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Rolipram potentiates bevacizumab-induced cell death in human glioblastoma stem-like cells

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ABSTRACT

Aims: Glioblastoma cancer stem-like cells (GCSCs) promote themselves proliferation by secreting the vascular endothelial growth factor A ($VEGF_A$) in an autocrine manner, positively regulated by phosphodiesterase IV (PDE4). In the current study, we investigated the putative cytotoxic effect of bevacizumab, a $VEGF_A$ blocker, alone and in combination with a specific inhibitor of PDE4 called rolipram on GCSCs isolated from human surgical tumor specimen with a focus on PI3K/AKT pathway.

Main methods: CD133+/CD15+ GCSCs were characterized by flow cytometry and expanded in a serum-free primary culture system. The cell survival, apoptosis, and protein expression values were measured using MTT assay, TUNEL staining and western blot, successively. Intracellular cAMP and free secreted $VEGF_A$ levels were assessed by cAMP enzyme immunoassay and ELISA, respectively.

Key findings: Bevacizumab suppressed GCSCs survival with IC_{50} ~ 6.5 μg/ml and enhanced the levels of apoptosis, p53 and cleaved-caspase3 along with a decrease in free $VEGF_A$ levels and ERKs activation. However, there was no significant modulation of AKT phosphorylation on serine 473, the intracellular PDE4A, $VEGF_A$ and cAMP levels. More cytotoxicity in co-treated cells coupled with a more substantial decline in the free $VEGF_A$ levels and a greater increase in the quantities of p53 and cleaved-caspase3 compared to those treated with bevacizumab alone. Co-treatment reduced phospho-AKT, endogenous $VEGF_A$ and PDE4A values but elevated cAMP levels.

Significance: This study highlighted a booster cytotoxic effect of combined rolipram and bevacizumab treatment on the GCSCs primary culture, suggesting that this approach is warranted in treatment of GBMs overexpressing $VEGF_A$ and PDE4A.

1. Introduction

Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor in adults [1]. Despite standard therapeutic interventions, the patients succumb to death within 12–15 months after diagnosis [2,3]. The evidence supports the existence of a sub-population of GBM cells known as glioblastoma cancer stem-like cells (GCSCs) having the potential of tumorigenesis [4–6] and chemo-resistance and radio-resistance [7,8]. These cells are characterized by some cell surface markers including CD133 and CD15 [9,10]. It is stated that CD133+/CD15+ GCSCs are capable of generating the sphere-like colonies in vitro [11,6,10]. Furthermore, overexpression of phosphodiesterase IVA (PDE4A) and vascular endothelial growth factor (VEGF) in human GBM tissues have been identified, associated with unfavorable clinical outcome [12] and intracranial tumor growth [13].

Indeed, an essential VEGF isotype to progress angiogenesis is $VEGF_A$, mostly secreted from GCSCs. $VEGF_A$-dependent growth promoting signal is mainly transmitted via VEGF receptor 2 (VEGFR2) [14–16]. GCSCs promote angiogenesis through the secretion of $VEGF_A$ in a paracrine manner on the endothelial cells [17,18]. In addition, GCSCs boosts the survival and proliferation themselves via $VEGF_A$-VEGFR2 interplay under a positive autocrine loop of $VEGF_A$ [19–21]. Recently, a research defined the stimulatory function of some PDE4 isoforms in upregulating of $VEGF_A$ expression [22]. Hence, it is of interest to clarify an ambiguity based on whether the combination of $VEGF_A$ blocker with the PDE4A inhibitor can confer a pronounced effectiveness on cell growth inhibition

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