

**Running title:** astrocyte and tumoral transformation

**Astrocytes reverted to a neural progenitor-like state with Transforming Growth**

**Factor alpha (TGF $\alpha$ ) are sensitized to cancerous transformation.**

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Gliomas, the most frequent primitive CNS tumors, have been suggested to originate from astrocytes or from neural progenitors/stem cells. However, the precise identity of the cells at the origin of gliomas remains a matter of debate because no pre-neoplastic state has been yet identified. TGF $\alpha$ , an EGF family member, is frequently over-expressed in the early stages of glioma progression. We previously demonstrated that prolonged exposure of astrocytes to TGF $\alpha$  is sufficient to trigger their reversion to a neural progenitor-like state. To determine whether TGF $\alpha$  de-differentiating effects are associated with cancerous transforming effects, we grafted intra-cerebrally de-differentiated astrocytes. We show that these cells had the same cytogenomic profile as astrocytes, survived *in vivo* and did not give birth to tumors. When astrocytes de-differentiated with TGF $\alpha$  were submitted to oncogenic stress using gamma irradiation, they acquired cancerous properties: they were immortalized, showed cytogenomic abnormalities, and formed high-grade glioma-like tumors after brain grafting. In contrast, irradiation did not modify the lifespan of astrocytes cultivated in serum-free medium. Addition of TGF $\alpha$  after irradiation did not promote their transformation but decreased their lifespan. These results demonstrate that reversion of mature astrocytes to an embryonic state without genomic manipulation is sufficient to sensitize them to oncogenic stress.

Gliomas are highly heterogeneous primitive tumors of the central nervous system and result in poor outcome despite new alkylating and anti-angiogenic treatments. The variability in clinical evolution of patients bearing tumors of similar histopathological appearances and the invasive nature of the tumors impair the development of curative treatments.

The cell at the origin of glioma remains a matter of speculation since no pre-neoplastic state has been identified <sup>1</sup>. Morphological and immunohistological similarities of glioma cells to macroglial cells of the CNS have led to the initial proposal that they derive from astrocytes or oligodendrocytes. Conversely, the coexistence within the same tumor of cells of different phenotypes, and the isolation of tumor stem cells from gliomas <sup>2,3</sup> favor neural progenitors or neural stem cells as the cell of origin of gliomas. The frequent development of gliomas in the vicinity of the sub-ventricular zone, which contains adult neural stem cells, as well as the migratory properties of these cells <sup>4</sup>, further support this hypothesis. Nevertheless, experimental development of gliomas in juvenile transgenic mice shows that cancerous transformation can be achieved with retroviral introduction of oncogenes in both progenitor/stem cells expressing nestin and differentiated cells expressing GFAP, a common astrocyte marker, although the transformation occurs with higher efficacy in immature cells <sup>5</sup>. A possible reconciliation between these opposite sets of data may be that gliomas result not only from alterations in the regulation of cell proliferation and survival, but also from alterations in the differentiation state of mature cells as proposed for other cancers <sup>6</sup>. We previously showed that prolonged exposure of differentiated astrocytes to Transforming Growth Factor ( $TGF\alpha$ ), a member of the Epidermal Growth factor (EGF) family <sup>7</sup>, results in their progressive

and functional reversion, first to neural progenitor-like cells akin to radial glia, and then to cells that exhibit properties of neural stem cells <sup>8</sup>. TGF $\alpha$  and its receptor erbB1 (or epidermal growth factor receptor, EGFR) are involved in the control of both gliogenesis and glioma development. They constitute the signaling module most frequently deregulated in gliomas. TGF $\alpha$  over-expression is found in about 80% of gliomas, and is observed from the initial steps of their development <sup>9</sup>, whereas the over-expression of erbB1 appears in 20–40% of gliomas in later phases <sup>10</sup>. Over-expression of TGF $\alpha$  and/or its receptor are, by themselves, insufficient to achieve cancerous transformation of astrocytes in transgenic mice <sup>11, 12</sup>.

In the context of regenerative medicine, the ability to revert somatic cells to an embryonic state without nucleic acid delivery of reprogramming factors <sup>13, 14</sup> and with a single modification of their environment <sup>8</sup> is of great interest. This interest is reinforced by the recent report that a subset of reactive astrocytes isolated from the brains of stab wound-injured mice may reacquire, at least *in vitro*, neural stem cell properties <sup>15</sup>, and by the known over-expression of TGF $\alpha$  by reactive astrocytes (reviewed in <sup>9</sup>). In light of the deregulation of TGF $\alpha$  expression in the early stages of glioma development, it is important to determine whether reversion of astrocytes to a neural progenitor state predisposes the cells to cancerous transformation. To evaluate this possibility, we used a classical mutagenic treatment, irradiation, to determine whether astrocytes de-differentiated into progenitor-like cells are more sensitive to cancerous transformation than their mature counterparts. Our results show that mature astrocytes, having regressed to a neural progenitor-like stage in response to a single change in their extra-cellular environment, can be the cell of origin of gliomas.

## Materials and methods

### Animals

C57Bl6/J and Nude mice (Charles River, France) were housed in an air-conditioned room with free access to water and food. NOD/SCID mice (Charles River, France) were maintained in microisolator cages in pathogen-free conditions.

### Cell culture and irradiation

Cultures of mouse astrocytes were prepared from cortices of 1-to-2-day-old C57Bl6/J mice as previously described <sup>16</sup>. Briefly, cultures were established in defined MEM:F12 medium containing 10% fetal calf serum (Biowest, Nuaille, France). Medium was changed every 3 days following washes with ice-cold phosphate-buffered saline (PBS, pH 7.4). When confluence was reached (8-10 DIV), cultures were shaken overnight (250 r.p.m.), trypsinised and seeded on poly-L-ornithine-coated glass slides (50,000 cell/cm<sup>2</sup>). The cells were further cultured for 4–6 days with washes with ice-cold PBS preceding each medium renewal, until an 80–90% confluent cell layer had formed. The cultures were then transferred to serum-free medium for 3 days, prior being cultivated for 7 days in defined medium supplemented or unsupplemented with TGF $\alpha$  (50ng/ml, AbCys, France). GFAP-immunoreactive astrocytes accounted for 91–96% of the cells in all cultures types. The remaining 4–9% of cells were CD11b receptor-immunoreactive microglia. TGF $\alpha$  levels present in these astrocyte culture lysates were determined using an anti-human TGF $\alpha$  sandwich ELISA (R&D systems, France) following the manufacturer's instructions. Assays of erbB1 levels and activation were performed as previously described <sup>8</sup> with the following antibodies: anti-erbB1 sc-03-G from Santa-Cruz (TEBU, France) for

immuno-precipitation, anti-erbB1 and anti-phosphotyrosine antibody 4G10 from UpstateBiotechnologies (Lake Placid, NY) for immuno-blotting. Expression of TACE, the enzyme that controls TGF $\alpha$  release from the cells <sup>17</sup>, was verified using Western blot analysis with an anti-TACE antibody from Santa-Cruz (1:250, sc-6416, TEBU, France), as previously described <sup>18</sup>.

Astrocytes cultivated with or without TGF $\alpha$  were irradiated with 5 Gy of  $\gamma$ -radiation using a <sup>137</sup>Cs irradiator (IBL 637, Institut Curie, Paris, France) at a dose rate of 1.57 Gy/min (Fig. 1a). Controls corresponded to sham-irradiated cultures. The cells were maintained at 37°C throughout the experimental procedure, except during irradiation, which was performed at 20°C in ambient air. Two hours after irradiation, the cells were washed with ice-cold PBS, and further cultured in their original medium with changes of media every 3 days. In addition, astrocytes cultivated with or without TGF $\alpha$  were treated with 1  $\mu$ M Tyrphostin AG1478 (La Jolla, CA) between minus 2, and plus 2 hours of the irradiation time-point. AG1478 is a specific inhibitor of erbB1 tyrosine kinase activity <sup>19</sup>, known to inhibit TGF $\alpha$  effects on astrocytes <sup>8, 20</sup>.

Changes in morphology or growth pattern were verified by visual inspection prior each medium renewal. After 40 DIV, medium was switched to a 1:1 mixture of DMEM and F12 nutrient (Invitrogen, France), containing 0.6% glucose, 2 mM glutamine, 13 mM sodium bicarbonate, 5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer, 5 IU/ml penicillin and 5  $\mu$ g/ml streptomycin, and B27, N-2 and G5 supplements (10  $\mu$ l/ml each, Invitrogen) (NS34 medium, <sup>8</sup>). This medium was renewed once per week. Experiments were repeated three times in an independent manner.

### **Proliferation and survival assays**



Seventy days post-irradiation, cultures were trypsinised and counted. Cell viability was determined by Trypan Blue exclusion. To construct growth curves, cultures were submitted to serial passages when reaching 70–90% confluence.

### **Array CGH**

The samples were homogenized, and genomic DNA was extracted according to Qiagen protocols with modifications. Restriction of DNA, quality control of restricted DNA by microanalysis, pooling, and clean-up, were performed following instructions from Agilent. The samples were hybridized in single versus a standard DNA (male or female, Promega) with Mouse Genome CGH 4x44K Agilent micro arrays (G4426B). The scanning and image analysis were performed with Agilent technology using default settings. Raw data .txt files from the Agilent Feature Extraction software 9.5 were then imported for analysis into CGH Analytics 3.4.40. Aberrations were obtained with the ADM2 algorithm and filtering options of a minimum of 5 probes and  $\text{abs}(\log_2 \text{ratio}) > 0.25$ . Sexual chromosomes were excluded from the analysis because male and female mice were indifferently used to establish the astrocyte cultures.

The microarray data analyzed in this paper have been submitted to the Array Express data repository at the European Bioinformatics Institute (<http://www.ebi.ac.uk/arrayexpress/>) under the accession number E-TABM-706.

### **Anchorage-independent Growth assays**

The cells (10,000) were uniformly suspended in 1.5 ml NS34 containing 66% methylcellulose medium (M3434; Stem Cell Technologies, France). The suspension was plated in 35 mm dishes. The plates were incubated at 37°C in a 5% CO<sub>2</sub>

humidified atmosphere after microscopic evaluation to ascertain that no cellular clusters have been seeded. The cells were cultured for 7 days prior to the evaluation of colony formation.

### **Subcutaneous and intracerebral grafting**

Cell suspensions in PBS were prepared prior to grafting from cultures treated with trypsin. Bilateral subcutaneous injections of either  $5 \cdot 10^6$  or  $5 \cdot 10^5$ -cells/200 $\mu$ l were performed into the flanks of 6-week-old NOD/SCID mice (n=10). Mice were screened twice weekly for tumor formation. Mice were sacrificed under anesthesia one month post-graft. The tumors were excised and fixed by immersion in paraformaldehyde for 24 hours prior to being embedded into paraffin. Stereotaxic injections of  $5 \cdot 10^5$  cells/5  $\mu$ l into the striatum of 6-week-old Nude mice (n=8) were performed using the following stereotaxic coordinates (antero-posterior = 0 from the bregma, lateral = 2.5 mm right, ventral = 3.8 mm deep from the dura <sup>21</sup>. Body-weights were monitored once a week, and mice were sacrificed 18–25 days post-graft with a transcardial paraformaldehyde perfusion as previously described <sup>22</sup>. Tissues were frozen in a 30% sucrose cryoprotective medium after a 24 hr incubation period.

### **Immunolabelling**

Ten  $\mu$ m-thick sections were prepared from paraffin-embedded subcutaneous tumors. Cryostat sections (20  $\mu$ m thickness) of the brains were cut in the frontal plane. Immunohistochemistry was performed on free-floating sections. Immunocytochemical and immunohistochemical procedures were performed as previously described <sup>23</sup> using the following primary antibodies: polyclonal rabbit or monoclonal anti-GFAP (1:500, Dako or 1:500 ICN, France), polyclonal rabbit anti-BLBP antibody (1:1000),

monoclonal anti-nestin (1:200, Chemicon, France), polyclonal rabbit anti-NG2 (1:2000, Tebu, France), polyclonal rabbit anti-TGF $\alpha$  (1:200, RGG-8040, Peninsula Laboratories, San Carlos, CA). Immunofluorescent labelings were viewed using an Axioplan 2 fluorescence microscope, and images were captured using the ApoTome (Zeiss), AxioCam MRm digital camera and AxioVision 4.2 software. Other immunolabelings images were acquired on a Digital still camera (DXM 1200, Nikon, USA) using the Lucia software (Laboratory Imaging, Ltd). The images were prepared for printing using Adobe Photoshop software (Adobe Systems, San Jose, CA).

## Results

### **Irradiation immortalizes astrocytes de-differentiated into progenitor-like cells**

Astrocytes maintained for a week in serum-free medium exhibit a flat, polygonal morphology (Fig. 1b), while TGF $\alpha$ -treated astrocytes acquire, after 7 days of treatment, a bipolar morphology (Fig. 1c). Irradiation did not modify the morphology or the life span of astrocytes cultured in serum-free medium. These cultures died within 3 weeks post-irradiation, as did their sister sham-irradiated counterparts (Fig. 1d). In contrast, TGF $\alpha$ -treated cultures survived beyond 3 weeks regardless of whether they had been irradiated (Fig. 1e).

Non-irradiated TGF $\alpha$ -treated astrocytes presented the previously described radial glia-like bipolar morphology <sup>8</sup>, with a thin layer of cytoplasm surrounding a fusiform nucleus, and two long thin processes extending in opposite directions (Fig. 1c). This morphology was maintained throughout the survival time of the cultures. On the contrary, irradiated TGF $\alpha$ -treated astrocytes exhibited progressive morphological changes. Forty days post-irradiation, irradiated TGF $\alpha$ -treated cultures contained

densely packed bipolar cells (Fig. 1e) that coexisted with giant stellar-shaped cells (Fig. 1f) and small rounded cells that covered large areas of the culture dish. Development of large masses of round, bright cells at the top of the cellular layer was observed in irradiated TGF $\alpha$ -treated astrocyte cultures maintained for 3 months after irradiation in TGF $\alpha$ -supplemented defined medium. Masses were collected from three independent cultures, and subsequently dissociated and further cultured. The cells kept growing in suspension, forming cellular spheres that occasionally attached to the culture flask (Fig. 1g). Eventually, all irradiated TGF $\alpha$ -treated cultures were composed, for the most part, of cells that formed floating spheres. In addition to these morphological changes, irradiated TGF $\alpha$ -treated cells exhibited a higher growth rate than sham-irradiated sister cultures (compare Fig. 1e with 1c). Unlike their sham-irradiated counterparts, irradiated TGF $\alpha$ -treated cells could be trypsinised and successfully re-seeded (Fig. 1h). These data show that irradiation immortalized astrocytes reverted to progenitor-like cells upon TGF $\alpha$  action.

Both ligand-independent and TGF $\alpha$ -dependent erbB1 activations have been shown to underlie the radioresistance of tumoral epithelial cells through enhanced survival and DNA repair<sup>24-27</sup>. ErbB1 expression has been previously characterized and shown to be functional in our mouse cortical astrocyte cultures (Fig. 2a, and<sup>20</sup>). These cells express TGF $\alpha$ , as detected by ELISA at a concentration of 5 pg TGF $\alpha$ /μg protein in our astrocyte cultures, and as observed using immunocytochemistry (Fig. 2c). They also express TACE, the metalloprotease required for TGF $\alpha$  release, as seen by immunoblotting (Fig. 2b). To evaluate whether irradiation survival of astrocytes depended on the mobilization of the endogenous TGF $\alpha$ -erbB1 pair, we repeated the irradiation procedure on cultures treated or untreated with TGF $\alpha$  and supplemented with 1 μM AG1478, a specific inhibitor of erbB1 tyrosine kinase activity

<sup>19</sup>, for 4 hours that included the irradiation time-point. Longer erbB1 inhibition was not attempted, as we previously observed that TGF $\alpha$ -treated astrocytes underwent apoptosis upon prolonged growth factor signaling inhibition <sup>20</sup>.

Blockade of erbB1 signaling had no effect on the survival of control or TGF $\alpha$ -treated astrocytes up to 40 days post-irradiation, the longest time point surveyed. To verify whether TGF $\alpha$  promoted DNA double-strand break repair or survival, we added the growth factor to control cultures within 30 minutes after their irradiation. Surprisingly, all cells died within 6 days, demonstrating that TGF $\alpha$  potentiates irradiation-triggered cellular stress.

Taken together, these results indicate that radioresistance of astrocytes, irrespective of whether they have been previously treated with TGF $\alpha$ , is independent from the erbB1-signalling pathway.

### **Tumoral transformation of irradiated TGF $\alpha$ -treated astrocytes**

In contrast with nuclei of sham-irradiated cells that possessed regular contours, nuclei of irradiated TGF $\alpha$ -treated cells exhibited atypia with irregular contours and segmentation (not shown). This observation raised the possibility that irradiation not only resulted in the immortalization of astrocytes de-differentiated into progenitor-like cells by TGF $\alpha$ , but also in their cancerous transformation. To evaluate this hypothesis, we first assayed their ability to grow in an anchorage-independent manner, a common landmark of cells' cancerous transformation <sup>28</sup>. Cells were seeded in culture medium containing 60% methylcellulose, and the formation of colonies was monitored. Numerous large colonies were observed in cultures of irradiated TGF $\alpha$ -treated astrocytes (Fig. 3a), whereas only rare and small colonies <50  $\mu$ m in diameter were formed by sham-irradiated TGF $\alpha$ -treated cultures (Fig. 3a,

insert), as expected for neural progenitors <sup>29</sup>. To assess *in vivo* tumorigenicity, we performed heterotopic and homotopic grafts. We first performed subcutaneous injections of irradiated TGF $\alpha$ -treated cells, growing either anchored to the culture flask or as floating spheres, into the flanks of 6-week-old NOD-SCID mice. Within 2 weeks, tumors became visible in all animals at the sites of injection. After the death of two mice, all the remaining animals were sacrificed 4 weeks post-injection. Dissection showed that all animals had developed tumors that ranged in size from 10–25 mm (Fig. 3b). Most of the tumors had developed beyond the subcutaneous space, and some grew within the peritoneal cavity. Histological analysis showed that the tumors were formed of bundles of spindle-shaped cells, and contained areas of pseudopalisading necrosis (Fig. 3c). They were highly destructive of the host tissues, as illustrated for the dermis (Fig. 3c), the cartilages (Fig. 3d), and the muscles (Fig. 3e). Their histological aspect evoked either a glioblastoma or a gliosarcoma. No difference was noted between tumors generated from anchored or floating cells (not shown). Immunohistological detection of Ki67 (Fig. 3f) revealed a 33% index of proliferation.

Homotopic grafts were achieved using stereotaxic injections of irradiated TGF $\alpha$ -treated cells growing in the form of floating spheres into the striatum of 6-week-old Nude mice. Striatal grafts of neural stem-like cells and progenitor-like cells derived from TGF $\alpha$ -treated astrocytes were used as controls. Recipients of grafts of neural-stem like cells and progenitor-like cells survived up to 6 weeks post-grafting without displaying any clinical sign. Upon histological examination, cellular grafts were found under the form of small and well-delineated cellular aggregates that remained localized along the injection track (Fig. 4a). In contrast, mice grafted with irradiated TGF $\alpha$ -treated cells displayed reductions in their body weight, and

behavioral alterations from around 2 weeks post-graft. All animals were sacrificed at 3–4 weeks post-graft and had developed tumors. Histological analysis showed vast bundles of spindle-shaped cells within the striatum, forming a densely packed cellular tumor (Fig. 4b-d), and patches of spindle-shaped cells could be observed throughout the ipsi-lateral brain, from the thalamus (Fig. 4b) to the olfactory bulb (not shown). Immunochemical analysis was used to determine whether the grafted cells maintained a similar molecular expression profile *in vivo* as *in vitro*. We previously reported that astrocytes de-differentiated into progenitor-like cells express markers of neural stem cells or progenitor cells (BLBP, nestin), and half of them express the astroglial marker GFAP<sup>8</sup>. In addition, we observed here that neither control nor TGF $\alpha$ -treated astrocytes express NG2, a glycoprotein recently identified in oligodendrocyte precursors<sup>30</sup> (data not shown). Most irradiated TGF $\alpha$ -treated cells maintained GFAP expression *in vitro* (Fig. 5a). GFAP-immunoreactive cells were observed within the core of the CNS tumors (Fig. 5b and 6a) although the pattern of GFAP-immunostaining showed the presence of reactive astrocytes around the tumor mass (Fig. 5b). Only a fraction of the irradiated TGF $\alpha$ -treated astrocytes retained *in vitro* expression of BLBP and nestin (Fig. 5c and e), and patches of BLBP- and nestin-immunoreactive cells were observed within the centre of the tumors (Fig. 5d and f). Interestingly, we found that a subset of irradiated TGF $\alpha$ -treated cells had acquired NG2 expression *in vitro* (Fig. 5g), and some NG2-immunoreactive cells were observed within the brain tumor core (Fig. 5h). Finally, no GFAP-immunoreactive tumor cells were observed in subcutaneous tumors (Supplementary Fig. 1b). Conversely, no cells expressing the neuronal marker synaptophysin were observed in CNS tumors (Supplementary Fig. 1d) while numerous synaptophysin-immunoreactive cells were observed within the subcutaneous tumors

(Supplementary Fig. 1c). These data indicate that the environment influences the molecular markers of the tumor cells, without affecting their tumorigenicity.

Taken together, these results demonstrate that irradiation triggered cancerous transformation of astrocytes if they had regressed to a progenitor-like status upon TGF $\alpha$  action.

### **Cytogenomic abnormalities of transformed TGF $\alpha$ -treated astrocytes**

Analysis of the karyotypes at 2 months post-irradiation revealed a hyperploidy in 50% of irradiated TGF $\alpha$ -treated astrocytes (data not shown). A high-resolution oligonucleotide CGH array was used to characterize the cytogenomic alterations of these cells *in vitro* and *in vivo*, compared with their non-irradiated sister cells. This analysis was performed on the cells used for subcutaneous grafting, i.e., at 9 months post-irradiation. The detailed results of the analysis are provided in supplementary Table 1. The profile of gains and losses for astrocytes maintained in serum-free medium were highly similar to those for TGF $\alpha$ -treated astrocytes. Both cell types exhibited polymorphisms compared with the control DNA sample provided by the manufacturer, most notably in chromosome 4qD1 (Fig. 6a, supplementary Table 1). A search for genomic imbalances appearing *de novo* in transformation was performed through the comparison of array CGH patterns of the samples. The profiles of irradiated TGF $\alpha$ -treated astrocytes growing either in the form of adherent (Fig. 6a) or floating cells (not shown) were very similar (Table 1), and differed from non-irradiated cultures most prominently through gains in chromosomes 6qA-E, 7qA-F, 11qB-E and 15qA-F (Fig. 6a and Table 1). Irradiated TGF $\alpha$ -treated astrocytes that grew as floating cells lacked the chromosome 15 and 7qF1-F4 alterations identified in irradiated TGF $\alpha$ -treated astrocytes growing as adherent cells.



The array CGH profiles of the different subcutaneous tumors derived from irradiated TGF $\alpha$ -treated astrocytes were overall comparable, and similar to the profile of their cells of origin. One marked exception was the segmental chromosomal copy number alteration (CNA) on chromosome 15; absent in irradiated TGF $\alpha$ -treated astrocytes growing as floating cells, it was acquired in all tumors derived from these cells (n=3). In addition, one tumor derived from irradiated TGF $\alpha$ -treated astrocytes growing as floating cells lacked CNAs on chromosome 7, whereas 2 other tumors exhibited CNAs only on chromosome 7qA1-B3 (Table 1). The profiles of tumors generated from TGF $\alpha$ -treated astrocytes growing as adherent cells exhibited a remarkable degree of similarity (Table 1). The most notable variation was seen in the 7qF1-F5 CNAs, present in irradiated TGF $\alpha$ -treated astrocytes growing as adherent cells and in all but one tumor generated from these cells (n=5).

These results show that irradiation-induced cancerous transformation of TGF $\alpha$ -treated astrocytes was accompanied by the acquisition of several CNAs, among which those located on chromosomes 6, 7 and 11 were conserved among all irradiated cells *in vitro*, and all tumors *in vivo*. Interestingly, gains in chromosome 6 and 11 segments encompassed the loci of the TGF $\alpha$  gene and the erbB2 proto-oncogene, an orphan receptor of the erbB1 family, respectively. No segmental alterations were observed in gene loci encoding other members of the EGF and erbB families, erbB1 included. Additional molecular and pharmacologic studies are required to determine which molecular pathways are instrumental in radiation-induced neoplastic transformation of these cells, and in the maintenance of their tumorigenicity.

## Discussion

Our results demonstrate that, unlike mature astrocytes, those that revert to neural progenitor-like cells upon  $TGF\alpha$  action can be submitted to cancerous transformation.

$TGF\alpha$  has been reported to induce cancerous transformation of cultured fibroblasts<sup>31</sup>, renal epithelial cells<sup>32</sup>, and mammary epithelial cells<sup>33</sup>. Transgenic mice over-expressing  $TGF\alpha$  develop, late in their life, malignant tumors in the liver and the mammary glands<sup>7, 34-36</sup>. However, sustained activation of the erbB1 signaling module is insufficient to trigger astrocyte neoplasia, unless astrocytes bear mutations in other oncogenes or tumor suppressor genes. For instance, primary cultures of astrocytes derived from *P53*<sup>-/-</sup> or *P16/P19*<sup>-/-</sup> mice, are immortal but not tumorigenic, but may progress to malignant transformation upon treatment with EGF, the structural and functional analogue of  $TGF\alpha$ <sup>12, 37, 38</sup>. In agreement with these results, we observed no sign of tumoral transformation of astrocytes either *in vitro*<sup>8</sup> or *in vivo* after intra-cerebral grafts (Fig. 4a), despite several months of continuous exposure to  $TGF\alpha$ . In addition, we observed that  $TGF\alpha$ -treated astrocytes are able to engage into a neuronal differentiation pathway *in vivo* (data not shown) as they do *in vitro*<sup>8</sup>. Finally, the genomic profiles of astrocytes maintained in serum-free medium are the same as those of astrocytes treated with  $TGF\alpha$ .

Our results show that astrocytes survive irradiation regardless of their exposure to  $TGF\alpha$ . Several studies demonstrated the participation of erbB1 activation in the cellular defense system against ionizing radiation, and clinical results indicate that erbB1 inhibition sensitizes different solid human tumors, including gliomas, to radiation<sup>39, 40</sup>. ErbB1 activation may be achieved within minutes via radiation-induced reactive oxygen/nitrogen species that induce phosphorylation of erbB1<sup>41-43</sup>, and

within 2 hours via increased proteolytic cleavage of the TGF $\alpha$  precursor <sup>26</sup>, resulting in the mobilization of intra-cellular pathways that promote cell survival and DNA repair <sup>44, 45</sup>. Although we ascertained that the astrocytes used in the present study express all the molecular components required for a functional endogenous TGF $\alpha$ -erbB1 signaling module, their radioresistance appears independent from erbB1 activity. Indeed, survival of irradiation is unaltered by the blockade of erbB1 tyrosine kinase activity within 4 hours of irradiation.

Although astrocytes resisted radiation, they demonstrated limited growth potential and entered into a senescence-like state 2–3 weeks post-irradiation, like their sham-irradiated counterparts. Addition of TGF $\alpha$  to control astrocytes after their irradiation did not promote their survival or cancerous transformation, but, on the contrary, induced their death. In contrast, irradiation of astrocytes reverted to progenitor-like cells upon TGF $\alpha$  action resulted in their immortalization and cancerous transformation. Sham-irradiated TGF $\alpha$ -treated cultures survived much longer in their original dish than untreated astrocytes, as previously reported <sup>8</sup>, but they did not sustain successive passages, indicating that TGF $\alpha$  treatment is insufficient to trigger astrocyte immortalization.

Cancerous transformation was ascertained by the cells' ability to form colonies in methylcellulose medium, their karyotype anomalies, and, most importantly, by the formation of subcutaneous tumors in NOD-SCID mice and high-grade glioma-like tumors in Nude mouse CNS. In both graft paradigms, the histological aspect of the tumors evoked either a glioblastoma or a gliosarcoma. Immunological characterization of the transformed cells *in vitro* and in brain grafts revealed, overall, a very immature phenotype, except for GFAP expression that was, at least *in vitro*, observed in most cells. Only a fraction of the cancerously transformed cells retained

neural progenitor markers like BLBP and nestin, which were expressed in the vast majority of non-irradiated TGF $\alpha$ -treated astrocytes<sup>8</sup>. In contrast, some had acquired the expression of NG2, a proteoglycan frequently associated with cancerous cells in glioma<sup>46</sup>, and involved in chemoresistance<sup>47</sup> and cell motility<sup>48</sup>. The high similarity of the genomic profiles of different subcutaneous tumors and the profile of their cells of origin, associated with their rapid development within 2–3 weeks in both graft paradigms, shows that the cells' tumorigenicity is independent of secondary alterations selected *in vivo*. In addition, the lack of genomic anomalies in TGF $\alpha$ -treated astrocytes prior to their irradiation shows that TGF $\alpha$  does not predispose the cells to cancerous transformation through the acquisition of an aneuploid state, a phenomenon recently shown to occur in a transient manner during normal differentiation of neural stem cells of the mouse sub-ventricular zone<sup>49</sup>.

## Conclusion

Our results show that regression of astrocytes to a progenitor-like stage sensitizes the cells to oncogenic events. Immediate exposure of irradiated-astrocytes to TGF $\alpha$  kills them instead of promoting their cancerous transformation. This further shows that the change in the differentiation status of the astrocytes is a prerequisite condition to their cancerous transformation. Whether this sensitization is of the same magnitude for “artificial” and “natural” progenitors remains to be determined. It is indeed possible to envisage that de-differentiated cells possess particular chromatin states and/or inappropriate DNA repair and apoptosis pathways. The present work indicates that alteration of the differentiation state of mature cells could be at the core of glioma development, although it does not allow eliminating neural stem or progenitor cells as parallel cells of origin of these primitive brain tumors.

In any case, our results suggest that the frequent TGF $\alpha$  over-expression observed at

the early stages of glioma development testifies to a participation of this growth factor in the earliest pathological events that result in glioma induction.

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## Figures legends

**Figure 1** Response to irradiation of astrocytes and astrocytes de-differentiated into progenitor-like cells by TGF $\alpha$ . **a.** Schematic representation of the experimental protocol. See text for details. **b.** Mouse astrocytes have a flat, polygonal shape in serum-free medium. **c.** After 7 days in the presence of TGF $\alpha$ , astrocytes acquire a bipolar shape. **d.** Irradiation does not affect the survival of astrocyte cultures maintained in serum-free medium. They die within 3 weeks post-irradiation, as do their sham-irradiated counterparts. **e.** Six weeks post-irradiation, TGF $\alpha$ -treated cultures contain densely packed bipolar cells. **f.** Giant stellar shaped cells (arrow) co-exist with bipolar cells in irradiated TGF $\alpha$ -treated cultures (6 weeks post-irradiation). **g.** Three months post-irradiation, cells growing as free-floating cellular spheres appeared in irradiated TGF $\alpha$ -treated cultures. Scale bar = 40  $\mu$ m in a–g. **h.** Growth



during serial passage of sham-irradiated TGF $\alpha$ -treated astrocyte cultures (black points) and irradiated TGF $\alpha$ -treated astrocyte cultures (red points). Serially passaged irradiated TGF $\alpha$ -treated astrocytes exhibited immortal growth while non-irradiated TGF $\alpha$ -treated astrocytes underwent proliferation arrest. Illustration of 3 independent experiments.

**Figure 2 a.** ErbB1 is expressed and activated in response to TGF $\alpha$  in mouse astrocyte cultures. Phosphorylated erbB1 was analyzed by Western blot in mouse astrocyte cultures under control conditions and following 5 min TGF $\alpha$  treatment. IPP: immuno-precipitation. IB: immuno-blotting. 4G10: monoclonal anti-phosphotyrosine antibody. **b.** TACE expression in mouse astrocyte cultured in serum-free medium. i: immature form of TACE; m: mature form of TACE. **c.** Immunofluorescent detection of TGF $\alpha$  in mouse astrocytes cultured in serum-free medium. Bar= 50  $\mu$ m.

**Figure 3 a.** Irradiated TGF $\alpha$ -treated astrocyte cultures form large and numerous colonies in semi-solid medium, while their sham-irradiated counterparts form only rare and small colonies (insert). Scale bar = 100  $\mu$ m. **b–f.** Subcutaneous tumors derived from irradiated TGF $\alpha$ -treated astrocytes. **b.** Illustration of the macroscopic appearance of the subcutaneous tumors. **c–d.** Hematoxylin-eosin staining illustrating the spindle-cell morphology of the tumor cells, and their destruction of the host dermis (**c**), the cartilages (**d**), and the muscles (**e**). Star in c marks an area of necrosis. Arrow in e points to a muscle fiber. **f.** The tumors contained numerous Ki67-immunoreactive cells. Scale bar = 100  $\mu$ m in c, d, e and f.

**Figure 4** Intra-cerebral tumors derived from irradiated TGF $\alpha$ -treated astrocytes.

Cresyl violet staining. **a.** Striatal grafts of neural stem-like cells derived from TGF $\alpha$ -treated astrocytes do not form tumors. **b–c.** Examples of tumors derived from irradiated TGF $\alpha$ -treated astrocytes. The tumor cells were spindle-shaped and invaded the host well beyond the transplantation point. LV: lateral ventricle. Scale bar = 500  $\mu$ m. **d.** High power view of tumoral cells invading the host tissue at the edge of the tumor. Scale bar = 60  $\mu$ m.

**Figure 5** Immunohistochemical characterization of irradiated TGF $\alpha$ -treated astrocytes in vitro and after intra-cerebral grafting. **a.** Most astrocytes de-differentiated into progenitor-like cells upon TGF $\alpha$  action and transformed by irradiation express the astroglial marker GFAP. **b.** Patches of GFAP-immunoreactive cells were observed within the core of the CNS tumors. **c and e.** Part of irradiated TGF $\alpha$ -treated astrocytes express *in vitro* BLBP and nestin, markers of neural stem cells or progenitor cells. **d and f.** Patches of BLBP and nestin-immunoreactive cells were observed within the tumor masse. **g.** A subset of irradiated TGF $\alpha$ -treated cells express NG2, a glycoprotein labeling a population of macroglial cells and of oligodendrocyte progenitors. **h.** NG2-immunoreactive cells were observed within the brain tumor core. Scale bars = 20  $\mu$ m in a, c, e, and g; 100  $\mu$ m in b, d, f and h; 10  $\mu$ m in the insets of f and h.

**Figure 6 a.** Profile of gains and losses of irradiated TGF $\alpha$ -treated astrocytes growing in the form of adherent cells (red), compared with their non-irradiated sister cells (grey), and non-irradiated astrocytes maintained in serum-free medium (black). Shown are log 2 ratios for each chromosome. Non-irradiated astrocytes exhibited the

same profile irrespective of exposure to TGF $\alpha$ . Irradiated TGF $\alpha$ -treated astrocytes exhibited gains in chromosomes 6, 7, 11 and 15. **b.** Profile of gains and losses of subcutaneous tumors derived from irradiated TGF $\alpha$ -treated astrocytes growing in the form of adherent cells (yellow) or floating cells (green).

**Table 1:** Chromosomal copy number alterations. Red and green areas indicate DNA gains and losses, respectively. Aberrations were obtained with ADM2 algorithm and filtering options of a minimum of five probes and  $\log_2(\text{ratio}) > 0.25$ . Genomic coordinates according to the mm9 build (July 2007) and values in  $\log_2(\text{ratio})$ .

**Supplementary Figure 1** Comparison of GFAP and synaptophysin expression in irradiated TGF $\alpha$ -treated astrocytes in intra-cerebral and subcutaneous grafts. **a.** GFAP-immunoreactive cells were observed within the core of the CNS tumors (arrow, 480 nm ApoTome optical slice). **b.** In contrast, no GFAP immunolabeling was detected in subcutaneous grafts. **c.** Synaptophysin immunolabeling was restricted to the host tissue in the case of intra-cerebral grafts. **d.** Numerous synaptophysin immunoreactive cells were observed within the subcutaneous tumors. Bar=100  $\mu\text{m}$  in a, b, d and 200  $\mu\text{m}$  in c.

**Supplementary table 1:** Chromosomal aberrations defined with the following parameters: ADM2 method for segmentation with threshold 6,  $\log_2$  ratio  $> 0.25$ , and numbers of probes  $\geq 5$ . Average values are provided for tumors derived from irradiated TGF $\alpha$ -treated astrocytes growing as adherent cells (n=5) and from irradiated TGF $\alpha$ -treated astrocytes growing as floating cells (n=3).

Genomic coordinates (mm9)	chromosome	band	ASTRO	ASTRO + TGF $\alpha$	IRRADIATED ASTRO + TGF $\alpha$ ADHERENT CELLS	IRRADIATED ASTRO + TGF $\alpha$ FLOATING CELLS	TUMOR 1 FROM IRRADIATED ASTRO + TGF $\alpha$ ADHERENT CELLS	TUMOR 2 FROM IRRADIATED ASTRO + TGF $\alpha$ ADHERENT CELLS	TUMOR 3 FROM IRRADIATED ASTRO + TGF $\alpha$ ADHERENT CELLS	TUMOR 4 FROM IRRADIATED ASTRO + TGF $\alpha$ ADHERENT CELLS	TUMOR 5 FROM IRRADIATED ASTRO + TGF $\alpha$ ADHERENT CELLS	TUMOR 1 FROM IRRADIATED ASTRO + TGF $\alpha$ FLOATING CELLS	TUMOR 2 FROM IRRADIATED ASTRO + TGF $\alpha$ FLOATING CELLS	TUMOR 3 FROM IRRADIATED ASTRO + TGF $\alpha$ FLOATING CELLS
chr2:81139123-101306651	2	2qD-E1	0	0	-0.28	0	-0.25	-0.26	0	0	0	-0.27	0	0
chr2:115846031-123819240	2	2qE5	0.84	0.86	1.04	0.92	0.78	0.87	0.83	0.82	0.78	0.69	0.78	0.84
chr3:83835375-93096260	3	3qF1	0	0	0	0.3	0	0	0	0	0	0	0	0
chr4:109852334-116510051	4	4qD1	2.65	2.63	2.38	2.42	2.12	2.44	0	0	2.25	2.19	2.46	0
chr4:132678793-140287612	4	4qD3	0	0	0	0	-0.54	-0.47	-0.58	0	-0.49	-0.43	-0.49	-0.55
chr4:140287612-146469778	4	4qE1	0	0	0	0.48	0	0	0	0	0	0	0	0
chr4:146469778-155029701	4	4qE2	0	0.08	0	0	0	0	0	0	0	0	0	0
chr5:81773799-91394246	5	5qE1	0	0	-0.26	0	-0.25	-0.28	0	0	-0.24	0	0	0
chr6:0-16613965	6	6qA1	0	0	-1.02	-1.03	-0.77	-0.85	-0.79	-0.84	-0.81	-0.61	-0.77	-0.85
chr6:16613965-116786401	6	6qA2-E3	0	0	0.29	0.43	0.33	0.36	0.37	0.38	0.36	0.29	0.35	0.39
chr6:125093383-139264118	6	6qF3-G1	0	0	-0.27	-0.27	-0.31	-0.31	-0.25	0	-0.25	-0.24	0	0
chr7:0-15170811	7	7qA1	0	0	0.3	0.3	0.26	0.24	0.34	0.3	0.28	0	0.22	0
chr7:15170811-37421334	7	7qA2-B2	0	0	0.36	0.45	0.26	0.31	0.34	0.3	0.28	0	0.28	0.25
chr7:37421334-47535208	7	7qB3	0	0	0.43	0.45	0.33	0.31	0.34	0.36	0.34	0	0.28	0.25
chr7:47535208-54109226	7	7qB4	0	0	0.3	0.3	0.26	0	0.34	0	0	0	0	0
chr7:54109226-60683244	7	7qB5	0	0	0.3	0.3	0	0	0.34	0	0	0	0	0
chr7:60683244-71302812	7	7qC	-0.72	0.3	-0.68	-0.4	0.26	-0.42	0.34	-0.36	-0.4	-0.69	-0.45	-0.5
chr7:71302812-90013479	7	7qD1-D3	0	0	0.3	0.22	0.26	0.24	0.34	0.3	0.28	0	0	0
chr7:90013479-102150128	7	7qE1-E2	0	0	0.3	0.22	0.26	0	0.34	0.3	0.28	0	0	0
chr7:102150128-11252615	7	7qE3	0	0	0.3	0.22	0.26	0.24	0.34	0.3	0.28	0	0.22	0
chr7:11252615-145134094	7	7qF1-F5	0	0	0.3	0	0.26	0	0.34	0.3	0.28	0	0	0
chr9:14343360-24059831	9	9qA2-A3	0	0	0	0	-0.32	-0.33	0	0	0	0	0	0
chr10:23539770-41194597	10	10qA4-B1	0	0	0	0	0	0	0	0	0	-0.27	-0.27	0
chr10:41194597-63753544	10	10qB2-B4	0	0	-0.3	0	0	0	0	0	0	0	0	0
chr10:95630316-111323496	10	10qC3-D1	0	0	-0.28	0	0	0	0	0	0	0	0	0
chr11:62758837-121798632	11	11qB3-E2	0	0	0.52	0.5	0.42	0.45	0.43	0.44	0.43	0.37	0.42	0.45
chr14:43144646-51575209	14	14qC1	0	0	-0.24	0	-0.31	-0.32	0	0	0	0	0	0
chr14:51575209-54550702	14	14qC2	0.62	0.63	0.56	0.55	0.53	0.51	0.55	0.55	0.57	0.5	0.56	0.58
chr14:59509857-68436336	14	14qD1	0	0.83	0.36	0.44	0	0	0	0	0	0	0	0
chr15:0-66107593	15	15qA1-D1	0	0	0.35	0	0.31	0.35	0.39	0.43	0.36	0.31	0.43	0.52
chr15:66107593-103492577	15	15qD2-F3	0	0	0.43	0	0.39	0.44	0.39	0.43	0.42	0.39	0.49	0.59
chr17:21829683-31434744	17	17qA3.3	-0.5	-0.54	-0.56	-0.51	-0.53	-0.49	-0.53	-0.49	-0.53	-0.49	-0.53	-0.56
chr19:0-16650124	19	19qA	0	0	-0.99	-0.99	-0.67	-0.7	-0.76	-0.81	-0.73	0.3	-0.67	-0.78
chr19:38173456-61321190	19	19qC3-D3	0	0	0	0	0	0	0	0	0	0.21	0	0

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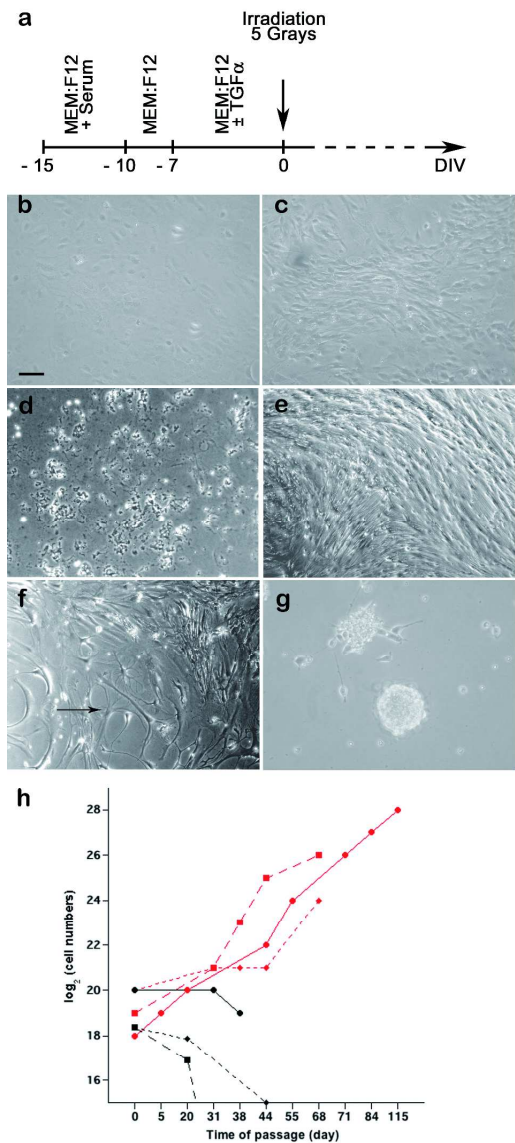


Figure 1

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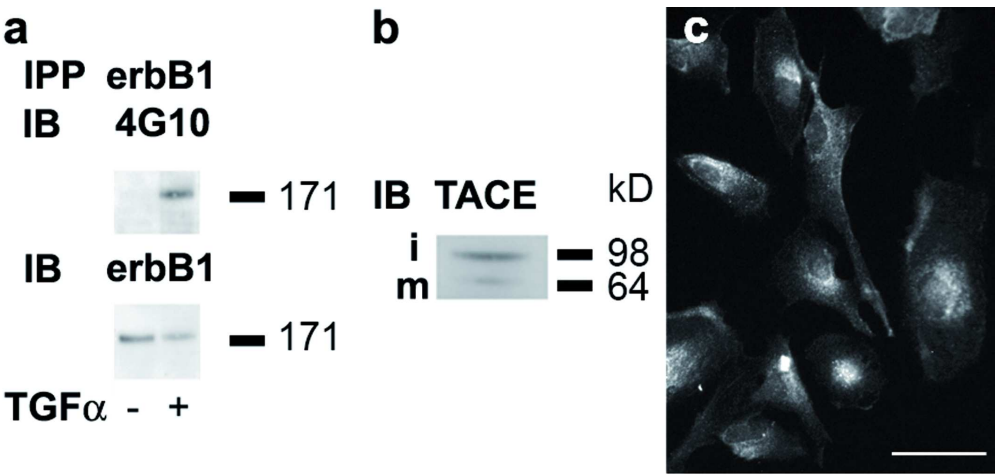


Figure 2

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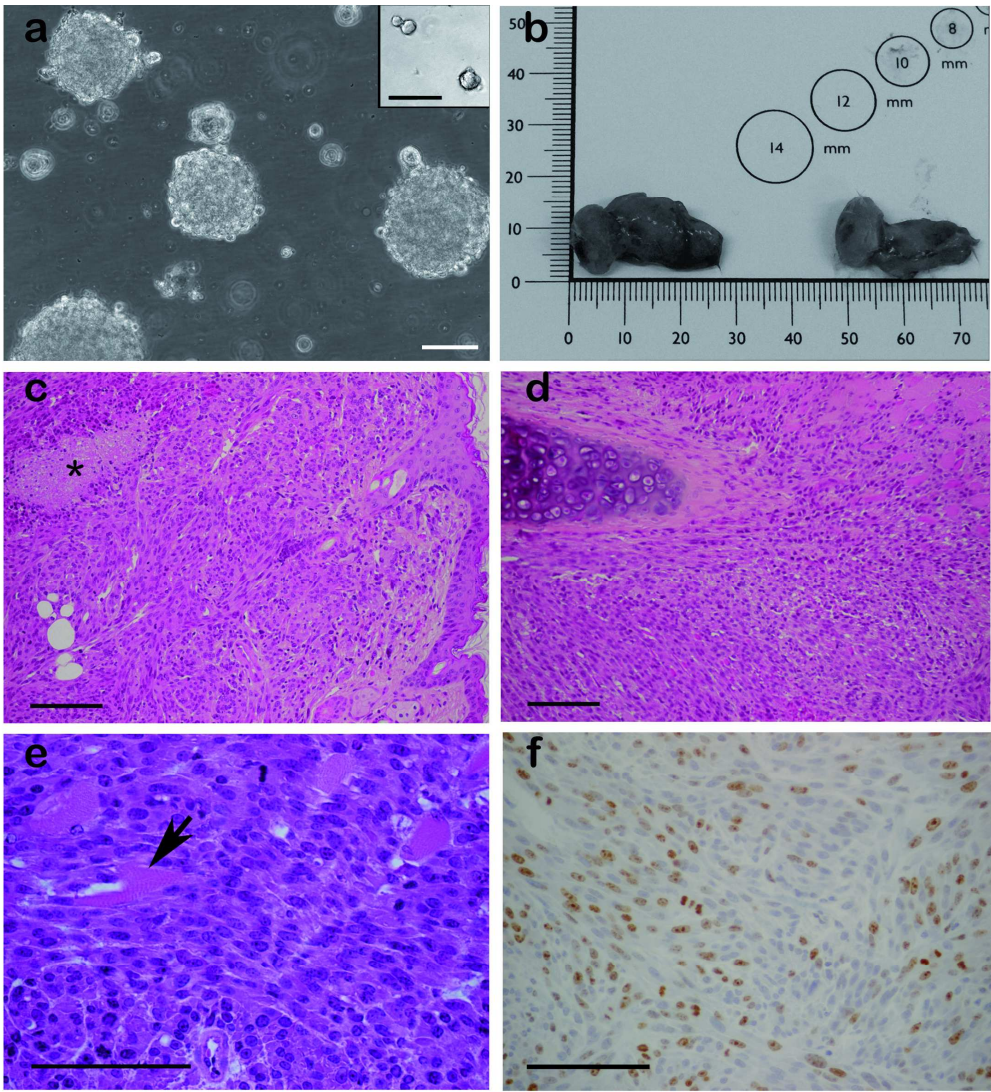


Figure 3

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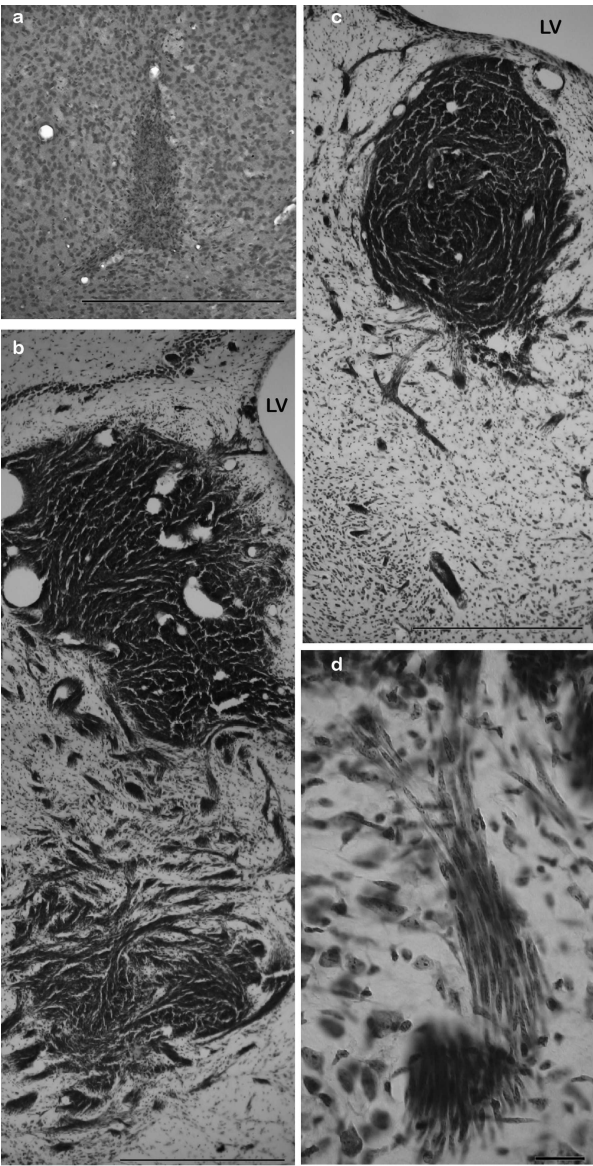


Figure 4

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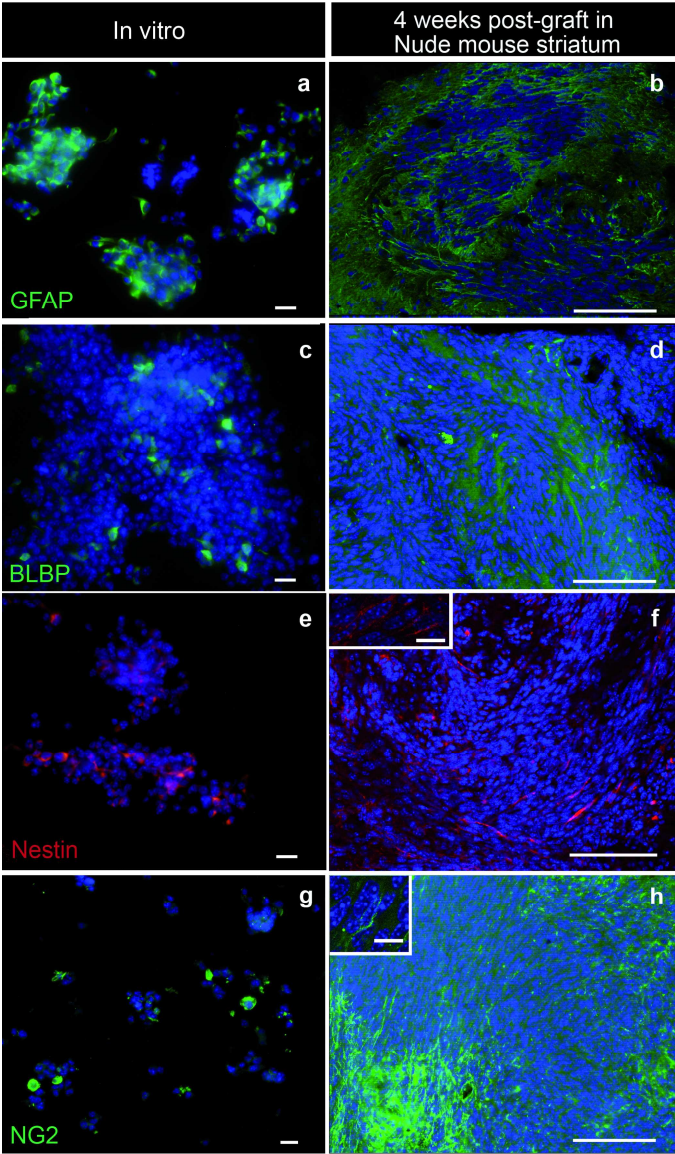


Figure 5

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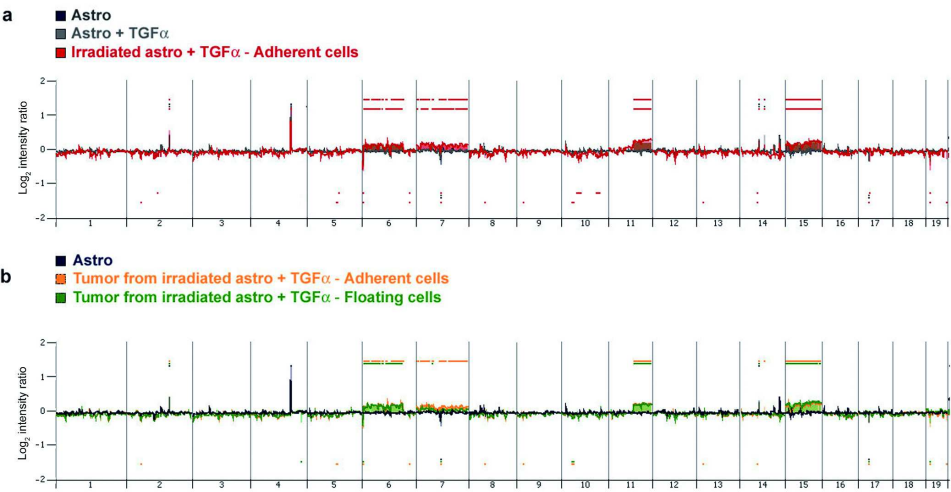


Figure 6

184x104mm (600 x 600 DPI)

#	#	#	#	#	#	ASTRO	ASTRO + TGF $\alpha$	IRRADIATED ASTRO + TGF $\alpha$ ADHERENT CELLS	IRRADIATED ASTRO + TGF $\alpha$ FLOATING CELLS	TUMORS FROM IRRADIATED ASTRO + TGF $\alpha$ ADHERENT CELLS	TUMORS FROM IRRADIATED ASTRO + TGF $\alpha$ FLOATING CELLS
n	chrxx	chr	arm	band	band2						
1	chr1:0-8912711	1	1q	1qA1	1qA1	0	0	0	0	0	0
2	chr1:8912711-12378766	1	1q	1qA2	1qA2	0	0	0	0	0	0
3	chr1:12378766-20301176	1	1q	1qA3	1qA3	0	0	0	0	0	0
4	chr1:20301176-22281779	1	1q	1qA4	1qA4	0	0	0	0	0	0
5	chr1:22281779-31194491	1	1q	1qA5	1qA5	0	0	0	0	0	0
6	chr1:31194491-43573257	1	1q	1qB	1qB	0	0	0	0	0	0
7	chr1:43573257-54961723	1	1q	1qC1	1qC1.1	0	0	0	0	0	0
8	chr1:54961723-	1	1q	1qC1	1qC1.2	0	0	0	0	0	0

	56447175										
9	chr1:56447175-59913229	1	1q	1qC1	1qC1.3	0	0	0	0	0	0
10	chr1:59913229-65855037	1	1q	1qC2	1qC2	0	0	0	0	0	0
11	chr1:65855037-75262900	1	1q	1qC3	1qC3	0	0	0	0	0	0
12	chr1:75262900-80709557	1	1q	1qC4	1qC4	0	0	0	0	0	0
13	chr1:80709557-88136817	1	1q	1qC5	1qC5	0	0	0	0	0	0
14	chr1:88136817-100515583	1	1q	1qD	1qD	0	0	0	0	0	0
15	chr1:100515583-103486487	1	1q	1qE1	1qE1.1	0	0	0	0	0	0
16	chr1:103486487-104476788	1	1q	1qE1	1qE1.2	0	0	0	0	0	0
17	chr1:104476788-113389500	1	1q	1qE2	1qE2.1	0	0	0	0	0	0
18	chr1:113389500-114874952	1	1q	1qE2	1qE2.2	0	0	0	0	0	0
19	chr1:114874952-126758568	1	1q	1qE2	1qE2.3	0	0	0	0	0	0
20	chr1:126758568-129729472	1	1q	1qE3	1qE3	0	0	0	0	0	0

21	chr1:129729472-140622786	1	1q	1qE4	1qE4	0	0	0	0	0	0
22	chr1:140622786-148545197	1	1q	1qF	1qF	0	1.2800	0	0	0	0
23	chr1:148545197-153001553	1	1q	1qG1	1qG1	0	0	0	0	0	0
24	chr1:153001553-153991854	1	1q	1qG2	1qG2	0	0	0	0	0	0
25	chr1:153991854-158448210	1	1q	1qG3	1qG3	0	0	0	0	0	0
26	chr1:158448210-161419114	1	1q	1qH1	1qH1	0	0	0	0	0	0
27	chr1:161419114-165380319	1	1q	1qH2	1qH2.1	0	0	0	0	0	0
28	chr1:165380319-166865771	1	1q	1qH2	1qH2.2	0	0	0	0	0	0
29	chr1:166865771-171322127	1	1q	1qH2	1qH2.3	0	0	0	0	0	0
30	chr1:171322127-177263935	1	1q	1qH3	1qH3	-1.5200	-1.5500	-1.5700	-1.6200	0.6200	-1.0700
31	chr1:177263935-183205743	1	1q	1qH4	1qH4	0	0	0	0	0	0
32	chr1:183205743-189642702	1	1q	1qH5	1qH5	0	0	0	-1.1000	-1.0500	-1.0100
33	chr1:189642702	1	1q	1qH6	1qH6	0	0	0	0	0	0

	-197069962										
34	chr2:0-14070368	2	2q	2qA1	2qA1	0	0	0	0	0	0
35	chr2:14070368-16415429	2	2q	2qA2	2qA2	0	0	0	0	0	0
36	chr2:16415429-29078760	2	2q	2qA3	2qA3	0	0	0	0	0	0
37	chr2:29078760-48308264	2	2q	2qB	2qB	0	0	0	0	-0.4200	0
38	chr2:48308264-60502583	2	2q	2qC1	2qC1.1	0	0	0	0	0	0
39	chr2:60502583-60971595	2	2q	2qC1	2qC1.2	0	0	0	0	0	0
40	chr2:60971595-68475791	2	2q	2qC1	2qC1.3	0	0	0	0	0	0
41	chr2:68475791-71758877	2	2q	2qC2	2qC2	0	0	0	0	0	0
42	chr2:71758877-81139123	2	2q	2qC3	2qC3	-2.4700	-2.5300	-2.5700	-2.6100	-2.5500	-2.5500
43	chr2:81139123-88643319	2	2q	2qD	2qD	0	0	-0.2800	0	0	0
44	chr2:88643319-101306651	2	2q	2qE1	2qE1	0	0	-0.2800	0	0	0
45	chr2:101306651-105058749	2	2q	2qE2	2qE2	0	0	0	0	0	0

46	chr2:105058749-113031957	2	2q	2qE3	2qE3	0	0	0	0	0	0
47	chr2:113031957-115846031	2	2q	2qE4	2qE4	0	0	0	0	0	0
48	chr2:115846031-123819240	2	2q	2qE5	2qE5	0.8400	0.8600	1.0400	0.9200	0.8200	0.7700
49	chr2:123819240-131792448	2	2q	2qF1	2qF1	0	0	0	0	0	0
50	chr2:131792448-134606522	2	2q	2qF2	2qF2	0	0	0	0	0	0
51	chr2:134606522-141172694	2	2q	2qF3	2qF3	0	0	0	0	0	0
52	chr2:141172694-146800841	2	2q	2qG1	2qG1	0	0	0	0	0	0
53	chr2:146800841-147738866	2	2q	2qG2	2qG2	0	0	0	0	0	0
54	chr2:147738866-152428988	2	2q	2qG3	2qG3	0	0	0	0	0	0
55	chr2:152428988-158995160	2	2q	2qH1	2qH1	0	0	0	0	0	0
56	chr2:158995160-163216271	2	2q	2qH2	2qH2	0	0	0	0	0	0
57	chr2:163216271-173534541	2	2q	2qH3	2qH3	0	0	0	0	0	0
58	chr2:173534541	2	2q	2qH4	2qH4	0	0	0	0	0	0

	-181976762										
59	chr3:0-18521769	3	3q	3qA1	3qA1	0	0	0	0	0	0
60	chr3:18521769-20471428	3	3q	3qA2	3qA2	0	0	0	0	0	0
61	chr3:20471428-35581293	3	3q	3qA3	3qA3	0	0	0	0	0	0
62	chr3:35581293-46791837	3	3q	3qB	3qB	0	0	0	0	0	0
63	chr3:46791837-56540137	3	3q	3qC	3qC	0	0	0.9000	0	0	0
64	chr3:56540137-60926871	3	3q	3qD	3qD	0	0	0	0	0	0
65	chr3:60926871-69700341	3	3q	3qE1	3qE1	0	0	0	0	0	0
66	chr3:69700341-72624831	3	3q	3qE2	3qE2	0	0	0	0	0	0
67	chr3:72624831-83835375	3	3q	3qE3	3qE3	0	0	0	0	0	0
68	chr3:83835375-93096260	3	3q	3qF1	3qF1	0	0	0	0.3000	0	0
69	chr3:93096260-97482995	3	3q	3qF2	3qF2.1	0	0	0	0	0	0
70	chr3:97482995-106256464	3	3q	3qF2	3qF2.2	0	0	0	0	0	0



71	chr3:106256464-108206124	3	3q	3qF2	3qF2.3	0	0	0	0	0	0
72	chr3:108206124-115029934	3	3q	3qF3	3qF3	0	0	0	0	0	0
73	chr3:115029934-126727893	3	3q	3qG1	3qG1	0	0	0	0	0	0
74	chr3:126727893-128677553	3	3q	3qG2	3qG2	0	0	0	0	0	0
75	chr3:128677553-138425853	3	3q	3qG3	3qG3	0	0	0	0	0	0
76	chr3:138425853-143787417	3	3q	3qH1	3qH1	0	0	0	0	0	0
77	chr3:143787417-148174152	3	3q	3qH2	3qH2	0	0	0	0	0	0
78	chr3:148174152-154023132	3	3q	3qH3	3qH3	0	0	0	0	0	0
79	chr3:154023132-159872112	3	3q	3qH4	3qH4	0	0	0	0	0	0
80	chr4:0-14742088	4	4q	4qA1	4qA1	0	0	0	0	0	0
81	chr4:14742088-17595395	4	4q	4qA2	4qA2	0	0	0	0	0	0
82	chr4:17595395-28057522	4	4q	4qA3	4qA3	0	0	0	0	0	0
83	chr4:28057522-	4	4q	4qA4	4qA4	0	0	0	0	0	0

	29959727										
84	chr4:29959727-43275161	4	4q	4qA5	4qA5	0	0	0	0	0	0
85	chr4:43275161-51359532	4	4q	4qB1	4qB1	0	0	0	0	0	0
86	chr4:51359532-54688391	4	4q	4qB2	4qB2	0	0	0	0	0	0
87	chr4:54688391-62772762	4	4q	4qB3	4qB3	-1.2200	-1.1700	-1.1700	-1.2100	-0.9900	-0.9400
88	chr4:62772762-68954928	4	4q	4qC1	4qC1	0	0	0	0	0	0
89	chr4:68954928-71332684	4	4q	4qC2	4qC2	0	0	0	0	0	0
90	chr4:71332684-83221465	4	4q	4qC3	4qC3	0	0	0	0	0	0
91	chr4:83221465-88928080	4	4q	4qC4	4qC4	0	0	-0.9100	-0.9700	-0.7100	0
92	chr4:88928080-96536899	4	4q	4qC5	4qC5	0	0	0	0	0	0
93	chr4:96536899-104621270	4	4q	4qC6	4qC6	0	0	0	0	0	0
94	chr4:104621270-109852334	4	4q	4qC7	4qC7	0	0	0	0	0	0
95	chr4:109852334-116510051	4	4q	4qD1	4qD1	2.6500	2.6300	2.3800	2.4200	3.8300	4.1700

96	chr4:116510051-119363358	4	4q	4qD2	4qD2.1	0	0	0	0	0	0
97	chr4:119363358-129825485	4	4q	4qD2	4qD2.2	0	0	0	0	0	0
98	chr4:129825485-132678793	4	4q	4qD2	4qD2.3	0	0	0	0	0	0
99	chr4:132678793-140287612	4	4q	4qD3	4qD3	0	0	-0.8400	-1.0600	-0.7500	-0.4900
100	chr4:140287612-146469778	4	4q	4qE1	4qE1	0	0	0	0.4800	0	0
101	chr4:146469778-155029701	4	4q	4qE2	4qE2	0	0.0800	0	0	0	0
102	chr5:0-14911692	5	5q	5qA1	5qA1	0	0	0	0	0	0
103	chr5:14911692-16354759	5	5q	5qA2	5qA2	0	0	0	0	0	0
104	chr5:16354759-25494184	5	5q	5qA3	5qA3	0	0	0	0	0	0
105	chr5:25494184-33671564	5	5q	5qB1	5qB1	0	0	0	1.2000	0	0
106	chr5:33671564-35595653	5	5q	5qB2	5qB2	0	0	0	0	0	0
107	chr5:35595653-50507346	5	5q	5qB3	5qB3	0	0	0	0	0	0
108	chr5:50507346-	5	5q	5qC1	5qC1	0	0	0	0	0	0

	58684726										
109	chr5:58684726-61089838	5	5q	5qC2	5qC2	0	0	0	0	0	0
110	chr5:61089838-71672330	5	5q	5qC3	5qC3.1	0	0	0	0	0	0
111	chr5:71672330-73596419	5	5q	5qC3	5qC3.2	0	0	0	0	0	0
112	chr5:73596419-77925620	5	5q	5qC3	5qC3.3	0	0	0	0	0	0
113	chr5:77925620-81773799	5	5q	5qD	5qD	0	0	0	0	0	0
114	chr5:81773799-91394246	5	5q	5qE1	5qE1	0	0	-0.2600	0	-0.2500	0
115	chr5:91394246-93799358	5	5q	5qE2	5qE2	0	0	0	0	0	0
116	chr5:93799358-99571626	5	5q	5qE3	5qE3	0	0	0	0	0	0
117	chr5:99571626-101976738	5	5q	5qE4	5qE4	0	0	0	0	0	0
118	chr5:101976738-107749006	5	5q	5qE5	5qE5	0	0	0	0	0	0
119	chr5:107749006-125065811	5	5q	5qF	5qF	0	0	0	0	0	0
120	chr5:125065811-126989900	5	5q	5qG1	5qG1.1	0	0	0	0	0	0

121	chr5:126989900-127951945	5	5q	5qG1	5qG1.2	0	0	0	0	0	0
122	chr5:127951945-130838079	5	5q	5qG1	5qG1.3	0	0	0	0	0	0
123	chr5:130838079-146230794	5	5q	5qG2	5qG2	0	0	0	0	0	0
124	chr5:146230794-152003063	5	5q	5qG3	5qG3	0	0	0	0	0	0
125	chr6:0-16613965	6	6q	6qA1	6qA1	0	0	-1.0200	-1.0300	-0.8300	-0.7500
126	chr6:16613965-21500425	6	6q	6qA2	6qA2	0	0	0.2900	0.4300	0.3600	0.3500
127	chr6:21500425-27364177	6	6q	6qA3	6qA3.1	0	0	0.2900	0.4300	0.3600	0.3500
128	chr6:27364177-28341469	6	6q	6qA3	6qA3.2	0	0	0.2900	0.4300	0.3600	0.3500
129	chr6:28341469-34205222	6	6q	6qA3	6qA3.3	0	0	0.2900	0.4300	0.3600	0.3500
130	chr6:34205222-41534912	6	6q	6qB1	6qB1	0	0	0.2900	0.4300	0.3600	0.3500
131	chr6:41534912-44466788	6	6q	6qB2	6qB2.1	0	0	0.2900	0.4300	0.3600	0.3500
132	chr6:44466788-45932726	6	6q	6qB2	6qB2.2	0	0	0.2900	0.4300	0.3600	0.3500
133	chr6:45932726-	6	6q	6qB2	6qB2.3			0.2900	0.4300	0.3600	0.3500

	50819187					0.9400	1.0000				
134	chr6:50819187-62546691	6	6q	6qB3	6qB3	0	0	0.2900	0.4300	0.3600	0.3500
135	chr6:62546691-74274196	6	6q	6qC1	6qC1	0	0	0.2900	0.4300	0.3600	0.3500
136	chr6:74274196-76717426	6	6q	6qC2	6qC2	0	0	0.2900	0.4300	0	0.3500
137	chr6:76717426-86001701	6	6q	6qC3	6qC3	0	0	0.2900	0.4300	0.3600	0.3500
138	chr6:86001701-94308683	6	6q	6qD1	6qD1	0	0	0.2900	0.4300	0.3600	0.3500
139	chr6:94308683-95774621	6	6q	6qD2	6qD2	0	0	0.2900	0.4300	0.3600	0.3500
140	chr6:95774621-103104312	6	6q	6qD3	6qD3	0	0	0.2900	0.4300	0.3600	0.3500
141	chr6:103104312-108479418	6	6q	6qE1	6qE1	0	0	0.2900	0.4300	0.3600	0.3500
142	chr6:108479418-109456710	6	6q	6qE2	6qE2	0	0	0.2900	0.4300	0.3600	0.3500
143	chr6:109456710-116786401	6	6q	6qE3	6qE3	0	0	0.2900	0.4300	0.3600	0.3500
144	chr6:116786401-122650153	6	6q	6qF1	6qF1	0	0	0	0	0	0
145	chr6:122650153-125093383	6	6q	6qF2	6qF2	0	0	0	0	0	0

146	chr6:125093383-131934427	6	6q	6qF3	6qF3	0.9500	1.0500	-0.2700	-0.2700	0.6400	0.8400
147	chr6:131934427-139264118	6	6q	6qG1	6qG1	0	0	-0.2700	-0.2700	-0.2600	0
148	chr6:139264118-142684640	6	6q	6qG2	6qG2	0	0	0	0	0	0
149	chr6:142684640-149525685	6	6q	6qG3	6qG3	0	0	0	0	0	0
150	chr7:0-15170811	7	7q	7qA1	7qA1	0	0	0.3000	0.3000	0.2900	0
151	chr7:15170811-18204973	7	7q	7qA2	7qA2	0	0	0.3600	0.4500	0.2900	0
152	chr7:18204973-28318847	7	7q	7qA3	7qA3	0	0	0.3600	0.4500	0.3600	0
153	chr7:28318847-34387172	7	7q	7qB1	7qB1	0	0	0.3600	0.4500	0.3600	0
154	chr7:34387172-37421334	7	7q	7qB2	7qB2	0	0	0.3600	0.4500	0.3600	0
155	chr7:37421334-47535208	7	7q	7qB3	7qB3	0.8900	0.9000	0.4300	0.4500	0.2900	0.2600
156	chr7:47535208-54109226	7	7q	7qB4	7qB4	0	0	0.3000	0.3000	0.2900	0
157	chr7:54109226-60683244	7	7q	7qB5	7qB5	0	0	0.3000	0.3000	0	0
158	chr7:60683244-	7	7q	7qC	7qC	-	-	0.3000	-0.4000	0.2900	-0.5500

	71302812					0.7200	0.6800				
159	chr7:71302812-76865443	7	7q	7qD1	7qD1	0	0	0.3000	0.2200	0.2900	0
160	chr7:76865443-80405299	7	7q	7qD2	7qD2	0	0	0.3000	0.2200	0.2900	0
161	chr7:80405299-90013479	7	7q	7qD3	7qD3	0	0	0.3000	0.2200	0.2900	0
162	chr7:90013479-99621660	7	7q	7qE1	7qE1	0	0	0.3000	0.2200	0.2900	0
163	chr7:99621660-102150128	7	7q	7qE2	7qE2	0	0	0.3000	0.2200	0.2900	0
164	chr7:102150128-111252615	7	7q	7qE3	7qE3	1.9800	2.0000	0.3000	0.2200	0.2900	1.7300
165	chr7:111252615-118332327	7	7q	7qF1	7qF1	0	0	0.3000	0	0.2900	0
166	chr7:118332327-122883570	7	7q	7qF2	7qF2	0	0	0.3000	0	0.2900	0
167	chr7:122883570-137042994	7	7q	7qF3	7qF3	0	0	0.3000	0	0.2900	0.9500
168	chr7:137042994-140582850	7	7q	7qF4	7qF4	0	0	0.3000	0	0.2900	0
169	chr7:140582850-145134094	7	7q	7qF5	7qF5	0	0	0.3000	0.3000	0.2900	0
170	chr8:0-16271352	8	8q	8qA1	8qA1.1	0	0	0	0	0	0



171	chr8:16271352-17228491	8	8q	8qA1	8qA1.2	0	0	0	0	0	0
172	chr8:17228491-20578475	8	8q	8qA1	8qA1.3	0	0	0	0	0	0
173	chr8:20578475-30149859	8	8q	8qA2	8qA2	0	0	0	0	0	0
174	chr8:30149859-34456982	8	8q	8qA3	8qA3	1.0500	0.7800	0	0	0.7900	0
175	chr8:34456982-44985504	8	8q	8qA4	8qA4	0	0	0	0	-0.3200	0
176	chr8:44985504-49292627	8	8q	8qB1	8qB1.1	0	0	0	0	-0.3200	0
177	chr8:49292627-51206903	8	8q	8qB1	8qB1.2	0	0	0	0	0	0
178	chr8:51206903-56949734	8	8q	8qB1	8qB1.3	0	0	0	0	0	0
179	chr8:56949734-60778287	8	8q	8qB2	8qB2	0	0	0	0	0	0
180	chr8:60778287-68435394	8	8q	8qB3	8qB3.1	0	0	0	0	0	0
181	chr8:68435394-69392533	8	8q	8qB3	8qB3.2	0	0	0	0	0	0
182	chr8:69392533-76092502	8	8q	8qB3	8qB3.3	0	0	0	0	0	0
183	chr8:76092502-	8	8q	8qC1	8qC1	0	0	0	0	0	0

	81835332										
184	chr8:81835332-86621024	8	8q	8qC2	8qC2	0	0	1.9500	0	0	0
185	chr8:86621024-91885285	8	8q	8qC3	8qC3	0	0	0	0	0	0
186	chr8:91885285-93320993	8	8q	8qC4	8qC4	0	0	0	0	0	0
187	chr8:93320993-97628115	8	8q	8qC5	8qC5	0	0	0	0	0	0
188	chr8:97628115-105285223	8	8q	8qD1	8qD1	0	0	0	0	0	0
189	chr8:105285223-106242361	8	8q	8qD2	8qD2	0	0	0	0	0	0
190	chr8:106242361-112942330	8	8q	8qD3	8qD3	0	0	0	0	0	0
191	chr8:112942330-126342267	8	8q	8qE1	8qE1	0	0	0	0.9900	0	0
192	chr8:126342267-132085098	8	8q	8qE2	8qE2	0	0	0	0	0	0
193	chr9:0-14343360	9	9q	9qA1	9qA1	0	0	0	0	0	0
194	chr9:14343360-19432940	9	9q	9qA2	9qA2	0	0	0	0	-0.3100	0
195	chr9:19432940-24059831	9	9q	9qA3	9qA3	0	0	0	0	-0.3100	0

196	chr9:24059831-37940503	9	9q	9qA4	9qA4	0	0	0	0	0	0
197	chr9:37940503-43955461	9	9q	9qA5	9qA5.1	0	0	0	0	0	0
198	chr9:43955461-46268906	9	9q	9qA5	9qA5.2	0	0	0	0	0	0
199	chr9:46268906-54597309	9	9q	9qA5	9qA5.3	0	0	0	0	0	0
200	chr9:54597309-62925712	9	9q	9qB	9qB	0	0	0	0	0	0
201	chr9:62925712-69403359	9	9q	9qC	9qC	0	0	0	0	0	0
202	chr9:69403359-77269073	9	9q	9qD	9qD	0	0	0	0	0	0
203	chr9:77269073-82358653	9	9q	9qE1	9qE1	0	0	0	0	0	0
204	chr9:82358653-84209409	9	9q	9qE2	9qE2	0	0	0	0	0	0
205	chr9:84209409-90687056	9	9q	9qE3	9qE3.1	0	0	0	0	0	0
206	chr9:90687056-91149745	9	9q	9qE3	9qE3.2	0	0	0	0	0	0
207	chr9:91149745-100403526	9	9q	9qE3	9qE3.3	0	0	0	0	0	0
208	chr9:100403526	9	9q	9qE4	9qE4	0	0	0	0	0	0

	-101328904										
209	chr9:101328904-107806551	9	9q	9qF1	9qF1	0	0	0	0	0	0
210	chr9:107806551-110582686	9	9q	9qF2	9qF2	0	0	0	0	0	0
211	chr9:110582686-119373778	9	9q	9qF3	9qF3	0	0	0	0	0	0
212	chr9:119373778-124000669	9	9q	9qF4	9qF4	0	0	0	0.9400	0	0
213	chr10:0-12750708	10	10q	10qA1	10qA1	0	0	0	0	0	0
214	chr10:12750708-17654827	10	10q	10qA2	10qA2	0	0	0	0	0	0
215	chr10:17654827-23539770	10	10q	10qA3	10qA3	0	0	0	0	0	0
216	chr10:23539770-33348007	10	10q	10qA4	10qA4	0	0	0	0	-0.2500	-0.2600
217	chr10:33348007-41194597	10	10q	10qB1	10qB1	0	0	0	0	-0.2500	-0.2600
218	chr10:41194597-48060364	10	10q	10qB2	10qB2	0	0	-0.3000	0	0	0
219	chr10:48060364-55906954	10	10q	10qB3	10qB3	0	0	-0.3000	0	0	0
220	chr10:55906954-63753544	10	10q	10qB4	10qB4	0	0	-0.3000	0	0	0

221	chr10:63753544-67676839	10	10q	10qB5	10qB5.1	0	0	0	0	0	0
222	chr10:67676839-68167251	10	10q	10qB5	10qB5.2	0	0	0	0	0	0
223	chr10:68167251-74542605	10	10q	10qB5	10qB5.3	0	0	0	0	0	0
224	chr10:74542605-88764550	10	10q	10qC1	10qC1	0	0	0	0	0	0
225	chr10:88764550-95630316	10	10q	10qC2	10qC2	0	1.3300	1.2100	1.2400	0	0
226	chr10:95630316-98572787	10	10q	10qC3	10qC3	0	0	-0.2800	0	0	0
227	chr10:98572787-111323496	10	10q	10qD1	10qD1	0	0	-0.2800	0	0	0
228	chr10:111323496-124074205	10	10q	10qD2	10qD2	0	0	0	0	0	0
229	chr10:124074205-129959148	10	10q	10qD3	10qD3	0	0	0	0	0	0
230	chr11:0-13016647	11	11q	11qA1	11qA1	0	0	0	0	0	0
231	chr11:13016647-17200570	11	11q	11qA2	11qA2	0	0	0	0	0	0
232	chr11:17200570-21849372	11	11q	11qA3	11qA3.1	0	0	0	0	0	0
233	chr11:21849372	11	11q	11qA	11qA3.	0	0	0	0	0	0

	-25568415			3	2						
234	chr11:25568415-30217217	11	11q	11qA3	11qA3.3	0	0	0	0	0	0
235	chr11:30217217-36260661	11	11q	11qA4	11qA4	0	0	1.5300	1.2400	0	0
236	chr11:36260661-43233865	11	11q	11qA5	11qA5	0	0	0	0	0	0
237	chr11:43233865-47882668	11	11q	11qB1	11qB1.1	0	1.2900	0	1.2000	0	0
238	chr11:47882668-49742189	11	11q	11qB1	11qB1.2	0	0	0	0	0	0
239	chr11:49742189-59969555	11	11q	11qB1	11qB1.3	0	0	0	0	0	0
240	chr11:59969555-62758837	11	11q	11qB2	11qB2	0	0	0	0	0	0
241	chr11:62758837-70661801	11	11q	11qB3	11qB3	0	0	0.5200	0.5000	0.4400	0.4200
242	chr11:70661801-73915963	11	11q	11qB4	11qB4	0	0	0.5200	0.5000	0.4400	0.4200
243	chr11:73915963-81818928	11	11q	11qB5	11qB5	0	0	0.5200	0.5000	0.2600	0.4200
244	chr11:81818928-90186773	11	11q	11qC	11qC	0	0	0.5200	0.5000	0.4400	0.4200
245	chr11:90186773-102273660	11	11q	11qD	11qD	0	0	0.5200	0.5000	0.4400	0.4200

246	chr11:102273660-110176625	11	11q	11qE1	11qE1	0	0	0.5200	0.5000	0.4400	0.4200
247	chr11:110176625-121798632	11	11q	11qE2	11qE2	0	0	0.5200	0.5000	0.4400	0.4200
248	chr12:0-17650279	12	12q	12qA1	12qA1.1	0	0	0	0	0	0
249	chr12:17650279-21180335	12	12q	12qA1	12qA1.2	0	0	0	0	0	0
250	chr12:21180335-26034162	12	12q	12qA1	12qA1.3	0	0	0	0	0	0
251	chr12:26034162-31770503	12	12q	12qA2	12qA2	2.4000	2.2000	2.0700	2.4200	0	0
252	chr12:31770503-39271872	12	12q	12qA3	12qA3	1.1800	1.2200	1.0500	1.1200	0.9700	1.0400
253	chr12:39271872-44125699	12	12q	12qB1	12qB1	0	0	0	0	0	0
254	chr12:44125699-45008213	12	12q	12qB2	12qB2	0	0	0	0	0	0
255	chr12:45008213-52068325	12	12q	12qB3	12qB3	0	0	0	0	0	0
256	chr12:52068325-66188548	12	12q	12qC1	12qC1	0	0	0	0	0	0
257	chr12:66188548-71483632	12	12q	12qC2	12qC2	0	0	0	0	0	0
258	chr12:71483632	12	12q	12qC	12qC3	0	0	0	0	0	0

	-81191286			3							
259	chr12:81191286-85603856	12	12q	12qD1	12qD1	0	0	0	0	0	0
260	chr12:85603856-88692655	12	12q	12qD2	12qD2	0	0	0	0	0	0
261	chr12:88692655-95752767	12	12q	12qD3	12qD3	0	0	0	0	0	0
262	chr12:95752767-106342935	12	12q	12qE	12qE	0	0	0	0	0	0
263	chr12:106342935-114726818	12	12q	12qF1	12qF1	0	0	0	0	0	0
264	chr12:114726818-120463159	12	12q	12qF2	12qF2	0	0	0	0	0	0
265	chr13:0-16312600	13	13q	13qA1	13qA1	0	0	0	0	0	0
266	chr13:16312600-21255812	13	13q	13qA2	13qA2	0	0	0	0	-0.3700	0
267	chr13:21255812-29659273	13	13q	13qA3	13qA3.1	0	0	0	0	0	0
268	chr13:29659273-33119521	13	13q	13qA3	13qA3.2	0	0	0	0	0	0
269	chr13:33119521-41522982	13	13q	13qA3	13qA3.3	0	0	0	0	0	0
270	chr13:41522982-44488909	13	13q	13qA4	13qA4	0	0	0	0	0	0



271	chr13:44488909-52892370	13	13q	13qA5	13qA5	0	0	0	0	0	0
272	chr13:52892370-59318546	13	13q	13qB1	13qB1	0	0	0	0	0	0
273	chr13:59318546-61790152	13	13q	13qB2	13qB2	0	0	0	0	0	0
274	chr13:61790152-69699292	13	13q	13qB3	13qB3	0	0	0	0	0	0
275	chr13:69699292-78597074	13	13q	13qC1	13qC1	0	0	0	0	0	0
276	chr13:78597074-81068680	13	13q	13qC2	13qC2	0	0	0	0	0	0
277	chr13:81068680-94909674	13	13q	13qC3	13qC3	0	0	0	0	0	0
278	chr13:94909674-106773383	13	13q	13qD1	13qD1	0	0	0	0	0.8100	0.8700
279	chr13:106773383-110727953	13	13q	13qD2	13qD2.1	0	0	0	0	0	0
280	chr13:110727953-116659808	13	13q	13qD2	13qD2.2	0	0	0	0	0	0
281	chr13:116659808-120614378	13	13q	13qD2	13qD2.3	0	0	0	0	0	0
282	chr14:0-14877464	14	14q	14qA1	14qA1	0	0	0	0	0	0
283	chr14:14877464	14	14q	14qA	14qA2	0	0	0	0	0	0

	-19340703			2							
284	chr14:19340703 -29754928	14	14q	14qA 3	14qA3	0	0	0	0	0	0
285	chr14:29754928 -43144646	14	14q	14qB	14qB	0	0	0	0	0	0
286	chr14:43144646 -51575209	14	14q	14qC 1	14qC1	0	0	-0.2400	0	-0.3000	0
287	chr14:51575209 -54550702	14	14q	14qC 2	14qC2	0.6200	0.6300	0.5600	0.5500	0.5500	0.5600
288	chr14:54550702 -59509857	14	14q	14qC 3	14qC3	0	0	0	0	0	0
289	chr14:59509857 -68436336	14	14q	14qD 1	14qD1	0	0.8300	0.3600	0.4400	0.3000	0
290	chr14:68436336 -72403660	14	14q	14qD 2	14qD2	0	0	0	0	0	0
291	chr14:72403660 -84305631	14	14q	14qD 3	14qD3	0	0	0	0	0	0
292	chr14:84305631 -88272955	14	14q	14qE 1	14qE1	0	0	0	0	0	0
293	chr14:88272955 -98191265	14	14q	14qE 2	14qE2. 1	0	0	0	0	0	0
294	chr14:98191265 -99183096	14	14q	14qE 2	14qE2. 2	0	0	0	0	0	0
295	chr14:99183096 -106621828	14	14q	14qE 2	14qE2. 3	0	0	0	0	0	0

296	chr14:106621828-110093236	14	14q	14qE3	14qE3	0	0	0	0	0	0
297	chr14:110093236-120011546	14	14q	14qE4	14qE4	0	0	0	0	0	0
298	chr14:120011546-123978870	14	14q	14qE5	14qE5	0	0	0	0	0	0
299	chr15:0-16412919	15	15q	15qA1	15qA1	0	0	0.3500	0	0.3400	0.4200
300	chr15:16412919-24163465	15	15q	15qA2	15qA2	0	0	0.3500	0	0.3400	0.4200
301	chr15:24163465-29634438	15	15q	15qB1	15qB1	0	0	0.3500	0	0.3400	0.4200
302	chr15:29634438-31914010	15	15q	15qB2	15qB2	0	0	0.3500	0	0.3400	0.4200
303	chr15:31914010-42855956	15	15q	15qB3	15qB3.1	0	0	0.3500	0	0.3400	0.4200
304	chr15:42855956-44679614	15	15q	15qB3	15qB3.2	0	0	0.3500	0	0.3400	0.4200
305	chr15:44679614-49694673	15	15q	15qB3	15qB3.3	0	0	0.3500	0	0.3400	0.4200
306	chr15:49694673-53341989	15	15q	15qC	15qC	0	0	0.3500	0	0.3400	0.4200
307	chr15:53341989-66107593	15	15q	15qD1	15qD1	0	0	0.3500	0	0.3400	0.4200
308	chr15:66107593	15	15q	15qD	15qD2	0	0	0.4300	0	0.4200	0.5000

	-68387165			2							
309	chr15:68387165-77049539	15	15q	15qD3	15qD3	0	0	0.4300	0	0.4200	0.5000
310	chr15:77049539-83432341	15	15q	15qE1	15qE1	0	0	0.4900	0	0.4200	0.5000
311	chr15:83432341-86623742	15	15q	15qE2	15qE2	0	0	0.4900	0	0.4200	0.5000
312	chr15:86623742-95286117	15	15q	15qE3	15qE3	0	0	0.4900	0	0.4200	0.5000
313	chr15:95286117-100757090	15	15q	15qF1	15qF1	0	0	0.4900	0	0.4200	0.5000
314	chr15:100757090-101668919	15	15q	15qF2	15qF2	0	0	0.4900	0	0.4200	0.5000
315	chr15:101668919-103492577	15	15q	15qF3	15qF3	0	0	0.4300	0	0.4200	0.5000
316	chr16:0-15439672	16	16q	16qA1	16qA1	0	0	0	0	0	0
317	chr16:15439672-16375409	16	16q	16qA2	16qA2	0	0	0	0	0	0
318	chr16:16375409-20586229	16	16q	16qA3	16qA3	0	0	0	0	0	0
319	chr16:20586229-26200655	16	16q	16qB1	16qB1	0	0	0	0	0	0
320	chr16:26200655-32282950	16	16q	16qB2	16qB2	0	0	0	0	0	0

321	chr16:32282950-38365245	16	16q	16qB3	16qB3	0	0	0	0	0	0
322	chr16:38365245-44915409	16	16q	16qB4	16qB4	1.0200	1.0000	0	0.9100	0	0
323	chr16:44915409-53804918	16	16q	16qB5	16qB5	0	0	0	0	0	0
324	chr16:53804918-58015737	16	16q	16qC1	16qC1.1	0	0	0	0	0	0
325	chr16:58015737-58951475	16	16q	16qC1	16qC1.2	0	0	0	0	0	0
326	chr16:58951475-66905245	16	16q	16qC1	16qC1.3	0	0	0	0	0	0
327	chr16:66905245-70648196	16	16q	16qC2	16qC2	0	0	0	0	0	0
328	chr16:70648196-79069836	16	16q	16qC3	16qC3.1	0	0	0	0	0	0
329	chr16:79069836-79537704	16	16q	16qC3	16qC3.2	0	0	0	0	0	0
330	chr16:79537704-91702295	16	16q	16qC3	16qC3.3	0	0	0	0	0	0
331	chr16:91702295-98252459	16	16q	16qC4	16qC4	0	0	0	0	0	0
332	chr17:0-13970997	17	17q	17qA1	17qA1	0	0	0	0	0	0
333	chr17:13970997	17	17q	17qA	17qA2	0	0	0	0	0	0

	-16153965			2							
334	chr17:16153965 -17463746	17	17q	17qA 3	17qA3. 1	0	0	0	0	0	0
335	chr17:17463746 -21829683	17	17q	17qA 3	17qA3. 2	0	0	0	0	0	0
336	chr17:21829683 -31434744	17	17q	17qA 3	17qA3. 3	- 0.5000	- 0.5400	-0.5600	-0.5100	-0.5200	-0.5300
337	chr17:31434744 -40166617	17	17q	17qB 1	17qB1	0	0	0	0.9400	0	0
338	chr17:40166617 -41476398	17	17q	17qB 2	17qB2	0	0	0	0	0	0
339	chr17:41476398 -45842335	17	17q	17qB 3	17qB3	0	0	0	0	0	0
340	chr17:45842335 -55883989	17	17q	17qC	17qC	0	0	0	0	0	0
341	chr17:55883989 -60249926	17	17q	17qD	17qD	0	0	0	0	0	0
342	chr17:60249926 -68108612	17	17q	17qE 1	17qE1. 1	0	0	0	0	0	0
343	chr17:68108612 -68981799	17	17q	17qE 1	17qE1. 2	0	0	0	0	0	0
344	chr17:68981799 -73347736	17	17q	17qE 1	17qE1. 3	0	0	0	0	0	0
345	chr17:73347736 -78586860	17	17q	17qE 2	17qE2	0	0	0	0	0	0

346	chr17:78586860-82952797	17	17q	17qE3	17qE3	0	0	0	0	0	0
347	chr17:82952797-89065108	17	17q	17qE4	17qE4	0	0	0	0	0	0
348	chr17:89065108-95177420	17	17q	17qE5	17qE5	0	0	0	0	0	0
349	chr18:0-19413462	18	18q	18qA1	18qA1	0	0	0	0	0	0
350	chr18:19413462-29542226	18	18q	18qA2	18qA2	0	0	0	0	0	0
351	chr18:29542226-35450671	18	18q	18qB1	18qB1	0	0	0	0	0	0
352	chr18:35450671-37138798	18	18q	18qB2	18qB2	0	0	0	0	0	0
353	chr18:37138798-45579434	18	18q	18qB3	18qB3	0	0	0	0	0	0
354	chr18:45579434-49799752	18	18q	18qC	18qC	0	0	0	0	0	0
355	chr18:49799752-54020070	18	18q	18qD1	18qD1	0	0	0	0	-0.6200	0
356	chr18:54020070-54442102	18	18q	18qD2	18qD2	0	0	0	0	0	0
357	chr18:54442102-60772579	18	18q	18qD3	18qD3	0	0	0	0	0	0
358	chr18:60772579	18	18q	18qE	18qE1	0	0	0	0	0	0

	-67947119			1							
359	chr18:67947119-75121660	18	18q	18qE2	18qE2	0	0	0	0	0	0
360	chr18:75121660-83562296	18	18q	18qE3	18qE3	0	0	0	0	0	0
361	chr18:83562296-90736837	18	18q	18qE4	18qE4	0	0	0	0	0	0
362	chr19:0-16650124	19	19q	19qA	19qA	0	0	-0.9900	-0.9900	-0.7500	-0.5600
363	chr19:16650124-25584337	19	19q	19qB	19qB	0	0	0	0	0	0
364	chr19:25584337-34924651	19	19q	19qC1	19qC1	0	0	0	0	0	0
365	chr19:34924651-38173456	19	19q	19qC2	19qC2	0	0	0	0	0	0
366	chr19:38173456-47513769	19	19q	19qC3	19qC3	0.9800	0.9900	1.0100	1.0400	0	1.2500
367	chr19:47513769-51574775	19	19q	19qD1	19qD1	0	0	0	0	0	0
368	chr19:51574775-58884586	19	19q	19qD2	19qD2	0	0	0	0	-0.7300	0
369	chr19:58884586-61321190	19	19q	19qD3	19qD3	0	0	0	0	-0.4200	0

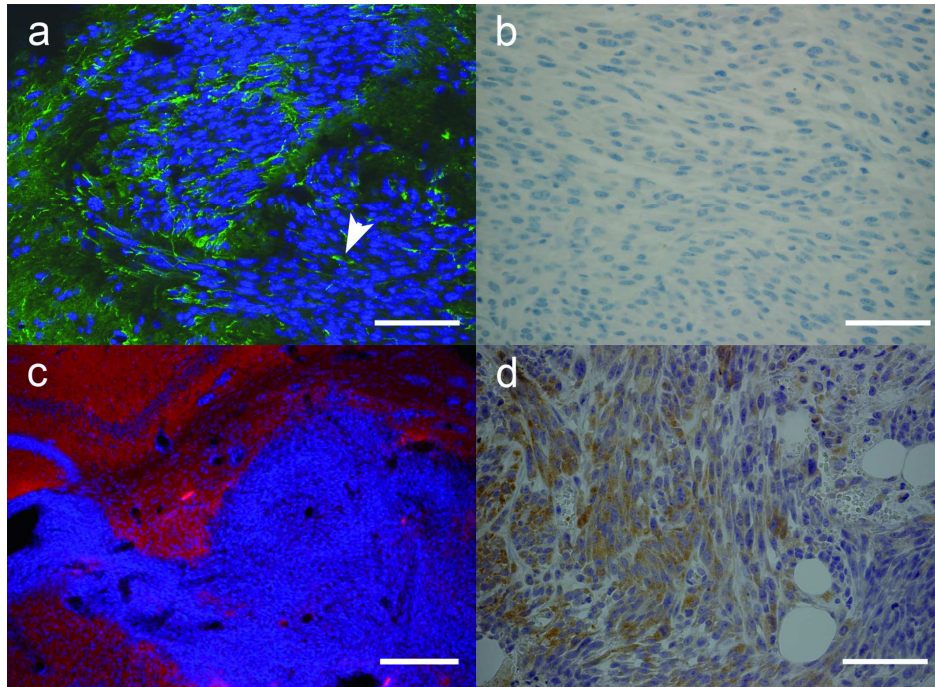


**Supplementary table 1: Chromosomal aberrations defined with the following parameters:**

ADM2 method for segmentation with threshold 6, Log2 ratio > 0.25, and numbers of probes  $\geq 5$ .

Average values are provided for tumours derived from irradiated TGF $\alpha$ -treated astrocytes

growing as adherent cells (n=5) and from irradiated TGF $\alpha$ -treated astrocytes growing as floating cells (n=3).



**Supplementary Figure 1: Comparison of GFAP and synaptophysin expression in irradiated TGF $\alpha$ -treated astrocytes in intra-cerebral and subcutaneous grafts. a. GFAP-immunoreactive cells were observed within the core of the CNS tumors (arrow, 480 nm thick ApoTome optical slice). b. In contrast, no GFAP immunolabeling was detected in subcutaneous grafts. c. Synaptophysin immunolabeling was restricted to the host tissue in the case of intra-cerebral grafts. d. Numerous synaptophysin immunoreactive cells were observed within the subcutaneous tumors. Bar=100  $\mu$ m in a, b, d and 200  $\mu$ m in c.**

90x114mm (600 x 600 DPI)