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Detailed analysis of two genetically altered regions of the genome in astrocytic gliomas by means of chromosomal tile path array-CGH

£147,000 over 3 years
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Summary of Research in Layman's Terms

Astrocytic tumours are the commonest type of human brain cancers. In the World Health Organisation (WHO) classification, they are subdivided into four malignancy grades based on their morphology. The least malignant form is the Pilocytic Astrocytoma grade I. These arise mostly among children, grow slowly, do not invade and do not become more malignant with time, as do the tumours of higher malignancy grade. Patients with Pilocytic Astrocytomas have generally a good prognosis. The astrocytic tumours that are most common in adults include the Astrocytoma (WHO grade II) the Anaplastic Astrocytomas (WHO grade III) and the Glioblastoma (WHO grade IV). The Glioblastoma, the most malignant form of astrocytic tumour, as well as the most common, may develop from an astrocytoma of lower malignancy grade or more commonly arise *de novo*. They grow rapidly, are highly invasive, and are only slightly responsive to both radiotherapy and current anti-cancer drug therapy. Their invasive nature makes surgical removal impossible. Survival at most centres is less than one year. Adult astrocytic tumours show particular patterns of genetic abnormality. The most frequently altered chromosomes are 7 and 10.

Three copies (instead of two) of chromosome 7 is very common in all grades of adult astrocytomas. In addition, multiple copies of the *EGFR* gene (a growth factor receptor gene that promotes cell proliferation and is normally located on the short arm of chromosome 7), are found in over 30% of glioblastomas. When tumour cells have aberrantly made multiple copies of a gene (known as amplification) they generally produce excess amounts of the protein encoded by the gene. Excess *EGFR* may stimulate proliferation. In addition, the multiple copies of the *EGFR* gene are often mutated in a particular way and these mutated gene copies code for an abnormal growth factor receptor that is constantly switched on. The identification of genes targeted by amplification in tumours has led to the discovery of many new cancer related genes. *EGFR* is not the only gene amplified on chromosome 7 in astrocytomas. We have preliminary data showing that the region of chromosome 7p12 amplified in glioblastoma is often large and encompasses many genes and in more than 10 of our glioblastomas the 7p12 region amplified does not include the *EGFR* gene, indicating that other adjacent genes are being targeted. Furthermore we have preliminary data indicating other novel amplified regions on chromosome 7.

Chromosome 10 is abnormal, mostly due to the loss of one copy, in the vast majority of glioblastomas and in a small percentage of anaplastic astrocytomas. The single retained copy of a gene located on the long arm of chromosome 10 and that is involved in growth control and programmed cell death called *PTEN* is mutated in only half of the tumours that have chromosome 10 loss, suggesting that there may well be other target genes on this chromosome. Recently, trials with a new drug called Temozolomide, have shown it to be particularly effective in patients whose glioblastoma cells lack a protein coded by the gene *MGMT* that repairs the damage to DNA cause by Temozolomide. *MGMT* is also located on the long arm of chromosome 10. In addition there is evidence that some tumours have gains of small regions of the short arm of chromosome 10.

In this study we aim to investigate, at a level of detail previously not possible, these two chromosomes in astrocytic tumours. We have a unique collection of over 300 astrocytic tumours that have been extensively studied for other abnormalities over the last 10 years. We believe there are as yet unidentified genes on chromosomes 7 and 10 that are involved in the development and/or progression of these tumours. To do this, we will first use CGH-microarray technology which we have successfully established in our laboratory over the last few years. This involves the printing of thousands of DNA fragments, with known sequence, from chromosomes 7 or 10 on glass slides (microarray). Using this microarray, we will be able to obtain a very detailed overview of any abnormalities of copy number at each of the fragments of the chromosome on the array very rapidly in a single experiment. This will identify at a higher resolution than previously possible any regions lost or amplified, where genes of interest for the development of these tumours may be located. We will make several purpose-built microarrays to do this, firstly arrays covering the whole of chromosomes 7 and 10 and then arrays with smaller fragments of DNA covering limited areas and permitting a higher resolution assessment of, for example, amplified regions such as that around the *EGFR* gene at 7p12. When critical regions are identified, candidate genes will be selected for further evidence of their being targeted, such as the presence of significant somatic mutation or consistent amplification and overexpression, using the more conventional molecular biological techniques we have been employing over the last few years.

We believe our study will help us better to understand the biology of this group of devastating brain tumours. The identification of the genes involved may assist in identifying new subgroups of astrocytic tumours, provide prognostic information as well as building the basis for specific, effective, targeted, molecular therapies.