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Topic: Investigation of the role of dysregulated mitochondrial dynamics in the progression and/or metastasis (EMT) of colorectal cancer

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Findings:

Mitochondria are highly dynamic organelles which remain in a continuous state of fission/ fusion dynamics to meet the metabolic needs of a cell. However, this fission/fusion dynamism has been reported to be dysregulated in most cancers. Such enhanced mitochondrial fission is demonstrated to be positively regulated by some activating oncogenic mutations; such as those of KRAS (Kristen rat sarcoma viral oncogene homologue) or BRAF (B-rapidly accelerated fibrosarcoma), thereby increasing tumor progression/ chemotherapeutic resistance and metabolic deregulation. However, the underlying mechanism(s) are still not clear, thus highlighting the need to further explore possible mechanism(s) of intervention. We sought to investigate how is $BRAF^{V600E}$ driven CRC (colorectal cancer) progression linked to mitochondrial fission/fusion dynamics and whether this window could be exploited to target CRC progression? Western blotting was employed to study the differences in expression levels of key proteins regulating mitochondrial dynamics, which was further confirmed by confocal microscopy imaging. Proliferation assays, soft agar clonogenic assays, glucose fermentation, ATP/ NADPH measurements were employed to study the extent of carcinogenesis. Genetic knockdown and/or pharmacologic inhibition of Dynamin related protein1/Pyruvate dehydrogenase kinase1 (DRP1/PDK1) and/or $BRAF^{V600E}$ were employed to study the involvement and possible mechanism of

these proteins in $BRAF^{V600E}$ driven CRC. Statistical analyses were carried out using Graph Pad Prism v 5. 0, data was analyzed by unpaired t-test and two-way ANOVA with appropriate post hoc tests.

Our results demonstrate that $BRAF^{V600E}$ CRC cells have higher protein levels of mitochondrial fission factor- DRP1/$pDRP1^{S616}$ leading to a more fragmented mitochondrial state compared to those harboring $BRAF^{WT}$. This fragmented mitochondrial state was found to confer glycolytic phenotype, clonogenic potential and metastatic advantage to cells harboring $BRAF^{V600E}$. Interestingly, such fragmented mitochondrial state seemed positively regulated by mitochondrial PDK1 as observed through pharmacologic as well as genetic inhibition of PDK1.

In conclusion, our data suggest that $BRAF^{V600E}$ driven colorectal cancers have fragmented mitochondria which confers glycolytic phenotype and growth advantage to these tumors, and such phenotype is dependent at least in part on PDK1- thus highlighting a potential therapeutic target.