Serum Concentration of Soluble Decoy Receptor 3 in Glioma Patients Before and After Surgery

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The suppression of immune responses in malignant gliomas is thought to be involved in glioma pathogenesis. The newly identified tumor-secreted soluble decoy receptor 3 (DcR3) can bind to the ligands CD95L and LIGHT, thereby neutralizing their pro-apoptotic actions. Little is known of the production of DcR3 by glioma cells. This study investigated the serum concentration of DcR3 in glioma patients before and after tumor removal. Blood samples were taken from 17 glioma patients and 10 control patients. The serum DcR3 concentration was measured using a DcR3 enzyme-linked immunosorbent assay. There was no statistically significant difference between preoperative (0.069 ± 0.027 ng/mL) and postoperative DcR3 concentrations (0.068 ± 0.022 ng/mL; $p = 0.951$). Similarly, there was no difference in preoperative DcR3 concentration between glioma patients (0.069 ± 0.027 ng/mL) and controls (0.063 ± 0.023 ng/mL; $p = 0.106$). Our study demonstrated no alteration in DcR3 concentration in glioma patients before and after tumor removal.

Key Words: decoy receptor 3, glioma
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Systemic and local immune responses are suppressed in malignant gliomas, and this suppression has been attributed a role in the pathogenesis of glioma. Soluble immunosuppressive cytokines are implicated as a cause of anergy in immune effector cells. The CD95 system may be involved in immune-modulating processes that could promote glioma cell proliferation *in vivo*. Down-regulation of CD95 expression at the cell surface, shedding of soluble CD95, and inhibition of the intracellular death-signaling pathway are mechanisms that may enable glioma cells to escape a CD95-mediated immune response [1].

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Recently, a soluble decoy receptor for CD95L, decoy receptor 3 (DcR3), has been identified [2–4]. DcR3 binds to the ligands CD95L and LIGHT, thereby neutralizing their pro-apoptotic actions [2,4]. DcR3 is frequently overexpressed by malignant tumors arising from the lung or gastrointestinal tract [2,3], and is amplified in colon and lung cancers [2]. Roth et al also demonstrated that DcR3 is expressed by malignant gliomas and suppresses CD95 ligand-induced apoptosis and chemotaxis [1]. In their studies, DcR3 expression was localized mainly in glioma cells in areas surrounding large ischemic necrosis. Wu et al demonstrated that the serum DcR3 concentration was increased in 82 of 146 patients with malignant tumors [5]. However, there are no reports about whether DcR3 can be detected in serum or whether the serum DcR3 concentration changes after surgical removal of malignant gliomas. In this study, we examined the serum concentration of DcR3 in glioma patients before and after surgery.

Materials and Methods

From 1999 to 2003, 17 patients were included in this study, 13 with astrocytomas and four with oligodendrogliomas. Tumors were totally removed in 11 patients (8 astrocytomas, 3 oligodendrogliomas). Blood samples (10 mL) were taken from glioma patients before and 8 days after tumor removal. Patients with hypertensive intracerebral hemorrhage were used as a control group. After formation of a clot, serum was carefully collected and centrifuged at 3,000 rpm (EBA 3S centrifuge; Hettich, Tuttlingen, Germany) for 5 minutes. The supernatant was carefully aspirated and stored at –30°C until analysis.

Serum DcR3 concentration was measured using the Anawrahta human DcR3 enzyme-linked immunosorbent assay (ELISA) kit (Anawrahta Biotechnology, Taipei, Taiwan). This employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for DcR3 was precoated onto a microplate before standards and samples were pipetted into the wells; the immobilized antibody bound to any DcR3 present. After washing away any unbound substances, a biotin-conjugated monoclonal antibody specific for DcR3 was added to the wells. Following a wash to remove any unbound biotinylated antibody reagent, streptavidin-horseradish peroxidase was added to the wells. Any unbound reagent was removed by washing, and a substrate solution was added to develop color in proportion to the amount of DcR3 bound in the initial step. Color intensity was measured at 450 nm absorbance. Mean values and standard errors of the mean were calculated. Data were analyzed using Student's *t* test, and *p* values of 0.05 or less were considered significant.

Results

Serum concentrations of DcR3 in both glioma patients and controls are shown in the Figure. The assay range was 0–243 ng/mL. The preoperative DcR3 concentration in glioma patients was 0.069 ± 0.027 ng/mL, which was not statistically significantly different from that in controls (0.063 ± 0.023 ng/mL; $p = 0.106$). The postoperative DcR3 concentration (0.068 ± 0.022 ng/mL) was not statistically different from the preoperative concentration (0.069 ± 0.027 ng/mL; $p = 0.951$).

Discussion

The newly identified tumor-secreted DcR3 neutralizes the biologic effects of CD95L [2]. DcR3 has been attributed an oncogenic role because it is preferentially produced by malignant tumor cells and, thus, might counteract CD95L-mediated immune responses. By a similar mechanism, DcR3 may contribute to immune escape from tumors by binding to the ligand LIGHT, which is highly expressed by activated T cells and induces apoptosis in tumor cells [4]. Roth et al demonstrated that human malignant gliomas express DcR3 *in vivo*, that enhanced expression of DcR3 suppresses CD95L-induced apoptosis *in vitro*, and that DcR3-expressing glioma xenografts are less prone to immune cell infiltration than non-DcR3-expressing glioma xenografts [1]. Conceivably, DcR3 secreted by tumors might protect them against apoptosis and, consequently, tumors secreting DcR3 would gain a survival advantage. Wu et al demonstrated that serum DcR3 should be considered as a novel parameter for the diagnosis, treatment, and prognosis of malignancies [5]. In their studies, 47 of 48 healthy individuals and patients with acute infection were serum DcR3-negative. In contrast, 82 of 146 tumor patients were serum DcR3-positive. Almost all serum DcR3-positive individuals (82 of 83 cases) had malignancy, excluding one case of liver cirrhosis.

In the study of Roth et al, DcR3 was detected in the supernatants of most glioma cell samples *in vitro* [1]. There are no reports in the literature of serum DcR3 concentrations in patients with brain tumors. Our study showed detectable serum concentrations of DcR3 in glioma patients. The preoperative serum concentration of DcR3 in glioma patients was not significantly different from that in control patients. Furthermore, the preoperative serum DcR3 concentration

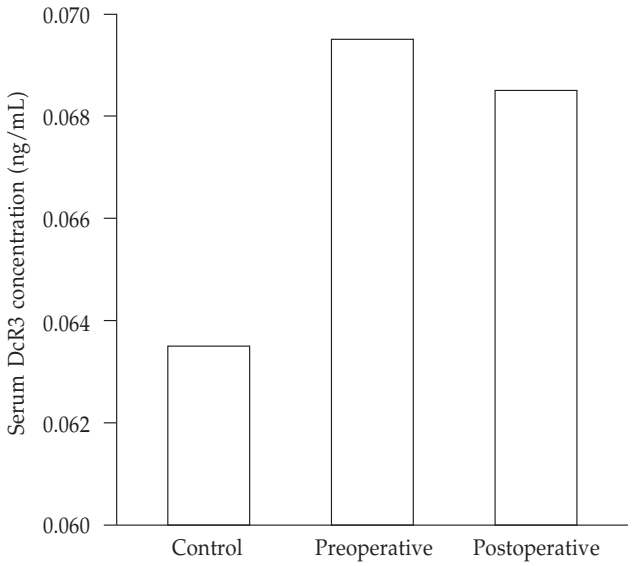

Figure. *Serum concentrations of decoy receptor 3 (DcR3) in both glioma patients and controls.*

in glioma patients was not significantly different from the postoperative concentration. However, the serum DcR3 concentration in glioma patients was very low. Wroblewski et al demonstrated that DcR3 was degraded rapidly to a major circulating metabolic fragment after subcutaneous administration in primates and mice [6]. Although the metabolism of DcR3 in brain tissue is unknown, our data suggest that DcR3 is rapidly degraded. Another explanation may be that the blood–brain barrier influences the transfer of DcR3 from brain tissue to the systemic circulation. DcR3 expression is localized mainly in glioma cells in areas surrounding large ischemic necrosis [1]. Interestingly, this expression pattern is similar to that of CD95 [7,8], suggesting the co-regulation of CD95 and DcR3 expression. DcR3 up-regulation may reflect a protective mechanism from CD95-mediated cell death. However, the relationship between the expression of DcR3 in tumor cells and serum DcR3 concentrations needs further investigation.

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腦神經膠質瘤患者手術前後 DcR3 血清濃度的變化

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免疫反應抑制作用於惡性腦神經膠質瘤中被証實，與腫瘤的發生也有相關。最近發現的 decoy receptor 3 (DcR3) 可以結合 CD95L 和 LIGHT ，抑制兩者的程式性自殺 (apoptosis) 的作用。目前只有少數文獻報導惡性腦神經膠質瘤分泌 DcR3 。本研究以 17 位惡性腦神經膠質瘤為對象，抽血檢查 DcR3 的血清濃度。手術前的 DcR3 的濃度 0.069 ± 0.027 ng/mL ，手術後為 0.068 ± 0.022 ng/mL ，兩者並無統計學的意義 (p = 0.951)。與控制組的 DcR3 濃度 (0.063 ± 0.023 ng/mL) 比較，惡性腦神經膠質患者的 DcR3 濃度也無統計學上的差異 (p = 0.106)。我們的研究顯示，惡性腦神經膠質瘤手術前後的 DcR3 血清濃度並無明顯差異，惡性腦神經膠質瘤患者與控制組 DcR3 血清濃度也並無差異，結果顯示 DcR3 雖是一種可溶解接受器（soluble receptor），其作用可能侷限於分泌細胞附近。因此，腫瘤細胞 DcR3 的表現與血清 DcR3 濃度的關係需進一步研究釐清。

關鍵詞：decoy receptor 3 ，神經膠質瘤

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