EXPRESSION OF TGFβ IN HUMAN GLIOMAS

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INTRODUCTION

Tumour Growth Factor β (TGFβ) isoforms belong to a super-family of cytokines that regulate cell growth and differentiation, cell motility, angiogenesis and apoptosis. Signal is transduced by a family of serine-threonine kinase receptors through activation of SMAD proteins. In cancer, activation primarily occurs after the formation of a complex between TGFβRI and TGFβRII.

Previous in vitro studies have suggested that microglia may promote tumour growth via production of TGFβ family members in the tumour microenvironment.

Our aim is to investigate the expression of TGFβ and TGFβR in astrocytomas and oligodendrogliomas, identify its cell source and discuss its possible role as therapeutic target in patients with.

METHOD

We first reviewed 6 microarray gene profile datasets available in GEO (http://www.ncbi.nlm.nih.gov/sites/entrez) assessing for expression of TGFβ I and II in human gliomas.

Fifty supratentorial adult gliomas (10 diffuse astrocytoma, 10 anaplastic astrocytoma, 20 glioblastoma, 4 low grade and 6 anaplastic oligodendrogliomas) were retrieved from the BTRC Tumour Registry at the Charing Cross Hospital. Patients ages ranged between 25 and 68 years; all received surgical debulking. High grade tumours were then treated with radiotherapy and chemotherapy.

TGFβ1 and TGFβ2 expression and the extent of microglial infiltration were assessed on tissue sections by immunohistochemistry using immunoperoxidase and double immunofluorescence. Antibodies used are shown in Table 1.

TABLE 1

	Manufacturer	Clone	Dilution	Pre-treatment
TGFβI	Santa Cruz Biotechnology	Poly-clonal (TB21)	1:1200	Citrate buffer + 30min MW
TGFβII	Santa Cruz Biotechnology	Poly-clonal (V)	1:400	*None*
TGFβRII	Santa Cruz Biotechnology	Poly-clonal (V)	1:200	*None*
Iba1	Santa Cruz Biotechnology	Mono-clonal (1022-5)	1:75	EDTA + 40min MW

RESULTS

Dataset analysis: The microarray dataset expression of TGFβ genes showed no significant correlation between relative gene expression and glioma grade, either between datasets or within separate probes themselves in each individual dataset.

Immunohistochemistry: In all cases, TGFβI expression was restricted to tumour endothelium and perivascular macrophages. TGFβII expression localised to tumour cells in a subset of 9 astrocytomas (1 low grade astrocytoma, 2 anaplastic astrocytomas and 6 glioblastomas) (Fig. 1). No TGFβI was observed in oligodendrogliomas and no expression was seen in microglial cell (Fig. 2).

Double immunofluorescence studies using Iba-1confirmed the lack of TGFβII in microglia across all tumour subtypes (Fig. 2).

The immunoreaction for TGFβRII was positive in endothelial cells. A non-specific nuclear immunolabelling was seen in neoplastic cells but no obvious expression was observed.

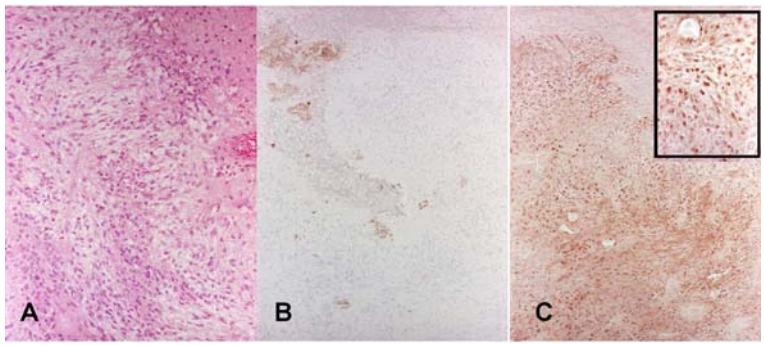

Fig 1: *This case of glioblastoma (A, HE 10x) shows no TGFβI expression in tumour cells (B, perox 10x) whereas TGFβII is extensively positive (C, perox 10x – inset 40x)*

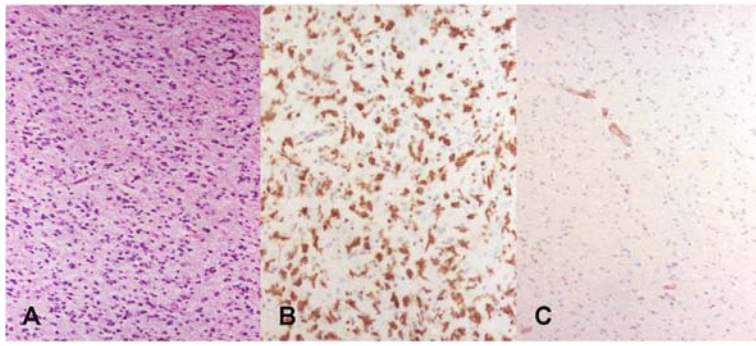

Fig 2: *This example of anaplastic astrocytoma (A, HE 20x) show considerable microglial activation (B, Iba1 perox 20x); no TGFβII is conversely present in tumour and microglial cells (C, perox 10x)*

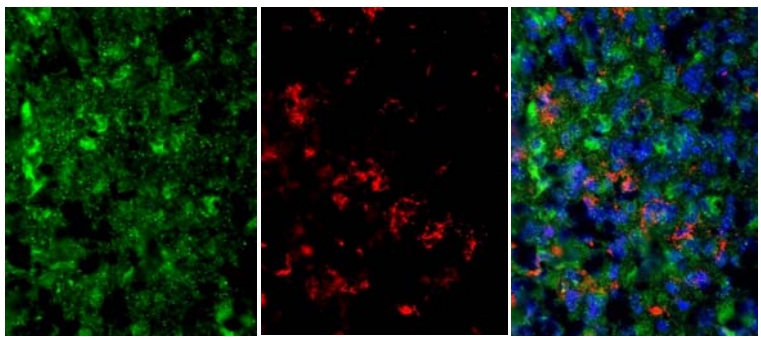

Fig 2: *The colocalisation of Iba1 (A, green, 20x) and TGFβII (B, red, 20x) confirms the lack of TGFβII expression in microglial cells (C, merge – nuclear DAPI, 20x)*

CONCLUDING REMARKS

Our results identify a subgroup of astrocytomas expressing TGFβII and confirm the variability of expression seen with the analysis of expression microarrays.

No autocrine/paracrine mechanism between neoplastic cells and microglial cells is observed. We are therefore unable to confirm *in vitro* studies suggesting that microglial-derived TGFβI promotes glioma growth and invasion *in vivo*.

Our results do not presently support the use of TGFβR antagonist in the treatment of human gliomas.

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