Expression of platelet derived growth factor and platelet derived growth factor receptor mRNA in a glioblastoma from a patient with Li-Fraumeni syndrome

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Abstract

Expression of platelet derived growth factor (PDGF) and PDGF-receptor mRNA was examined from a glioblastoma taken from a patient with Li-Fraumeni syndrome. Northern blot analysis and in situ hybridisation showed very high concentrations of both PDGF-A and PDGF α -receptor mRNA in the tumour. The overall pattern of PDGF expression was similar to those found in sporadic glioblastomas. Mutations in p53 has been implicated as an early pathogenic event leading to sporadic low grade astrocytomas, and is the third most common tumour type in patients with Li-Fraumeni syndrome, where they are predisposed due to a germline mutation in the p53 tumour suppressor gene. This study suggests that progression towards a glioblastoma in both the general population and in patients with Li-Fraumeni syndrome may involve potential autocrine and paracrine stimulation by growth factors such as PDGF.

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Patients with Li-Fraumeni syndrome harbour a germline mutation in the p53 tumour suppressor gene, and have a 50% chance of developing at least one malignancy by age 30.¹ Astrocytomas, occurring at a younger age than in the general population, are the third most common of the six cancer types presently associated with the syndrome. Malignant astrocytomas from patients with Li-Fraumeni syndrome and those from the general population are pathologically indistinguishable.

Both PDGF and PDGF receptors are expressed by most malignant astrocytomas cell cultures, and their tumorigenic growth can be reversed by blocking PDGF autocrine and paracrine stimulation.² Recently we have identified increased concentrations of PDGF and PDGF receptors at both the mRNA and protein level in sporadically occurring glioblastoma specimens taken at operation.³ Whether PDGF is also important in the development of glioblastomas where there is a

cancer predisposing germline defect, such as in the Li-Fraumeni syndrome, is not known and is the subject of this study.

Case and family history

A 26 year old man presented with a three month history of headaches. Brain CT showed a left frontal enhancing mass, which was subtotally resected and diagnosed as a glioblastoma (specimen OR1). Five months after external radiation, progressive symptomatic enlargement of the residual tumour necessitated a second debulking (specimen OR2). Lesions were noted in both hemispheres by 18 months, and were confirmed by biopsy to be multifocal glioblastomas. Members of four successive generations in the patient's family had succumbed to malignancies at an early age, in an autosomal dominant pattern of cancer transmission with high penetrance (fig 1). On this clinical-epidemiological basis, a diagnosis of Li-Fraumeni syndrome was made. Our patient was the first member with a verified CNS tumour in this family.

Methods

TISSUE COLLECTION

Specimens for northern analysis (OR1 OR2) were snap frozen. For in situ hybridisation, the specimens (OR2 only) were immediately immersed in freshly prepared 4%

Figure 1 Family pedigree (courtesy of F P Li and M Dreyfuss) with an autosomal dominant pattern of cancer transmission through multiple generations, leading to a clinical-epidemiological diagnosis of Li-Fraumeni syndrome. The patient is identified with an arrow. B = breast carcinoma; BB = bilateral breast carcinoma; S = sarcoma; OS = osteosarcoma; MESO = mesothelioma; SCC = squamous cell carcinoma; CN = CNS tumour (glioblastoma).

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Figure 2 Northern blot analysis for PDGF-*A* and *B* (A) and PDGF α and β -receptor (B) mRNA. Two specimens of glioblastoma from the patient with Li-Fraumeni syndrome (OR1 and OR2) and cell lines serving as positive controls [U343 (glioblastoma) for PDGF-*A* and *B*; U373 (glioblastoma) for PDGF α -receptor and SK76 (human fibroblast; FIBR) for PDGF β -receptor] were examined. A specimen labelled BRAIN, from a patient diagnosed with mesial temporal sclerosis was used to represent non-neoplastic brain. Roughly equal amounts of RNA were transferred on the blots as seen by the corresponding ethidium bromide stained 28S ribosomal RNA bands. PDGF α -receptor mRNA was greatly increased in the glioblastomas, especially in the OR1 specimen, whereas PDGF β -receptor mRNA was barely detected. PDGF-*A* (2.8, 2.2, and 1.6 kb) and to a lesser degree PDGF-*B* mRNA were both increased in the glioblastoma specimens.

paraformaldehyde/phosphate buffered saline (PBS) solution, and then immersed in a 30% sucrose/PBS buffer overnight. Cryosections of 6 μ m thickness were made on gelatin pre-coated slides.

NORTHERN BLOT ANALYSIS

Total cellular RNA (20 μ g) was isolated on a caesium chloride gradient, separated on a formaldehyde denaturing gel and transferred to Duralon (Stratagene, CA). The RNA was cross linked, prehybridized for two hours at 42°C, and subsequently hybridised with [32 P] labelled cDNA PDGF probes overnight. The random hexamer priming technique was used for labelling the human cDNA PDGF (A and B) and PDGF (α and β) receptor probes. Filters were washed in 0.1M tri-sodium citrate plus 0.1% sodium dodecyl sulphate at 50°C for 30 minutes and exposed to x ray film for either 44 (fig 2A) or 24 hours (fig 2B).

IN SITU HYBRIDISATION

Around 400–500 base pair fragments from the coding regions of human cDNA PDGF clones were cloned into Bluescript plasmid (Stratagene, CA). T7 RNA polymerase generated antisense and T3 RNA polymerase generated sense [35 S] UTP (New England Nuclear) labelled riboprobes. The protocol was similar to that outlined in Current Protocols of Molecular Biology,⁴ with minor modifications. The slides were developed

after one week and counter stained with haematoxylin and eosin.

Positive controls for each in situ experiment included cells that were shown to express the appropriate RNA by northern blot analysis (fig 2A and 2B). Negative controls included RNAase pretreated sections and sense strand riboprobe (fig 3A and 3B). Immunohistochemistry with specific antibodies to the two PDGF ligands and receptor subunits were undertaken on cryosections from the same block used for in situ experiments (data not shown).

Results

The most striking result was the amount of PDGF α -receptor mRNA expression by the glioblastoma specimen taken at OR1 (fig 2B). Many areas from OR2, analysed by in situ hybridisation, consisted of necrotic glial tissue with scattered large pleomorphic cells, expressing PDGF α -receptor mRNA (arrow in fig 3B). By contrast, PDGF β -receptor was undetectable in OR1 by northern analysis. The β -receptor signal was detectable by northern blot analysis from OR2, but was much lower compared with the PDGF α -receptor signal (fig 2B). In situ hybridization for PDGF β -receptor on the OR2 specimen, however, showed that the number of grains localising to the large pleomorphic cells was not increased compared with the negative

Figure 3 In situ hybridisation with [35 S] labelled PDGF-A and B (A) and PDGF α and β -receptor (B) antisense riboprobes on cryosections of OR2 glioblastoma specimen. Sense strand riboprobes served as the negative control. Increased PDGF-A and α -receptor mRNA signal were localised to the large pleomorphic tumour cells (arrows), which were GFAP positive on sections taken from the same tissue block (data not shown). PDGF-B mRNA signal in these cells was also increased, but to a lesser extent when compared with the background signal from the sense strand. PDGF β -receptor mRNA signal was hardly detectable.

control (fig 3B). The overall pattern of PDGF receptor expression was similar in six sporadically occurring glioblastomas previously analysed.³

Three PDGF-A mRNA transcripts (2.8, 2.2 and 1.6 kb), the result of alternate splicing, were detected (fig 2A). In situ hybridisation corroborated the increased PDGF-A mRNA in the tissue from OR2 (fig 3A), localising to the same larger pleomorphic cells expressing PDGF α -receptor mRNA (arrows in fig 3A and 3B). By comparison, PDGF-B

mRNA expression was minimal, a difference that was most evident in the specimen from OR1 (fig 2A). PDGF-B mRNA was detected by in situ hybridisation in the large cells from OR2; however, the concentration of the grains were much less than for PDGF-A. Similar to the patient with Li-Fraumeni syndrome most sporadically occurring glioblastomas had increased concentrations of both PDGF-A and PDGF-B mRNA.³ Immunohistochemical analysis of adjacent tumour sections used for in situ experiments with antibodies specific to PDGF ligands and PDGF receptor subunits, showed expression similar to the data for the mRNA (data not shown).

Discussion

The identification of PDGF and its receptors in glioblastoma cell cultures and specimens from operation suggests that PDGF is an important mitogen for astrocytomas.^{3,5} In sporadic low grade astrocytomas, amounts of PDGF and PDGF receptors are lower than their malignant counterpart, suggesting that PDGF is important in the malignant progression of these tumours. This was supported by our findings that blocking PDGF stimulation of malignant human astrocytoma cell lines with PDGF dominant negative mutants, reverted their tumorigenic growth in cell cultures and nude mice.² This study shows that the overall expressions of PDGF and PDGF receptor mRNA are similar in a patient with Li Fraumeni syndrome with a glioblastoma, and in those previously found in sporadic high grade tumours.³ Abundant PDGF-A and PDGF α -receptor mRNAs were expressed and localised to the large pleomorphic tumour cells, with the potential for PDGF stimulated growth. The tumour cells also expressed PDGF-B that could activate the PDGF α -receptor, although at lower levels than PDGF-A. In sporadic glioblastoma specimens, overexpression of PDGF α -receptors may reflect amplification of the α -receptor gene.⁶ In one reported case the PDGF α -receptor was also rearranged, resulting in the truncation of a portion of the extracellular domain involved in binding of PDGF.⁷ It is not known if altered or the normal overexpressed PDGF α -receptors in malignant astrocytomas are active, either constitutively or through binding of the indigenously expressed PDGF.

Li-Fraumeni syndrome was recognised in 1969 from clinical-epidemiological data of patients with sarcoma in childhood, with a high incidence of familial cancers.⁸ The cancers associated with the syndrome present at a young age, are often multiple, and are transmitted in an autosomal dominant fashion from one generation to the next. These characteristics of Li-Fraumeni syndrome are clearly shown in the pedigree of our patient. Presently, six types of cancers are associated with the syndrome (soft tissue sarcoma, osteosarcoma, breast cancer, acute leukaemia, adrenocortical carcinoma, and astrocytomas), all of which are pathologically similar to their

more common sporadic counterparts.¹ Astrocytomas are the third most common associated with the syndrome, often occurring before the age of 15, with the rest usually before the age of 45. Most of these are malignant in nature and do not differ pathologically from those present in the general population.

Germ line mutations of one p53 allele were initially detected in fibroblasts from six Li-Fraumeni families.¹ Exon 5-8 was examined, as it encoded the highly conserved and DNA binding region of the p53 protein, which is probably vital for its function. Most of the p53 mutations reported from a variety of sporadically occurring cancers are clustered within this region of the p53 gene.⁹ Loss of activity from the remaining normal p53 allele, through a second somatic mutation or deletion, would lead to tumours in these patients at a higher rate than those not carrying the germ line defect.

Further examination of larger numbers of clinically diagnosed Li-Fraumeni families, however, showed germ line p53 mutations in only 50% of the families.¹⁰ The family in this report illustrates such a case, as no germ line p53 mutations were detected within the conserved exons 5-8 by the polymerase chain reaction (Li, personal communication). The underlying defect may still reside in mutations of the p53 gene, but perhaps outside the conserved regions first examined. Presently, a more extended search is being undertaken over a wider region of the p53 gene in this and other Li-Fraumeni families. In addition, mutations in cancer susceptible genes other than p53 may be transmitted in a subset of Li-Fraumeni families. Amplifications of genes such as MDM2,¹¹ the protein products of which bind and block the function of normal p53, is yet another possible genetic mechanism for a subset of Li-Fraumeni families where germ line p53 mutations cannot be identified.

Loss of one copy of the short arm of chromosome 17, near the p53 gene, occurs in 30% to 50% of sporadic astrocytomas.¹² Mutations in the p53 gene between exons 5-8 (conserved region of p53), are present in about one third of all astrocytomas, and two thirds of those tumours with loss of one p53 allele. Although some reports suggest that p53 deletions are only present in higher grade astrocytomas, recent reports show p53 mutations to be a relatively early event. Sidransky *et al*¹³ reported that a small subpopulation of low grade astrocytoma cells had p53 mutations. The growth advantage provided from inacti-

vation of p53 resulted in these cells becoming the dominant cell type as the tumour progressed to a higher grade.

The molecular pathogenesis of sporadic astrocytomas still remains unclear. Previous results suggest that PDGF provides mitogenic stimulation for malignant astrocytomas, a pathogenetic mechanism that is possibly common to development of these tumours in a genetically predisposed patient. Whether other growth factors or receptors (epidermal growth factor, fibroblast growth factor, etc) implicated in the pathogenesis of sporadic malignant astrocytomas are also found in these tumours associated with Li-Fraumeni syndrome needs to be studied. Examination of rare familial cancer syndromes should allow greater insights into their more common sporadic counterparts, as their molecular pathogenesis are likely to be similar.

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