ImmunoTools *special* Award 2013

Fermín E. González, Ph.D., Assistant Professor

Faculty of Dentistry, University of Chile, Santiago, Chile

Proteomic and immunological analysis of cancer cell lysates: Identification of new danger signals for immune cells activation

Cancer is an epidemiological problem in both developed and developing countries. Radio and chemotherapy approaches have showed significant improvements only in specific types of cancer, for example hematological malignancies, but very limited ones in solid tumors. During the last decade, immune-therapeutic approaches have been used as an alternative treatment against both solid and hematological malignancies. Dendritic cell (DC)-based immunotherapy takes advantage of the enormous DC's phenotypic and functional plasticity. DC's ability to induce distinct adaptive immune responses is based on their strong capacity to sense different molecular inputs from pathological microenvironments through a range of innate-related receptors, for example pathogen recognition receptors (PRR), and the elaborated signals pathways generated by them after proper ligands engagement.

Damage associated molecular patterns (DAMP) are endogenous factors, originating from necrotic or stressed cells, that can act as "danger signals" inducing an inflammatory response. We have previously described the importance of specific DAMPs belonging to cancer cell lysates in the process from immature DCs to mature DCs. However, the complete amount of cancer cell lysates' DAMPs and their relative contributions to the observed phenotype of the DCs remain unknown.

The overall aim of our proposal is to characterise different cancer cell lysates´ proteomic profiles, in order to identify new danger signal molecules from biologically relevant candidates belonging to the lysates.

In order to verify our hypothesis, we will perform a high-throughput proteome analysis of different types of cancer cell-lysates. In addition, we will test the phenotypic and functional maturation capacity on human DC and THP-1 cells of stressed cancer cell-derived relevant proteins. To this, we will perform an extensive analysis by flow cytometry, among others techniques, of several maturation-associated DC markers on *in vitro* generated monocyte-derived human DCs as well as cytokine-stimulated THP-1 cells. DCs and THP-1 differentiation will be induced with rh IL-4 and rh GM-CSF. DCs and THP-1 cells will be characterized phenotypically by using the following maturation markers: MHC-I, MHC-II, CD11c, CD1a, CD80, CD83, CD86, CCR7, and CD40. Different fluorophores-conjugated anti-human antibodies and recombinant

human cytokines will be used during these analyses: anti-HLA-ABC-FITC, HLA-DR-FITC, CD1a-FITC, CD40-FITC, CD80-FITC, CD83-FITC, CD86-FITC, CD11c-PE, CCR7-FITC, rh IL-4, rh GM-CSF. Moreover, the validation of protein candidates as new DAMPs will be tested by different functional assays like phagocytosis capacity, secretion of pro-inflammatory/Th1 related cytokines by DCs; *in vitro* and *in vivo* migration capacity of stimulated DCs; and DCs' cross-presentation assays to T cells.

Determination of new DAMPs present in cancer cells and their immune effects, will allow us to better understand the activation process of DCs during the immune response against tumors and, in turn assist the development of improved and more effective approaches in the cancer immunotherapy field.