Evaluation of mTOR as a new therapeutic target in meningiomas

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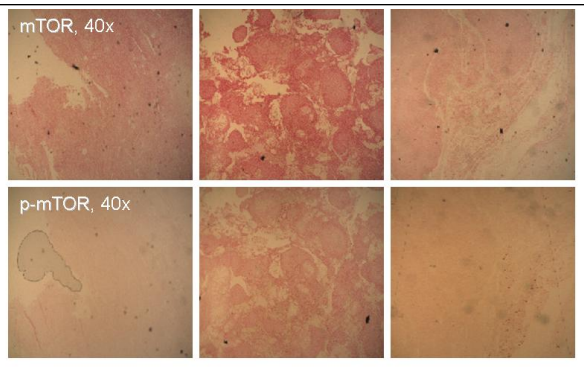
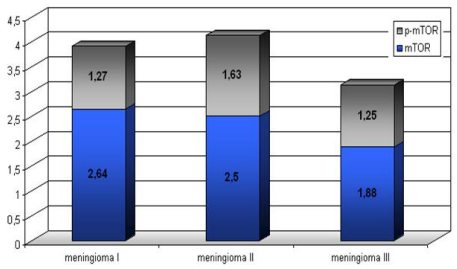
Introduction

The protein kinase mammalian target of rapamycin (mTOR) is known to play an important role in the regulation of cell growth and has been evaluated as a target for the treatment of a variety of tumors. Increasing evidence even suggests it's involvement in glioma cell survival, but to date little is known about mTOR in more benign tumor diseases like meningiomas.

Methods

Primary meningioma cells cultured from human meningiomas (WHO grade I-III) and the permanent meningioma cell lines BenMen 1, and HBL 52 were evaluated using immunofluorescence (IF), real time PCR, and Western blot analysis for expression of mTOR and it's phosphorylated active isoform p-mTOR. Dose-response-curves were compiled with the inhibitors Everolimus and Temsirolimus to determine the half maximal inhibitory concentration (IC50) for both meningioma cell lines. The inhibition of meningioma cell growth by the specific mTOR inhibitors Everolimus (20µM, 40µM) and Temsirolimus (25µM, 50µM) was further assessed using proliferation and viability assays on primary meningioma cultures over 24-72 hours.

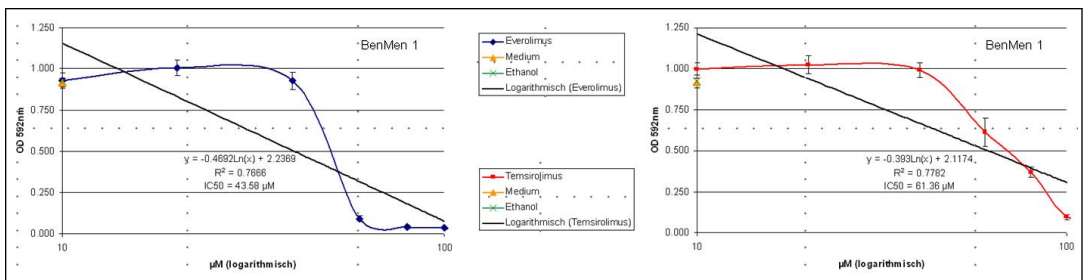




Immunohistochemical analysis of mTOR and p-mTOR expression in meningiomas

Results

IF demonstrated the presence of mTOR and p-mTOR in the vast majority of all primary meningioma cells with a strong cytoplasmic and mostly weak nuclear distribution. Furthermore IF showed a clear positivity for mTOR and p-mTOR in BenMen 1 and HBL 52 meningioma cell lines. These findings were supported by positive immunoblotting and real time PCR. The half maximal inhibitory concentration (IC50) for Everolimus in both meningioma cell lines averages 45.5µM and for Temsirolimus 57.4µM. Depending on these results meningioma cell growth inhibition by Everolimus and Temsirolimus in their highest concentration was demonstrated for different primary cell lines by a significantly decreased cell viability (MTT assay) and cell proliferation (BrdU incorporation) after 72h.



MTT viability assay, calculation of IC50 of Temsirolimus & Everolimus

Learning Objectives

Expression of mTOR in human meningiomas and evaluation of mTOR as a therapeutic target for the inhibition of meningioma cell growth.

Conclusions

These recent data show that mTOR and p-mTOR are expressed in human meningiomas. In vitro, meningioma cell growth can effectively be inhibited by the specific mTOR inhibitors Everolimus and Temsirolimus.