Investigating mechanisms for Invasion associated with Upregulated cMET using Tumor Organoids

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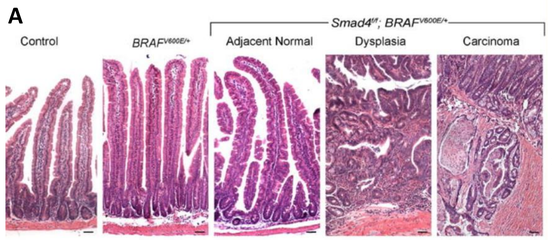
ABSTRACT

Colon cancer is the third most prominent cancer in the US, and the second leading cause of cancer-related deaths. While most colorectal tumors arise from the WNT-driven adenoma pathway, approximately 10% of these CRC tumors are due to mutations in the BRAF oncogene (Powell et al., 1992). When initiated by the BRAF oncogene, colon cancers are classified as serrated tumors, which are associated with an especially poor prognosis (De Sousa et al., 2013). Thus, it is important to study the conditions that contribute to the arise of these tumors. In previous research published through the Verzi Lab, the BRAF oncogene alone was inefficient in producing serrated tumors in the small intestine of mice. However, when paired with the loss of the tumor suppressor SMAD4, serrated tumorigenesis was accelerated by creating a high WNT environment (Tong et al. 2017). Furthermore, the Smad4$^{f/f}$ BRAF$^{V600E/+}$ mouse model also aggressively developed invasive tumors. Tumor organoids derived and cultured from these invasive tumors show a unique capability to form directional protrusions through the Matrigel to form networks with neighboring tumor organoids, which is a behavior characteristic of invasive cancer cells (Sibony-Benyamini, Gil-Henn., 2012). Furthermore, these invasive phenotypes also result in an upregulation of cancer invasion-associated genes such as ivadapodia and angiogenesis. cMET has an important role in cell functions including wound healing, cell proliferation and migration which can lead to metastasis and invasion when overexpressed (Safaie Qamsari, E. et al., 2017). By using lentiviral transfection to develop organoid lines with cMET overexpression, we were able to see an exaggerated invasive phenotype as cMET organoids develop fully formed networks much faster than mock organoids. qPCR data also shows that overexpression of cMET further upregulates invasion-associated genes, providing additional evidence to support the role of cMET in invasion and metastasis. The phenotype displayed in our cMET organoid model emphasizes the impact cMET has in migration and cell proliferation and thus can be associated with invasion.

BACKGROUND

BRAF$^{V600E/+}$ plays a significant role in inducing cell migration and invasion in colon cancer cells (Makrodouli et al., 2011). Upregulation of cMET has been correlated with invasion in colon cancers and has shown to cause resistance in BRAF mutant targeted therapies (Bradley et al., 2016). Smad4$^{f/f}$ BRAF$^{V600E/+}$ tumor organoids were derived from serrated tumors of our mouse model (Tong et al 2017). When cultured in Matrigel, these tumor organoids developed 3-dimensional, flat projections that protrude off one organoid and extend in the direction of neighboring organoids. This behavior is characteristic of invasive cancer cells (Sibony-Benyamini, Gil-Henn., 2012). Thus, the tumor organoid system can be an effective tool in understanding the mechanisms in which tumors become invasive. Our studies will address whether cMET expression in tumor organoids will contribute to an accelerated invasive phenotype. These findings are important because the MET signaling pathway can be targeted in therapies to prevent metastasis.



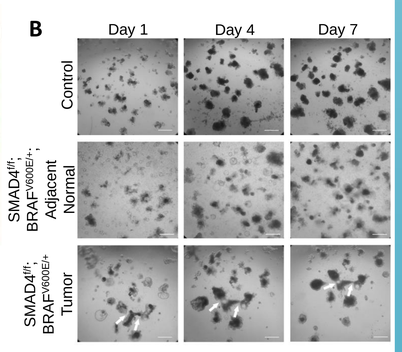


Figure 1 (A) H&E stain shows morphology of adjacent normal and tumor tissue of control, BRAF$^{V600E/+}$, and SMAD4$^{f/f}$; BRAF$^{V600E/+}$ tissue of mice. Dysplasia are characteristic of serrated morphology. Carcinomas are shown to be invasive, penetrating the muscle layer.

(B) Organoid line WT control and SMAD4$^{f/f}$; BRAF$^{V600E/+}$ adjacent normal organoids form no connections. SMAD4$^{f/f}$; BRAF$^{V600E/+}$ organoids derived from tumors do exhibit this invasive phenotype. Connections made between organoids are emphasized by arrows. 4x images on Day 1, 4, and 7 are shown. The scale bar is equivalent to 0.5 mm. Images are representative of four technical replicates. Images are representative of three biological replicates for SMAD4$^{f/f}$; BRAF$^{V600E/+}$ tumor and adjacent normal organoids and one biological replicate for WT organoids.

RESULTS

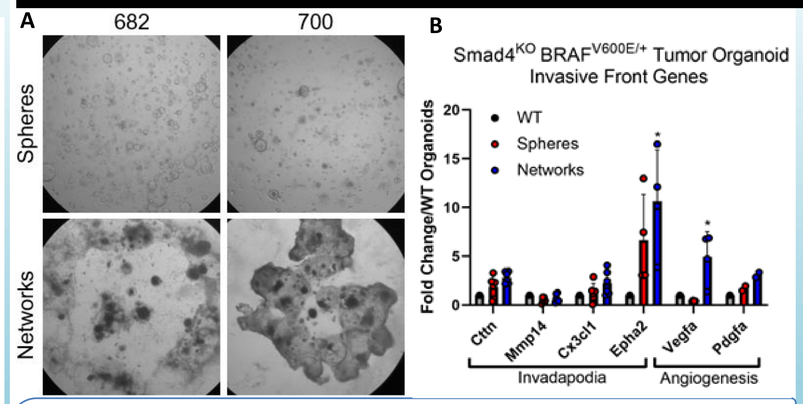


Figure 2 (A) Biological replicates of Smad4$^{f/f}$ BRAF$^{V600E/+}$ tumor organoids. Sphere images of tumor organoids at Day 3 compared to tumor organoids after developing networks at Day 29. (B) qPCR analysis of invasive front genes in Smad$^{f/f}$ BRAF$^{V600E/+}$ tumor organoid spheres and networks. Results normalized to gene expression levels of Day 3 WT organoids. * = $p < 0.05$, Two-Way ANOVA. Invadapodia and angiogenesis genes are upregulated in networked organoids compared to spheres and control which supports our hypothesis that networking is an invasive behavior.

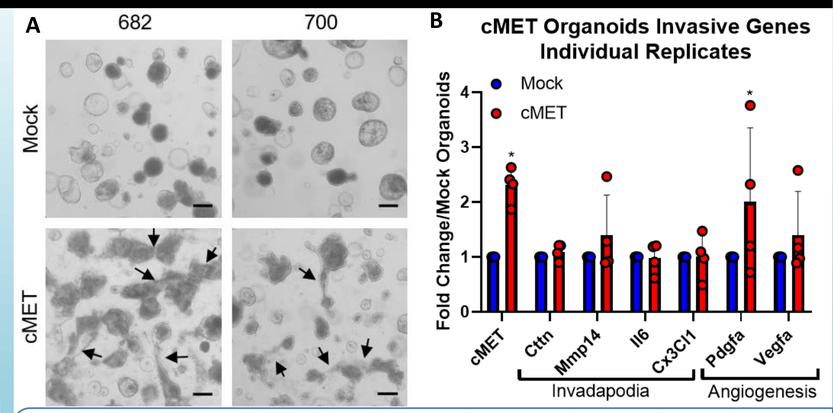


Figure 3 Representative images of Mock organoids compared to cMET lentiviral transfected organoids at Day 8. Networks are present in cMET organoids while Mock organoids remain individual spheres. (B) Graph shows qPCR analysis of Mock and cMET organoids. Results were normalized to genes expression levels of Mock organoids. * = $p < 0.05$, Two-Way ANOVA. . Invadapodia and angiogenesis genes are upregulated in cMET organoids compared to Mock organoids which supports our hypothesis that cMET contributes to invasion.

CONCLUSION and FUTURE DIRECTIONS

The progression of invasive tumors seen in our Smad4$^{f/f}$BRAF$^{V600E/+}$ mouse model translated into an invasive phenotype in our tumor organoids, which has led us to investigate the mechanisms behind invasion which allow for tumors become metastatic. One pathway that has shown to be a key player in invasion is the cMET signaling pathway which is involved in migration and cell proliferation but can lead to metastasis (Moser et al., 2007). Expression of cMET in our Smad$^{f/f}$BRAF$^{V600E/+}$tumor organoids revealed that this pathway contributes to an accelerated development of the invasive networking phenotype. Furthermore, we also found that expression of cMET upregulates pathways associated with cancer invasiveness such as the PDGF signaling pathway, invadaopdia pathway, and wound healing. These findings potentially identify specific targets that contribute to invasion, which can be targets for potential therapeutics.

Using our cMET organoids, one possible next step is to organize a drug assay using the PDGFa inhibitor Crenolanib. Crenolanib is a type I inhibitor which works by binding to and inhibiting PDGFR isoforms. This inhibits the PDGFR-related signal transduction pathways that contribute to angiogenesis and tumor cell proliferation (Heinrich et al., 2012). Treating our cMET organoid model with working concentrations of Crenolanib (Heinrich et al., 2012), we would then assess the efficacy of Crenolanib based on organoid viability and networking. Viability and networking can be assessed by observing qualitative characteristics that indicate organoid health and networking capability which includes the presence of a distinct structural border and the formation of directional protrusions. If we see a change in the networking phenotype displayed by these organoids, this will provide evidence that the PDGF signaling pathway is a key component of invasion which would reinforce our existing qPCR data which suggests that Pdgfa is an important gene in invasion.

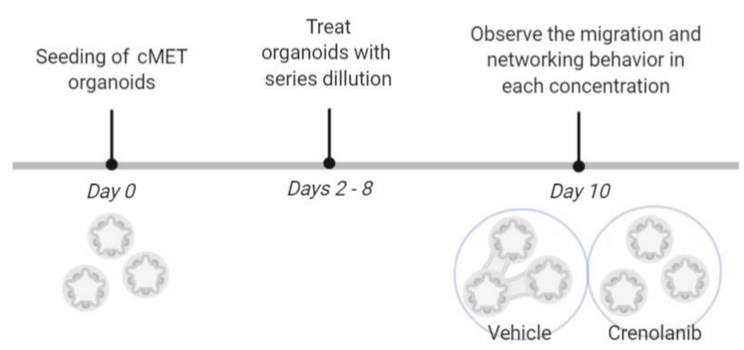


Figure 4 cMET organoids are seeded at Day 0. Treatment with Crenolanib dilution series starting from day 2 and continuing until day 8-10. On day 10 the phenotype would be assessed. According to our hypothesis which predicts that Crenolanib could prevent an invasive networking phenotype from forming, we expect to see cMET organoids treated with Vehicle to fully network and cMET organoids treated with a working concentration of Crenolanib to maintain a spherical phenotype.

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