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Characterization of cancer stem cells (CSC) isolated from LI, a human glioblastoma (GBM) cell line

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It is becoming increasingly clear that tumour development is due to a small fraction of mutated stem cells (CSC). Established cancer cell lines represent a good source of CSC with respect to tissue tumour samples because they do not contain any contaminating normal stem cells and it is easy to obtain large quantities of them. We isolated putative CSC via a non-adherent neurosphere (NS) assay from LI. Using a clonal assay, we selected from the primary NS two clones named F11 and D2.

LI, F11 and D2 cells and these clones under differentiation were examined for expression of stem cell markers (CD133, Nestin, Musashi-1 and Sox-2), markers of differentiation (βIII-Tubulin and GFAP) and Ca2+-channels, by immunocytochemistry, western blot analysis and confocal Ca2+ imaging. Both F11 and D2 clones expressed higher levels of stem cell markers with respect to LI cells. Markers of differentiation were expressed at high levels in both LI cells and clones. The expression of Nestin, Sox-2, and βIII-Tubulin was down-regulated in clones under differentiation, whereas Musashi-1 was increased. LI, F11 and D2 cells did not exhibit Ca2+ signals following KCl-induced membrane depolarization, thus suggesting that they do not express functional voltage-dependent Ca2+-channels. Nevertheless, transient increases in intracellular Ca2+ levels were observed after cell exposure to ATP in both F11 and D2 during differentiation. The ATP treatment did not affect cell proliferation. The increased expression of stem cell markers and their decrease in cells under differentiation demonstrate the stem characteristics of clones we selected. The findings regarding expression of differentiation markers and of Ca2+-channels require further investigation. After a deeper characterization, both F11 and D2 could represent a good model to improve the knowledge on CSC and to identify new therapeutic approaches in GBM.

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Keywords: LI cell line, CSC, differentiation, Ca2+-channels.