

Karolina Janczar, Emilie Croisier, Bob Homapour, Kevin O'Neill, Amanda Forsyth, Federico Turkhemier, Manuel Deprez*, Federico Roncaroli.
BTRC / Imperial College of London, London, United Kingdom & *Department of Neuropathology, University of Liege, Belgium

Abstract #6022

BACKGROUND

Human astrocytomas express the translocator protein (TSPO), which transfers cholesterol through the mitochondrial membrane and initiates Steroidogenesis.

Previous *in vitro* studies have reported steroid biosynthesis by glioma cell lines.

Synthetic glucocorticoids may interfere with the apoptotic effects of chemotherapeutic drugs.

Glucocorticoids may suppress microglial immune response *in vitro*.

	WHO grade	Number of cases	Age at diagnosis ^a	Male/Female
Diffuse astrocytoma	II	10	37.5 (25-56)	8/2
Anaplastic astrocytoma	III	10	39 (25-61)	5/5
Glioblastoma	IV	10	57 (35-68)	5/5
Oligodendroglioma	II	10	33 (24-49)	5/5
Anaplastic oligodendroglioma	III	10	46.5 (21-69)	3/7

Table 1. Distribution and demographic data of the 50 patients
^a values represent medians; the range of values in brackets

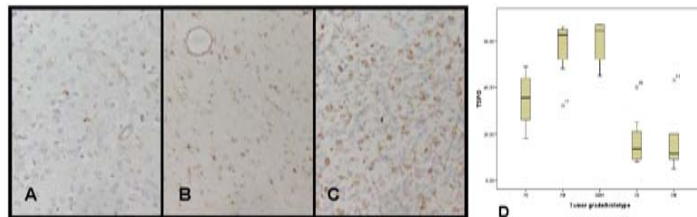


Figure 1. TSPO in gliomas
Grade II oligodendroglioma (a); Diffuse astrocytoma (b); Glioblastoma (c); (ABC x20)
Boxplot (d) represents the distribution of TSPO in tumors of each grade and histotype - o represents outliers and the divider of the box indicates the median.

METHOD

We investigated 30 human infiltrating astrocytomas and 20 oligodendrogliomas (10 examples of each WHO grade) (Table 1) for the expression of the proteins involved in cortisol biosynthesis peroxidase immunohistochemistry

The expression of TSPO, P450scc, P450c17A1 and P450c11B1 in formalin fixed paraffin embedded tissue samples was assessed immunohistochemically using an avidin-biotin peroxidase method with a 3,3'-diaminobenzidine chromagen. For pGR, the Super Sensitive Polymer HRP IHC Detection System (BioGenex Laboratories) was used.

Using immunohistochemistry on frozen sections, 24 were examined for the presence of cortisol with a polyclonal anti-cortisol antibody (Sigma Aldrich) intended for radioimmunoassay. Sections were post-fixed in formalin after 2 hr incubation in primary antibody. The reaction product was revealed using Super Sensitive Polymer HRP IHC Detection System (Biogenex Laboratories).

Immunoblotting was performed on one histologically representative tissue sample from each tumor grade and histotype

Antibody	Clone	Supplier	Dilution
TSPO	8D7	Dr Casellas, Sanofi	1:400
P450scc	D-16	Santa Cruz Biotech	1:50
P450c17A1	N-17	Santa Cruz Biotech	1:50
P450c11B1	N-12	Santa Cruz Biotech	1:50
Cortisol	Polyclonal	Sigma Aldrich	1:50
pGR	Polyclonal	Cell Signaling Tech	1:1000

Table 2. Details of the primary antibodies used for the study (Antigen retrieval procedure for TSPO, P450scc, P450c11B1 and pGR MW in 1mM EDTA buffer pH 8)

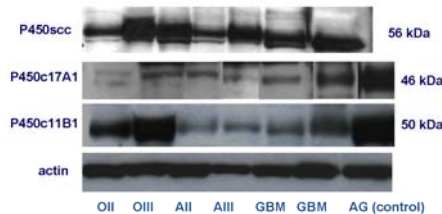


Figure 2. Western blotting analysis of P450scc, P450c17A1 and P450c11B1 in gliomas (OII – oligodendroglioma, OIII – anaplastic oligodendroglioma, AII - diffuse astrocytoma, A III - anaplastic astrocytoma, GBM – glioblastoma, AG – adrenal tissue)

RESULTS AND CONCLUDING REMARKS

- The expression of corticosteroidogenic molecules, cortisol and glucocorticoid receptors increases with grade in astrocytic tumors but remained low in oligodendrogliomas
- Our results unveil a novel autocrine / paracrine mechanism Endogenous cortisol synthesis in neoplastic cells of human high grade
- Endogenous cortisol synthesis may explain chemoresistance of high grade astrocytomas
- Endogenous production of cortisol may explain the suppression of microglial immune response against tumor cells.

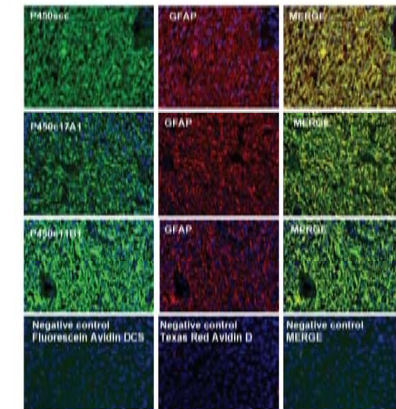


Figure 3. Key steroidogenic enzymes in glioblastoma P450scc, P450c17A1 and P450c11B1 colocalize with GFAP - Bottom panel – negative control (omitting the primary antibody), original magnification 20 x

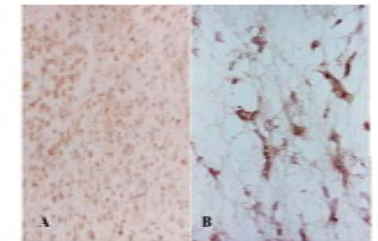


Figure 4. Phosphorylated glucocorticoid receptors (a, x20) and cortisol in a glioblastoma (b, x40)



Acknowledgments

This work is funded by the Brain Tumour Research Campaign.