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TGF-β1, TNF-α and cytochrome *c* in human astrocytic tumors: A short-term follow up and correlation with survival

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Abstract

Objectives: To evaluate the association of signals of apoptosis namely, TGF-β1, TNF-α and cytochrome *c* release in cytoplasm with survival rate to determine the potential use of such parameters as predictive markers for patients with astrocytomas.

Design and methods: We measured TGF-β1, TNF-α and cytoplasmic cytochrome *c* in 30 astrocytic tumors Grade II, III and IV.

Results: We found that TNF-α and cytochrome *c* release in Grade IV tends to be significantly lower than those in Grade II, whereas TGF-β1 did not significantly change in the different grades. Patients with astrocytic tumors having elevated cytochrome *c* showed a better survival rate compared to those with less release. There is neither a correlation shown between TNF-α and cytochrome *c* release nor between TNF-α and patient survival. TGF-β1 was positively correlated with cytochrome *c* release. Patients showing such correlation had increased survival rate over 18 months follow up period.

Conclusion: These data suggest that TGF-β1 and cytochrome *c* may be useful prognostic markers that help patients' stratification and in adjusting the disciplines of therapy.

Keywords: TGF-β1; TNF-α, Cytochrome *c*; Apoptosis; Gliomas; Survival

Introduction

Glial tumors present with general signs and symptoms of increased intracranial pressure and/or focal manifestations related to the specific area of the brain occupied by the lesion. Gliomas are considered the most common occurring malignancies in the central nervous system [1].

Astrocytic tumors, one type of gliomas, may be categorized into four groups according to World Health Organization (WHO): pilocytic astrocytomas (Grade I); including lesions with low proliferative potential, diffuse astrocytomas (A; Grade II), anaplastic astrocytomas (AA; Grade III) and glioblastoma (GBM; Grade IV) [2]. The latter is a highly malignant astrocytic tumor that is resistant to current antineoplastic strategies such as radiation and chemotherapy. Generally, the prognosis of patients with Grades III and IV is poorer than that of patients with Grade II (low Grade) astrocytoma. However, there are some exceptions as some patients with glioblastomas show better prognosis, whereas others with diffuse astrocytomas show bad prognosis. Glioblastomas are also divided clinically and biologically into two types: primary glioblastoma or de novo that progresses rapidly, 95% more common [3] and affects patients of different ages [4] and secondary glioblastomas which are other different disease entities that develop more slowly and progressively from low grade tumor diffuse or anaplastic astrocytomas [5]. This necessitates the detection of new predictive prognostic markers that help differentiate the variable grades and the different subtypes. To our knowledge, there are no widely recognized or validated biological markers that predict outcomes of patients with these tumors. This limits

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both the ability of clinicians to optimally prognosticate and the clinical researches to effectively stratify patients for therapy [6].

Apoptosis has been examined previously in pre-treatment specimens of patients with a variety of tumors including brain tumors [6–8]. High levels of apoptosis may predict a good outcome for patients with glioblastoma and medulloblastoma [7,8]. Schiffer et al. [9] found that high levels of apoptosis correlated weakly with poor survival for patients with oligodendroglioma. They suggest that the prognostic value of apoptosis might be tumor-specific.

The study of apoptotic changes in these tumors may give a prognostic marker for these malignancies [6]. Apoptosis is one of the common findings in several tumor types [10,11]. The inability of tumor cells to undergo apoptosis may be an important mechanism of resistance to current antineoplastic strategies. Low levels of spontaneous apoptosis might correlate with enhanced resistance to cytotoxic treatment strategies [12]. In most tumor types, apoptosis is thought to be contributing significantly to the response of tumors to these modalities [6,12]. The degree of apoptosis observed in tumor specimens obtained prior to the initiation of cytotoxic therapy might predict prognosis, especially in tumors generally resistant to therapy [13]. Previous studies have shown that the degree of apoptosis detectable in tumors correlates with patient survival with various glial tumors [7,9]. Other studies did not find such correlation between apoptosis and the grade of malignancy [14,15] or outcome of patient [16–18]. Such controversy is marked also in those studies that demonstrated a negative correlation [19,20].

The apoptotic process is characterized by signals that are transmitted from various receptors, eventually resulting in the activation of protein-splitting enzymes called caspases [21]. Gliomas are characterized by deregulation of growth factor production and growth factor receptor expression, e.g. over-expression of cytokine transforming growth factors (TGF-β) and over-expression/constitutive activation of receptors for epidermal growth factor (EGF). TGF-β enhanced, inhibited or had no significant effect on proliferation [22].

Transforming growth factor β signal leads to a number of biological end points that involve cell growth, differentiation, morphogenesis and apoptosis [23]. Gliomas synthesize and secrete TGF-β1, 2 and 3 which down-regulate monocytes surface marker expression, cytokine secretion and cytotoxicity and T-cell responsiveness [24]. TGF-β1 is an antiproliferative and proapoptotic cytokine for astrocytoma cells [25]. TGF-β1 rapidly induces apoptosis through activating caspases. TGF-β1 induced apoptosis also occurs via the release of cytochrome *c* and the subsequent oligomerization of Apaf-1 into apoptosome complex. TGF-β1 induction of apoptosis is a receptor-mediated event that operates through the mitochondrial/Apaf-1 caspase activation pathway [21].

Among the cytokines that mediate the induction of apoptosis is tumor necrosis factor (TNF-α), the prototypic member of a family of cytokines that interact with a corresponding set of a receptor (TNFR) family. TGF-β and TNF-α were expressed in glial cells or extracellular spaces in the area of peri-tumoral edema. The expression of TNF-α in the area of peri-tumoral edema may indicate that these proteins are not utilized for tumor growth, but may be used to guard the brain against tumor invasion [26].

In order to understand the link between apoptotic changes and clinico-pathological parameters of patients with astrocytomas, we proposed to measure TGF-β1, TNF-α levels and mitochondrial cytochrome *c* release in tumor tissues excised from these patients. This will help the identification of some helpful prognostic parameters that assist in modulation of the post-surgical therapeutic intervention.

Materials and methods

Patients

This study was conducted on 30 patients with cerebral astrocytomas diagnosed at the Department of Neurosurgery, Al-Azhar University Hospitals. The diagnosis was made using exclusion criteria. Tumors affecting the brain stem and cerebral regions were excluded, and we focused on tumors of cerebral origin. All specimens were obtained from the initial surgery performed from April 2003 to March 2004. The patient ages ranged from 5 to 65 years with mean ± SD (40.8 ± 20.07). They were 12 males (40%) and 18 females (60%) patients. The distribution of tumor grades (according to the World Health Organization distribution system) [5] was as follows: Grade II, $n=12$ (40%); Grade III, $n=8$ (26.67%); and Grade IV, primary glioblastoma multiform, $n=10$ (33.33%). No cases were found to be Grade I or secondary glioblastoma. Survival rates were calculated using clinical follow up until 18 months.

All gliomatous tissues were excised without any thermal or chemical agents as much as possible with the precaution of conserving neurological function. The tissues obtained are mainly from the active tumor around the center, were divided equally in size, half of them was processed for histopathological evaluation and the second was washed with ice-cold saline and stored immediately at −70°C for further biological studies (measurement TNF-α, TGF-β1 and cytochrome *c* release). Patients were then divided into two groups based on the median values obtained for each analyzed parameters and were followed up for survival over 18 months of duration.

Materials

Human transforming growth factor (TGF-β1) enzyme immunoassay (EIA) and human tumor necrosis factor (TNF-α) EIA Kits were purchased from Quantikine (R&D system, USA). Nitrocellulose (NC) membrane was obtained from Bio-Rad (USA). Mouse monoclonal antibody clone 4CYTC-21 against heart human cytochrome *c* was purchased from R&D system (USA). Rabbit anti-mouse IgG1-ALP and the 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitroblue tetrazolium (NBT) kit were purchased from Zymed Laboratories INC, (San Francisco, CA, USA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).