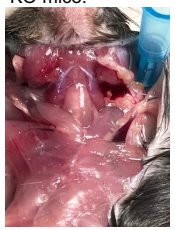

Figure 2. Thyroid Dissection: A vertical incision was continued from inferior to superior to expose the neck. The parotid glands were reflected superior and laterally and excised. The sternohyoid and sternothyroid were then traced inferiorly, cut, reflected superiorly and then removed. All tissue superior to the manubrium and inferior to the hyoid including the trachea was collected.

Tissue samples were processed in lysis buffer with protease inhibitors, crushed three times, and rotated for one hour at 4℃. Samples were centrifuged and the supernatants were collected.

Western Blot Analysis

Protein concentrations were measured against bovine serum antigen standard curves. Consistent protein amounts were loaded on SDS-PAGE gels. The gels were transferred to PVDF using standard Western Blot technique. The blots were stained with Ponceau and probed for either β-Actin or α-tubulin as a loading control. *Tmem127*, RET, EGFR, HER2, FGFR, and PDGFR-α were probed, and levels were quantified relative to control using ImageJ.

Pooled Liver and Adrenal Gland Results

Western Blot analysis of pooled data from 3 WT and *Tmem127* KO mice revealed increased RTKs in KO mice with increased EGFR in PDGF-α levels liver and increased FGFR and PDGF- α levels in adrenal glands.

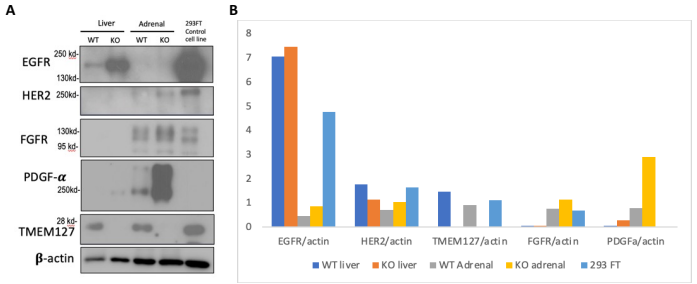

Figure 3. (A) Western Blot analysis of RTK levels in liver and adrenal glands of WT and *Tmem127* KO mice. **(B)** Graph of quantification of RTK levels.

Adrenal Gland and Spleen Results

Western Blot analysis of individual samples from WT and *Tmem127* KO mice showed increased RET levels in KO mice in both the adrenal glands and spleens.

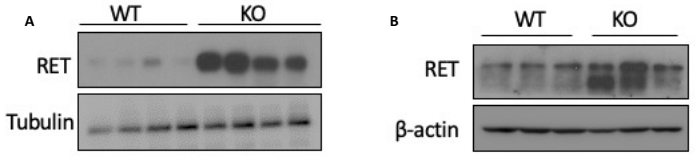

Figure 4. (A) Western Blot analysis of RET levels in adrenal glands of WT and *Tmem127* KO mice. **(B)** Western Blot analysis of RET levels in spleen of WT and *Tmem127* KO mice.

Thyroid Results

Western Blot analysis revealed increased RET expression in thyroids of *Tmem127* KO mice relative to their WT counterparts.

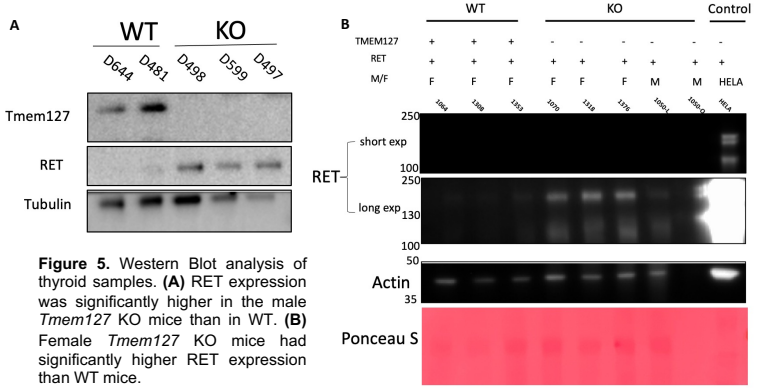

Figure 5. Western Blot analysis of thyroid samples. **(A)** RET expression was significantly higher in the male *Tmem127* KO mice than in WT. **(B)** Female *Tmem127* KO mice had significantly higher RET expression than WT mice.

Conclusion and Next Steps

We found that *Tmem127* KO mice have higher levels of RTKs and importantly RET, the oncoprotein associated with the MEN2 syndrome. MEN2 is a neuroendocrine cancer syndrome that is always associated with either medullary thyroid carcinoma or the pre-malignant C-cell hyperplasia, pheochromocytomas/paragangliomas half the time, and either primary hyperparathyroidism (MEN2A) or mucosal neuromas and marfanoid habitus (MEN2B). Interestingly, despite downregulating RET, TMEM127 mutations have not been tied to thyroid cancer. For future work, we will evaluate whether receptor kinase overexpression in TMEM127 KO is tissue-specific, and the mechanisms underlying these effects of TMEM127. Recent work from our lab suggests that TMEM127 may affect traffic and degradation of RTKs.

References

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3. Shim HK, Kim SG, Kim TS, Kim SK, Lee SJ. Total Thyroidectomy in the Mouse: the Feasibility Study in the Non-thyroidal Tumor Model Expressing Human Sodium/Iodide Symporter Gene. Nucl Med Mol Imaging. 2011 Jun;45(2):103-10. doi: 10.1007/s13139-011-0076-x. Epub 2011 Feb 15. PMID: 24899988; PMCID: PMC4043020.
4. Guo Q et al. TMEM127 suppresses tumor development by promoting RET ubiquitination, positioning and degradation. Cell reports in press (2023).