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Rapamycin dose-dependently promotes differentiation and cell death in an in vitro model of malignant gliomas

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Glioblastoma (GB, grade IV astrocytoma) is the most common and lethal brain tumor characterized by increased proliferation and resistance to chemotherapy and radiotherapy. GB infiltrates the brain, always relapses and leads to death within 2 years from diagnosis. At cellular level, relapse and infiltration are correlated with the presence of GB precursors stem-like cells. At molecular level, relapse and infiltration are correlated with upregulation of the mammalian target of rapamycin, mTOR which constantly characterizes malignant gliomas.

By definition mTOR is strongly inhibited by rapamycin and rapalogs. Several studies were carried out to evaluate the effects of rapamycin in experimental models of GB. However, it remains controversial whether rapamycin and rapalogs produce cell differentiation or cell death.

Therefore, in the present study we used a wide range of doses of rapamycin to address the issue of differential effects obtained by rapamycin in various experimental setting. In particular, rapamycin was administered ranging from 1 nM up to 1microM to assess cell viability in GB cell lines (U87MG). All the experiments were carried out exposing the cells for 24 h to rapamycin.

We found that rapamycin produced dose dependently cell death which was significant for doses starting at 100 nM and reaches a plateau at 1 microM (50% cell death).

Interestingly, when using rapamycin at doses between 1 nM and 100 nM we described only slight cell death while the prominent effect induced by rapamycin consisted of cell differentiation. In detail, beta 3 tubulin increased, while nestin decreased under rapamycin exposure. These markers were related to phenotypic changes consisting of an increased number and length of cell processes along with the loss of fusiform cell shape.

In the process of characterizing the effects of mTOR up-regulation and its pharmacological inhibition we also measured the accumulation of an mTOR-dependent protein such as alpha-synuclein. In fact, mTOR up-regulation is known to inhibit the autophagy pathway which removes alpha-synuclein. Consistently, in U87MG cells we found accumulation of alpha-synuclein which was removed dose dependently by rapamycin exposure.

Our data show that inhibiting mTOR with rapamycin is a powerful tool to induce cell differentiation in U87MG cells, whereas cell death significantly occurs only at the highest doses.

Keywords: U87MG, alpha-synuclein, mTOR inhibition, immunocytochemistry.