ImmunoTools *special* Award 2015

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Anti-inflammatory and differentiative approach applied to cutaneous melanoma

Cutaneous melanoma is one of the most aggressive tumors characterized by high malignancy. Many risk factors are involved in melanoma and very often genomic variations are involved in cancer development, progression and drugs resistance. Current therapies for human cutaneous melanoma include surgery, chemotherapy, radiotherapy, immune-based therapy, target therapy and the combination of these approaches. However, their effectiveness is limited in time due to the development of resistance mechanisms. A promising therapeutical approach to melanoma is differentiation therapy. Its application halts the progression of cancer, allowing the transformed cells to regain the morphology and functions of a mature cell. Recently, a crucial role of the cytosolic enzyme transglutaminase, whose expression and activity is known to represent a differentiation marker in many cancer types including melanoma, was reported (*Facchiano et al., 2013*). Further, the important role of pro-differentiative drugs as new therapeutic approach against melanoma has been reported (*Beninati et al., 2014*) opening new interesting perspectives.

The aim of my project is to apply anti-inflammatory and differentiative agents to counteract the growth and metastatic potential of melanoma.

Theophylline, a methylxanthine drug used in therapy for its potent anti-inflammatory and differentiation activities, will be used on human melanoma models (melanoma cell lines and melanospheres) by monitoring their differentiative stages and metastatic potential as previously described (*Sette et al., 2013; Beninati et al., 2014*).

The close correlation between chronic inflammation and cancer development and progression is already well known. Cytotoxic $CD8^+$ T lymphocytes (CTLs) can infiltrate the tumor microenvironment and destroy tumor cells but often the acquisition by the tumor of an immunological tolerance, linked to the production and

accumulation of inflammatory factors, results in a lack of response of the immune system. The molecular mechanism of theophylline's anti-inflammatory effect is not completely understood, although adenosine receptor antagonism, phosphodiesterase, NF-Kb and PI3K inhibition were suggested. Furthermore, the anti-inflammatory action can be an effect of interaction between theophylline and HDAC (Nuclear Histone Deacetylase), involved in inhibition of transcription of many cytokines genes in proinflammatory cells. Further, the theophylline ability to reduce cancer cell growth, invasiveness and differentiation were demonstrated (*Lentini et al., 2010*).

In this context, different melanoma experimental models, treated with theophylline, will be characterized through the study of differentiation and stemness markers expression by flow cytometry analysis (**CD20, CD24, CD38 and CD44**).

The reduction of the engraftment capacity of melanoma models after theophylline treatment will be studied using melanoma-endothelial cells co-cultured and monitoring the expression of **CD18, CD61, CD47, CD54, CD62p** by flow cytometry; the expression of markers related to melanoma growth and aggressiveness (**CD36, CD63 and CD71**) and apoptosis (**Annexin-V; Control-IgG1**) will be also assessed.

Preliminary data of our laboratory indicate that melanoma cells express several cytokine receptors. This strongly suggests to test theophylline in combination with cytokines with anticancer activity as **TNF-α, IP-10, IL-2, IL-12, IFN-γ, IL-7 and GM-CSF** to evaluate tumor cells survival. ImmunoTools conjugated antibody against **IFN-gamma, IL-6, IL-8, TNF-α** will be used to study the differential expression of related receptors after theophylline treatment compared to the control.

I will be very pleased to use ImmunoTools reagents for the analyses presented in this project.

References:

Beninati *et al*., Inhibition of cell proliferation, migration and invasion of B16-F10 melanoma cells by α-mangostin. Biochem Biophys Res Commun. 2014 Aug 8;450(4):1512-7.

Facchiano *et al*., Tissue transglutaminase activity protects from cutaneous melanoma metastatic dissemination: an in vivo study. Amino Acids. 2013 Jan;44(1):53-61.

Lentini *et al*., Antitumor activity of theophylline in combination with Paclitaxel: a preclinical study on melanoma experimental lung metastasis. Cancer Biother Radiopharm. 2010 Aug;25(4):497-503.

Sette *et al*., Mek inhibition results in marked antitumor activity against metastatic melanoma patient-derived melanospheres and in melanosphere-generated xenografts. J Exp Clin Cancer Res. 2013 Nov 16;32:91.

ImmunoTools *special* AWARD for **Martina Cordella** includes 25 reagents

FITC - conjugated anti-human CD36, CD47, CD54, CD62P, CD63, CD71,

PE - conjugated anti-human CD18, CD20, CD24, CD38, CD44, CD61, IFN-gamma, IL-6, IL-8, TNFa, Control-IgG1, Annexin V,

recombinant human cytokines: rh GM-CSF, rh IFNgamma, rh IL-2, rh IL-7, rh IL-12, rh IP-10 /CXCL10, rh TNFα

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