

## Characterization and gene expression of cancer stem cells grown as sarcospheres from human primary sarcomas

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This study aimed to identify, isolate and characterize cancer stem cells from human primary sarcomas. We performed cytometric analyses for stemness and differentiation antigens including CD29, CD34, CD44, CD90, CD117 and CD133 on 21 human primary sarcomas on the day of surgery. From sarcoma biopsies, we obtained two chondrosarcoma stabilized cell lines and two osteosarcoma stabilized cell lines on which spheres formation, side population profile, stemness gene expression and in vivo and in vitro assays were performed. On a chondrosarcoma cell line, the whole genome microarray analyses were performed (sarcospheres versus adherent cells).

All samples expressed the CD133, CD44 and CD29 markers. We selected a CD133+ subpopulation from stabilized cell lines that displayed the capacity to grow as sarcospheres able to initiate and sustain tumour growth in NOD/SCID mice, to express stemness genes including OCT3/4, Nanog, Sox2 and Nestin and to differentiate into mesenchymal lineages. Microarray analyses pointed out a huge gene expression difference between sarcospheres and adherent cells. About 2000 genes, including ones related to cell cycle, stemness and epigenetic regulation, resulted to be very differentially expressed in the two population considered. With signed ratio of 2,806 genes were over-expressed and 1,029 under-expressed on sarcospheres when compared with adherent cells.

The most highly overexpressed gene were thioredoxin interacting protein (fold +25) that modulates the cellular redox state, growth differentiation factor 15 (fold +10), member of the TGF- $\beta$  superfamily, histone cluster 1, H2ad (fold +9) and solute carrier family 2 member 14 that facilitate glucose transport (fold +8,9).

Our findings evidence the existence of cancer stem cells in human primary bone sarcomas and highlight CD133 as pivotal marker for identification of these cells. This may be of primary importance in the development of new therapeutic strategies and new prognostic procedures against these highly aggressive and metastatic tumours.

Keywords: cancer stem cells, CD133, sarcospheres, gene expression