ImmunoTools *special* Award 2015

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Extra-cell communication: Comparative characterization and proteomic analysis of trophoblast-derived nanovesicles, including exosomes

Human placenta is compound by trophoblast cells. The correct development of embryo depends on trophoblast function. These cells invade the uterus and remodel the maternal spiral arteries. The intrauterine environment of the first trimester plays an important role in the regulation of trophoblast function and capacities (Lunghi et al. 2007). Trophoblast shares several skills with cancer cells, defining it as “pseudo-malignant” type of tissue or “physiological metastasis” – despite its spatial and temporal regulation (Ferretti et al. 2007).

As tumour microenvironment determine the tumor cell progression, decidual and endometrium tissues control the trophoblastic performance. Each micro-environment regulates the cell behavior by a crosstalk or “molecular communication” in which several signal molecules, as hormones, growth factors and cytokines, are exchanges, giving a paracrine signaling among the cells. Particularly, TGF-β, IGF and EGF signaling pathways play a key role, regulating process such as proliferation, migration, invasion and angiogenesis (Fitzgerald et al. 2005). TGF-β differently affects the trophoblast and choriocarcinoma behavior, activating not only the TβR/SMAD canonical signaling pathway, but also others as MAPK and PI3K/Akt signaling pathways (Jones et al. 2006).

In addition, trophoblast tissue produces deportation and microshedding, including exosomes delivery, and is suggested as mechanisms to modulate the maternal immune system (Pantham et al. 2011). Exosomes are proposed as communication extracellular organelles, rich on signal molecules, which could mediate paracrine signaling (Mathivanan et al. 2010).

We hypothesize that trophoblast and choriocarcinoma cells modulate the response and regulation of its micro-environment by exosomes delivery, in which these exosomes are an interesting source of regulatory molecules, as signaling proteins and miRNAs. Aim of this project is to analyze trophoblast-derived micro and nano-vesicles and to evaluate its protein profile, regarding to target biological networks. HTR8/SVneo immortalized trophoblast cell line and JEG-3 choriocarcioma cell line are used as models, treating it with TGF-β cytokine. The trophoblast-derived micro- and nano-vesicles, including exosomes, will be analyzed by Nanoparticle Tracking Analysis (NTA) and its protein profile will be assay by Proteomic Techniques.

Additional to vesicle obtaining and analysis, it is necessary to characterize the obtained preparation by immunoblot techniques as Western Blot and flow cytometry, as well as to determine if TGF-β would affect its production and protein profile. In general, the exosome markers include tetraspanins (CD9, CD37, CD63, CD81, CD82), MHC molecules, adhesion molecules as ICAM and Integrins (CD11a, CD15, CD34, CD44, CD47, CD54), or milk-fat globule-EGF-Factor VIII (MFGE8, or lactadherin), and cytosolic proteins as stress proteins, Tsg101, Alix, or cytoskeletal proteins (Mathivanan et al. 2010). Tetraspanins interact with other transmembrane and intracellular proteins, as membrane metalloprotease (CD10, CD156/ADAM8), receptors (CD27, CD95/FasR) and other molecules related with signaling pathways as endoglin (CD105) which are incorporate to exosomes and release in microenvironment, acting over extracellular matrix or target cells (Mazurov et al. 2013; Yoshioka et al. 2013; Zoller 2009).

Additional, trophoblastic cells and its derived vesicles are characterized by CD10, syncytin, placental alkaline phosphatase (PLAP), cytokeratin 7 and 18, HAI-1 and hCG, among others (Soares and Hunt 2006).

By other hand, it is necessary to determine the presence of other vesicles as apoptotic bodies, by mean of Annexin V. The conjugated antibody offered by Immunotools would help me in to characterize the obtained micro and nanovesicles and to determine a possible effect of TGF-β over its release.

This project is a first approach to understand extra-cell communication between trophoblast cells and its micro-environment, compared to choriocarcinoma growths, and its interactions, and how the trophoblast-derived micro and nanovesicles might modulate its neighbor cells behavior, giving a restrictive or permissive micro-environment.

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