ImmunoTools *special* Award 2013

Cindy Gallerne, PhD,

Inserm UMR1014, Hôpital Paul Brousse, Bâtiment Lavoisier,
14 Avenue Paul Vaillant Couturier, 94807 Villejuif, France

Renal cancer stem cells microenvironment and homeostasis: effects of hypoxic stress on Interleukine-15-induced epithelial differentiation

The clear cell renal cell carcinoma (RCC) is the most lethal form of genitourinary tumors with an important metastatic index. This highly aggressive radio- and chemoresistant cancer was associated with the tumor microenvironment characteristics and the identification of a population of "cancer stem cells" (CSC) or “tumor-initiating stem cells”, identified by the **CD105** surface marker expression and named CSC/CD105+. These undifferentiated cells, characterized by their property of self-renewal and their ability to generate tumors in immunocompromised mice, have been identified as responsible for the development and spread of tumors. Currently, a major therapeutic challenge consists in depleting or differentiating this CSC pool resistant to conventional therapeutic treatments and responsible for tumor relapse.

In this context, our group showed that **Interleukin-15 (IL-15)**, a cytokine involved in renal pathophysiology, induces *in vitro* stable epithelial differentiation of CSC/CD105+, associated with a tumorigenic potential loss and increased sensitivity to anticancer agents and Natural Killer. Moreover, IL-15 is currently tested for metastatic renal cancer treatment due to its immuno-stimulatory properties (Phase I study, NIH protocol NCT01021059). Therefore, IL-15 represents a promising candidate as an adjuvant tool for future therapeutic strategies in kidney cancer treatment. However, the differentiating action of IL-15 could be impeded *in vivo* by tumor microenvironment. Indeed, the RCC are characterized by large regions of hypoxia caused by an inefficient neovascularization and, in addition, approximately 60% of RCC exhibit Von Hippel-Lindau (VHL) gene inactivation, causing oxygen-independent pseudo-hypoxia status. Yet, intratumoral hypoxia is considered as a key factor of tumor aggressiveness as it induces stem-like cells emergence through epithelial-mesenchymal transition.

Our project aims to evaluate if hypoxia/pseudo-hypoxia 1) preserves or enhances the CSC/CD105+ stemness, 2) impedes IL-15 differentiating action. We will find out the mechanisms involved in hypoxia-induced cellular response in order to identify molecular targets able to sensitize CSC to drugs and NK.

Among the different methods used to rule this project, CSC will be cultivated in a medium supplemented with **rhEGF** and **rhFGF-b** to generate tumorospheres in presence or absence of IL-15. The percentage of apoptotic cells will be measured by flow cytometry using an **Annexin V** labeling in response to chemotherapeutic agents. Stemness properties will be studied by flow cytometry experiments by the detection of the expression of markers such as **CD24**, **CD29**, **CD44**, **CD45** and **CD33**, whose expression has been observed in CSC under normoxic conditions. Finally, the capacity of CSC to generate tumors in mice after IL-15 differentiation in normoxia or hypoxia will be evaluated. Human cancer cells will be detected in these tumors using an **anti-HLA-ABC** antibody, and cancer stem cells will be labeled with the specific antibodies described above.

These findings will allow us to improve the *in vitro* and *in vivo* IL-15-differentiating action with the aim to develop therapeutic approaches to selectively eliminate cancer stem cells.