

Isolation and molecular characterization of Glioma stem cells

Liquid Biopsy = CellprediKt® + Diagnostic Apheresis

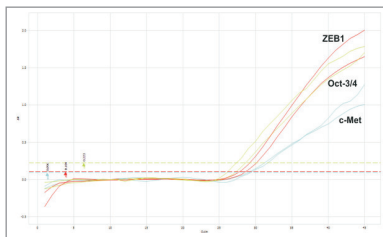
Dr. U. Kübler, Dr. J. Schnepel

Brain Tumor 2013, Berlin

The underlying cause of glioblastoma is the loss of controllability of normal glial cells and the formation of tumor stem cells in a molecular niche. The Dr. Kübler GmbH has a patented system available for isolation, quantification and molecular characterisation of these cells. After dissolution of neuroectodermal cell layers GFAP expressing Cancer Stem Cells (CSCs) can be found in the bloodstream, which have been undergone epithelio mesenchymale transition (EMT) and which represent the heterogeneity of both the primary tumor and disseminated cells. Therefore, the cells change their cell-specific characteristics and thus gain migratory capability and invasiveness. They already circulate in the bloodstream before a primary tumour gets visible. The early detection of these cells is a revolution in prevention, diagnosis and treatment. ^[1-10]

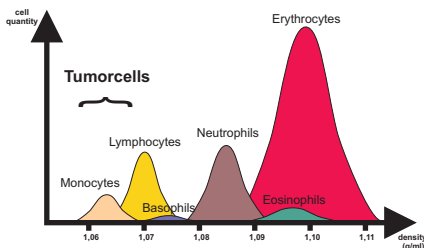
CellprediKt®

The CellprediKt® test allows detection of circulating cells with deranged epithelio-mesenchymal transition in the bloodstream via Real-Time RT-PCR (reverse transcriptase-polymerase chain reaction). The epithelio-mesenchymale character of these cells is documented by overexpression resp. amplification of Oct-3/4, c-Met and ZEB1. Quantification and precise characterization of the cancer stem cells detected with CellprediKt® follows by diagnostic apheresis. ^[7, 8, 11-13]



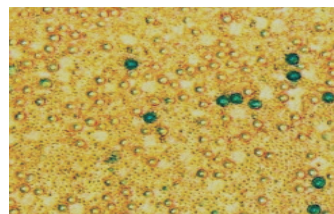
Diagnostic Apheresis

The Diagnostic Apheresis enables a quantitative extraction of metastasis initiating Cancer Stem Cells (MICs) from the bloodstream and their complete molecular-pathological characterization without any biopsy (c-Met, Oct-3/4, GFAP, EGFR, erbB2, erbB3, myc, ras, p53m, MDR, CD44v5/v6, VEGF, Akt/mTOR, IDO, Survivin, Urokinase). ^[10]



Detection

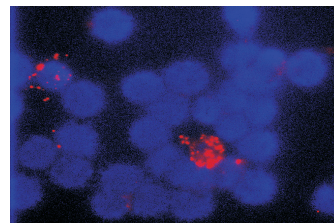
A specifically developed ELISA test (enzyme linked immuno-sorbent assay) as well as FISH techniques (fluorescence in situ hybridisation) provide a single cell detection and consequently a quantification. Furthermore an expression profile of circulating tumor cells is created by determination of different biomarkers. ^[10, 14, 15]



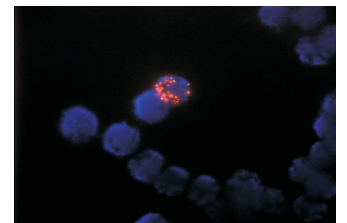
GFAP positive cells



Oct-3/4 positive cells



MET gene amplification



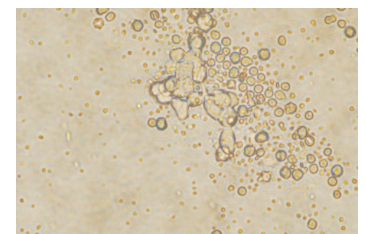
c-erbB2 gene amplification

Therapeutic consequences

A combined immunotherapy consisting of Natural Killer cells (NK cells) and heat-shock proteins can specifically attack and destroy Cancer Stem Cells. ^[14, 15]



NK cells



^[1] Glioblastoma: therapeutic challenges, what lies ahead, Lima, F.R. *et al.*, 2012, Biochim. Biophys. Acta, 1826(2), 338-349.

^[2] Glioblastoma multiforme: overview of current treatment and future perspectives, Anton, K. *et al.*, 2012, Haematol. Oncol. Clin. North Am., 26(4), 825-853.

^[3] New molecularly targeted therapies for glioblastoma multiforme, Polivka, J. *et al.*, 2012, Anticancer Res., 32(7), 2935-2946.

^[4] EMT, cancer stem cells and drug resistance, emerging axis of evil, Shing, A., Settleman, J., Oncogene 2010, 29, 4741-4751.

^[5] p53 regulates epithelial mesenchymal transition and stem cell properties through modulating mi-RNAs, Chang, C., *et al.*, Nature Cell Biology 2011, 13, 317-323.

^[6] Cancer Stem Cells, Jordan, C.T., Guzman, M.L., Noble, M., N. Engl. J. Med. 2006 355;12, 1253-61.

^[7] c-MET expression level in primary colon cancer: a predictor of tumor invasion and lymph node metastases, Takeuchi, H. *et al.*, Clin Cancer Res 2003, Vol.9, 1480-1488.

^[8] Tumor cells circulate in the blood of all major carcinomas, but not in healthy subjects or patients with nonmalignant diseases, Allard, W.J., *et al.*, Clin Can Res 2004, 10, 6897-6904.

^[9] The evidence for Cancer Stem Cells, Niederhuber, J., (NCI, Bethesda) 5th Int. H.F.C. Behr-Symposium 2008, DKFZ Heidelberg.

^[10] Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing, Gerlinger, M. *et al.*, 2012, N. Engl. J. Med., 366(10), 883-892.

^[11] Epithelial-mesenchymal transitions in development and disease, Thiery, J.P., *et al.* 2009, Cell 139, 871-890.

^[12] The epithelial-mesenchymal transition generates cells with properties of stem cells, Mani, S.A., 2008, Cell 133, 704-715.

^[13] Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits, Polyak, K., Weinberg, R.A., 2009, Nat Rev Cancer 9, 265-273.

^[14] Process for the *in vitro* diagnosis of a glioma or an astrocytoma and a pharmaceutical mixture for the treatment, Kübler, U., 2011, European Patent EP1486787B1.

^[15] Isolation and Characterization of Circulating Cancer Cells as Vaccine Candidates, Dr. Kübler GmbH, Cancer Vaccines 1996, New York.