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PLEKHG5 regulates autophagy, survival and MGMT expression in U251-MG glioblastoma cells

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A signalling pathway involving PLEKHG5 (guanine exchange factor) for the Ras superfamily member RAB26 to transcription factor NF-κB was discovered in autophagy. PLEKHG5 was reported in glioblastoma multiforme (GBM) and correlates with patient survival. Thus, the generation of a cellular model for understanding PLEKHG5 signalling is the study purpose. We generated a CRISPR/Cas9-mediated knockout of PLEKHG5 in U251-MG glioblastoma cells and analysed resulting changes. Next, we used a mRFP-GFP-LC3^{+} reporter for visualisation of autophagic defects and rescued the phenotype of *PLEKHG5* wildtype via transduction of a constitutively active RAB26QL-plasmid. Effects of overexpressing RAB26 were investigated and correlated with the O^{6}-methylguanine-DNA methyltransferase (MGMT) and cellular survival. *PLEKHG5* knockout showed changes in morphology, loss of filopodia and higher population doubling times. Accumulation of autolysosomes was resulted by decreased LAMP-1 in PLEKHG5-deficient cells. Rescue of *PLEKHG5*$^{-/-}$ restored the downregulation of RhoA activity, showed faster response to tumour necrosis factor and better cellular fitness. MGMT expression was activated after RAB26 overexpression compared to non-transduced cells. Survival of *PLEKHG5* knockout was rescued together with sensitivity to temozolomide by RAB26QL. This study provides new insights in the PLEKHG5/RAB26 signalling within U251-MG cells, which suggests potential therapeutic strategies in other glioma cells and further in primary GBM.

Glioblastoma multiforme (GBM) is the most frequent malignant primary human brain tumour in adults[1]. Despite radiation and temozolomide (TMZ) chemotherapy, the average survival time of GBM patients is no longer than 15 months[2]. A frequently used model of glioblastoma is the cell line U251-MG, which was derived from a grade III-IV astrocytoma isolated from a male patient in 1973[3,4]. Furthermore, U251-MG cells are commonly used for examining the role of various genes in tumour pathogenesis[5] and the efficacy of therapeutic agents[6].

Recently, Pleckstrin homology containing family member 5 (PLEKHG5) has been described as a prognostic biomarker in glioblastoma patients[7]. PLEKHG5 is a guanine exchange factor (GEF), which is highly expressed in endothelial cells of the nervous system and in different cancer cells[5,8,9]. PLEKHG5 functions as a GEF for Rho GTPases, which are key regulators for cellular dynamics. Activation of Rho by binding a GEF like PLEKHG5, was also described to be involved in polarity-orientated cell migration in U251-MG cells[5]. Thus, Dachsel and colleagues published that the signalling via the PLEKHG5/RhoA pathway is a major contributor in the dissemination and poor outcome of GBM.

Direct expression of PLEKHG5 has been shown to activate nuclear factor "kappa-light-chain-enhancer" in activated B-cells (NF-κB) signalling in different cell lines in vitro as well as in spinal cord and brain in vivo[10]. RhoA was also discovered to be an activator of the transcription factor NF-κB[11]. These findings provide an additional link between cancer and PLEKHG5 signalling, since NF-κB is a key player in cancer development and progression[12,13]. It also plays a major role in various cellular functions like inflammation, immune response, apoptosis and proliferation[11,12,14], particularly in the nervous system[15,16]. Of note, an activation of the NF-κB transcription factor is frequently observed in GBM[17]. Soubannier and Stifani reported that the canonical NF-κB

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