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

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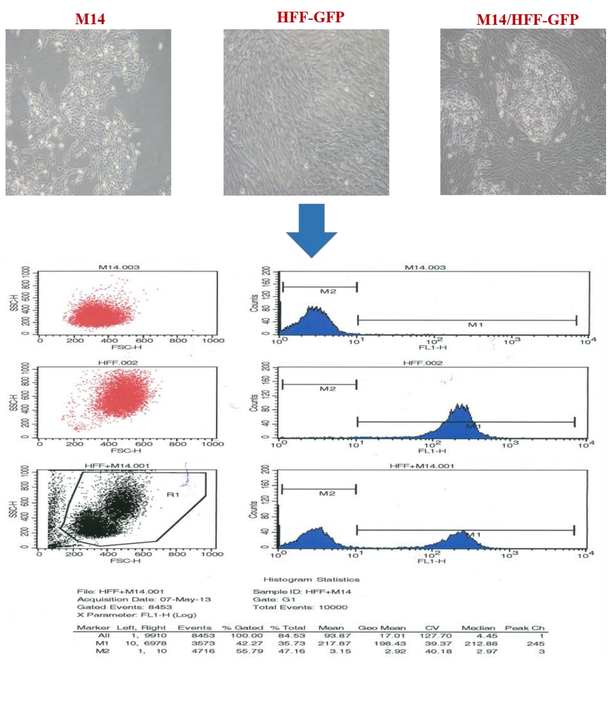
Abstract

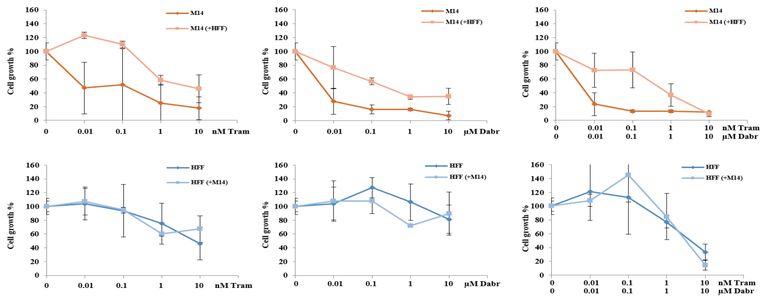
Background: In BRAF-mut melanoma combined BRAF/MEK inhibition increases survival; however, pharmacological effects on the genetically "normal" tumor microenvironment (i.e. paradox MAPK activation) may set the stage for the development of drug resistance.
Methods: To assess the functional relevance of 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 inhibitors (alone and combined) has been evaluated 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 transient/stable transfection of constitutively active gene constructs or RNA interference, as appropriate. Previously, we discovered that HFF significantly protected BRAF-mut M14 melanoma cells from the growth inhibitory activity of BRAF/MEK inhibitors (dabrafenib and trametinib), alone or combined, by "direct cell-cell contact". To ascribe a functional role to SEMA6A and AXL in tumorprotective 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 silencing, performed in BRAF-mut 2/59 melanoma cells, and/or AXL overexpression, performed in BRAF-mut M14 melanoma cells, abrogated the protective effect derived from melanoma/stroma interactions; viceversa after AXL silencing BRAF-mut SKMEL24 melanoma cells became more resistant to BRAF/MEK inhibitors 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 cytofluorimetric analysis



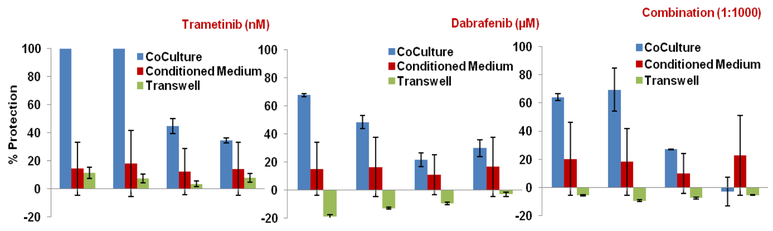
Flow cytometric analysis is used to monitor viability and drug responseof melanoma (M14) and immortalized skin fibroblast (HFF+GFP) cell lines in the context of 2D co-culture model.

Stroma protects M14 melanoma cells from the growth inhibitory activity of BRAF and MEKi .....



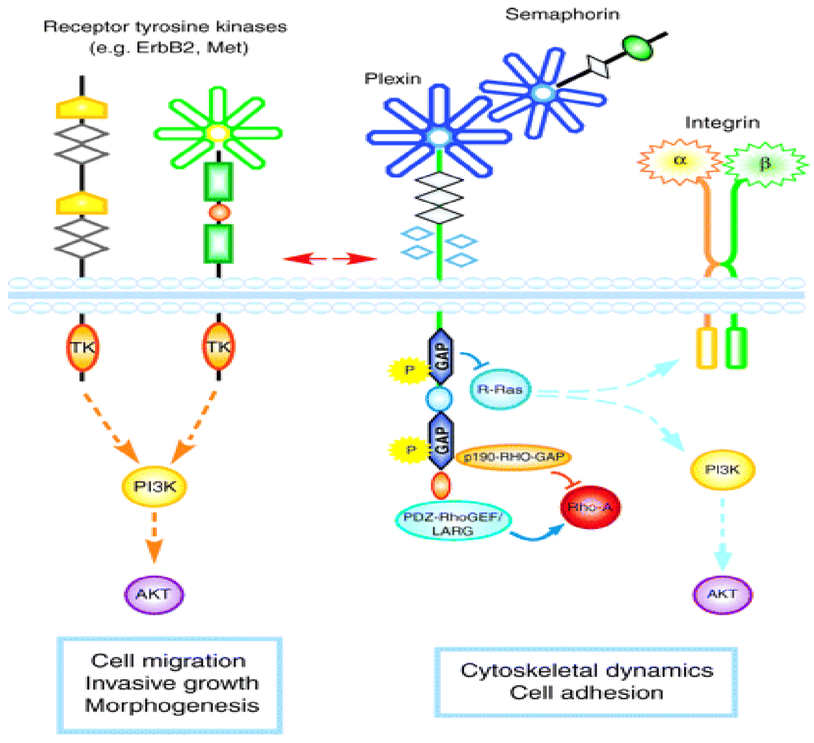
M14 alone and in co-culture with HFF cells, were treated with Trametinib (Tram) and Dabrafenib (Dabr) alone and in combination at a fixed 1:1000 ratio. After 72h cells were counted and the number of GFP positive and negative cells were calculated using cytofluorimetric analysis.

....and drug sensitivity is modulated by cell to cell contact



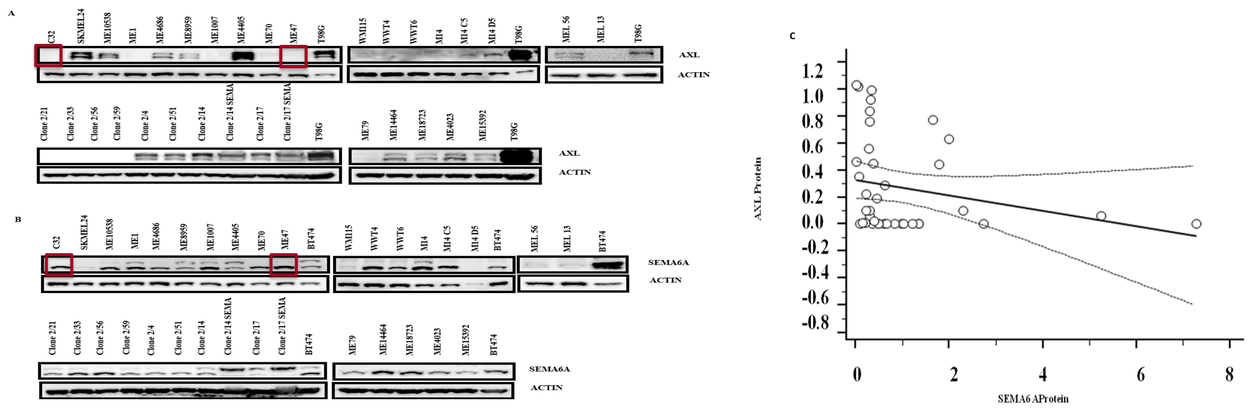
M14 melanoma and HFF+GFP cells were cultured alone or in combination. In a context of 2D co-culture, growth inhibitory activity of both BRAFi and MEKi is significantly decreased (i.e. melanoma cells are "protected" by stroma). This effect is mediated by cell to cell contact: in fact, co-culture experiments performed in trans-well Boyden chambers or on isolated melanoma cells cultured in HFF-CM (Conditioned medium) showed a much lower, if any, degree of protection, as compared with direct-contact 2D co-culture.

AXL and SEMA6A: two possible mediators of cell to cell contact



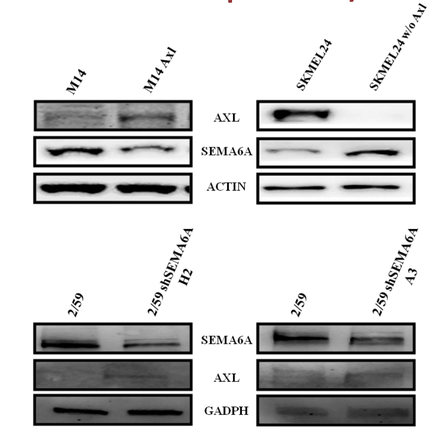
Capparuccia L and TamagnoneL . Journal of Cell Science 2009

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



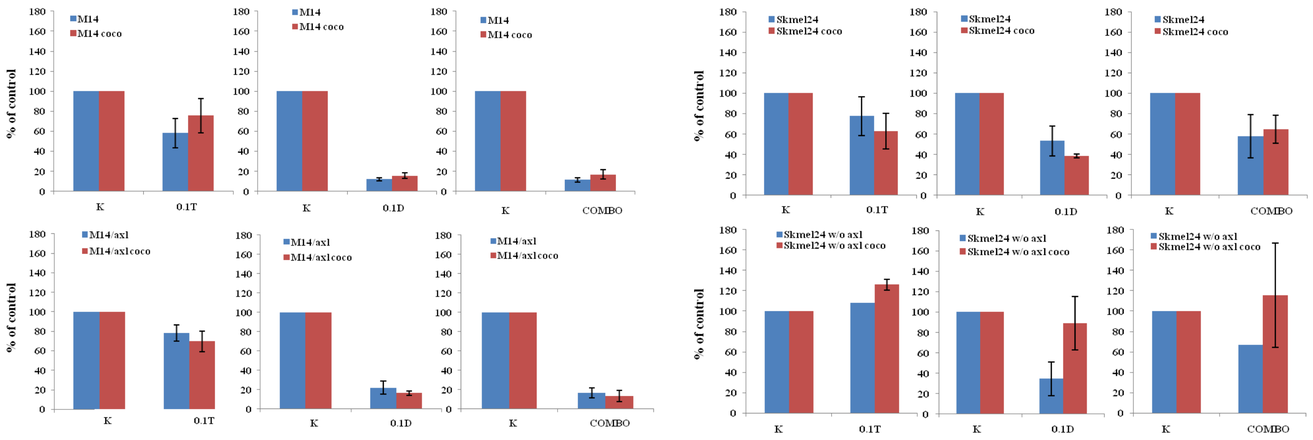
In Figure A-B, we analyzed the basal expression of AXL and SEMA6A in a panel of melanoma cell lines. The cells were lysed and analyzed by Western Blotting analisys using specific antibodies. T98G and BT474 cells were used as positive control for AXL and SEMA6A protein expression, respectively. In Figure C , statistical analysis of AXL and SEMA6A basal expression demostrated an inverse correlation between the two proteins.

… and the same inverse correlation is observed when AXL and SEMA6A are overexpressed e/o silenced.

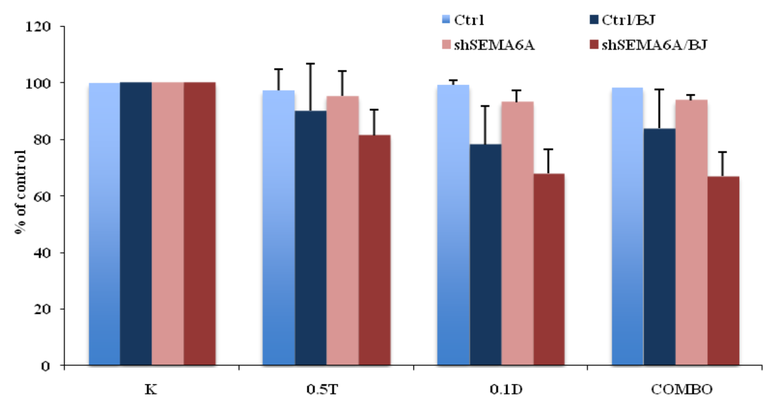


AXL and SEMA6A were overexpressed e/o silenced in M14 and SKMEL24 and 2/59 cells. The cells were lysed and analyzed by Western Blotting analisys using specific antibodies for AXL and SEMA6A. Western blot with antibodies specific for β-actin and GAPDH are shown as protein loading and blotting control.

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



AXL was overexpressed e/o silenced in M14 and SKMEL24 cells. The cells were cultured alone or in combination and were treated with Trametinib 0.1nM (0.1T) and Dabrafenib 0.1µM (0.1D) alone and in combination at a fixed 1:1000 ratio. After 72h cells were counted and the number of GFP positive and negative cells were calculated using cytofluorimetric analysis.



SEMA6A was silenced in 2/59 cells. The cells were cultured alone or in combination with BJ cells and were treated with Trametinib 0.5nM (0.5T) and Dabrafenib 0.1 µM (0.1D) alone and in combination. After 72h the cells were counted and the number of GFP positive and negative cells were calculated using cytofluorimetric analysis.

Conclusions

- In Braf-mut melanoma, the interaction between stromal and tumor cells protects melanoma cells from MAPK inhibitors; such protection may be overcome by combined BRAF/MEK inhibition at full doses of each inhibitor.
- This protection against MAPK inibitors 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 reciprocal relationships in a cohort of melanoma patients.
- To create new models of 3D co-culture system with melanoma and stroma cells in which AXL and SEMA6A are overexpressed and/or silenced .

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