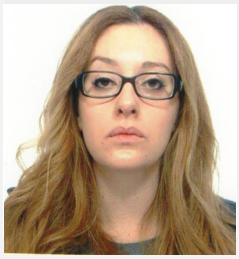
ImmunoTools *special* Award 2015



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Deciphering microenvironmental factors leading to enrichment of aggressive phenotypic subpopulations and angiogenesis formation during Non Small-Cell Lung Cancer Progression

Lung cancer is the leading cause of cancer related death worldwide with poor survival rates of patients and with unsatisfactory available treatments. Based on the size of the transformed cells, lung cancer can be classified as small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC spreads more slowly than SCLC but it is the most aggressive and most common types of lung cancer which accounts 85 to 90% of the diagnosed cases.

Although patient management and treatment of NSCLC has been improved in the past years, still patient prognosis remains poor even when the disease is detected at the early stage. The mechanisms leading to tumour development are not yet clearly elucidated; however, several studies demonstrated tumour microenvironment as an active player of cancer cells plasticity and angiogenesis formation.

Indeed, the tumour microenvironment, which is composed of various growth factors, cytokines and chemokines and stromal cells, enhances the spread of cancer cells. The cancer stem cell (CSC) theory is specifically related to tumour cell subpopulations with unique properties of tumour initiation, propagation and metastasis; nevertheless, which factors within the tumour microenvironment contribute to the enrichment of specific subpopulations of cancer cells is still under investigation.

Furthermore, the relationship of CSC subpopulations with tumour angiogenesis formation and metastasis is not yet well understood. Hence, by means of various in vitro assays, the aim of the present project is to identify key growth factors, cytokines and chemokines that specifically are involved in the promotion of distinct phenotypic subpopulations of NSCLC and angiogenesis.

To understand the mechanism by which these specifics subpopulations of NSCLC cells are linked to directing metastatic cancer cells and vascular formation in the tumour tissue will be very important to find novel effective therapeutic targets and improve patient prognosis.

To achieve the objective of this study different lung cancer cell lines (e.g. A549, NCI-H23) will be used under a specific microenvironment conditioned media with selected ImmunoTools growth factors (EGF, FGF-b, BMP-7, HGF, PDGF-AA, PDGF-BB, TGF-beta3, VEGF-121, VEGF-A), cytokines (Gal-1, Gal-3, IL-1a, Il-6, IL-8, VEGFR2) and chemokines (CCL2, CCL5, CCL19, CXCL4).

Phenotypic characterization of specific subpopulations of tumour cells will be performed by analysis of markers such as CD34, CD24-/CD44+, CD105, CD133+, CD147, $ALDH^{high}$, known to have a role in aggressiveness, metastasis and therapeutic resistance of NSCLC using Flow Cytometry.

The supernatant from cultured cells in different conditioned media will be used to perform angiogenesis assay using HUVEC cell lines, endothelial cells will be evaluated for CD31 and CD62P expression variation; migration /invasion assays will be performed to analyze the metastatic potential of different NSCLC cell lines in the presence and absence of chemokines using transwell chamber. Analysis by qRT-PCR will be performed to profile specific gene expressions (SOX9, VEGF and Gal-1) that are associated with NSCLC cells plasticity and tumour vascularization.

Therefore, the chance to use these multiple type of ImmunoTools antibodies, growth factors and cytokines will help me to achieve the objectives of this research project; moreover, the outputs of this study will have very important implications in understanding the cross-talk among distinct subpopulation of NSCLC cells and metastatic and angiogenesis driving factors to improve or discover novel therapeutic strategies for patients suffering with NSCLC tumour.

Essential bibliography:

Akunuru et al. (2012) Non-small cell lung cancer stem/progenitor cells are enriched in multiple distinct phenotypic subpopulations and exhibit plasticity. *Cell death & disease, 3(7), e352*; Rivas-Fuentes et al. (2015) Role of Chemokines in Non-Small Cell Lung Cancer: Angiogenesis and Inflammation. *Journal of Cancer, 6(10), 938*; Lamalice et al. (2007) Endothelial cell migration during angiogenesis. *Circulation research, 100(6), 782-794*; Chen et al. (2015) Non-small-cell lung cancers: a heterogeneous set of diseases. *Nature Reviews Cancer, 15(4), 247-247*; Andrade de Mello et al. (2012) Insights into angiogenesis in non-small cell lung cancer: molecular mechanisms, polymorphic genes, and targeted therapies. *Recent patents on anti-cancer drug discovery, 7(1), 118-131*; Reck et al. (2013) Management of non-small-cell lung cancer: recent developments. *The Lancet, 382(9893), 709-719*; Gottschling et al (2012) Are we Missing the Target?–Cancer Stem Cells and Drug Resistance in Non-small Cell Lung Cancer. *Cancer Genomics-Proteomics, 9(5), 275-286*.

ImmunoTools *special* AWARD for **Ilaria Bucci** includes 24 reagents

FITC - conjugated anti-human CD31, CD34, CD44, CD62P, CD105, CD147

recombinant human cytokines: EGF, FGF-b, BMP-7, HGF, PDGF-AA, PDGF-BB, TGF-beta3, VEGF-A, Gal-1, Gal-3, IL-1a, IL-6, IL-8, VEGFR2, CCL2 (MCP-1), CCL5 (Rantes), CCL19 (MIP-3b), CXCL4 V1 (Platelet Faktor-4 Variant1/ PF-4V1)

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