Cancer Biology & Therapy 2010; 9: 56-65

CXCL12/CXCR4 promotes motility and proliferation of glioma cells

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Background

Glioblastoma is the most malignant and common primary brain tumor among all central nervous system neoplasm. Despite significant advances in surgical and imaging techniques as well as chemo- and radio-therapeutic regimes, the survival of patients suffering with glioblastoma remains gloomy and ranges from 12-14 months. The development of a viable therapeutic strategy for glioblastoma, therefore, requires understanding of the factors that govern the cellular proliferation, robust angiogenesis, extensive infiltration and resistance to apoptosis. Role of chemokine receptor 4 (CXCR4) and its ligand CXCL12 has been described in glioma cell proliferation and motility, therefore this receptor could be a favourable target aimed towards controlling aggression of glioblastoma.

Study Design

Earlier reports in glioblastoma have stated that malignant gliomas significantly over-express CXCR4. To evaluate the possible contribution of CXCR4/CXCL12 to the development of glioblastoma, various in vivo and in vitro experiments have been performed. But several discrepancies have been described regarding the role of this signalling pathway for proliferation, apoptosis and migration ability of glioma cells. To understand the role of CXCR4 in glioblastoma pathology, various strategies have been put together by Carmo et al to study the apoptosis, proliferation, survival and motility after CXCR4/CXCL12 signalling pathway activation.

Expression of CXCR4 by glioma cell line U-118 was confirmed using western blot and immunofluorescence. To ascertain that expression of CXCR4 is CXCL12 dependent, glioma cells were incubated with ligand CXCL12 and antagonist AMD3100, and results indicated a positive role of CXCL12 on CXCR4 expression by glioma cells. Also, in control sets, CXCR4 expression was observed, which suggests constitutive expression of CXCL12 by glioma cell line and it was proved by ELISA for CXCL12. However,

binding of CXCL12 to CXCR4 is not sufficient to induce the proliferation, motility and survival. To confirm the functionality of this complex, P13K/Akt signaling activation was evaluated by measuring the pAkt using western blot. Although basal levels of pAkt were observed in control cells, but CXCL12 significantly increased the pAkt observed in western blot. pAkt expression dependency on CXCL12 was further confirmed by the co-incubating with CXCL12 and Akt inhibitor LY294002.

In addition, authors have checked the role of CXCL12 in glioma cell proliferation. Increased cell proliferation was evaluated by quantification of the BrdU incorporation. Glioma cell proliferation was stimulated significantly by CXCL12 and inhibited by AMD3100. Resistance to apoptosis is one of the major negative factors in cancer progression. In glioblastoma, glioma cells also show apoptosis resistance, and it was confirmed by fluorescence microscopy and flow cytometry. PI was used as a marker for cell integrity and Hoechst stain details about the chromatin condensation in cells. AMD3100 was found to induce the increase in chromatin condensation. PI flow cytometry showed that after treatment with AMD3100 cells appeared in subG1 phase of cell cycle, which is representative of early apoptosis.

Invasiveness and increased motility is one more positive factor which helps in glioblastoma progression. Chemotaxis and cell migration was studied in vitro by trans-well and scrap assay respectively. Results of both assays suggested the increased migratory potential of cells in the influence of CXCL12 and these properties were significantly blocked in the presence of CXCL12. Moreover, this migration and invasive attribute acquired by glioma cells was further evaluated in terms of change in cytoskeleton. Arrangement of F-actin filaments was observed under the influence of CXCL12 and AMD3100, which further support the results obtained in migration and chemotaxis studies. Although authors have studied the cytoskeleton studies, but what is governing this migration at molecular level, was not studied completely.

Implication

In order to understand the role of CXCR4/CXCL12 axis in glioblastoma pathogenesis, Carmo et al showed that proliferation of glioma cells has been increased significantly by the activation of CXCR4 receptor by its ligand CXCL12. On the whole, results of current study describe the active participation of CXCR4/CXCL12 axis in glioblastoma pathogenesis and could

be a possible adjuvant in therapeutic regimen for glioblastoma. Current study assumes a direct relationship between CXCR4/CXCL12 activation and materialization of cancer hallmarks, but this must be confirmed by studying downstream signalling pathway. Further, whole study revolved around the glioma cell line in vitro, and therefore in vivo experiments can be the future.

doi : 10.5214/ans.0972-7531.1017207