B-16

Imipridone ONC201 Blocks Tumor Growth by Inhibiting RET/IGFBP2 Pathway in Medullary Thyroid Cancer

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BACKGROUND: Medullary thyroid carcinomas is a neuroendocrine tumor arising from the calcitonin-secreting C cells. RET tyrosine kinase receptor is a driver oncogene and frequently mutated in MTC. Activating mutation of RET prevents apoptosis through inhibition of ATF4, a transcriptional regulator of endoplasmic reticulum (ER) stress. While tyrosine kinase inhibitors (TKIs) can delay progression, the responses to these agents are incomplete. Innovative drugs are needed that are effective against refractory MTC. ONC201 is a first-in class small molecule with favorable safety profile that activates p53-independent apoptosis through induction of ATF4.

METHODS: MTC cells were treated with ONC201 and cell viability was measured by colorimetric assay and cleaved caspase3/PARP staining followed by flow cytometry. MTC xenografts were treated with ONC201 at a weekly dose of 120 mg/kg for 8 weeks. Real-time RT-PCR, western blot, and immunohistochemistry were used to examine mRNA and protein levels.

RESULTS: Here, we report that ONC201 caused apoptosis in MTC cells and abrogated MTC tumor growth in mice without toxicity. ONC201 Induced mRNA levels of apoptotic genes, including ATF4, DDIT3 (CHOP) and BBC3. ONC201 also inhibited RET expression at both mRNA and protein levels. Functional proteomic identified IGFBP2 as a RET target that is known to promote survival, metastasis, and resistance to MAPK inhibitors. The treatment of MTC cells with ONC201 or ATF4 overexpression downregulated IGFBP2 mRNA and protein levels and decreased the secretion of IGFBP2 into the conditioned media. Immunohistochemical and western blot analysis of MTC xenografts

demonstrated decreased IGFBP2 protein levels in mice treated with ONC201 that was associated with decreased proliferation. The combination of ONC201 with TKIs inhibited cell survival in a synergistic manner.

CONCLUSION: These results demonstrate that ONC201 inhibits RET-mediated signaling and support the further investigation of ONC201 as a therapeutic agent for the treatment of MTC alone or in combination with thyrosine kinase inhibitors.