Investigating the Activity of Three Truncated Isoforms of MET Receptor

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MET receptor is a receptor tyrosine kinase (RTK) that is important in various biological processes such as wound healing and liver regeneration. The biological activity of MET is initiated when its ligand, Hepatocyte Growth Factor (HGF), binds to the receptor's extracellular domain, induces receptor dimerization and activates signaling pathways such as PI3K-AKT and MAPK. These pathways are responsible for cellular proliferation, migration, and invasion. For this reason, aberrant MET activation is implicated in various types of cancers and is an attractive therapeutic target.
In Dr. Cartegni's lab, antisense oligonucleotides (ASO) are utilized to target RTKs in cancer. ASO are designed to prevent U1-snRNP from binding to an exon's 5' splice site (ss) and activate intronic polyadenylation (IPA) to generated stable mutant mRNA encoding a therapeutic RTK variant. For MET, this strategy leads to a reduction of full length MET receptor (FL-MET) and generation of soluble decoy MET isoform (sdMET) which lacks transmembrane and kinase domain but retains its ability to bind HGF; hence, its sequesters ligand and prevent downstream signaling pathway. Our lab has identified endogenous sdMET isoforms including Intron 6 variant (In6-IPA). Because In6-IPA is expressed at low levels, we targeted Exon 6 (Ex6) 5'ss with ASO and demonstrated an increase in In6-IPA. However, in addition to IPA, we detected usage of cryptic 5'ss in Ex6 (Ex6_del-19) and Ex6 skipping. Because In6-IPA, Ex6_del-19 and Ex6 skipping isoform are similar with minor difference of the c-terminus, we hypothesized that Ex6_del-19 and Ex6 skipping will have comparable inhibitory activity as In6-IPA. For my summer project, I used a c-terminal flag tag recombinant variant for Ex6_del-19 and Ex6 skipping to compare their activity with In6-IPA. First, I transfected all three constructs into Hek293T cells, collected conditioned medium (CM) and demonstrated that Ex6_del-19 and Ex6 skipping proteins are secreted into extracellular space just as In6-IPA. To compare their inhibitory activity, I collected CM from transfected Hek293T cells, add 10 ng/ml of HGF to CMs and treat HeLa cells with HGF/CM for 5 minutes. After 5 minutes, I collected the cell lysate and performed western blot analysis for MET, AKT, and ERK1/2 phosphorylation status. The results demonstrated that the flag recombinant variants were able to block FL-MET phosphorylation and prevent PI3K-AKT and MAPK pathway activation. In conclusion, Ex6_del-19 and Ex6 skipping have comparable activity as In6 IPA. Supported by NIH R25ES020721.