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Leptin enhances the invasive ability of glioma stem-like cells depending on leptin receptor expression

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ABSTRACT

Glioma stem-like cells have been demonstrated to have highly invasive activity, which is the major cause of glioma recurrence after therapy. Leptin plays a role in glioma invasion, however, whether and how leptin contributes to the biological properties of glioma stem-like cells, such as invasion, remains to be explored. In the current study, we aimed to explore the role of leptin during glioma stem-like cells invasion as well as the signaling pathway. We found that glioma stem-like cells exhibited high invasive potential, especially in the presence of leptin, Ob-R coexpressed with CD133 in glioma stem-like cells was showed to be responsible for leptin mediated invasion of glioma stem-like cells. Our results indicated that leptin served as a key intermediary linking the accumulation of excess adipokine to the invasion of glioma stem-like cells, which may be a novel therapeutic target for suppressing tumor invasion and recurrence.

1. Introduction

Glioma is the most common primary brain tumor in human, with poor median survival time despite the improvement in therapy of early diagnosis, surgery and radiation (Tabatabai et al., 2010). The aggressive invasion by malignant glioma cells into surrounding normal brain tissues leads to high mortality, which attributed to the poor survival of glioma patients. A growing body of evidence has demonstrated that cancer stem cells (CSCs), as a subpopulation of tumor cells with the ability to undergo self-renewal and the potential for multi-differentiation, giving rise to tumor heterogeneity, are responsible for tumor invasion and recurrence (Reya et al., 2001). Similar to CSCs from other types of cancers, glioma stem-like cells have been demonstrated to have highly invasive activity, which is the major cause of glioma recurrence after therapy (Inoue et al., 2010; Molina et al., 2010; Yu and Bian, 2009). Thus, approaches that target glioma stem-like cells offer a novel strategy in addressing the spread of glioma tumors.

A large number of studies on cancer incidence link the propensity of cancers with obesity (Bianchini et al., 2002; Calle et al., 2003). Leptin, the most studied adipokine, was a 16-kDa polypeptide that plays an important role in the regulation of body weight homeostasis by affecting food intake, energy expenditure and thermogenesis (Friedman

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and Halaas, 1998). Leptin was once thought to be produced by adipose tissue only, however, leptin and overexpressed with its receptor (Ob-R) has since been demonstrated in several normal and cancerous tissues including glioma. Because it stimulates three pathways well known for their roles in cell growth: proliferation, survival and migration, leptin has been classified as a growth factor (Lawrence et al., 2012).The recent study showed that leptin enhanced migration and invasion of the rat glioma cells, which provides strong evidence that leptin plays a role in glioma invasion. Feldman et al. (2012) have demonstrated that the expression of Ob-R was a characteristic feature of tumor-initiating stem cell (TISCs) (Feldman et al., 2012). Whether and how leptin contributes to the biological properties of glioma stem-like cells, such as invasion, remains to be explored.

In the current study, we aimed to explore the role of leptin during glioma stem-like cells invasion as well as the signaling pathway. We found that glioma stem-like cells exhibited high invasive potential, especially in the presence of leptin, Ob-R coexpressed with CD133 in glioma stem-like cells was showed to be responsible for leptin mediated invasion of glioma stem-like cells. Our results indicated that leptin served as a key intermediary linking the accumulation of excess adipokine to the invasion of glioma stem-like cells.

2. Results

2.1. *Leptin enhances the invasiveness of CD133+ glioma stem-like cells*

After sorting CD133+ and CD133- cells from human glioma cell line U87 cells by flow cytometry, CD133+ cells were maintained in serum-free condition and the purity was evaluated more than 90% (Supplement Fig. 1A). To establish colony formation capabilities of CD133+ and CD133- cells, freshly sorted cells were seeded in 24-well plates at 300 cells per well. After 14 days culture in DMEM with serum-free, CD133+ cells displayed a higher capability for tumor colony formation than CD133- cells (Supplement Fig. 1B).

It has been reported that leptin promotes the survival of mammary cancer stem cells (CSCs) *in vivo*. We decided to compare the motility and invasive ability of CD133+ glioma cells with that of CD133- cells induced by leptin. As shown in Fig. 1A and B, on application of human recombinant leptin (200 ng/mL) to cells, we found that leptin significantly increased migration of CD133+ glioma stem-like cells and reached to 2.7 ± 0.23 fold (Fig. 1A). Moreover, matrigel coated transwell invasion test showed that the invasive ability of CD133+ cells through matrigel basement membrane matrix was increased up to 3.5 ± 0.15 fold by leptin at 200 ng/mL (Fig. 1B). In contrast, leptin has no obvious effect on motility and invasive abilities of CD133- cells. We then examined the expression of genes that are known to be involved in cell migration and invasion. It was found that the expression of matrix metal proteinase-9 (MMP-9), β-integrin, neuronal (N)-cadherin and Vimentin were significantly increased in leptin-induced CD133+ cells compared to that in control cells ($p < 0.005$), while the expression of these genes in CD133- cells has no changes in response to leptin (Fig. 1C). These results demonstrated that leptin could increase migration and invasion of CD133+ glioma cells notably, but not CD133- cells.

2.2. *High level of leptin receptor coexpressed with CD133+ in glioma cells and tissue samples*

Leptin acts via transmembrane receptors (Ob-R), which are found to be expressed on diverse cancer cells derived from different tissues such as breast (Frankenberry et al., 2006), colon (Ratke et al., 2011) or prostate (Somasundar et al., 2004). Subsequently, Ob-R expression has been confirmed in human primary glioma tissue as well as established human glioma cell lines (Riolfi et al., 2010). To explore further the underlying mechanism by which leptin mediated CD133+ glioma cells invasion, we chose two glioma cell lines (U87 cells and U251 cells) and two samples of fresh giloma tissues to examine the expression of Ob-R. The mRNA level of Ob-R was measured by real-time PCR. As shown in Fig. 2A, Ob-R expression was found to be much higher in CD133+ cells than that in CD133- cells from U87 and U251 human glioma cell lines. Next, we examined the expression of Ob-R in two human primary glioma samples. Similar to what we observed in glioma cell lines, Ob-R expression was higher in CD133+ cells but not CD133- cells (Fig. 2A). In parallel to their mRNA levels, higher protein level of Ob-R was detected in the CD133+ cells derived from both human glioma cell lines and primary glioma samples than that in their CD133- counterparts (Fig. 2B). The immunofluorescence assay showed that Ob-R and CD133 were coexpressed in the CD133+ cells (Fig. 2C). These data indicate that the high level of Ob-R coexpressed with CD133+ both in glioma cell lines and tissue samples.

2.3. *Leptin-induced invasiveness of CD133+ glioma stem-like cells are dependent on Ob-R*

In order to determine the role of Ob-R in leptin-induced invasiveness of CD133+ glioma stem-like cells, three pairs of Ob-R siRNAs were individually transfected into CD133+ cells that were derived from U87 glioblastoma cells to knock down endogenous Ob-R. Ob-R mRNA in CD133+ cells transfected with siRNA1, siRNA2, or siRNA3 were reduced by 56.8%, 52.3%, or 48.5%, respectively (Fig. 3A). siRNA-mediated knockdown of Ob-R protein was confirmed by Western blot (Fig. 3A, inset). Because Ob-R protein was remarkably decreased in the CD133+ tumor cells that were transduced with siRNA3, we therefore chose siRNA3 for the next experiments.

As shown in Fig. 3B, after being stimulated by leptin, CD133+ cells from U87 glioblastoma cells overexpressing Ob-R siRNA3 displayed decreased migration compared to control cells ($P < 0.05$), indicating a role for Ob-R expression in CD133+ glioma cells migration. To determine the effect of Ob-R on glioma cell invasion, we again utilized the Matrigel invasion assay (Fig. 3C). After Ob-R knockdown, significantly fewer CD133+ cells invaded through the matrix when compared to cells expressing an empty control vector ($P < 0.05$), The real-time PCR showed that loss of invasiveness capability of the CD133+ cells with Ob-R knockdown was also correlated to the significant reduction of MMP-9, β-integrin, N-cadherin