B-2

TMEM127 exerts a tumor suppressive role in pheochromocytoma by mediating RET ubiquitin-dependent degradation

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BACKGROUND

TMEM127 encodes for a ubiquitously expressed transmembrane protein with limited knowledge into its role. TMEM127 germline loss-of-function is a driver of pheochromocytoma and paraganglioma (PPGLs), tumors derived from the adrenal medulla and extra-adrenal paraganglia, respectively. Molecularly, TMEM127 mutant PPGLs belong to the kinase cluster, characterized by kinase signaling transcriptional programs. Receptor tyrosine kinase RET, a driver of PPGLs via germline or somatic gain-of-function mutations similarly belongs to the kinase cluster. Previously, we reported that TMEM127 loss led to mTOR signaling activation, suggesting that TMEM127 loss had an impact on kinase signaling pathways associated with mTOR, such as RET. Compellingly, and in line with the shared kinase signaling pathway signatures, TMEM127 mutant PPGLs display high levels of RET at the protein level, a phenomenon which is conserved in murine and cell line models. Here we sought to mechanistically interrogate the impact of TMEM127 loss on RET in an oncogenic context.

METHODS

Primary tumor samples harboring different mutations, engineered Tmem127 KO mice, and engineered TMEM127 KO cell lines were analyzed for the abundance, localization, turnover, and signaling of RET as impacted by TMEM127 loss. Additionally, we interrogated the impact of TMEM127 functional motifs in targeting RET for degradation via recruitment of an E3 ligase. Lastly, we interrogated the RET-dependent oncogenic impact of TMEM127 loss on RET by investigating viability, proliferation, and transformation features in vitro and in vivo.

RESULTS

Compellingly, and in line with the shared kinase signaling pathway signatures, TMEM127 mutant PPGLs display high levels of RET at the protein level, a phenomenon which is conserved in murine and cell line models. Further investigation revealed that TMEM127 impacted RET degradation.

Mechanistically, we showed that TMEM127 was critical for the recruitment of NEDD4, an E3 ligase, to ubiquitinate RET, targeting it for endosomal trafficking and lysosomal degradation, with TMEM127 loss impacting RET localization and abundance. Our experiments with functional TMEM127 mutants determined that its C-terminal PxxY motifs were necessary to recruit NEDD4 to target RET for degradation. Lastly, in vitro and in vivo models of TMEM127 loss were found to be sensitive to RET clinical grade inhibitors.

CONCLUSIONS

Our data supports a novel tumor suppressive role of TMEM127 in PPGLs by targeting RET for degradation via recruitment of NEDD4, establishing the RET accumulation in TMEM127 mutant PPGLs as a dysregulation of this mechanism. Translationally, our work supports the clinical benefit of RET targeted therapy in TMEM127 mutant PPGLs.

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