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AP-1 and heat shock protein 27 expression in human astrocytomas

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Abstract Purpose: In previous work we have shown that the expression of heat shock protein 27 (Hsp27) is associated with anaplastic potential of astrocytomas (Anticancer Res 1997, 17:2677–2682). Heat shock protein-coding genes have been found to have a putative AP-1 (activator protein-1)-binding site in their promoter region and the synthesis of these proteins is induced by the same extracellular stimuli that also activate AP-1 (a homo/heterodimer of members of the Jun and Fos families). In order to detect the putative relation of Hsp27 with AP-1 activation in human astrocytomas we examined eighty astrocytic tumors with different grades of malignancy for c-Jun, c-Fos, and Hsp27 expression. Methods: Six pilocytic astrocytomas (WHO grade I), 15 diffuse fibrillary astrocytomas (WHO grade II), 19 anaplastic astrocytomas (WHO grade III), and 40 glioblastomas multiforme (WHO grade IV), were studied by immunohistochemistry using monoclonal and polyclonal antibodies directed against human Hsp27, c-Fos, and active (phosphorylated) forms of c-Jun (p-c-Jun). Monoclonal antibodies against the phosphorylated forms of the over-expressed MAP kinases JNK (c-Jun N-terminal kinase) (p-JNK) and p38 (p-p38) were also used. Results: Overexpression of p-c-Jun, c-Fos and p-JNK was observed in the majority of glioblastomas (grade IV) whereas only minimal expression was noted in diffuse fibrillary astrocytomas (grade II). Hsp27 expression was observed only in the tumor specimens where c-Jun and c-Fos were co-expressed. AP-1/Hsp27 co-expression was associated with ascending grading of malignancy and it was independent of the proliferation index of the tumors. Conclusions: Our findings suggest that during malignant progression of astrocytomas there is activation of signal transduction cascade(s) culminating in AP-1 induction.

Keywords Astrocytoma · AP-1 · Hsp27 · MAP kinases · Glioma progression

Introduction

Some astrocytomas, e.g., pilocytic astrocytomas, are usually indolent neoplasms which only exceptionally undergo anaplastic (malignant) transformation or exhibit aggressive behaviour. On the other hand, diffuse fibrillary astrocytomas have a well-known tendency for malignant progression (Russell and Rubinstein 1989). A large number of factors have been used to determine the anaplastic potential and the prognosis of these tumors (Smith and Jenkins 2000). Recently, it has been found that Hsp27, a member of the small heat shock protein family (Ciocca et al. 1993) is consistently expressed in poorly differentiated astrocytomas (Khalid et al. 1995; Hitotsumatsu et al. 1996; Assimakopoulou et al. 1997) but the underlying mechanism of this expression is still obscure.

Heat shock protein-coding genes have been found to have a putative AP-1-binding site (Hosokawa et al. 1993; Dale et al. 1996) in their promoter region and the synthesis of these proteins is induced by the same extracellular stimuli that also activate AP-1 (Munell et al. 1994; Harada et al. 1997; Gilby et al. 1997; Dubé et al. 1998). Activation of transcription factor AP-1 is the last step in a signal transduction pathway which is stimulated – via reversible phosphorylation events – by members of the MAPK (mitogen-activated protein kinase)/SAPK (stress-activated protein kinase)/JNK (c-jun N-terminal kinase) family (Whitmarsh and Davis 1996).

AP-1, a homo/heterodimer of members of the Jun and Fos families of bZIP nuclear proteins (Papavassiliou...
1995), is characterized by its ability to modulate gene expression in response to growth factors but also to various other stimuli including thyrotropic hormone, neurotransmitters, heat shock and tumor promoters, carcinogens, ultraviolet light or ionizing radiation (Angel and Karin 1991). Overexpression of AP-1 has been implicated in the pathogenesis and in malignant progression (Arbuthnot et al. 1991; Magrisso et al. 1993; van der Burg et al. 1995; Franchi et al. 1998; Volm et al. 1998) of several human tumors, but there are only a few reports of c-Jun or c-Fos expression in human glial tumors (Orian et al. 1992; Ferrer et al. 1996).

In this work, we examined the expression of c-Jun and c-Fos (AP-1) and JNK, p38 kinases in human astrocytomas of different grades of malignancy and the putative relation of AP-1 with Hsp27 expression in these tumors.

Materials and methods

Tissue samples

Specimens from 80 patients (25 female and 55 male) with astrocytomas who underwent surgery at the Neurosurgery Department of Patras University School of Medicine during a 5-year period (1991–1996) were retrieved from the files of the Department of Pathology, University of Patras Hospital, Patras, Greece. All specimens were originally diagnosed according to conventional histopathologic criteria based on the 1993 World Health Organisation (WHO) “Histological Typing of Tumours of the Central Nervous System” (Kleihues et al. 1993) and classified as ploidy astrocytoma (WHO grade I) (n = 6) (age range, 2–25 years, mean age, 16 years), diffuse fibrillary astrocytoma (WHO grade II) (n = 15) (age range, 11–56 years, mean age, 30 years), anaplastic astrocytoma (WHO grade III) (n = 19) (age range, 11–67 years, mean age, 43 years), and glioblastoma multiforme (WHO grade IV) (n = 40) (age range, 3–77 years, mean age, 52 years). Seventy-five tumors were supratentorial and five were cerebellar (all ploidy). Of the 80 astrocytic tumors, 68 were primary excisions upon initial clinical presentation and 12 had received prior radiotherapy.

Antibodies

Hsp27 (Clone G3.1; Biogenex, San Ramon, Calif., USA) is a mouse monoclonal antibody raised against a 24–27 KD estrogen-regulated heat shock protein. p-c-Jun (KM-1, Santa Cruz) is a mouse monoclonal IgG1 antibody raised against a peptide corresponding to amino acids 56–69 of c-Jun of human origin which reacts with c-Jun p39 phosphorylated on Ser-63. p-Jun (060659 Biomol) is a polyclonal antibody against the active form of c-Jun (phosphorylated on Ser 73 within the transactivation domain). It is known that these phosphorylations of c-Jun – on Ser-63 and Ser-73 – are both necessary for c-Jun activation following a variety of extracellular stimuli (Whitmarsh and Davies 1996). c-Fos (sc-52-G, Santa Cruz) is a goat affinity-purified polyclonal antibody raised against a peptide mapping to the amino terminus of human c-Fos p62. p-JNK (G-7, Santa Cruz) is a mouse monoclonal IgG1 antibody raised against a peptide corresponding to amino acids 183–191 mapping at the carboxy terminus of JNK1 of human origin phosphorylated on Thr-183 and Tyr-185 (identical to corresponding JNK2 sequence). p-p38 (D-8, Santa Cruz) is a mouse monoclonal IgM antibody corresponding to a short amino acid sequence containing phosphorylated Tyr-182 of p38 of human origin. Antibodies were used at dilution of 1:100 except of c-Fos (1:200). All tumors examined in this study were also stained with mouse monoclonal antibodies to glial fibrillary acidic protein (GFAP) (clone 6F2) (IgG1) (Dako, Santa Barbara, Calif., USA) and Ki-67 (MIB-1, Ylem, Italy).

Immunohistochemistry

Immunoperoxidase studies were performed on formalin-fixed, paraffin-embedded tumor sections according to the biotin–strep-tavidin peroxidase method as previously described (Assimakopoulou et al. 1997), using StrAvigen Multilink, Super Sensitive Immunodetection System B-SA (Biogenex, San Ramon, Calif., USA). For c-Jun, p-JNK, and p-p38 antigen retrieval was performed additionally following a microwave-based method as previously described (Assimakopoulou et al. 1998). Negative controls included unrelated primary antibodies. As positive controls, specimens of osteosarcomas (Franchi et al. 1998) known to express c-Jun and c-Fos and specimens of breast carcinomas (Ciocca et al. 1993) known to express Hsp27 were used.

Analysis of staining and statistical methods

Tumor cellularity, staining intensity, and proportion of positive tumor cells were assessed by light microscopy. Counting of immunolabeled tumor cells was performed manually. Using a final magnification of 400× (objective × eyepiece), ten non-overlapping fields were chosen at random and a total of 100 tumor cells were counted in every field with the aid of an ocular grid. To assess the fraction of immunolabeled cells in specimens from each patient case and each antibody, the labeling index (LI), defined as the percentage of positive (labeled) cells out of the total number of tumor cells counted, was determined. Tumors with LI≥1 (1% immunopositive cells) were considered as positive.

The statistical significance of differences in LIs between groups was evaluated by one-way analysis of variance. Relationships between LIs as well as relationships between LI and patient’s age were evaluated by linear regression analysis and Pearson’s correlation coefficient. The Student’s t-test was performed in order to compare the mean LIs for male and female. P values less than 0.05 were considered significant (Fleiss 1981).

Results

Expression of AP-1

The expression of c-Jun was linked to a specific nuclear distribution of its phosphorylated, fully active form (p-c-Jun) and all data regarding c-Jun expression concern this active form of c-Jun. Positive c-Jun and c-Fos staining was mainly localized in the nucleus, although in some tumors both nuclear and/or cytoplasmic staining was observed. Only the nuclear staining was evaluated for both c-Jun and c-Fos oncoproteins. Nuclear immunopositivity (LI≥1) was observed in 59/80 (74%) tumor specimens for c-Jun and c-Fos (Table 1).

There was considerable staining heterogeneity among different tumors but also within individual tumors (intratumoral heterogeneity). Diffuse fibrillary astrocytomas (grade II) contained a small number of scattered (Fig. 1d) and occasionally small clusters of immunoreactive tumor cells and in most instances these cells showed cytologic atypia. In anaplastic astrocytomas, a small number of scattered immunoreactive tumor cells was also observed. Gemistocytic cells were always positive. Strong c-Jun and c-Fos immunostaining was