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Rapamycin induces neuronal differentiation and decreases prion-like proteins in human glioblastoma cell cultures

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Glioblastoma (GB, grade IV astrocytoma) is highly proliferating, infiltrating, and relapsing. So far, no therapy cures the disease and on average lethality occurs within 2 years from diagnosis. Despite the intimate molecular mechanisms of GB remain unknown, the mammalian Target Of Rapamycin (mTOR) is constantly upregulated within GB and in other astrocytomas. Consistently, mTOR was proposed as a target to cure GB. In fact, the mTOR inhibitor rapamycin was tested in order to prevent the growth of GB in human primary cultures and in mouse xenograft. Cytopathology of GB is highly heterogeneous for the co-existence of various cell types and the occurrence progressive steps in cell differentiation. Remarkably, the non differentiated stem-like cells represent primary GB precursors which initiate the tumor growth. Altered transmission of pathological proteins was recently claimed to underly the growth of GB and its infiltration. These proteins are controlled by the mTOR pathway and spread from cell to cell suggesting a prion-like mechanism in GB. Therefore, in the present study, using the human GB cell line U87MG, we evaluated the effect of a wide range of rapamycin concentrations (between 1 nM to 1 x 10^3 nM) at 24 h on: cell survival; cell morphology and electrophysiology; cell differentiation and migration; expression of PrP and other prion-like proteins.

We found that effects of rapamycin on U87MG are dose-dependent. While high doses (100 nM up to 1000 nM) reduce cell viability, lower rapamycin doses (1nm up to 10 nM) produce cell differentiation consisting of morphological and immunohistochemical changes. Rapamycin promotes a neuronal shape with process elongation and increased cell size. This associates with the loss of the staminal marker nestin while the early neuronal marker betaIII-tubulin increases. At 24 h rapamycin inhibits U87MG cell migration dose-dependently. The neuronal morphology associates with altered intracellular calcium flux but not neuronal electrophysiological properties. Finally, expression of prionoids which we found to be high in baseline conditions was suppressed by rapamycin. This is consistent with our ultrastructural findings which substantiate the dramatic effects induced by mTOR inhibition. Our results suggests pathological cell communication in GB while providing cellular evidence supporting the use of rapamycin as well rapalogs as effective drugs to treat malignant astrocytoma.

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Key words

U87MG cell cultures, nestin, betaIII-tubulin, prionoids, morphology.

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