This abstract was submitted to DTRF for the September 2022 Int'l Research Workshop.

Growth factor signaling and kinase inhibitors regulate oncogenesis in desmoid fibromatosis by modulating activity of the beta-catenin transcription target ABL1

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Introduction: While activation of beta-catenin is associated with desmoid fibromatosis (DF), mechanisms by which this oncogene initiates tumorigenesis are unclear as are factors underlying variable biologic behavior in the disease and vulnerability to targeted therapies. This study sought to define downstream pathways dysregulated by beta-catenin that may be modulated to potentially affect patient outcome.

Methods: Multiple primary DF cell lines were developed from surgical specimens, validated by Sanger sequencing, and immortalized by ectopic expression of *TERT*. Gene expression was assessed in DF tumors (n=45) with U133A arrays and by RNA-seq in cells. Direct targets of beta-catenin were identified by CHIP-seq. Lentiviral systems were used to deliver shRNA (or scramble control) and overexpression constructs. Cell proliferation, protein levels/phosphorylation and gene expression were assessed by CyQuant DNA quantification, immunoblot, and RT-PCR, respectively. Endothelial cell (HUVEC) tube formation was quantitated using light microscopy.

Results: Gene set enrichment analysis performed on RNA-seq data comparing DF cells treated with shRNA directed against *CTNNB1* showed downregulation of hypoxia-regulated genes, and unsupervised analysis clustered 45 DF tumors separately from normal mesenchymal tissue based on the expression levels of these genes. *CTNNB1* knockdown (KD) was associated with reduction in HIF1A and ability of DF cells to induced endothelial tube formation in HUVEC co-cultures (71%, $p<0.001$); ectopic expression of *HIF1A* in *CTNNB1* KDs rescued this effect. *HIF1A* KD itself inhibited DF induction of HUVEC tube formation (49%, $p<0.001$), but did not affect DF cell proliferation. CHIP-seq nominated *ABL1*, a known regulator of HIF1 translation, as a direct target of beta-catenin. *CTNNB1* KD caused 65% ($p=0.01$) decrease in *ABL1* expression, and reduction in levels of c-ABL, its downstream target p-CRKL, and HIF1-alpha. Unlike *HIF1A*, *ABL1* KD also reduced proliferation in multiple DF cell lines (up to 90%) as did direct inhibition of c-ABL with its inhibitor dasatinib (IC_{50} <50nM). Dasatinib and sorafenib, a PDGFR-beta inhibitor of clinical benefit in DF, both reduced cellular levels of p-ABL, p-CRKL and HIF1a expression in DF cells. Sorafenib also inhibited HUVEC tube-formation (59% at 1uM, $p<0.05$) induced by DF. Conversely, exogenous PDGF-BB stimulated DF proliferation (53% increase at 20ng/ml, $p<0.05$), increased p-ABL, p-CRKL and HIF1a in DF and promoted endothelial cell tube formation (2-fold, $p<0.05$) when added to DF and HUVEC co-cultures but not HUVEC cell cultures alone.

Conclusion: *ABL1* is a transcriptional target of beta-catenin in DF cells and is necessary for proliferation and maintenance of HIF1-alpha levels. Regulation of c-ABL activity by PDGFR-beta and targeted therapies modulates DF cell proliferation and paracrine signaling, suggesting a reason for variable biologic behavior between tumors and a mechanism for sorafenib activity in DF. This finding may point to markers predictive of outcome in patients.