AXL TYROSINE KINASE RECEPTOR AS A KEY REGULATOR OF PROLIFERATION AND SURVIVAL IN COLORECTAL CANCER (CRC)

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Background: Novel therapeutic strategies are evolving in CRC to selectively inhibit oncogenic pathways. In this scenario we identified the tyrosine kinase receptor AXL as a potential target.

Methods: We evaluated the expression and activation of AXL kinase and its ligand GAS6 in a panel of CRC cell lines by receptor tyrosine kinase array (RTK), western blot and Real Time PCR. In the AXL expressing cell lines we analyzed cell survival and drug sensitivity (using the AXL inhibitor foretinib) by MTT assay. Finally we generated stable short hairpin RNA (shRNA)-sh-AXL cell line in order to evaluate the effect of AXL specific knockdown in a model of CRC in vitro and to analyze the mechanism underlying AXL activation. Moreover, TGFβ-expression was measured by Luminox assay.

Results: We found AXL expression in LOVO, HCT116, SW480 and SW620 CRC cells whereas the HT29, SW48, SW48-CR (Cetuximab Resistant), Colo205, GEO, GEO-CR (Cetuximab Resistant) and HCT15 CRC cells were AXL-negative. AXL-expressing cell lines were sensitive to foretinib with an IC₅₀ range from 0.5 to 2 mM. AXL inhibition caused a significant phosphorylation decrease of MAPK, AKT and S6 ribosomal protein. This data were further confirmed by stable silencing of AXL with sh-RNA in LOVO cells that resulted in a dramatic reduction of cell proliferation and activation of downstream pathway. Since no GAS6 levels were found by ELISA in the supernatant of cells tested, we tried to investigate the possible mechanism of AXL activation in our model. We found highest levels of TGFβ in HCT116 and SW620 cells in medium. The stimulation of AXL expressing cells with a recombinant TGFβ, for 20 and 120 minutes, resulted in increase of downstream pathway.

Conclusions: This data highlighted AXL as a potential target in CRC. AXL-expressing cells are resistant to anti-EGFR therapies, thus it could be a relevant target for cancer treatment, in fact AXL inhibition by foretinib or sh-RNA resulted in a significant cell proliferation and survival reduction. Finally, we identified the TGFβ as a potential activator of AXL-mediated signaling pathway. The in vivo study is currently ongoing on HCT116 ortotopic xenografts in nude mice treated with foretinib and final results will be presented.

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