PROJECT

Tutor's Name	Andrea Cignarella
Cotutor's Name	

1) Project title

Sex differences in immune checkpoint expression and release by human endothelial cells and monocyte-derived macrophages: implications for combined personalized therapies

2) Abstract (max 500 words)

Background

Immune check-points (ICs) including PD-1 or PD-L1 are expressed in various cell types including lymphocytes, endothelial cells (ECs) and monocyte-derived macrophages, and can be upregulated as a suppressive signal by a number of mediators (e.g. cytokines, growth factors [GF]) that characterize the inflammatory microenvironment of several disease conditions. Notably, emerging evidence highlights the immunosuppressive role of endothelial PD-L1 in different settings. However, little is known about gender differences in the functional role of ICs, which may have relevant implications for the response to IC inhibitors as single agents or combined therapies.

Soluble forms of PD-1 (sPD-1) and PD-L1 (sPD-L1), released either by membrane ICs or by exosomes, have been detected in the serum of patients with inflammatory arthritis, acute coronary syndrome and cancer. However, the functional significance of soluble ICs is largely unknown.

Preliminary data

Data from our lab showed that: 1) expression and release of PD-L1 by human umbilical vein ECs (HUVECs) in response to cytokines and GFs including VEGF are sexually dimorphic; and 2) ECs may represent a major source of sPD-L1.

Objective

Based on this background, the general objective of the project is to further investigate factors (e.g. estrogen) and functional mechanisms underlying the sexual dimorphism of PD-1/PD-L1 in human ECs of different origins as well as in monocyte-derived macrophages. The specific aims will be as follows: 1) to assess gender differences and the response to estrogen in EC models and monocytes exposed to inflammatory mediators and GFs; 2) to define mechanisms of IC release (exosomes, matrix metalloproteinases); and 3) to define the functional role of endothelial PD-L1 and its pharmacological modulation.

Methods

Experiments will be carried out in primary cultures of human cells such as HUVECs, microvascular ECs and monocyte-derived macrophages from male and female donors. Monocytes will be isolated from peripheral blood mononuclear cells. The expression and trafficking of ICs will be investigated by

western blot, flow cytometry and ELISA. The exosome content of ICs will be also measured after sequential centrifugation of cell culture medium and further spun on a sucrose gradient. Functional assays will include endothelial-leukocyte co-culture experiments and cytokine release.

Anticipated output

The project may lay the ground for sex-specific combined anti-cancer therapies targeting immune and endothelial cells. The long-term goal will be to identify sex-specific biomarkers of drug response.