5142

Detection of genomic changes in glioblastoma stem cell subpopulations by array comparative genomic hybridisation

Daniela Loetsch[1], Christine Pirker[1], Sabine Spiegl-Kreinecker[2], Michael Grusch[1], Johannes Fischer[2], Michael Micksche[1], Walter Berger[1]

[1]Institute of Cancer Research, Vienna, Austria; [2]Departments of Neurosurgery-Wagner Jauregg Hospital, Linz, Austria

Introduction:

Glioblastoma multiforme (GBM) is one of the most aggressive tumors characterized by bad prognosis and limited treatment response. Stem-cell like GBM subpopulations are believed to cause tumor initiation and treatment resistance. Aim of this study was to clarify whether the GBM stem-cell like subpopulations (GSCLS) present in GBM cell cultures are characterized by specific genomic alterations and/or by differences concerning gene expression.

Results:

Fourteen GBM primary cultures and 6 cell lines were analyzed for the presence of GSCLS compartments by investigating their ability to grow in serum-free neurobasal medium (NBE-medium). Growth in neurospheres was compared to the expression of the stem cell markers CD133, nestin and SOX2 . 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. 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 that grew in NBE-medium as a monolayer or died expressed only one (30%) or no stem cell marker (70%).

Genomic changes in selected primary cultures (N=8) and a GBM cell line grown in parallel for 6 to 60 weeks in spheres and monolayer cultures were comparably analyzed by (array)CGH. In all cases analyzed, GSCLS grown as neurospheres contained widely the identical chromosomal gains/amplifications and losses as the respective parental cell cultures. Differences concerned only random low-level gains/losses of large chromosomal regions or whole chromosome arms.

Further microarray-based gene expression analysis of GBM neurospheres cultured for 4 weeks identified significantly up- or downregulated genes relative to the respective monolayer cultures. The signature of significantly changed genes (monolayer culture versus sphere culture) was very stable between GBM samples (cell lines and tumors) able to grow as neurospheres, but entirely different in those that grew adherently in NBE medium.

Material and Methods:

Cell culture

GBM cell lines, primary cultures and fresh tumor samples were dissected, resuspended in serum-free NBE-medium and grown in parallel to the respective monolayer cultures in RPMI containing 10% serum.

Array CGH (aCGH)

Tumor- and reference-DNA were digested, labeled and hybridized to 4X44K oligonucleotide based arrays (Agilent) according to protocols provided by Agilent. Arrays were scanned on the Micro Array Scanner G2505B (Agilent). Data were analyzed using the Feature Extraction and DNA Analytics software. Cy5/Cy3 ratios are given as log2 values.

Gene expression analysis

RNA of corresponding pairs of neurospheres and monolayer cultures were labeled with the Two-Color-Gene Expression Analysis Kit (Quick Amp Labeling) according to protocols provided by Agilent. Arrays were scanned on the Micro Array Scanner G2505B (Agilent). Data were analyzed using GeneSpring software.

Ability for sphere formation of primary cultures and cell lines in NBE-medium

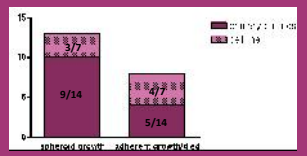


Figure 1.: Nine out of 14 GBM primary cultures and 3 out of 7 GBM cell lines were able to grow as stable non-adherent neurospheres in NBE-medium for 3 up to 14 months.

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

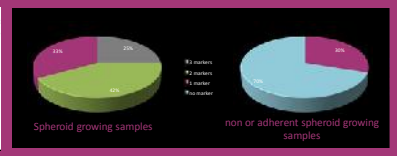


Figure 2.: From those GBM samples, able to grow as neurospheres in NBE-medium, 33% expressed all 3 stem cell markers, 42% two markers and 25% one stem cell marker.
The samples that grew in NBE-medium as monolayers or died, expressed only one (30%) or no stem cell marker (70%).

Growth pattern of GBM primary cultures and a GBM cell line

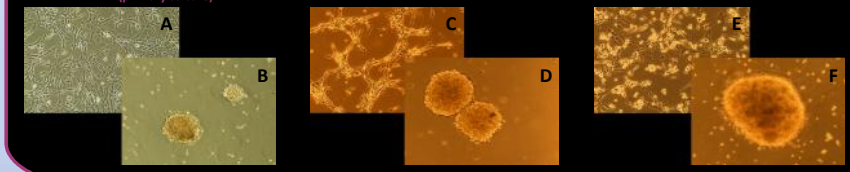


Figure 3.: A, C, E Glioblastoma cells cultured in RPMI medium containing 10% serum grew adherently and formed monolayers.

B, D, F In NBE- medium they started to detach and formed non-adherent spheres.

aCGH: Detection of differences between KIMA, RAEW and CRL2020 (GBM primary cultures and /or cell lines) grown as monolayers versus neurospheres at the gene level: Chromosome 4, 7 and 10 as examples.

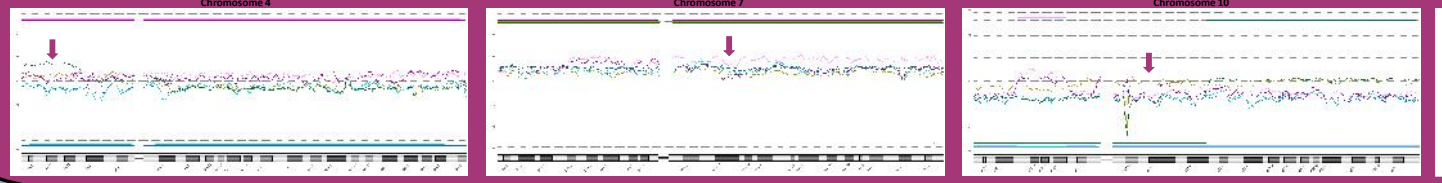
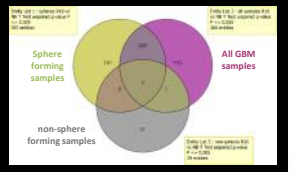
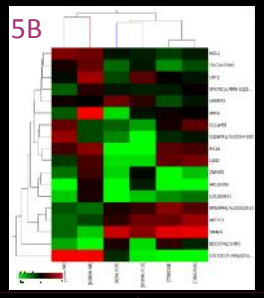


Figure 4.: Neurospheres, stable growing in NBE-medium from three up to 14 months, were analyzed by array CGH and compared to the respective parental cells. The genome profiles showed widely identical chromosomal gains/amplifications and losses as the respective parental cell cultures. Differences concerned only random low-level gains/losses of large chromosomal regions or whole chromosome arms (marked with magenta arrows).





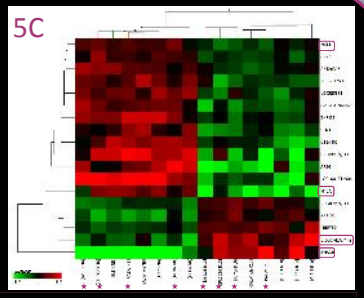


Figure 5.: A. Venn Diagramm comparing significantly changed mRNAs of all GBM samples cultured in NBE-medium (green), with those able to form spheres (magenta) and those that grew adherently in NBE-medium (grey). B, C. Heatmap representing the top 18 signifcantly up-and downregulated mRNAs in the GBM-sphere forming samples (N=8). Neurospheres clustered against the monolayer cultures only in sphere-forming samples (cell lines and tumors) (5C) but not in those that grew adherently in NBE-medium (N=3) (5B).

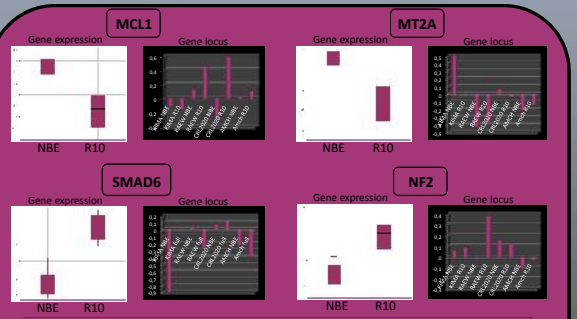


Figure 6.: box plots showing the 4 most significantly changed mRNAs in neurospheres (NBE) as compared to the corresponding monolayers (R10). Bar graphs depict the dose of the respective gene loci in NBE-cultured GBM samples opposed to the corresponding monolayer cultures (marked with stars in the heatmap in 5C).

Conclusion:

- Long-term cultured neurospheres of GBM cell lines and primary cultures showed widely the identical genomic changes as compared to the respective partental cells cultured in RPMI with 10% serum.
- Gene expression analysis of neurospheres compared to the respective monolayer cell cultures identified significantly regulated genes.
- The signature of significantly changed entities of GBM samples able to form spheres clustered neurospheres against the adherent-growing cultures. An unsupervized clustering of GBM samples, not able to form spheres, did not lead to those clusters.

In conclusion, we demonstrate that the majority of human GBM primary cell cultures/cell lines contain stem cell-like subpopulations. These sphere-forming cell clones harbor widely identical gene amplifications/deletions but a significantly different mRNA signature as compared to the respective monolayer cell cultures.
These data suggest that epigenetic mechanisms rather than genomic changes are the driving force determining the cancer stem cells phenotyp in glioblastoma.

Sponsored by