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Autophagy Activation, a Survival Mechanism for pNETs Treated with mTOR Inhibitors

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Background: The effects of the mTOR allosteric inhibitor RAD001 are more pronounced in pancreatic neuroendocrine tumors (pNETs) than in other tumor types; however resistance occurs, underscoring the need for novel lines of therapy. Based on our published studies in hepatocellular carcinoma, we propose to improve the clinical response to RAD001 in pNETs and in parallel counteract the development of RAD001 resistance by combining RAD001 with a dual PI3K/mTOR ATP-site-competitive inhibitor, which acts synergistically to completely inhibit mTOR.

Methods: Human BON cells (gift from C. Townsend at UTMB) were generated to stably express a doxycycline (Dox)-inducible shRNA targeting the autophagy gene ATG3 (shATG3) or a non-silencing control (shNS).

Results: The inhibitory synergy on proliferation we noted previously between RAD001 and BEZ235 is not unique to RAD001/BEZ235, as we can recapitulate this response in BON cells treated with RAD001 or rapamycin combined with the PI3K/mTOR ATP-site competitive inhibitor PKI-587 (gift from Pfizer). In BON cells the rapamycin/PKI-587 combination profoundly inhibited phosphorylation of key downstream mTOR targets including 4E-BP1, Akt and ULK1 and activated AMPK. To determine if the induction of autophagy by rapamycin/PKI-587 promotes or inhibits tumorigenesis in pNETs, we disrupted autophagy by depleting ATG3 in BON cells using an inducible shATG3-expression system. Impaired autophagy partially rescued the proliferation inhibition conferred by the combination treatment, compared to shNS control cells. Conversely, activation of autophagy using the disaccharide trehalose impaired BON cell proliferation.

Conclusions: These data support that the induction of autophagy in pNETs is anti-tumorigenic and that AMPK functions potentially as a tumor suppressor, not a resistance mechanism to mTOR/PI3K inhibitor treatment in these tumors. A deeper mechanistic understanding is warranted to determine if combining mTOR inhibitors with activators of autophagy or AMPK would be beneficial for the treatment of pNETs.