ImmunoTools *special* Award 2014

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Functional features, stemness and aggressiveness of Vemurafenib-resistant melanoma cells

Melanoma is rapidly increasing in incidence throughout the world. Patients with early-stage melanoma can be treated successfully with surgical resection; however, once cutaneous melanoma has metastasized, it is extremely refractory to conventional antineoplastic treatments. Despite progresses in drug development programs and molecular approaches to identify the drug targets, incidence and mortality rates due to melanoma continues to rise at an alarming rate. The worldwide incidence of cutaneous melanoma has steadily increased over last decades. Recently, however, the treatment of melanoma has been revolutionized by therapies targeting the RAF-MEK-ERK MAPK pathway. This pathway is constitutively activated in the majority of cutaneous melanomas via oncogenic mutations in the BRAF kinase (1). The most common mutation in BRAF is a substitution at amino-acid position 600 (V600E) in exon 15. Potent and selective BRAF-inhibitor, vemurafenib (PLX-4032), has produced response rates above 50% and improved progression-free survival in BRAF-mutant melanoma patients. Unfortunately most patients show disease progression within 6-8 months (2).

Many studies support the presence and involvement of cancer stem-like cells in tumor initiation and progression, as well as chemo-resistance and therapeutic failure of anti-tumor strategies. In fact, it has been suggested that within tumour, multiple subpopulations of cancer cells may coexist: some of these cells may exhibit differentiative features, others grow, whereas some may have stem cell-like properties (3). Therefore, novel therapies directed toward cancer stem-like cells represent a very promising anticancer strategy.

Therefore, the aim of this project is to identify the mechanisms of acquired resistance to BRAF inhibition, with particular attention to the differentiative stages, secretory pathways and aggressiveness of melanoma cells. As tumor model, we will use human melanoma cell lines, including a primary human stem-like cell line, with acquired *in vitro* resistance to vemurafenib, previously generated in our laboratory.

Melanoma cells (control and with acquired resistance to vemurafenib) will be characterized by the expression of antigens that have been associated with melanoma differentiation or stemness: CD20, CD24, CD29, CD38 and CD44, in agreement with previous reports (4). The expression of the selected markers will be monitored by flow cytometry analysis. We will also investigate the induction of apoptosis (using Annexin-V).

It is well known that BRAF mutation is an abnormal change in a gene that causes some melanoma to grow and spread more aggressively. Therefore, the analysis of aggressiveness of vemurafenib-resistant melanoma cells appears of some interest. ImmunoTools human SDF-1α/CXCL12a, MCP2/CCL8, RANTES/CCL5 and PDGF-BB will be used as chemotactic factors to test migration ability of melanoma cells using a transwell assay (Boyden chambers technique) (5). Melanoma cells will be also characterized by the expression of selected markers related to melanoma growth and aggressiveness (CD36, CD63 and CD71).

Finally, to investigate the possible secretory pathways involved in vemurafenib-resistance, we will analyse cytokine concentration in supernatants and cell lysates by using ImmunoTools ELISA-set (IFN-gamma, human IL-8, TNF-a).

ImmunoTools reagents will be essential for the analyses presented in this project.

References

1) Davies et al., Mutations of the BRAF gene in human cancer. Nature 417(6892):949-54, 2002.
2) Sullivan and Flaherty, Resistance to BRAF-targeted therapy in melanoma. Eur J Cancer 49(6):1297-304, 2013.
3) Maccalli and De Maria, Cancer stem cells: perspectives for therapeutic targeting. Cancer Immunol Immunother (in press), 2014.
4) Medema, Cancer stem cells: the challenges ahead. Nat Cell Biol 15(4):338-44, 2013.
5) Roussos et al., Chemotaxis in cancer. Nat Rev Cancer 11(8):573-87, 2011.

ImmunoTools *special* AWARD for **Claudio Tabolacci** includes 25 reagents

FITC - conjugated anti-human CD29, CD38, CD63, CD71, Control-IgG1, Annexin V,

PE - conjugated anti-human CD20, CD24, CD44, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V,

human ELISA-set (for one 96 plate), human IFN-gamma, human IL-8, human TNF-a, human TSLP (each ELISA set contain 3 reagents),

recombinant human cytokines: rh MCP2 / CCL8, rh PDGF-BB, RANTES / CCL5, rh SDF-1α / CXCL12a

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