Genomic Changes of Glioblastoma Stem Cell Subpopulations are Widely Identical with those of the Original Parental Cell Line

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Introduction:

Human glioblastoma multiforme (GBM) represent the most frequent form of primary brain tumors and is characterised by poor prognosis. Chemotherapeutic treatment is mainly limited by the blood-brain-barrier and the expression of chemoresistance-related proteins. There is evidence that GBMs contain **stem-cell like subpopulations**, responsible for tumor initiation, tumor formation and therapy resistance. This subpopulation is characterized by stem cell markers, e.g. CD133, nestin and SOX2 and insensitivity against chemotherapeutic agents and irradiation.

Aim of this study was to clarify whether the GBM stem-cell like subpopulations (GSCLC) present in GBM cell cultures are characterized by specific genomic alterations. Fourteen GBM primary cultures and 7 cell lines were analyzed for the presence of GSCLC compartments by investigating their expression of different stem cell markers (CD133, nestin and SOX2) and ability to grow in serum-free neurobasal (NBE) medium.

Growth ability and sphere formation of primary cultures and cell lines in NBE-medium

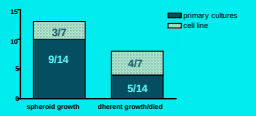

Figure 1.: Nine out of 14 GBM primary cultures and 3 out of 7 GBM cell lines were able to grow as stable non-adherent neurospheres in NBE-medium for 3 up to 14 months.

Distribution pattern of three stem cell markers (CD133, nestin and SOX2)

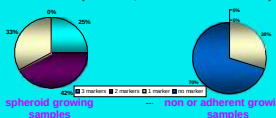

Figure 2.: From those GBM samples, able to grow as neurospheres in NBE-medium, 33% expressed all 3 stem cell markers, 42% two markers and 25% one stem cell marker.
The samples who grew as monolayers or died in NBE-medium, expressed only one (30%) or no stem cell marker (70%).

Growth pattern and expression of CD133 and Nestin: GBM primary culture vs. GBM cell line

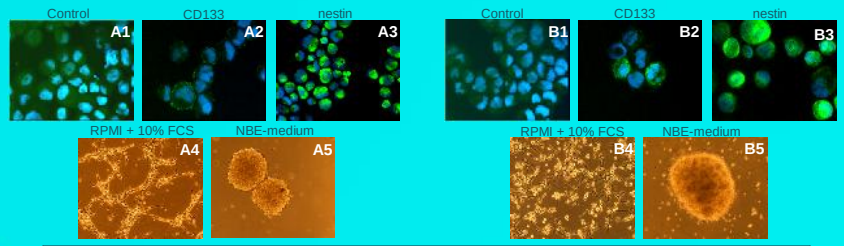

Figure 3.: **A2, B2** Immunofluorescence staining with an antibody against CD133 showed CD133 expression on the cell surface in the GBM primary culture RAEW and the cell line CRL2020, respectively. Both samples expressed nestin (**A3 and B3**). **A1, B1** were the respective negative controls.
A4, B4 Glioblastoma cells cultured in RPMI medium containing 10% serum grew adherently and formed monolayers. **A5, B5** In NBE- medium they started to detach and formed non-adherent spheres.

Classical CGH – comparison of U373 GBM cells grown in medium as monolayers with serum (left) vs. neurospheres (right)

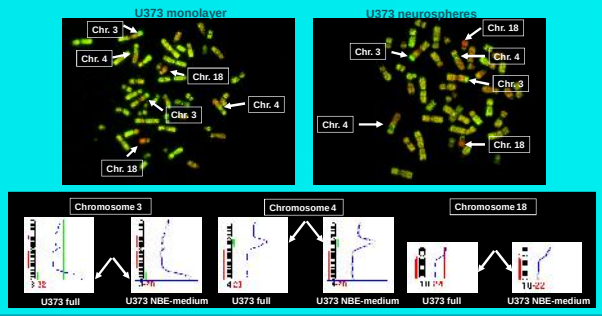

Figure 4.: Overview of chromosomal gains and losses in the U373 GBM cell line. Selected gains (green) and losses (red) are marked with white arrows and their profiles are shown below. No significant genomic changes were seen between the mother cell line grown in medium with serum and the respective stable neurospheres.

aCGH: Detection of differences between RAEW (GBM) primary culture grown as monolayer (blue) vs. RAEW neurospheres (purple) at the gene level

Figure 5A.: Neurospheres, stable growing in NBE-medium from three up to 14 months, were analyzed by array (a)CGH and compared to the respective parental cells. Again, widely identical profiles (marked with red boxes) were seen. However differences at distinct gene loci were found and are marked by red circles (see Figure 5B).

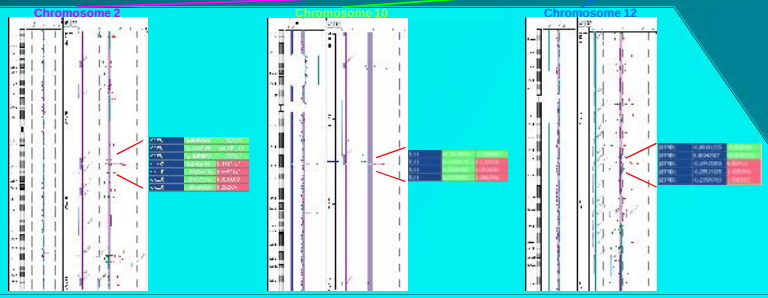

Figure 5B.: Distinct differences (amplifications) on Chromosome 2 (part of CREB1 locus), Chromosome 10 (TLX1) and Chromosome 12 (LETMD1) were seen (compare genomic profiles in Figure 5A).

Summary: 14 GBM primary cultures and 7 GBM cell lines were analyzed for their ability to grow as neurospheres in NBE-medium. Those able to survive as non-adherent spheres over a prolonged time period expressed at least one stem cell marker (CD133, nestin and/or SOX2) as analyzed by western blot, immunofluorescence, RT-PCR and/or FACS analyses. The majority (70%) of GBM cell cultures incapable of growth as spheres expressed non of the investigated stem cell markers. Selected primary cultures (N=8) and two GBM cell lines, grown in parallel for 3 up to 14 months in spheres and monolayer cultures, were comparably analyzed for genome-wide genomic gains and losses by (array)CGH.
Typical genomic changes for GBM were generally found, including gains of chromosome 7 and losses of 9p and 10. In all cases analyzed, **GBM stem-cell like subpopulations** (GSCLC) grown as neurospheres contained **widely the identical chromosomal gains and losses** as the respective parental cell cultures as detected by classical CGH. Only occasional differences observed concerned random low-level gains/losses of large chromosomal regions or whole chromosomes. On closer inspection by aCGH **small but distinct amplifications/deletions** could be found in the GSCLC. These affected several known cancer-related genes which are currently investigated in ongoing gene expression experiments.
In conclusion, we demonstrate that most GBM cell cultures/cell lines harbor GSCLC with widely identical larger genomic alterations as the respective monolayer cell cultures. However, small amplicons/deletions might be characteristic for the cancer stem cell compartment and contribute to its specific role in the carcinogenic process.

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