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

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A B S T R A C T

Aims: Glioblastoma cancer stem-like cells (GCSCs) promote themselves proliferation by secreting the vascular endothelial growth factor A (VEGFA) in an autocrine manner, positively regulated by phosphodiesterase IV (PDE4). In the current study, we investigated the putative cytotoxic effect of bevacizumab, a VEGF 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 levels were measured using MTT assay, TUNEL staining and western blot, successively. Intracellular cAMP and free secreted VEGFα levels were assessed by cAMP enzyme immunoassay and ELISA, respectively.

Key findings: Bevacizumab suppressed GCSCs survival with K50 = 6.5 μg/ml and enhanced the levels of apoptosis, p53 and cleaved-caspase3 along with a decrease in free VEGFα levels and ERKs activation. However, there was no significant modulation of AKT phosphorylation on serine 473, the intracellular PDE4A, VEGFα and cAMP levels. More cytotoxicity in co-treated cells coupled with a more substantial decline in the free VEGFα 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 VEGFα 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 GBM overexpressing VEGFα and PDE4A.

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1. Introduction

Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor in adults [1]. Despite standard therapeutic interventions, 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 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 [11,10]. 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 VEGFα mostly secreted from GCSCs. VEGFα-dependent growth promoting signal is mainly transmitted via VEGF receptor 2 (VEGFR2) [14–16]. GCSCs promote angiogenesis through the secretion of VEGFα in a paracrine manner on the endothelial cells [17,18]. In addition, GCSCs boosts the survival and proliferation themselves via VEGFα-VEGFR2 interplay under a positive autocrine loop of VEGFα [19–21]. Recently, a research defined the stimulatory function of some PDE4 isoforms in upregulating of VEGFα expression [22]. Hence, it is of interest to clarify an ambiguity based on whether the combination of VEGFα blocker with the PDE4A inhibitor can confer a pronounced effectiveness on cell growth inhibition

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