TUMOR-STROMA INTERACTIONS AS A DETERMINANT OF DRUG RESISTANCE IN BRAF-MUT MELANOMA.

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Abstract

Background: In BRAF-mut melanoma combined BRAF/MEK inhibition increases survival; however, pharmacological effects on the genetically “normal” tumor microenvironment (i.e. paracrine MAPK activation) may set the stage for the development of drug resistance.

Methods: To assess the functional relevance of AXL and SEMA6A expression, in the regulation of melanoma/stroma interactions and sensitivity/resistance to pathway inhibitors, we silenced or overexpressed the two proteins by genetic manipulation, and the response of melanoma models to BRAF/MEK inhibition (alone and combined) has been investigated in 2D co-culture systems.

Results: SEMA6A and AXL expression in a panel of genetically characterized melanoma cell lines, short-term primary melanoma cultures and patient-derived melanoma initiating cells, were inversely correlated. Furthermore, we observed the same inverse correlation when we overexpressed or knocked-down SEMA6A and AXL expression in different BRAF-mut melanoma cell lines, by transfections/transfections of constitutively active gene constructs or RNA interference, as appropriate. Preliminarily, we discovered that AXL significantly protects BRAF-mut M14 melanoma cells from the growth inhibitory activity of BRAF/MEK inhibitors (idarubicin and trametinib), alone or combined, by “direct cell-cell contact”. To ascribe a functional role to SEMA6A and AXL in tumor-promotive melanoma/stroma interactions, correlative co-culture experiments have been conducted using melanoma cells characterized for high or low/undetectable expression of the protein of interest. In this context, SEMA6A overexpression, performed in BRAF-mut M14 melanoma cells, abrogated the protective effect derived from melanoma/stroma interactions; conversely after AXL silencing BRAF-mut SAENL24 melanoma cells became more resistant to BRAF/MEK inhibition than control cells.

Conclusions: Our data suggest that tumor-stroma interactions protect BRAF-mut melanoma from MAPK inhibition; such functional protection is mediated by cell-cell contact. SEMA6A and AXL are mediators of this interaction and their reciprocal relationships are being further studied in melanoma cell line models and clinical series.

Co-culture system and cytometry analysis

Flow cytometry analysis is used to monitor viability and drug responsive melanoma (M14) and immortalized skin fibroblast (HFF-HFP) cell lines in the context of 2D culture models.

AXL and SEMA6A basal expression are inversely correlated in a large panel of melanoma cell lines...

In Figure 4A, we analyzed the basal expression of AXL and SEMA6A in a panel of melanoma cell lines. The cells were lysed and analyzed by Western blotting analysis using specific antibodies. M14 and MEL144 cells, which were tested as positive control for AXL and SEMA6A protein expression, respectively. In Figure 4C, detection analysis of AXL and SEMA6A basal expression demonstrated an inverse correlation between the two proteins.

2D co-culture system with melanoma and stroma cells in which AXL and SEMA6A are overexpressed and/or silenced

AXL and SEMA6A were overexpressed (e/o silenced) in M14 and MEL144 cell lines. The cells were lysed and analyzed by Western blotting analysis using specific antibodies for AXL and SEMA6A. Western blot with antibodies specific for β-actin and GAPDH are shown as protein loading and blotting control.

AXL was overexpressed (e/o silenced) in M14 and MEL144 cells. The cells were cultured alone or in combination and were treated with Transwell substrate (1.5 μL of 0.5% DAB and 0.5% lipid) alone or in combination at a fixed 1:5 ratio. After 72 h, cells were counted and the number of DAPI positive and negative cells were calculated using cytometry analysis.

SEMSE was silenced in M14 cells. The cells were cultured alone or in combination with 36 cells and were treated with Transwell substrate (1.5 μL of 0.5% DAB) alone or in combination at 1:5 ratio. After 72 h, cells were counted and the number of DAPI positive and negative cells were calculated using cytometry analysis.

Conclusions

In BRAF-mut melanoma, the interaction between stromal and tumor cells protects melanoma cells from MAPK inhibition through reciprocal intercellular signaling, as we demonstrated in BRAF-mut melanoma cell lines at all doses of each inhibition.

• This protection against MAPK-kinases is associated with cell-to-cell contact.
• AXL and SEMA6A proteins, two possible mediators of tumor/stroma interactions in melanoma, are differentially expressed and inversely correlated in a large panel of melanoma cell lines.

Future Perspectives

• To evaluate the AXL and SEMA6A and their reciprocal relationships in a cohort of melanoma patients.
• To investigate the effect on cell co-culture system with melanoma and stroma cells in which AXL and SEMA6A are overexpressed and/or silenced.