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Both mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinases (ERK) 1/2 and phosphatidylinositide-3-OH kinase (PI3K)/Akt pathways regulate activation of E-twenty-six (ETS)-like transcription factor 1 (Elk-1) in U138 glioblastoma cells☆

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ABSTRACT

Epidermal growth factor (EGF) and its receptor (EGFR) have been shown to play a significant role in the pathogenesis of glioblastoma. In our study, the EGFR was stimulated with EGF in human U138 glioblastoma cells. We show that the activated mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinases (ERK) 1/2 pathway phosphorylated the E twenty-six (ETS)-like transcription factor 1 (Elk-1) mainly at serine 383 residue. Mitogen-activated protein kinase kinase (MEK) 1/2 inhibitor, UO126 and ERK inhibitor II, FR180204 blocked the Elk-1 phosphorylation and activation. The phosphatidylinositide-3-OH kinase (PI3K)/Akt pathway was also involved in the Elk-1 activation. Activation of the Elk-1 led to an increased survival and a proliferative response with the EGF stimulation in the U138 glioblastoma cells. Knocking-down the Elk-1 using an RNA interference technique caused a decrease in survival of the unstimulated U138 glioblastoma cells and also decreased the proliferative response to the EGF stimulation. The Elk-1 transcription factor was important for the survival and proliferation of U138 glioblastoma cells upon the stimulation of EGFR with EGF. The MAPK/ERK1/2 and PI3K/Akt pathways regulated this response via activation of the Elk-1 transcription factor. The Elk-1 may be one of the convergence points for pathways located downstream of EGFR in glioblastoma cells. Utilization of the Elk-1 as a therapeutic target may lead to a novel strategy in treatment of glioblastoma.

1. Introduction

Glioblastoma multiforme (GBM) is the most malignant and common primary brain tumor. Epidermal growth factor receptors (EGFRs) are overexpressed or mutated in about 40% of GBMs (Batra et al., 1995; Ekstrand et al., 1991; Libermann et al., 1985; Nishikawa et al., 1994; Wong et al., 1987). The EGFR is a trans-membrane tyrosine kinase that stimulates a number of downstream signal transduction pathways to transmit messages of proliferation, invasion and resistance to apoptosis in GBMs (Feldkamp et al., 1999; Montgomery et al., 1995; Moscatello et al., 1998; Prigent et al., 1996; Rodrigues et al., 2000; Wu et al., 1999).

The most widely studied signal transduction pathways for EGFR messages are the Raf/mitogen-activated protein kinase kinase (MEK)/mitogen-activated protein kinase (MAPK) and the phosphatidylinositide-3-OH kinase (PI3K)/3-phosphoinositide-dependent protein kinase-1 (PDK1)/Akt (Treisman, 1996). A member of the MAPK family, extracellular-signal-regulated kinases (ERK) 1/2, has a role in regulating cell proliferation and promoting cell survival in GBMs (Amos et al., 2006; Bhatt and Ferrell, 1999; Chang and Karin, 2001; Treinies et al., 1999; Uht et al., 2007). The PI3K/Akt pathway is also essential in GBM cell survival, invasion, inhibition of apoptosis and transmitting the messages from the growth factor receptors to the nuclei. Phosphatase and tensin homolog (PTEN) is the major control mechanism in the PI3K pathway and PTEN mutations are commonly seen in *de novo* formation of GBMs (Chakravarti et al., 2004; Choe et al., 2003; Klingler-Hoffmann et al., 2003).

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One of the suggested downstream transcriptional targets of EGFR is E-twenty-six (ETS)-like transcription factor-1 (Elk-1), a member of the ETS domain superfamily of transcription factors (Yordy and Muise-Helmericks, 2000). Elk-1 can be activated by different pathways. Elk-1 is one of the main nuclear targets of activated MAPK/ERK1/2 after growth factor stimulation (Huang et al., 2000; Marais et al., 1993). Moreover, the PI3K/Akt pathway via the MAPK/ERK1/2 pathway activates Elk-1 in lung carcinoma cells to induce their proliferation (Zhang et al., 2006). EGF induces the transactivation of the Elk-1 in the hepatocellular carcinoma cells (Pusl et al., 2002). It has been previously reported that the MAPK/ERK1/2 and PKC η pathways may stimulate the proliferation in GBMs and those pathways may interact with the Elk-1 (Uht et al., 2007; Amos et al., 2005; Hussaini et al., 2000; Martin et al., 2007); however, the interaction of the PI3K/Akt pathway and the Elk-1 has not been studied in GBM cells previously.

Therefore, we decided to assess the role of the MAPK/ERK1/2 and PI3K/Akt signal transduction pathways on regulating the Elk-1 activation upon the stimulation by EGF and whether the Elk-1 plays a role in the proliferation and survival of the U138 glioblastoma cells.

2. Results

2.1. Proliferative response to EGF is related to the Elk-1 transcriptional activity in the U138 glioblastoma cells

The EGF stimulates the proliferation of a subgroup of several GBM cell lines both *in vitro* and *in vivo*. In our study, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) proliferation assay showed that the U138 glioblastoma cells had a high basal proliferative rate and the basal proliferation slowed down after longer starvation periods (24 *vs.* 48 h). As expected, the proliferation assay confirmed that the EGF increased the proliferative rate significantly in the U138 glioblastoma cells after both short duration (24-h) and long duration (48-h) starvation periods despite the fact that the EGF effect was weaker after 48-h starvation (*t* test; $t = 2.90$, $p = 0.006$; $t = 2.13$, $p = 0.04$; respectively) (Fig. 1a). Moreover, inhibition of either the MAPK/ERK1/2 pathway by UO126 or FR180204 or the PI3K/Akt pathway by LY294002 or wortmannin decreased the proliferative rates induced by the EGF stimulation significantly after a 24-h starvation period. However, this effect was not observed after a 48-h starvation period and the inhibitors of either pathway were unable to reverse the marginal increase in proliferation induced by the EGF stimulation. When the inhibitors of either the MAPK/ERK1/2 or PI3K/Akt pathways were utilized alone without concomitant administration of EGF, the basal proliferation rate did not differ significantly after 24-h or 48-h starvation periods. These results showed that the EGF stimulation resulted in an increase in the proliferation of the U138 glioblastoma cells and the EGFR-mediated proliferative response was related to both the MAPK/ERK1/2 and PI3K/Akt pathways. Next, we sought to determine if the Elk-1 had a role in the survival and proliferative response of the U138 glioblastoma cells to the EGF stimulation. The U138 glioblastoma cells were transfected with siRNA directed against Elk-1 (Demir and Kurnaz, 2008) and transfection of 100 ng Elk-1 siRNA reduced levels of the Elk-1 by more than 68%. Knocking-down the expression of the Elk-1 transcription factor significantly decreased the basal proliferative rate of the U138 glioblastoma cells compared to the cells transfected with the scramble RNA as demonstrated by an adenosine 5′-triphosphate-based viability assay (*t* test, $t = 10.62$, $p = 0.00004$) (Fig. 1b). A significant increase at survival and proliferation rates was obtained after the administration of EGF to the U138 glioblastoma cells transfected with the scramble RNA (*t* test, $t = 4.23$, $p = 0.0055$). However, the EGF stimulation was

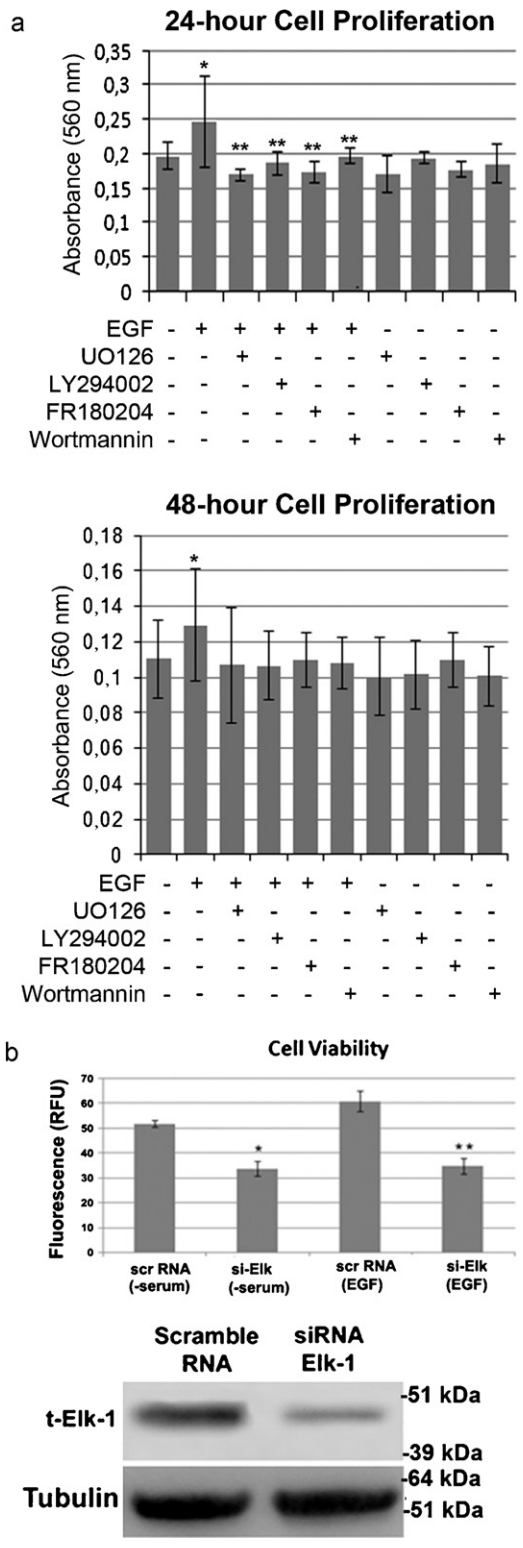

Fig. 1. The MAPK/ERK1/2 and PI3K/Akt pathways and the Elk-1, are important in the survival of the U138 glioblastoma cells. (a) Cell proliferation rates were assessed with MTT cell proliferation assay. Two starvation groups were prepared; cells were kept in a serum free medium either for 24 h or for 48 h. After starvation periods, the inhibitors were applied 1 h prior to the EGF stimulation for 24 h and then 20 μl of MTT solution (5 mg/mL in PBS) was added to each of the wells and the plates were incubated for 4 h, after which the medium was removed and 200 μl of dimethyl sulfoxide (DMSO) was added to each well. The plates were mixed to dissolve the formazan in the DMSO. Optical density was read at 560 nm. (b) The U138 glioblastoma cells were transfected with si-elk-1 plasmids and a 68% decrease in Elk-1 levels was obtained as depicted by the Western blot analysis. Cells were treated with U0126 or LY294002 for 1 h and then EGF was added. After 48 h of drug addition, ATP-based cell viability assay was utilized.