TUMOR-STROMA INTERACTIONS INFLUENCE THE RESPONSE TO PI3K-TARGETED AGENTS IN PRECLINICAL MODELS OF COLORECTAL CANCER (CRC)

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Abstract:
Background: Identification of the precise mechanisms of drug action in relationship to molecular disease drivers is crucial to the successful development of new therapeutic strategies. Recent high-throughput molecular analysis is being used to identify putative biomarkers to provide personalized cancer therapy. However, such analysis is usually focused on tumor cellular autonomous determinants of sensitivity to drug treatment and overlooks microenvironmental interactions. The aim of the study is to investigate how PTEN expression/function in CRC cells modulates the response to signaling inhibitors in the context of complex microenvironmental interactions.

Methods: Genetically engineered CRC cell lines (x-MAN™ HCT116 and HCT116 Pten⁻) were treated with MAPK1 and PI3K/mTORC inhibitors alone or in combination, in the presence or absence of stromal fibroblasts or fibroblast/endothelial cell conditioned medium (CM). Cytofluorometric analysis and Crystal Violet assay were used to analyze functional response to targeted agents, pathway activation and cytokine/chemokine profile were analyzed using Western blot (WB) and ELISA assay respectively.

Results: While response of CRC cells to MEK1 is dictated mainly by the tumor genetic background, response to PI3K/mTORC is strongly influenced by TME/CM interactions. Fibroblast CM selectively increases sensitivity of PTEN-dependent HCT116 cells to PI3K/mTORC double inhibitor by modulating PTEN function. Indeed, despite increased levels of total PTEN protein, phosphorylation of its C-terminal tail disables PTEN from plasma membrane and makes it inactive, resulting in increased P38, levels. PTEN inactivation, in turn, shifts the balance towards the formation of Raptor-containing mTORC2 complexes, resulting in downstream activation of mTORC2, but not mTORC1. The same results were obtained with different fibroblast and endothelial CM; stromal CM, indeed, upregulates pathway leading to greater response to PI3K/mTORC double inhibitor. The analysis of cytokine/chemokine production revealed a similar pattern in CM from different stromal cells, however this could not be sufficient to explain the observed response.

Conclusions: Understanding the mechanisms underlying microenvironmental interactions (tumor, stroma, soluble factors) may be of fundamental importance to overcome therapeutic resistance and develop more effective therapies for patients affected by CRC.

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