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Regulation of glioblastoma cell proliferation in dependence of cell density and growth factors in vitro

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Glioblastoma (GBM) cells are characterized by uncontrolled proliferation and resistance to apoptosis, which leads to undesirable clinical outcome, specifically poor prognosis in patients. Previous work has showed that passage of exponentially growing U251 cells (U251-E) and re-seeding at high density resulted in complete downregulation of ERK1/2 phosphorylation, whereas ERK1/2 activation was more strongly upregulated when U251 cells were harvested from plateau phase (U251-P). This suggested that releasing cells from density arrest provides provided a strong mitogenic signal which had an effect on GBM cell proliferation.

In the present study, ERK1/2 activation suggested increased proliferation upon re-plating of U251-P cells at high density, while ERK1/2 downregulation led to limited proliferation with cell apoptosis when re-plating of U251-E cells at high density (EH). The limited proliferation was associated with G2 accumulation and downregulation of cell cycle proteins. Upregulation of p27 in U251-E cells upon starvation enabled the cells to survive after reseeded at high density, thus supporting a role for uncontrolled cell-cycle progression in inducing apoptosis. Although FAK activation was suppressed, EH cells were not rescued from apoptosis by the induction of FAK expression, indicating the downregulation of FAK signaling pathway was not responsible for the decreased p-ERK1/2. In addition, a fundamental difference of EKR1/2 regulation was observed in parent cultures. ERK1/2 activation in U251-P cells was strongly upregulated upon loss of attachment via autocrine signaling. Furthermore, the increase in JNK activation was partially mediated via IGF-1R signaling in U251-E cells, and contributed to apoptosis after reseeding at high density. Although the clonogenic survival curves were very similar for immediate plating of cells from both phases, surviving fraction at 2Gy was slightly higher in cells from U251-P than those from U251-E. Immunostaining with γH2AX showed a significantly higher foci number at 1h and 3h after 2Gy irradiation in cells from U251-P, indicating that re-plating of U251-P cells exhibited a relatively more radio-resistant phenotype. A reversible contact inhibition was detected in GBM cells. Besides, the combination of IGF-1 and FGF-2 provided a growth advantage in GBM cells *in vitro*.

Taken together, these results highlight certain molecular regulations preserved in GBM cells, specifically, (a) that a balance between mitogen activated ERK1/2 and stress activated JNK pathways mediated via IGF-1R pathway regulate GBM cell proliferation and survival, (b) that cyclin-dependent kinase inhibitors remain some functionality in GBM cells, which guarantees the proper cell cycle progression coordinated with mitogenic signaling, (c) and that these regulatory mechanisms contributes to cellular homeostasis and can provide a better understanding of the aggressiveness in GBM.