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# Characterization of immune microenvironment in cancer stem cells in non-small cell lung cancer. Analysis of immunosuppressive and immunoregulatory factors

Susana Torres[1] , Silvia Calabuig Fariñas[2] , Rafael Sirera[3] , Alejandro Herrero Pomares[1] , Cristobal Aguilar Gallardo[1] , Marais Mosqueda[1] , Andrea Moreno[1] , Eva Escorihuela[4] , Eloisa Jantus-Lewintre[5] , Carlos Camps[6]

1) Laboratorio de Oncología Molecular, Fundación para la Investigación Hospital General de Valencia 2) Laboratorio de Oncología Molecular, Fundación para la Investigación Hospital General de Valencia; CIBERONC; Departamento de Patología, Universitat de València 3) Departamento de Biotecnología,Universitat Politècnica de Valencia 4) Laboratorio de Oncología Molecular, Fundación para la Investigación Hospital General de Valencia; CIBERONC 5) Laboratorio de Oncología Molecular, Fundación para la Investigación Hospital General de Valencia; CIBERONC; Departamento de Biotecnología,Universitat Politècnica de Valencia 6) Laboratorio de Oncología Molecular, Fundación para la Investigación Hospital General de Valencia; CIBERONC; Servicio de Oncología Médica, Consorcio Hospital General Universitario de Valencia; Departamento de Medicina, Universitat de València

**Background:** Cancer Stem Cells (CSCs) are a small subpopulation of cells with self-renewal and the ability to grow forming tumourspheres under non-adherent conditions. They are involved in recurrence, metastasis and resistance. The interactions between CSCs and immune cells are involved in the down-regulation of the immune response, which allows tumours to escape from immunosurveillance. Therefore, a better understanding of how CSCs interact with their immune microenvironment in non-small cell lung cancer (NSCLC) would result in an improved characterization of patient's immune contexture and new immunotherapeutic protocols, which may overcome the limitations of conventional therapeutic strategies.

**Objectives:** The aim of this work was to compare the release and expression of immunoregulatory cytokines between monolayer cells and tumourspheres.

**Methods:** We analysed four soluble factors with immunosuppressive (IL-4, IL-10), and immunoregulatory (IL-6, IL-8) capacity. The study of relative gene expression was performed on tumour cells from eight resected NSCLC patients samples and 12 NSCLC cell lines (A549, H1650, H460, H23, H358, H2228, HCC827, PC9, H1993, H1395, H1975 and SW900) grown in monolayer and as spheroids by RTqPCR. The study of secretion was performed on a medium supernatant of cells from two of these NSCLC tumour patients samples and ten of these cell lines grown in monolayers and tumourspheres at three different densities ($10^4$, $5{\cdot}10^5$ and $10^5$ cells/ml) by bead-based multiplex assay using the Millipore kit 8 (Millipore's Milliplex® MAP Kit HSTCMAG-28SK panel) and Luminex 100/200.

**Results:** All the human tumour primary cells and cell lines secreted and expressed detectable levels of IL-6 and IL-8. Conversely IL-10 and IL-4 were beyond the detectable range (<1.83 and <1.46 pg/ml, respectively) for most of these cell lines and NSCLC tumour samples, according to the expression. We observed differences between levels IL-6 and IL-8, secreted by adherent cells and tumourspheres, and also between samples (Table 1). We also observed differences between the expression of IL-6 and IL-8 by monolayer cells and spheroids. Adherent cells showed increased IL-6 levels (at both the mRNA and protein levels) compared to lung-tumourspheres, while lung-tumourspheres expressed significantly higher IL-8 levels compared to monolayer cells. No significant differences were found for the IL-8 secretion between adherent cells and spheroids.

**Table 1**. Differences in the secretion of IL-6 and IL-8 in adherent cells and tumourspheres by a multiplex analysis. Detection range of IL-8(0.28-2273.3 pg/ml). Detection range of IL-6 (0.17-1750.12 pg/ml).

| | ***pg/ml x $10^5$ cells IL-6*** | ***pg/ ml x ·$10^5$ cells IL-8*** |
|---|---|---|
| ***Samples*** | **Adherent cells** | **Tumourspheres** |
| ***A549*** | <0.17 | 1.3 |

| | | |
|---|---|---|
| ***H2228*** | 1506,62 | 578,30 |
| ***HCC827*** | 348.2 | >1750.12 |
| ***H358*** | 10.9 | 27.8 |
| ***H1993*** | 34.4 | 2.2 |
| ***PC9*** | 144.4 | 5.4 |
| ***SW900*** | 121.7 | 5.4 |
| ***H460*** | 359.8 | 227.8 |
| ***H23*** | 3.7 | 2.8 |
| ***H1650*** | 995.9 | 352.2 |
| ***Patient 1*** | 17.4 | 0.6 |
| ***Patient 2*** | >1750.12 | >1750.12 |

**Conclusions:** Our preliminary results suggest that lung CSCs secrete IL-8, whose cloud be involved in the modulation of the immune microenvironment while lung-tumourspheres avoid the secretion of IL-6. The next step is to extend the cohort to validate our results and to study others immunoregulatory factors.

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