Introduction

A myth of futility

In Greek mythology, Sisyphos, king of Corinth, was punished in Hades for his trickery against Thanatos, the God of death. When his time had come and Thanatos came to fetch him, Sisyphos had him chained up so that no one on earth died until Ares, the God of War, came to free Thanatos. Before Sisyphos was taken to the underworld with the help of Ares, he asked his wife to leave his body unburied. When he reached Hades he was permitted to go back to earth to punish his wife, and he lived to a ripe old age before dying a second time. His trickery resulted in his eternal punishment in Hades. The cunning Sisyphos had to roll a huge stone up a mountain over and over again (Britannica Concise Encyclopedia 2006).

Overwhelming dynamics …

Not unlike Sisyphos repeatedly rolling his rock up the mountain, contemporary disciples of Askeplios engage into a fight against malignant brain tumors that repeatedly recur following surgical removal. Tumor cells rapidly proliferate and insidiously infiltrate the human brain, imposing anarchic rule onto one of the most complex and vulnerable organs. Among the various types of brain-intrinsic tumors, glioblastoma is the most malignant and prevalent form that occurs at an annual frequency of some 300 new cases in Switzerland. Mean patient survival is less than 1 year. Neurosurgeons can control the rapidly proliferating tumor mass by repetitive resections, reducing increased intracranial pressure and thereby delaying imminent death. Despite usage of most modern tools of the operating theatre, such as online magnetic resonance imaging or computer-assisted navigation, the infiltrative nature of...
the disease eventually puts an end to these efforts. The problem of glioblastoma can be reduced to the fact that there is no specific treatment for tumor infiltrating cells that migrate throughout wide areas of the brain into regions where the blood barrier is still intact. Moreover, it is the blood brain barrier that represents a major obstacle for most cancer drugs to reach their target following systemic application.

Attacking the problem

From a scientific point of view, I will subdivide the problem “glioblastoma” to 4 major subtopics: 

i) the molecular genetics and biology of the tumor cell, 

ii) the complex interactions between the tumor cell and the host organ and its immune cells, neurons and astrocytes, 

iii) the specific nature of the blood brain barrier which imposes unique requirements on the biodistribution of candidate drugs, and 

iv) resistance of GBM cells to cancer drugs. In recent years, the development of lipophilic drugs that penetrate the blood brain barrier to a certain degree has been complemented by local neurosurgical strategies to directly deliver drugs into the tumor mass and adjacent areas of tumor cell infiltration. The four topics cover important aspects of the new field of preclinical and clinical neuro-oncology, a new discipline that is interconnected with fields such as neurosurgery & neurology, radio-oncology, oncology, basic and applied cancer research, developmental biology and especially neuroscience.

The malignant cell

Human malignant gliomas arise from neural progenitor cells or dedifferentiated astrocytes. Among the countless mutations in the genome of tumor cells, a few key genes and associated pathways have been well characterized. This allowed to create several distinct murine glioma models that faithfully reiterate the histological hallmarks of the disease. This similarity with the human disease phenotype does not prove that these models also exhaustively mimick the altered genotype of the human disease. That is the reason why animal models for cancer are limited to test therapeutic concepts for clinical use. Nonetheless,
these models can be utilized to test a biological hypothesis and help to define a hierarchy of genetic alterations involved in tumorigenesis. For instance, activated PKB/Akt and Ras alleles alone could not induce glioblastomas, but the combined activation did. Likewise, oncogenic EGFR did only induce glioblastomas in a background genotype characterized by tumorigenic deletions of the Ink4a/arf locus. Interestingly, astrocytes harboring homozygous deletions at the Ink4a/arf locus displayed accelerated growth curves similar to neural stem cells.

Cancer is a stochastic disease in which mutation patterns vary between different tumors. The relatively monotonous histological phenotype is caused by a great variety of ever new combinations of genetic alterations. Mutations that convey a growth advantage to a cell are selected for, leading to overgrowth of precursor cell populations with increasingly malignant tumor cell clones. We now understand that a limited number of mutations are sufficient to give rise to brain tumors. Growth control pathways (EGFR, PDGFR, c-Met and others) activating mitogen activated kinases are deregulated. The activity of a central signaling protein, the protein kinase B (PKB/Akt), is frequently out of balance, either due to mutations of an upstream regulatory protein, e.g. phosphatidylinositol-3 kinase (PI3KCA), the tumor suppressor PTEN, or the regulatory factor CTMP. A mutation of the PTEN gene in GBM cells is shown that leads to activation of the protein kinase B (PKB) (Figure 1). PKB is a central signaling molecule that is interconnected to key cellular functions like survival, migration or energy metabolism.

Figure 1
PTEN reduces PKB/Akt phosphorylation (Ser-473) in U87MG cells and does not modulate FAK phosphorylation in cell adhesion assays. Cellular lysates from U87MG cells transfected with empty vector (Vector), vector containing wild-type PTEN (WT), or mutant PTEN (C124S) were immunoblotted with anti-phospho PKB/Akt (Ser473, top panel) and reprobed with anti-PKB/Akt (second panel, top). [CANCER RESEARCH 59, 5479–5482, 1999]
As protection against physical and chemical stress factors, Nature bestowed a safeguard mechanism to every cell for the sake of the entire organism. Cancer cells are characterized by resistance to pro-apoptotic stimuli. If a cell accumulates genetic damage which is beyond its repair capacity, a highly controlled program of self-destruction (also called cellular suicide or apoptosis) is elicited. Tumor cells carry mutations in the regulatory network of apoptosis. For example, many cancer cells harbor mutations in the p53 gene or in functionally related genes like p14ARF or HDM2. The structural organization of the p14ARF/p16INK4a locus on chromosome 9p21 is curious because the same nucleotide sequence gives rise to 2 distinct protein products by using a different promoter associated with a single nucleotide frame shift. The multifaceted p53 protein can be viewed as a cellular “apopostat” that sets the threshold for the induction of the cell-intrinsic death program.

Pro-apoptotic stimuli (physical energy, radiation, cytotoxic drugs, targeted interventions) can still induce cell death, however, higher amounts of energy or drug doses have to be used to destroy these cells in comparison to tumor cells that are wild-type for the p53 pathway. Mutations of the p53 gene have not found to be frequent in GBM, however, p14INK4a – an upstream regulatory protein of p53 – is often inactivated.

In cancer cells, stop signals controlling cell proliferation are inactivated. For example, the onset of the S-phase of the cell cycle to synthesize new DNA is held in check by a multi-protein complex consisting of p16INK4a, cyclin D, the cyclin-dependent kinase 4 and the retinoblastoma protein Rb. In GBM, genetic alterations can be found in at least one of these four cell cycle regulatory factors. The tumor suppressor p16INK4a is frequently inactivated by homozygous deletions in many cancers. p16INK4a can also be inactivated by epigenetic alterations. In this case, the gene promoter which lies within a dense CpG island, is hypermethylated resulting in gene silencing.

Invasiveness of tumor cells is regulated by a number of factors, including EGFR and focal adhesion kinase (FAK) and the tumor suppressor PTEN. A curiosity of cancer is that malignant cells carry a severe mutator phenotype. Genes that control the genomic integrity
of a cell can also be mutated, leading to the accumulation of countless epigenetic and genetic alterations. A rare example is the mutation of mismatch repair genes MSH2 or MLH1 in Turcot’s syndrome, which is defined by the co-manifestation of a GBM and a colorectal cancer. Genes that control genomic integrity in ordinary GBM are still to be discovered.

The altered microenvironment in the brain

The vision to use a patient’s own immune defense system to fight cancer cells is also being tested in malignant brain tumors. In an early study, we analyzed the T cell receptor profile in tumor-derived lymphocytes and the gene expression profile of critical cytokines. Interestingly, transcription of genes encoding for inhibitory factors (IL-10, TGF-b2) appears to prevail in primary brain neoplasm. Comparing the patterns of subpopulations of T-cells by characterizing their molecular composition disclosed distinct enrichment of T-cell within the tumor as compared to the distribution pattern in peripheral blood lymphocytes, indicating a selection of some T-cell families possibly due to recognition of tumor antigens. Ex vivo expanded tumor infiltrating lymphocytes that were harvested following stimulation with interleukin-2 and antibodies against CD3 showed nearly complete loss of their cytotoxic potential, likely to be a consequence of the expression of immunosuppressive factor by the tumor cells. It became clear that cellular adoptive immune therapy has to overcome functional inactivation of these cytotoxic T cells in order to become a useful anti-tumor tool.

The invasion of healthy brain tissue by tumor cells proceeds along the extracellular matrix of white matter tracts and the perivascular space, which contain ligands for the integrin family of transmembrane receptors. Focal adhesion kinase (FAK) is required for integrin-dependent signaling and modulates cellular adhesion, migration, and survival. By blocking FAK activity, levels of activated caspase-3, a cell death effector, were found to be increased which sensitized tumor cells to apoptotic stimuli and reduced their invasiveness. Loss of substrate
attachment in normal cells leads to the induction of apoptosis, or anoikis (greek: homeless). Tumor cells are generally resistant to anoikis, reflecting independence from integrin signaling. We found that introducing functional PTEN into glioma cells was sufficient to restore the anoikis reaction by reverting activating phosphorylation of PKB/Akt on Ser-473.

Tenascin-C, an extracellular matrix glycoprotein, is expressed in the stroma of most solid tumors, including GBM. Acting like an oncogene, tenascin-C stimulates tumor growth by various mechanisms including promotion of proliferation, escaping immuno-surveillance and positively influencing angiogenesis. Clinically, radiolabelled monoclonal antibodies against tenascin-C are being evaluated as new therapeutic options for GBM patients. In a large genetic study comparing deletion pattern on chromosome 1p between glioblastoma and oligodendroglioma (a much less malignant brain-intrinsic tumor with a mean survival of some 10 years), we detected a unique and highly consistent recombination site on 1p11 that involved the developmental gene Notch2. By studying the role of Notch2 in brain tumorigenesis, we found that gene expression of tenascin-C is regulated by Notch2. Notch2 mediates this effect via the canonical transcription factor CSL. We have detected a mutation in an malignant oligodendroglioma cell line that inactivates the binding domain between Notch2 and CSL (so called ram23 domain). This mutation leads to a significant reduction of tenascin-C expression. Inactivation of Notch2 appears to be linked to a glioma subtype (oligodendroglioma) that has a much better prognosis than GBM and does not express tenascin-C. We found in a large epidemiological study that the prognosis of brain tumor patients is linked to expression of Notch2 and Tenascin-C (Figure 2). In GBM, both proteins are abundantly expressed while they are absent in the more benign oligodendroglioma that usually have lost chromosomal arms 1p and 19q (manuscript in review).
Beta- and alphaKnife: targeted radiopeptides for malignant gliomas

There is no satisfactory surgical therapy for malignant gliomas because of their infiltrative nature and exponential growth rate that annihilates the best surgical result. Furthermore, malignant brain tumors are often critically located adjacent to or within functionally important areas that lead to severe neurological morbidity when aggressively treated by debulking surgery. The general principle of tumor surgery, which demands resection around the tumor mass respecting a safety margin of 1–2 cm, cannot be respected in neurosurgery for obvious reasons. In a longstanding collaboration with the Clinic and Institute of Nuclear
Medicine and the Radiochemistry Unit, we have advanced technology that allows neoadjuvant tumor treatment prior to resection. Initially, the octreotide analog DOTATOC was used which was not found to be suitable for the treatment of prevalent GBM because of inconsistent expression of somatostatin receptors type 2. We therefore developed a modified version of the 11-mer peptide substance P that is conjugated to DOTAGA, a chelator that entraps the radionuclides Yttrium-90 (pure beta-emitter, mean tissue range 5 mm) or Bismuth-213 (alpha-emitter, mean range 84 micrometers). Such an approach could have several advantages over conventional debulking strategies. First of all, the tumor mass is saturated with the therapeutic agent by local injection prior to resection which induces widespread tumor necrosis and tumor shrinkage (Figure 3). Secondly, spreading of malignant and invasive tumor cells by the surgical procedure (violating the principle of circumferential tumor surgery) can be significantly reduced. Furthermore, tumors at critical location can be treated with ultra low range-alpha emitters sparing adjacent neuronal tissue. Preliminary experience using peptidic vectors labeled with alpha- and beta-emitting metallic radionuclides has shown that many of these goals have been achieved.

Figure 3
Radiological response in deep-seated GBM. Coronal planes of T1-weighted MR-images with the contrast agent gadolinium-DOTA (1, 3) and of [18F]-deoxy-glucose-PET images (2, 4) show an inoperable large GBM in the left thalamus (case 12, Table 1). A partial response (3, 4) was observed 3 months after targeted intratumoral \( ^{90} \text{Y} \)-DOTAGA-SP, leading to clinical improvement (15 to 65 points in Barthel-Index). [Clin Cancer Res 2006;12, 2006]
can be achieved. Using diffusible, small drug like vectors, development of a new dosimetry protocol allows to perform precise dosimetry on a Gray/voxel basis, representing the scientific basis for future calculations of dose-response curves (Kneifel et al, submitted). Glioblastomas are highly vascularized lesions that tend to bleed during resection, rendering 3D-orientation difficult for the surgeon. Neoadjuvant targeting of NK-1 receptor positive tumor cells that also grow around the neo-vasculature induces an additional anti-angiogenic effect that transforms the highly vascularized tumor into a non-hemorrhagic radionecrotic mass that forms a pseudocapsule. Neoadjuvant treatment (“betaKnife”) thus improves respectability of glioblastoma. For critically located tumors, alpha protocols are being established (“alphaKnife”).

New systemic approaches as combinatorial strategy

In collaboration with Novartis Oncology, we have tested a number of new cancer drugs as single agents and specifically in drug combinations, looking for additive or synergistic effects. Many regulatory genes are epigenetically silenced by methylation and acetylation. Cardinal feature of malignant gliomas, as well as many other cancers, is a high rate of ATP consumption via upregulation of glycolysis. These metabolic as well as the classical cancer pathways represent potential targets for interference with specific drugs. Recently, small lipo-soluble molecules were designed as a new class of drugs more specifically targeting these cancer pathways. These drugs were applied to human GBM cells for induction of apoptosis as a readout of drug efficacy. Single applications of the protein kinase inhibitors against EGFR, PDGFR and mTOR were tested as single drugs and in various combinations. Even though these new drugs showed cytostatic effects to variable degrees, apoptosis of tumor cells could only be induced if an additional cytotoxic compound was added. This observation supports the argument that blocking signalling of well established cancer pathways may not be sufficient to achieve a lasting effect. Induction of cell death may be a prerequisite to obtain a profound effect.
In addition, the glycolytic inhibitor 2-deoxyglucose (2-DG) was used to exert a metabolic stress onto the cancer cells, reducing the availability of ATP. 2-DG alone was not able to induce cell death and had only a cytostatic effect at dose ranges from 1–25 mM. In contrast, cytotoxic drugs like the microtubule inhibitor EPO906 or histone deacetylase inhibitors like trichostatin A, sodium butyrate and LAQ824 were able to efficiently trigger apoptosis, but are known to lead to toxicity with increased dose ranges in mice and humans. We therefore tested combinations of low doses of cytotoxic drugs with cytostatic drugs, 2-DG and indomethacin. As p21 was shown to be re-expressed upon exposure to histone deacetylase inhibitors and to protect cancer cells against apoptotic stimuli, 2-DG was used as a sensitizer for apoptosis following treatment with histone deacetylase inhibitors, since 2-DG strongly inhibits protein expression including p21. Synergistic induction of apoptosis was observed in glioma and other cancer cell lines upon this combined treatment (Figure 4). Thus, drug combinations that inflict a

![Figure 4](image)

2-DG and HDAC inhibitor LAQ824 synergize to induce apoptosis in cancer cell lines. Hs683 and LN401 glioma and MCF-7 and HeLa cancer cell lines were cultured in triplicates in standard medium during 48h. Cells got fresh medium before to be treated with increasing doses of LAQ824 (10, 30, 60, 90, 180nM), and/or 25mM 2-DG during 72h after which time floating and viable cells were pooled and cell death was measured using FACS analysis (pro-G1 phase). HeLa cell line was exposed to 25mM 2-DG and 60nM for 72h.
cytotoxic stress to cancer cells by inducing widespread re-expression of silenced genes (which leads to expression of p21 of the stressed cell to halt the cell cycle machinery) and simultaneously reduce the availability of ATP (thwarting the p21 response) may trigger a profound pro-apoptotic effect selectively to cancer cells. The combination of an antimetabolic and cytotoxic stress may represent a promising new way to treat certain cancers in the future including glioblastoma.

Outlook

Instead of living the myth of Sisyphos, new building blocks are constantly integrated into the growing wall of scientific progress that will eventually lead to a new house representing the future way to attack the clinical problem of glioblastoma. Local treatment modalities will improve resectability of these tumors. The goal is to reduce the visible tumor mass to less than 1 cm³, a value which is proven to be critical to obtain lasting clinical responses in medulloblastoma, a malignant brain tumor of children. New systemic approaches will eventually lead to a better control of the infiltrative component of the disease. Given the combinatorial “genius” of the system GBM (mutator phenotype), it is likely that singular approaches will rapidly be thwarted by emergence of resistance mechanisms. For this reason, combinatorial strategies have to be tested and set up to tackle the problem from several angles. Targeted biological interventions will sensitize tumor cells to physical and chemical stress (irradiation, cytotoxic agents), enhancing the degree of tumor cell apoptosis that will lead to lasting clinical responses.

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REFERENCES


