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Regulation of tumor initiating cells in glioblastoma multiforme. The role of Aldehyde Dehydrogenase 1 and cellular hypoxia.

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1 INTRODUCTION

1.1 Glioblastoma multiforme

Glioblastoma multiforme (GBM), the most common type of primary tumor of the central nervous system (CNS) in adults, remains one of the most devastating and fatal pathologies, with most patients succumbing to disease within less than sixteen months (Hegi et al. 2005; Stupp et al. 2005; Parsons et al. 2008).

Glioblastoma exhibit rapid and extensive cell invasion throughout the human brain (Louis et al. 2007) and despite tremendous advances in the understanding of underlying mechanisms in tumor biology, the poor prognosis for GBM patients could not be improved substantially over decades (Stupp et al. 2009).
The cells of origin of glioblastomas could not been definitively identified yet, nevertheless the identification of tumorigenic stem-cell like precursor cells suggests that they may have a neural stem cell origin (Canoll & Goldman 2008; Dirks 2006; R. Chen et al. 2010; Galli 2004).

This investigation aims to identify and illuminate some of the core cellular regulatory mechanisms, that seem to control these stem-cell like precursor cells. The following chapter provides an introduction into general Glioblastoma biology, the current knowledge of Glioblastoma markers and meaning of mutations, the current standard of diagnosis and care and also introduces to the principles of the cancer stem cell theory, with a special emphasis on the GBM stem cell marker Aldehyde Dehydrogenase 1 and the role of cellular hypoxia.

1.1.1 Epidemiology

Brain tumors account for 2 percent of all cancer in human. The average annual age-adjusted incidence rate for malignant primary brain tumors is 7.3 per 100.000 person-years with an age adjusted mortality rate of 4.6 per 100.000 person-years (Jemal et

al. 2010). Gliomas constitute more than 80% of primary malignant brain tumors among astrocytomas and 53% of astrocytomas are GBM (Adamson et al. 2009) (Fig. 1). Patients average age at onset is 54 years for all primary brain tumors and 62 years for GBM (Dolecek et al. 2012). The vast majority of GBM are diagnosed as de-novo or also termed primary GBM. Only a subset of 5% of GBM arise from lower-grade brain tumors (grade II or grade III) (Adamson et al. 2009).

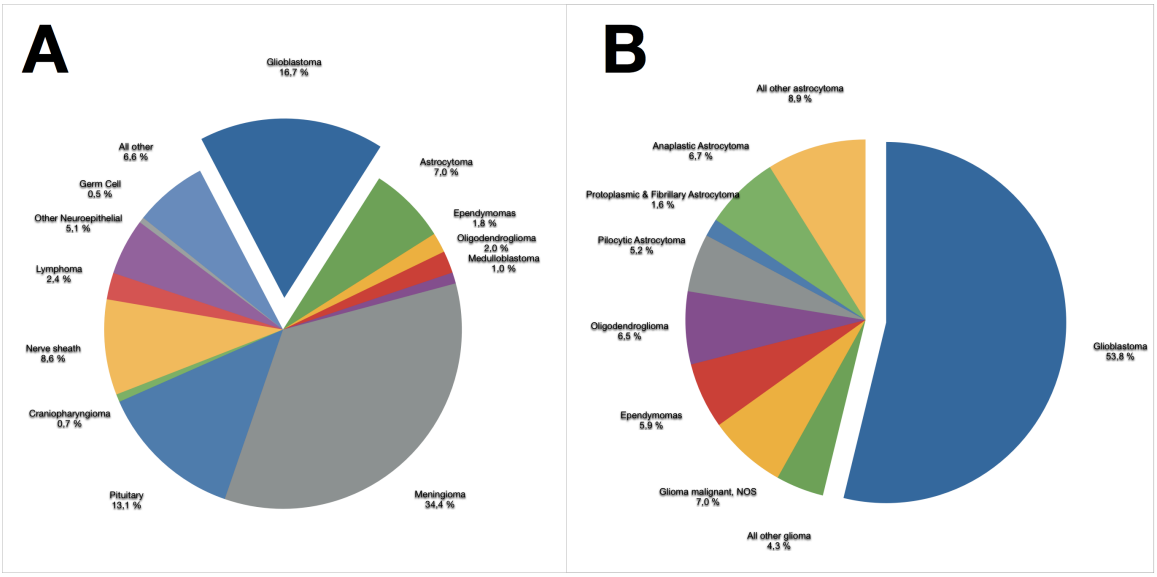

Figure 1: Primary brain tumors by histology and subtypes of astrocytic tumors
Left side (A): Distribution of primary brain and CNS tumors by histology. *Right side (B):* Distribution of primary brain and CNS gliomas by subtype (modified from: CBTRUS Statistical Report 2012)

1.1.2 Classification

Attempts to classify brain tumors date back to 1863, when Rudolf Virchow (Virchow R., 1863) first introduced the term glioma to separate them from other tumors of the brain.

In 1926, Percival Bailey and Harvey Cushing characterized the term Glioblastoma multiforme, based on the idea that GBM arise from glioblasts (glial precursor cells) and due to the heterogenous appearance, which is a hallmark of GBM with necrosis, hemorrhage and cysts (Bailey, P., Cushing, HW. 1926).

Studies by Kernhoan (1949) and Ringertz (1950) refined the classification system and incorporated that different brain cells give rise to histologically distinct brain tumors. Furthermore, the group of astrocytic tumors were divided into three grades: astrocytoma, anaplastic astrocytoma and glioblastoma (Kernohan & Mabon 1949; Ringertz 1950).

The World Health Organization (WHO) published a classification scheme of central nervous system tumors in 1979, that was revised in 1999 and 2007 and combines tumor nomenclature with a tumor grading system, based on the histopathological features and clinical presentation.

In its current version, the WHO system recognizes seven gliomas as distinct tumor entities, characterized by cytological and immunohistochemical evidence of differentiation along astrocytic, oligodendroglial or both lineages and divides CNS neoplasms into 4 grades. The prognostic power of the WHO grading scheme yielded in widespread acceptance throughout the clinical environment.

WHO grade I and II are generally considered as low grade and grade III and IV as high grade astrocytomas. Grade I tumors show low proliferative potential and may be cured following surgical resection. Lesions categorized as grade II harbour likewise low proliferation capacity, but contain atypical cells and tend to recurrence and grow invasively. Malignant transformation to higher tumor grades might also occur.

In WHO grade III lesions, histopathological investigation typically reveals atypic nuclei and high mitotic activity. The designation WHO grade IV describes highly malignant lesions, containing necrotic foci or extensive microvessel proliferation, high mitotic activity and usually rapid disease progression with dismal prognosis (Table 1). Astrocytic WHO grade IV tumors are subdivided in Glioblastoma (95%), Small-cell GBM, Giant cell GBM, GBM with primitive neuronal features (primitive neuroectodermal tumor [PNET]-like) and Gliosarcoma (2%) (Miller & Perry 2007). Following a WHO consensus, GBM that contain foci resembling oligodendroglioma

are associated with a significantly worse prognosis and classified as glioblastomas with oligodendroglioma component (Miller et al. 2006).

The WHO grade also reflects response to treatment and overall survival. Therefore the tumor grade is a key factor in the decision making process regarding the use of adjuvant radiotherapy and choice of a specific chemotherapeutic protocol.

	WHO I	WHO II	WHO III	WHO IV
Tumor dignity	benign	low-malignant	high-malignant	highly-malignant
Median overall survival	more than 5 yrs.	3 - 5 years	1 - 3 years	below 15 months
Neuronal origin	Gangliocytoma			Medulloblastoma
Glial origin	Pilozytic astrocytoma	Diffuse astrocytoma Oligodendroglioma Ependymoma	Anaplastic astrocytoma Anaplastic oligodendroglioma Anaplastic ependynoma	Glioblastoma
Meningeal origin	Meningioma			
Nerve sheath origin	Schwanomma			

Table 1: Classification Scheme of Primary Brain Tumors
(modified from a scheme by J. Schlegel)

1.1.3 Gene Expression Profiling and GBM sub-classification

Molecular and genetic determinants contributed additional refinements to the existing morphology based classifications and some of them may be incorporated into future revisions of the WHO classification of tumors of the central nervous system.

Genetic alterations of TP53, PTEN, EGFR, RB1 NF1, ERBB2, PIK3R1 and PIK3CA in GBM are already well known in GBM biology (McLendon et al. 2008). Primary GBM in particular, exhibit frequent EGFR amplification and PTEN mutation (Kleihues & Ohgaki 1999). Secondary GBM on the other hand often show TP53 and IDH1

mutation (Ohgaki & Kleihues 2007). With reference to the chromosome, primary GBM can be separated from secondary GBM by regular trisomy of chromosome 7, monosomy of chromosome 10, gains of chromosome arms 129, 19q and 20q (Toedt et al. 2011).

In an effort to elucidate distinct genetic fingerprints in GBM, several groups analyzed hundreds of glioma tissue samples with Gene Expression Profiling (GEP) (Fig. 2) (Shirahata et al. 2007; A. Li et al. 2009a; Freije et al. 2004; Phillips et al. 2006). In a recent work (Verhaak et al. 2010), Verhaak et al. and the Cancer Genome Atlas (TCGA) Research Network suggested a gene-expression based molecular classification of GBM into 4 distinct subtypes: proneural, neural, classical and mesenchymal. In the classical subtype, Endothelial Growth Factor Receptor (EGFR) amplification and EGFRvIII mutation, Cyclin-dependent kinase inhibitor 2A (CDKN2A) deletion and a lack of TP53 mutations were found, whereas the mesenchymal subtype showed mutations in Neurofibromatosis Gene 1 (NF1), TP53 and the Phosphatase and Tensin homolog (PTEN). In the proneural subtype Alpha-type Platelet Derived Growth Factor Receptor (PDGFRA) amplification and mutations in Isocytrate Dehydrogenase I (IDH1), TP53 and PIK3CA/PIK3R1 genes were found. In consistence with previously published data, the proneural subtype is notably more frequently detectable in younger patients (Phillips et al. 2006; Lee et al. 2008). Finally, the neural type expressed neuronal markers, such as NEFL, GABRA1, SYT1 and SLC12A5 (Verhaak et al. 2010).

Unlike previous GEP studies, Verhaak and the TCGA group used a data set solely consisting of GBM, whereas aforementioned studies also included lower-grade gliomas, that might have led to a misinterpretation. The clinical meaning of this suggested sub-classification is illustrated by a significant increase in survival of the Mesenchymal and the Classical Subtype, following Temozolomide (TMZ) and radiation therapy.

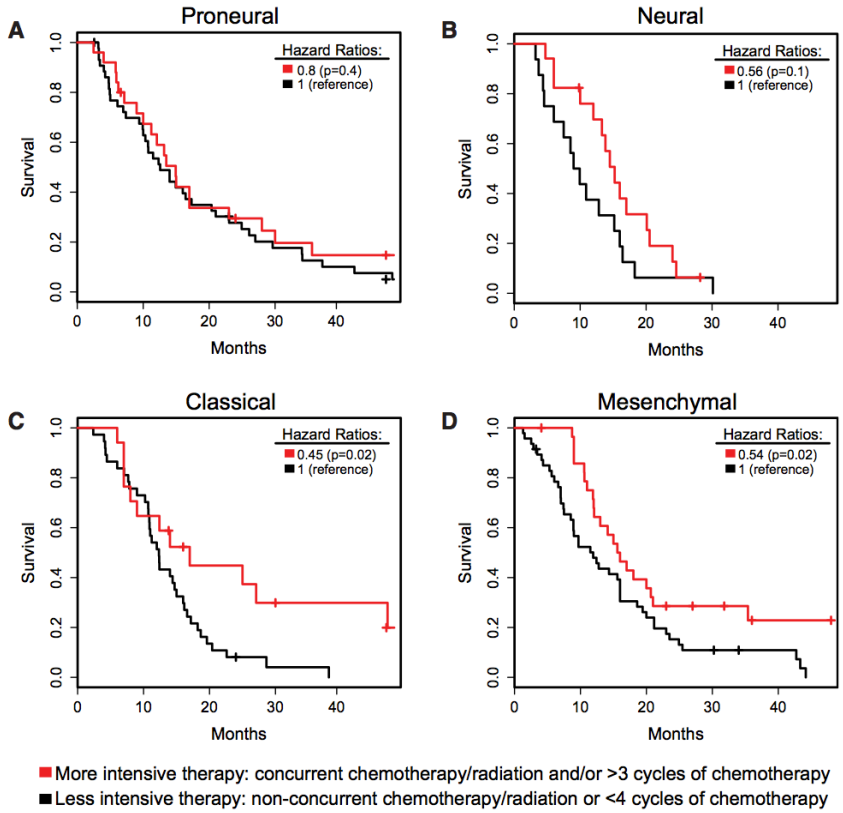

Figure 2 : Gene expression profiling (GEP) and survival (Verhaak et al. Cancer Cell, 2010)

1.1.4 Other prognostic meaningful marker

1.1.4.1 LOH 1p19q

The prognostic variability in overall survival, particularly among lower-grade gliomas, could be improved by the implementation of prognostic markers. One of the most utilized tests detects a co-deletion of chromosomal arms 1p and 19q, associated with a more favourable therapy response and overall survival in oligodendroglial (Jeuken et al. 2004)} and oligoastrocytic anaplastic gliomas, treated with radiotherapy or radiotherapy plus chemotherapy (Cairncross et al. 1998). Numerous investigators have described co-deletions of 1p19q in up to 80% of oligodendroglioma and 60% of anaplastic oligodendroglioma. The numbers reported in tumors of mixed lineage are lower and range from 30-50% of oligoastrocytomas to 20-30% of anaplastic oligoastrocytoma (Jenkins et al. 2006; Griffin et al. 2006). In GBM, the rate is

reported to be < 10%. Three prospective randomized clinical trials confirmed the prognostic significance of this genetic aberration (Intergroup Radiation Therapy Oncology Group Trial 9402 et al. 2006; van den Bent et al. 2006; Wick et al. 2009).
Notably only a combined and complete loss of 1p and 19q are prognostic favourable. A partial loss is even associated with a worse prognosis (Felsberg et al. 2004).

1.1.4.2 MGMT promotor gene methylation

O6-methylguanine-DNA methyltransferase (MGMT) encodes for an O6-methylguanine methyltransferase that removes alkyl groups from the O-6 position of guanine. GBM and anaplastic glioma patients with MGMT hypermethylation were found to be more sensitive to alkylating agents such as temozolomide (Esteller et al. 2000; Hegi et al. 2005; Hegi et al. 2008; Weller et al. 2009).
MGMT encodes for a DNA repair protein, that removes alkyl groups from the O6 position of guanine, which are appended by alkylating reagents, such as temozolomide or nitrosourea compounds (Gerson 2004). Thus, hypermethylation of the MGMT promotor results in increased chemotherapeutic effectivity.

Other mechanisms underlying the prognostic improvements associated with MGMT promotor methylation are not yet fully understood. To note, most anaplastic glioma with MGMT methylation likewise contain mutations in the IDH1 gene (Sanson et al. 2009) or homologous loss of 1p/19q (van den Bent et al. 2009). Both aberrations are associated with better prognosis. Consequently, in long term survivors of GBM (OS > three years) MGMT promotor methylation could be detected significant more frequently than in other GBM samples (Krex et al. 2007).

1.1.4.3 IDH1/IDH2 mutation

Recently, sequencing analysis of 22 GBM by Parsons and coworkers revealed somatic mutations in the Isocytrate Dehydrogenase (Parsons et al. 2008). A large body of literature suggest, that IDH1 mutations are much more frequent in secondary GBM (70-80%) (Nobusawa et al. 2009) and in younger patients, as well as in a vast

majority of grade II and III astrocytomas and oligodendrogliomas, but can be rarely encountered in primary GBM (5%) (Balss et al. 2008; Hartmann et al. 2009; Yan et al. 2009; Parsons et al. 2008).

The mutation results in a gain of enzyme function. IDH catalyzes a-ketoglutarate to R(-)-2-hydroxy-glutarate (Dang et al. 2011). Several studies have proven, that IDH1/ IDH2 mutation is associated with prolonged overall survival, (Parsons et al. 2008; Yan et al. 2009) whereas other investigators reported, that mutational status lacks significance in response to chemotherapy (Dubbink et al. 2009).
A monoclonal antibody, detecting IDH1 mutation on position R132H is available, making routine IDH1 mutation testing feasible (Capper et al. 2010).

The molecular pathways, yielding to aberrant DNA and histone methylation of IDH1 mutated tumor cells are not thoroughly clear.
However, the results of most studies indicate, that IDH1 mutation is correlated with TP53 mutations, co-deletion of 1p19q and MGMT promotor methylation (Ichimura et al. 2009; van den Bent et al. 2010; Watanabe et al. 2009; Sanson et al. 2009). Indicating the genetic heterogeneity of primary and secondary GBM, previous studies described inverse correlation with loss of chromosome 10 and EGFR amplification (Sanson et al. 2009; van den Bent et al. 2010).

Some studies have suggested that enzymatic gain of function mutations in IDH1 and IDH2 lead to markedly increased levels of 2-hydroxyglutarate (2HG) (Yan et al. 2009).

1.1.4.4 EGFR receptor amplification in GBM

Amplification of the epidermal growth factor receptor gene (EGFR) at 7p12 is the most frequent genetic aberration in primary GBM (40%) (Yip et al. 2008). A rearrangement of EGFR, namely a 801-bp in-frame deletion of exons 2-7 is encountered regularly, with EGFRvIII being the most common variant (Sugawa et al. 1990). Clinically meaningful, EGFR amplification or rearrangements indicate poor

prognosis and an aggressive tumor phenotype (Pelloski et al. 2007; Shinojima et al. 2003).

To date, results of most clinical trials, which evaluated the feasibility of targeting EGFR pathways to increase adjuvant therapeutic sensitivity has been unsatisfactory (Preusser et al. 2008; Halatsch et al. 2006).

1.1.4.5 BRAF mutation

Interest has recently been focused on the BRAF gene. A proto-oncogene at7q34, that is found duplicated or mutated in 60-80% of WHO grade I pilozytic astrocytoma (Bar et al. 2008; Jones et al. 2009). Since BRAF gene mutations are rarely detectable in higher grade astrocytic or oligodendrocytic tumors, (Pfister et al. 2008) testing for BRAF mutations contribute to differential diagnosis between pilocytic astrocytoma and other brain neoplasms in uncertain histopathological cases (Korshunov et al. 2009).

1.1.5 Current therapeutic guidelines

As it would go well beyond the scope of this introduction to discuss implications of current therapeutic guidelines in meticulous detail, the following chapters review briefly the current state of the art recommendations for treating newly diagnosed GBM.

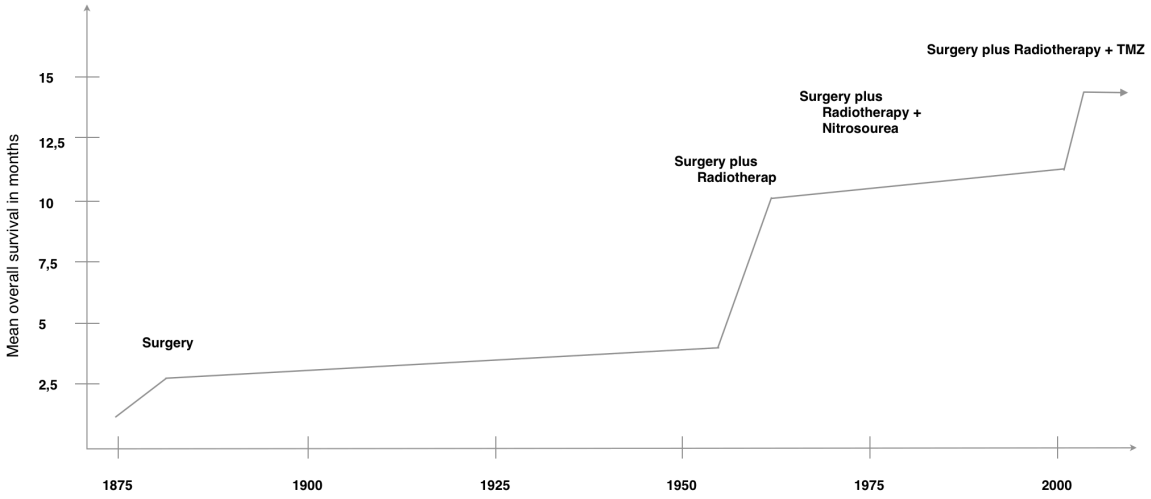

Figure 3: GBM standard of care development and mean overall survival from 1875 to present. First surgical cases date back to last quarter of 19th century with a high peri- and postoperative mortality. Whole brain radiation therapy (WBRT) was introduced around 1950 and doubled survival times from 4-6 months to 10-11 months. Few years later, nitrosourea compounds added marginal additional survival benefit (2 months). In 2005 a single study introduced a new standard of care, by using the alkylating agent Temozolomide, concomitant with postoperative RT and continuation for 6-12 months. The mean overall survival rose to 14.6 months in selected study populations.

1.1.5.1 Neurosurgical Treatment

In newly diagnosed GBM, the current neurosurgical paradigm is „maximal safe resection“, i.e. most possible aggressive resection, while preventing new postoperative neurological deficits. This standard has particular implications for tumors located near to eloquent areas.

Remarkably, until to date there is no straight forward recommendation between biopsy only and surgical resection and likewise ongoing controversy pertaining the extent of resection. The single conducted randomized clinical trial lacks level 1 evidence due to inadequate methodology for reliable conclusions (Hart et al. 1996).

However, at Class 2 evidence level, aggressive resection is associated with improved functional status and prolonged survival (Devaux et al. 1993; Laws et al. 2003). Demonstrating that remaining tumor mass after surgery is of prognostic importance,

independent of other variables, a study by Wood and colleagues concluded that the amount of tumor before and 9 weeks following radiotherapy is highly prognostic (Wood et al. 1988).
Similar findings in the field of pediatric malignant brain tumors were reported by Bucci et al. (Bucci et al. 2004).
Further support for the concept of survival benefit through more aggressive resection, were provided in 2006 by Stummer and coworkers with the introduction of fluorescence-guided resection using 5-aminolevulinic acid (5-ALA) (Stummer et al. 2006).

In line with this trial, Pichlmeier et al. have investigated the influence of surgical extension with and without 5-ALA on survival depending on RPA class. Stratified for degree of resection, progression free survival of patients in RPA classes IV and V was significantly longer (Pichlmeier et al. 2008).

One group of authors suggested a cut-point for survival benefit regarding the volumetric extent of GBM resection. In this work, Sanai and coworkers found, that survival benefit was significantly larger above 78 percent resected tumor based on the gadolinium-enhancing portions of the tumor (Sanai et al. 2011).

In contrast to the apparent advantages of total resection, other studies found no increases in overall survival (Coffey et al. 1988; Quigley & Maroon 1991; Kreth et al. 1993). To note, many of these studies were conducted before the era of high-resolution MRI, neuronavigation and other complementary techniques, that are employed today in many neurosurgical centers.

Finally, neurosurgical tumor debulking may be beneficial in other aspects. Morbidity and acute neurologic complications are fewer in patients who undergo resection, compared with biopsy. Surgery also facilitates sooner discontinuation of corticosteroids and therefore reduces steroid-related complications (Fadul et al. 1988). Tumor resection before radio-chemotherapy treatment may also improve postoperative adjuvant therapy response (Salcman 1988; Glantz et al. 1991).

Lastly, tumor resection regularly yields larger and thus more representative samples for appropriate neuropathological diagnosis, a prerequisite for accurate therapeutic management.

1.1.5.2 Radiotherapy

Radiotherapy is a treatment standard for GBM with a total radiation dose of 54 - 60Gy, in single fractions of 1,8 - 2 Gy. If possible, the dose should be 60 Gy rather than 54 Gy, but dose escalation over 60 Gy could not prove to provide further benefits.
Abbreviated schemes of radiation therapy were found to be applicable for elderly patients or in palliative settings (Roa et al. 2004). In contrast to other therapeutic modalities, radiation therapy significantly prolonged overall survival in patients older than 70 years of age (Keime-Guibert et al. 2007).

1.1.5.3 Chemotherapy

A class I evidence trial by Stupp et. al in 2005 recommended concurrent and post-irradiation Temozolomide, a DNA alkylating agent for patients from 18 - 70 years with adequate systemic health. In this landmark study, 573 patients, diagnosed with GBM between 2000 and 2002 were randomized to standard radiation therapy (60Gy in 30 daily fractions of 2 Gy) only and concomitant temozolomide (75mg per square meter of body-surface area per day, 7 days per week) and adjuvant temozolomide (150-200mg per square meter for 5 days during each 28-day cycle). In the temozolomide group, median survival could be increased by 2.5 months and two year survival was almost three times higher over radiation therapy alone (Stupp et al. 2005).

In an accompanying study in the same year, median survival of patients with GBM and methylated MGMT promotor was 21.7 months and 46% of 2-year-survivors compared with 15.3 months and 22.7%, respectively in patients without the promotor methylation (Hegi et al. 2005; Hegi et al. 2008).

To date, there is no recommendation regarding continuation of chemotherapy longer than six months or in alternative dosage schemes. For patients older than 70 years of age, no evidence based therapeutic guideline exist.

Since the introduction of a the temozolomide chemotherapy, the importance of nitrosourea compounds as a primary therapeutic option has been largely replaced.
The Neuro-Oncology Working group 01 trial compared the efficacy of nimustine (ACNU) plus VM26 and ACNU plus cytarabine (Ara-C) chemotherapy with standard radiotherapy and reported a survival of 17.3 months for GBM patients treated with ACNU and VM26. However, no study arm comparing radiation therapy with chemotherapy was conducted (Weller et al. 2003).

Interstitial chemotherapy with BCNU wafers (Gliadel), the only FDA-approved treatment for intracerebral chemotherapy, is discussed controversially, since the reported overall survival of 13,9 months in the study arm treated with interstitial BCNU vs. 11,6 in the untreated group failed to prove statistical significance in the subgroup of GBM patients.

However, in a long-term follow-up study of 240 wafer-treated patients with 59 patients who were available for long-term follow-up, significant improvements of survival could be reported 1,2 and 3 years after implantation (Westphal et al. 2006).

In recent studies, other groups have taken a combined approach, by adding biodegradable carmustin wafers to the standard multimodal treatment approach with promising results, but also a higher rate of treatment associated toxicity and adverse events (Salvati et al. 2011; Bock et al. 2010).

Alternative chemotherapeutic application procedures, like intracerebral catheter based systems or convection enhanced delivery schemes yet failed to demonstrate supremacy over other medical treatments and face severe technical difficulties (Bock et al. 2010).

1.2 Cancer Stem Cells

1.2.1 Introduction

The existence of cancer stem cells is widely accepted and has clear and numerous clinical implications and promises (Cheng et al. 2010; Dietrich et al. 2010). The deepening of understanding in molecular and tumor biology have made it obvious, that stem cells might not only play a crucial role in organogensis, but may also have a harmful involvement in oncogenesis.

The classic model of cancer suggests that malignant cell transformation can occur in all cells of a tumor and these altered cells give subsequent rise to temporarily homogeneous cell populations, that develop into heterogenous cells only later in the tumor evolution process (Hanahan & Weinberg 2011).

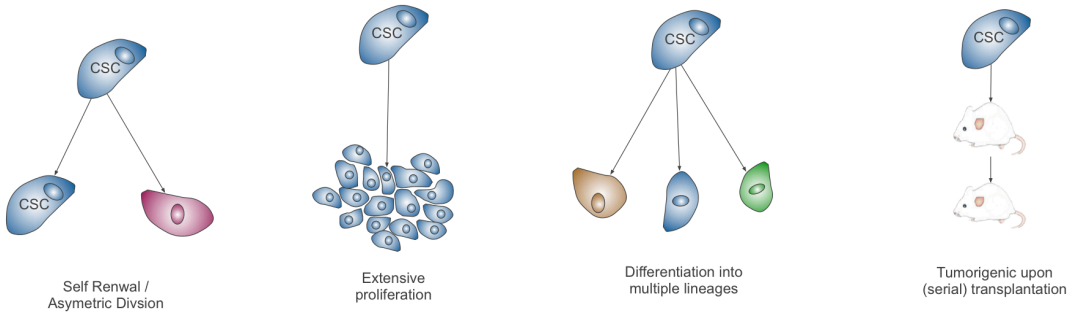

Figure 4: Characteristics of Cancer Stem Cells

Challenging this view, the paradigm of the cancer stem cell model presumes that oncogenic mutation appear particular in tissue stem or progenitor cells, that have acquired stem-cell like characteristics by yet not clearly understood mechanisms (Bonnet & Dick 1997; Glinsky 2007; Jaiswal et al. 2003). The theory hypothesizes, that a hierarchical organization exist inside a tumor, similar to the cellular order in normal tissues. Thus, the tumor bulk mainly consists of more differentiated progeny cells of much less numerous CSCs (Sánchez-García et al. 2007) (Fig. 5).

To date, CSCs can best be defined by functional criteria, resembling stem cell characteristics like extensive proliferation, the capacity for self-renewal (i.e., that

daughter cells retain the same biologic characteristics as the parent cell), the potential to different into multiple lineages and importantly in the field of oncology, the potential to initiate tumor growth after implantation, while highly recapitulating the originary tumor (Reya et al. 2001) (Fig. 4). Tumor heterogeneity thus is the result of nontumorigenic daughter cells, that differentiate into various cancer cell phenotypes. Despite the fact, that evidence of cancer stem cells is still incomplete and detected frequencies of CSCs in human tumor samples exhibit large variations, CSCs may be responsible for tumor progression, relapse and therapy resistance (Dean et al. 2005; Jordan et al. 2006).

Therefore current cancer treatment, that often targets rapidly dividing cells and thus leaves remaining cancer stem cells untouched and path the way for tumor regrowth. Treatment strategies causing CSCs to differentiate were proposed by several groups and showed great promise (Bar et al. 2007; Fan et al. 2009; Piccirillo et al. 2009; Clément et al. 2007; Sarangi et al. 2009; Chigurupati et al. 2010).

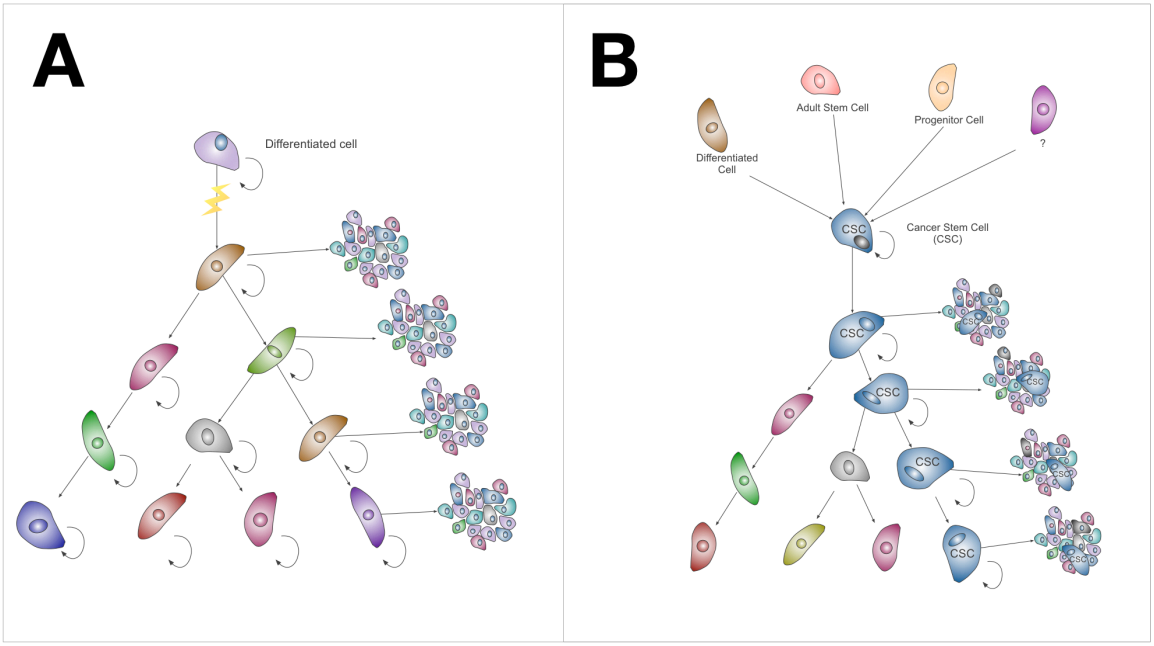

Figure 5: Comparison of models of cancer origin (modified from J. Visvader).
Left side (A): Classical concept of tumor development by mutated differentiated cell. *Right side (B):* Cancer stem cell concept of tumor development.

1.2.2 Cancer Stem Cells in other tumors

The first isolation of CSCs was performed in tumors of the hematopoetic system, in which stem-cell biology is well studied (Lapidot et al. 1994). Evidence about CSCs were first described by Bonnet and Dick in acute myeloid leukemia (AML) (Bonnet & Dick 1997; Jaiswal et al. 2003; Wulf et al. 2001) and was later found in chronic myelegous leukemia (CML) (Wulf et al. 2001) and acute lymphoblastic leukemia (ALL) (Cox et al. 2004).
A large body of literature today suggests the existence of cancer stem cells in a growing number of solid tumors. Breast cancer cells, expressing high levels of CD44 but low or undetectable levels of CD24 are highly tumorigenic and resistant to chemotherapy (Al-Hajj et al. 2003; X. Li et al. 2008; Glinsky 2007; Charafe-Jauffret et al. 2009).

Other solid tumors with stem cell fractions are colorectal cancer (CRC) (Ricci-Vitiani et al. 2006; Todaro, Francipane, et al. 2010a), hepatocellular carcinoma (HCC) (Ma, Lee, et al. 2008b), pancreatic adenocarcinoma (C. Li et al. 2007), bladder urothelial cell carcinoma (Su et al. 2010), small cell lung cancer (Eramo et al. 2008), ovarian carcinoma (Chang et al. 2009), prostate cancer (Collins et al. 2005), osteosarcoma (L. Wang et al. 2010), melanoma (Boonyaratanakornkit et al. 2010), thyroid carcinoma (Todaro, Iovino, et al. 2010b) and retinoblastoma (Seigel et al. 2005).

1.2.3 Cancer Stem Cell Marker and Identification Methods

Although CSCs share certain markers with normal stem cells, the identification of distinct and unique CSC marker is of high clinical importance, since these markers could finally lead the way to a drugable target.
As CSCs are no homogeneous group of cells, but rather seem to vary in each different tumor entity and even within a single tumor, the expression of markers is a field of ongoing investigation.

Commonly, CSC populations are defined by expression or absence of cellular surface proteins or combination of these.

1.2.3.1 CD133

Human CD133 or Prominin-1 was discovered in 1997 as a target of the AC133 monoclonal antibody in hematopoietic stem cells (Yin et al. 1997). CD133 is a 120kDa five transmembrane protein with two extracellular loops (Weigmann et al. 1997). The definitive biological role of CD133 has yet to be determined. Coexpression with ß1-Integrin may indicate its role in stem cell maintenance in epidermal basal cells and ganglioside-binding motifs on the first extracellular domain may regulate cell-cell contacts (Yu et al. 2002).

CD133 positive tumor cells, alone or in combination with other surface proteins, were detected in various cancers, but most publications propose a role of CD133 in Glioblastoma (Singh et al. 2004). Notably, Uchida and coworkers first isolated CD133 positive human neural stem cells from fetal brain tissue (N. Uchida et al. 2000). In 2007, O´Brien and Ricci-Vitiani described CD133 as a marker for cancer initiating cells in colorectal carcinoma (Ricci-Vitiani et al. 2006). In a later study, the same group described a combination of $CD133^+$ with $CD24^+$ for colon cancer stem cells (Vermeulen et al. 2008).
In prostate cancer, a combinatorial use of $CD44^+$, $\alpha 2\beta 1^{high}$ and $CD133^+$ isolated self-renewing and tumor initiating cells from various prostate cancer specimen (Collins et al. 2005). Chen et al. found $CD133^+$ lung cancer cells from primary tumors and lung cancer cell lines highly co-expressing stem cell marker Oct-4 and multiple drug resistance marker ABCG2 (Y.-C. Chen et al. 2008).

Contradictory to these findings, several studies showed that CD133-negative cells also contain tumorigenic potential upon transplantation into immunodeficient mice (J. Wang et al. 2008; Ogden et al. 2008, Shmelkov et al. 2008).

1.2.3.2 CD44

The CD44 surface marker is a 85-kDa transmembrane glycoprotein with 10 exons and at least ten tissue-specific splice variants (CD44v1-10). The interactions of CD44 in cell-cell communication are abound and recently has been the focus of a large number of investigations (Bourguignon et al. 2009; Afify et al. 2009). It has become clear, that CD44 has implications in cell adhesion, cell motility and proliferation (Afify et al. 2009; Marhaba & Zöller 2004). Pointing to CD44 as specific marker in cancer stem cells, CD44 positive prostate cells expressed increased levels of Nanog, BMI1 and SHH and other stem cell specific genetic signatures (Klarmann et al. 2009).

A study by Al-Hajj and colleagues in 2003 showed that $CD24^{-}/CD44^{+}$ breast cancer cells contained cells with the potential to re-capitulate the parental tumor upon implantation in very low cell numbers (Al-Hajj et al. 2003). In prostate cancer, a combination of the previously described CSC marker with $\alpha2\beta1^{high}$ and $CD133^{+}$ with CD44 led to the isolation of self-renewing cell populations with multi-lineage differentiation potential (Collins et al. 2005). Later, Patrawala et al. used only CD44 with comparable results (Patrawala et al. 2006). Dalerba and coworkers identified CSC from colorectal cancer cells based on the combined presence of Epithelial cell adhesion molecule (EpCAM) and CD44 (Dalerba et al. 2007).
In disagreement with these studies, Mack et al. demonstrated that CD44 failed to differentiate between malignant and normal epithelial cells (Mack & Gires 2008). Other studies reported CD44 to be associated with reduced metastasis and more favourable prognosis (Diaz et al. 2005; Lopez et al. 2005).

1.2.3.3 CD24

The role of CD24 as a marker for CSCs is discussed very controversially (Fang et al. 2010). CD24 is a O- and N-glycolysated extracellular bound 27-amino-acid protein (Pirruccello & LeBien 1986; Aigner et al. 1997). Presence or abscence of CD24 is also tissue specific and has been identified in breast cancer cells ($CD44^{+},CD24^{-}$) (Kai

et al. 2010), prostate cancer ($CD44^+$,$CD24^-$) (Hurt et al. 2008), and colon cancer ($CD133^+$/$CD24^+$) (Vermeulen et al. 2008).

Interestingly, in Glioblastoma cells, truncated glioma associated oncogene homologue 1 (tGLI1), a zinc finger transcription factor, were found to activate several carcinogenesis related pathways, among them K-RAS mediated signaling, tumor growth factor-beta and protein kinase A. Furthermore, the promotor of CD24 was activated by tGLI1, resulting in increased expression (Lo et al. 2009).

1.2.3.4 Side-Population

Goodell and coworkers demonstrated that a distinct subpopulation of bone marrow cells are capable to actively exclude the Hoechst 33342 dye (Goodell et al. 1996), a DNA dye used for flow cytometric analysis of DNA (Hamori et al. 1980). This dye can pass intact cell membranes, but gets actively extruded by ATP-dependent binding cassette (ABC) transporters (Zhou et al. 2001). In the initial study by Goodell, highest fractions of Hoechst 33342 extruding cells were found in hematopoietic stem cells. Since then, the side-population (SP) assay was used for identification and isolation of diverse normal and cancer stem cell populations in hematologic and solid neoplasms (Challen & Little 2006; Wu & Alman 2008). SP cells showed to be refractory to chemotherapeutic compounds and so could contribute to tumor recurrence and therapy resistance (Hirschmann-Jax et al. 2004).

1.2.4 Glioma stem cell markers

To note, a high number of published work on glioma stem cell isolation has been performed on the base of CD133 expression. However, in addition to CD133 (described above), a number of other promising markers were introduced in the last years.

A2B5 is a cell surface ganglioside, that is expressed on glial and neuronal progenitor cells (Nunes et al. 2003) and on neural stem cells from the subventricular zone.

$A2B5^+/CD133^+$ and $A2B5^+/CD133^-$ implantation experiments revealed, that both fractions can initiate tumor growth. Interestingly, $A2B5^-$ cells were not tumor initiating (Ogden et al. 2008). It remains to be determined, which role A2B5 possesses for reliable identification, given the high number of 33 - 90% A2B5 expressing cells in anaplastic astrocytoma and GBM (Ogden et al. 2008).

CD15, an adhesion protein, has been detected in primary GBM cells in variation of 2.4 - 70% and demonstrated stem cell characteristics like self-renewal, tumor initiating and multi-lineage differentiation potential. CD15 expression have also been detected on $CD133^-$ cells (Son et al. 2009). Mao and colleagues made corresponding observations in tumor sphere cultures, suggesting that $CD133^-$ cells may constitute a distinct subset of glioma stem cells (Mao et al. 2009). Other markers, recently suggested for brain tumor stem cell identification are podoplanin, originally detected on podocytes. Podoplanin is a mucin type transmembrane glycoprotein, widely associated with metastasis and tissue invasiveness (Mishima et al. 2006).
A few studies suggested Integrin alpha6 as a marker for enhanced tumorigenesis in glioma and reported coexpression with other alleged glioma stem cell markers (Delamarre et al. 2009).
A solution to identify tumor stem cells without any usage of intra- or extracellular markers has been proposed recently by Clément and coworkers by exploiting intrinsic autofluorescence properties and distinctive morphologies of cells obtained from tumor samples and glioma cell cultures (Clément et al. 2010).

In addition to the markers described above, the neurosphere generation assay is one of the most widely used models for growing stem cells or tumor stem cells in order to study their proliferation and multi-lineage potential. Neurosphere based culture requires the addition of basic Fetal Growth Factor (bFGF) and Epidermal Growth Factor (EGF) into serum-free media. Only mitogen responsive stem cells proliferate and build clusters, whereas more differentiated cells die (Reynolds & Rietze 2005; J. B. Jensen & M. Parmar 2006). Self renewal, as one of the key distinct characteristics of a stem cell, can be demonstrated by serial passaging of spheres, i.e. dissociation with subsequent re-plating to initiate secondary and tertiary spheres upon passage.

Given the massive proliferation potential of stem cells, in proliferation stimulating media, serial passaged stem cells should soon give rise to a large cell population. Further on, multi lineage potential can be tested in differentiation assays followed by morphological assessment or immunohistochemical analysis, e.g. detection of GFAP for glial lineage (Bramanti et al. 2010) or nestin, an intermediate filament protein expressed by neural progenitor cells (Walcott & Provis 2003).

1.3 Aldehyde Dehydrogenases

1.3.1 Introduction

Cytosolic, mitochondrial or nuclear located Aldehyde dehydrogenase (ALDH) are enzymes, that metabolize endogenous and exogenous aldehydes and belong to a superfamily of enzymes, consisting of at least 19 tissue specific isoforms with distinct functions and implications (E. H. Huang et al. 2009; Marchitti et al. 2008). Retinoic acids are metabolized by some ALDHs via oxidization of retinol to retinoic acid (RA) (Black & Vasiliou 2009) ALDHs are also involved in the detoxification of lipid peroxidation derived products, therefore playing an important role in the cellular defense against oxidative stress (Makia et al. 2011). ALDH Isoform 1 has been found to be overexpressed in cancer stem cells in numerous human hematopoietic and solid tumors, suggesting ALDH1 as a novel intracellular marker for cancer stem cells (Pearce et al. 2005).

1.3.2 ALDH nomenclature

The aldehyde dehydrogenase gene superfamiliy resource center has created a publicly available database for nomenclature and functional information of the ALDH gene superfamily (Black & Vasiliou 2009).
The root symbol ALDH is followed by a number, indicating the respective family. ALDH proteins from one family (e.g. ALDH1) are defined by having less than 40% amino-acid identity to that from another family. The next digit is a letter, designating the subfamily (e.g. ALDH1A). Subfamilies are defined as sharing more than 60%

amino-acid identity. The individual gene constitutes the last digit (e.g. ALDH1A1). Genes for mouse and fruit fly are italicized (Black & Vasiliou 2009).

1.3.3 Physiological function of ALDH1

Given, that aldehydes are mostly cytotoxic and need to be detoxified, one important role of ALDH is the NAD(P)-dependent oxidation of aldehydes to carboxylic acids (Marchitti et al. 2008). Aldehydes can occur upon various metabolic events like membrane lipid degradation, amino acid and carbohydrate catabolism (Marcato et al. 2011). Other functions of Aldehyde Dehydrogenase include ester hydrolysis, androgen and cholesterol binding, antioxidant production, ultraviolet light absorption and hydroxyl radical scavenging (Marcato et al. 2011; Black & Vasiliou 2009; Brocker et al. 2011). Some isoforms, most of them belonging to the ALDH1 family (ALDH1A1, ALDH1A2, ALDH1A3 and ALDH8A1), oxidize retinal to retinoic acid (Penzes et al. 1997; X. Wang et al. 1996). Retinal is the product of retinol dehydrogenases by Retinol (Vitamin A) oxidation (Zhao et al. 1996). Retinoic acid has important implication as it initiates transcription via the retinoic acid receptor (RAR) and retinoid x receptor (RXR), that build the retinoic acid response element (RARE) receptor complex after activation. Transcriptional products control normal growth, development (Kashyap et al. 2011), differentiation, (Gudas & Wagner 2011) apoptosis (Noy 2010), cell cycle arrest and were discussed for their implications in stemness (Ross et al. 2000; Ginestier et al. 2009).
ALDH1A1 is also involved in the metabolism of acetaldehyde, an ethanol metabolite. Low enzymatic activity may contribute to alcohol sensitivity (Ueshima et al. 1993).

1.3.4 ALDH as cancer stem cell marker

Initially, enhanced ALDH activity was detected by Cheung et al. in acute myeloid leukemia studies. The authors demonstrated, that ALDH activity could better identify tumor stem cells, that expressed the previous suggested marker CD34 (Cheung et al. 2007). Studies by Ginestier and coworkers, later in the same year, isolated CSCs from breast cancers based on high ALDH expression (Ginestier et al. 2007). Since

then, an ever growing body of publications clearly suggested the applicability of ALDH as a novel and reliable intracellular CSC marker in many other cancers, including lung (Moreb et al. 2007; Jiang et al. 2009), bladder (Su et al. 2010), colon (Chu et al. 2009; E. H. Huang et al. 2009), head and neck (Clay et al. 2010), osteosarcoma (L. Wang et al. 2010), melanoma (Boonyaratanakornkit et al. 2010), thyroid cancer (Todaro, Iovino, et al. 2010b), prostate (van den Hoogen et al. 2010), pancreatic cancer (Rasheed et al. 2010), liver (Ma, Chan, et al. 2008a), retinoblastoma (Ma, Chan, et al. 2008a), ovarian cancer (Chang et al. 2009) and GBM (Rasper et al. 2010). The overwhelming majority of studies comparing ALDH1 overexpression retrospectively with prognosis and chemoresistance found worse prognosis and more aggressive cancer biology correlated with aberrant high ALDH activity (Marcato et al. 2011; Gong et al. 2010; Ginestier et al. 2007), whereas one study by Chang et al. reported a more favourable prognosis in ovarian cancer patients (Chang et al. 2009).

1.3.5 Aldefluor assay for detection and isolation of ALDH^{high} cells

The Aldefluor reagent system is a commercially available kit for identification of cells based on their ALDH activity. The ground work for the development of this assay were laid by Storms and colleagues (Storms et al. 1999) by engineering of a fluorescent substrate, termed BODIPY aminoacetaldehyde (BAAA), an uncharged substrate, that diffuses freely into viable cells by passive diffusion and gets converted by intracellular ALDHs into a negatively charged carboxylate ion (BODIPY aminoacetate, BAA^-), that subsequently gets trapped intracellularly. Thus, cells that express high levels of ALDH could be identified by higher fluorescent intensity in flow cytometry. By inhibition of ALDH with the inhibitor diethylamino-benzaldehyde (DEAB) in 10-fold molar excess, a negative staining control sample can be created to set up the flow cytometer and to differentiate between ALDH^{high} cells and ALDH^{low} cells.

1.4 Hypoxia

1.4.1 Tumor angiogenesis and VEGF-Signaling

Whereas alveolar oxygen concentration is about 14% (Guyton, 2006), physiologic oxygen levels in the human brain range from 2,5% to 12,5% (pO_2 = 100 to 200 mm Hg) (Evans 2004a; Evans 2004b). Hypoxic regions, with even lower oxygen levels are a hallmark of many cancers. Thus, in a rapidly growing neoplasm, angiogenesis, i.e. the formation of new blood vessels from pre-existing vessels, is a prerequisite for sufficient nutrition of the tumor with metabolites and oxygen and for removal of cellular waste and carbon dioxide (Hanahan & Weinberg 2011). Tumor blood vessels typically lack proper vessel architecture and structure, characterized by underdeveloped capillary sprouting, inconsistent blood flow, short-circuits, microhemorraghes, tortuous and disproportionate branching, leakiness and endothelial thickening (Ricci-Vitiani et al. 2010; Heath & Bicknell 2009).
Thus, oxygen concentration can be decreased fiercely in the tumor microenvironment (Vaupel & Hockel 2000).

In the course of tumor progression and apparently yet in the very early premalignant phase of tumor formation (Raica et al. 2009), angiogenesis is continually activated by constant secretion of growth factors and pro-angiogenic signals like the fibroblast growth factor (FGF), erythropoietin (EPO), plasminogen activator inhibitor-1, angiopoietins, platelet derived growth factor (PDGF) and matrix metalloproteinases (MMP) (Grunewald et al. 2006). Thereby, the tumor needs to release enough pro-angiogenic factors to compete with anti-angiogenic factors (angiogenic switch) (Rafii & Lyden 2008). A well studied angiogenic signalling pathway gets activated by vascular endothelial growth factor (VEGF) (Baeriswyl & Christofori 2009). Upon binding of a VEGF ligand to a vascular endothelial growth factor receptor (VEGFR1-3), a tyrosine kinase on the cell surface of vascular endothelial cells, the receptors dimerize and become activated by autophosphorylation of intracellular tyrosine residues, further activating intracellular proteins resulting in second

messenger generation and activation of pathways leading to increased vessel permeability, migration, proliferation and survival (Holmes & Zachary 2008).

1.4.2 Hypoxia in GBM

In GBM, hypoxic regions are frequent and the presence is linked to worse prognosis (R. L. Jensen 2009; Sathornsumetee et al. 2008). In the current WHO classification for tumors of the central nervous system, hypoxic necrotic foci with pseudopalisading tumor cells are characteristics of high grade glioma, defining an astrocytic tumor as a GBM (WHO grade IV astrocytoma) (Rong et al. 2006; Louis et al. 2007).

1.4.3 Hypoxia Inducible Factors and Hypoxia Target Genes

Hypoxia can occur during acute or chronic vascular diseases, pulmonary disease and can be found frequently in the tumor microenvironment. In tumors, hypoxia is associated with a more aggressive growth pattern and therapeutic resistance. Target genes of hypoxia related pathways have numerous implications in the regulation of normal and cancer stem cells.

The cellular response to hypoxia is complex and has been studied for decades. Among the mediators of low oxygen supply are the hypoxia-inducible factors 1α, 2α and 3α (HIF-1α - HIF-3α) (Harris 2002; Park et al. 2003; Keith & Simon 2007). In mammals, HIF-1α and HIF-2α are considered as the main regulators of hypoxia. HIF-1α is apparently ubiquitous expressed in all cell types, whereas HIF-2α is only expressed on certain endothelial cells, kidney interstitial cells, hepatocytes and certain tumor stem cells (Silván et al. 2009). Nevertheless, both subtypes are crucial for development and survival of the organism, as knockout experiments have revealed (Ramírez-Bergeron & Simon 2001). The physiological basics concerning HIF-signaling are described for the universally expressed HIF1 subtype.

Under normoxic conditions, HIF-1α is posttranslationally modified by hydroxylation on the proline residues P402 and P564, located in the oxygen-dependent degradation

domains (ODDDs) by three prolyl hydroxylase domain (PHD) proteins (Ivan et al. 2001), whose activity depends on normal oxygen levels. This is a prerequisite for ubiquitination of a complex, consisting of the von Hippel-Lindau (vHL) protein (Iliopoulos et al. 1996), elongin-B and -C, CUL2, RBX1 and two other ubiquitin-activating enzymes to be finally subjected to degradation by the 26S proteasome (Kallio et al. 1999). HIF-1 inhibiting factor (FIH-1), constitutes another inhibiting mechanism via hydroxylation of the Asn-803-aminoacid and thus inhibits binding of HIF-1α to the co-activator E1A binding protein p300/CREB-binding protein (p300/CBP).

In hypoxic states, the PHD proteins are inhibited and the affinity of VHL to HIF-1α is reduced. Thus, stabilized HIF-1α is translocated to the nucleus and heterodimerizes with the constitutively expressed HIF-1b or aryl hydrocarbon receptor nuclear transporter (ARNT) and interacts with CBP/p300 and DNA polymerase II (Pol II) (Semenza 2009). This complex subsequently binds to hypoxia responsive elements (HRE) in the promotor region of hypoxia-regulated genes (Harris 2002).
Hypoxia related downstream genes assist the cell to adapt to hypoxic condition by inducing angiogenesis, glycolytic metabolism, growth-factor expression and pH adaptation. Many pathways overlap with oncogenic signaling.

1.4.4 Hypoxia and Stem Cells

Studies in embryology and perinatal neurogenesis revealed the regulatory implications of low oxygen conditions for stem cells. Meanwhile it is well known, that cell proliferation, survival and differentiation of stem and progenitor cells strongly depend on hypoxia related signaling (Mazumdar et al. 2009; Vieira et al. 2011).
In fetal and embryo development, placental oxygen is physiologically limited and development occurs in a hypoxic environment (Ottosen et al. 2006). Studies have shown, that low oxygen level are crucial for brain development, vessel formation and neural fold closure (Iyer et al. 1998; Panchision 2009). Human embryonic stem cells (hESC) are able to maintain their pluripotent differentation and self renewal capacity when cultured in low-oxygen atmosphere (Ezashi et al. 2005). In line with these

findings, Yoshida et al. reported enhanced generation of induced pluripotent stem cells under hypoxia (Yoshida et al. 2009).

Similar observations were reported in the field of adult stem cells. Hematopoietic stem cells and neural precursor cells show enhanced survival, clonogenic growth and proliferation under hypoxic culture conditions (Studer et al. 2000). These results are in keeping with more recent studies about increased in vitro expansion of human postnatal brain $CD133^+$ $nestin^+$ precursor cell in reduced oxygen tension by Pistolatto et al. (Pistollato et al. 2007).

1.4.5 Physiologic Hypoxic niche

The initial suggestion, that stem cells favour regions with diminished perfusion and oxygen tension, called niches was established nearly four decades ago (Schofield 1978). In this specific microenvironment, oxygen level, matrix proteins and temperature regulate stem cell function and differentiation (Danet et al. 2003; Yoshida et al. 2009). Parmar et al. studied the distribution of hematopoietic stem cells in the bone marrow and found them predominantly located at the lowest end of an oxygen gradient, enabling them to maintain an undifferentiated state (K. Parmar et al. 2007). In the human brain, neural stem cells reside in hypoxic niches, e.g. located in the subventricular zone (SVZ) in the lateral ventricles and the subgranular zone (SGZ) in the dentate gyrus of hippocampus with oxygen concentration estimated at 2.5 - 3% oxygen (Santilli et al. 2010). Stem cell niches have been described in various anatomic locations, among them skin (Draheim & Lyle 2011), testis (Voog et al. 2008), intestine (Mathur et al. 2010), bone marrow (Orkin & Zon 2008) and brain (Ihrie & Alvarez-Buylla 2011).

1.4.5.1 Tumor stem cell niche

For more than a decade, hypoxia is generally considered to play a key role in tumor progression by stimulating core tumor pathways like angiogenesis, adapted metabolism, invasiveness and survival (Bertout et al. 2008). Concordant with that,

tumor stem cells could be isolated from distinct anatomical niches, resembling the hypoxic microenvironment of physiological stem cell niches. In addition to molecular cues that might be driven by hypoxic conditions, the low perfusion of stem cell niches might shield tumor stem cells from chemotherapeutic agents, immune answer (Moore & Lemischka 2006) and by induction of multidrug resistance proteins (Comerford et al. 2002).

1.4.6 Hypoxia and Signaling

Beyond tumor angiogenesis, it is becoming more evident, that HIFs affect tumor growth on more facets by crosstalk with other oncogenic pathways. Notch is a transmembrane receptor with an extracellular and an intracellular domain (Nicd) (Bray 2006). After Jagged or Delta family ligands bind to the extracellular domain, HIF-1α can bind to the Nicd. Subsequently, this complex can interact with Notch-response-elements (NRE), giving rise to Hes3 and Shh proteins, both of them known to be involved in increased survival of neuronal stem cells (Gustafsson et al. 2005). Moreover, Notch signalling blocks stem cell and progenitor cell differentiation and induces c-Myc (Satoh et al. 2004).

The Ocatamer binding transcription factor 4 (Oct4), a protein encoded by the POU5F1 gene is a homeodomain transcription factor of the Pituitary/Octamer/Unc (POU) family. Oct4 is a core regulator in the self-renewal of undifferentiated stem cells and along with Sox2 and Nanog, a key signature for human embryonic stem cells (hESCs) (Du et al. 2009). Knock-in studies by Covello et al. revealed that HIF-2α, even if only detectable in certain tissues, is crucial for development and survival of the organism. Furthermore HIF-2α was identified as a direct upstream regulator of Oct-4 (Covello 2006).

1.5 Oxidative Stress and the role of ALDH1 in detoxification

Cells produce energy in form of adenosine triphosphate (ATP) mainly through mitochondrial oxidative phosphorylation. In this process electrons pass a number of redox reactions at the inner mitochondrial membrane. On this way, adenosine diphosphate (ADP) and phosphate are finally synthesized to ATP.

Five complexes of proteins form the electron transport chain (ETC) and are located at the inner mitochondrial membrane, acting as a proton pump, that transports protons out of the mitochondrial matrix into the intermembrane space. Thus establishing an electrochemical gradient from Complex I to Complex IV and in a final step, the ATP synthase complex generates ATP via oxidative phosphorylation. In more detail, complex I (NADH-ubiquinone oxidoreductase) and complex II (succinate dehydrogenase) accept electrons from nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) and pass them to coenzyme Q (ubiquinone). Electrons are then transferred by complex III (cytochrome c reductase) to cytochrome c, where electrons are passed on to complex IV (cytochrome c oxidase), which uses electrons and hydrogen ions to reduce molecular oxygen to water.

1.5.1 Reactive oxygen species

During mitochondrial oxidative phosphorylation, the majority of molecular oxygen is reduced to water at complex IV as described above, nonetheless, 1-4% of oxygen is incompletely reduced to anion superoxide (O_2^-), also called reactive oxygen species (ROS). The sites of ROS-production inside mitochondria are complex I and in particular complex III (Zhang & Gutterman 2007).

Under hypoxic conditions, cellular metabolism gradually switches from oxidative phosphorylation to less efficient anaerobic glycolysis (Cairns et al. 2011). Paradoxically and poorly understood, despite less electrons that are transported along the ETC during cellular hypoxia, the generation of ROS is increased under hypoxia (Jezek & Hlavatá 2005). Besides the implications, reactive oxygen species

might have in cell and stem cell signaling (Cho et al. 2006), highly reactive hydroxyl radicals react with cellular components like polyunsaturated fatty acids in membrane lipids (lipid peroxidation). These radicals are formed from Hydrogen peroxide in the presence of metal ions or upon reaction of nitric oxide with anion superoxide (Nigam & Schewe 2000; Adibhatla & Hatcher 2008). Peroxidization of unsaturated fatty acids leads to generation of phospholipid aldehydes such as maliodialdheyde (MDA), 4-hydroxynonenal (HNE) (K. Uchida 2003) and acrolein (Stevens & Maier 2008). With respect to the role of aldehyde dehydrogenases as potent detoxifying enzymes and the putative cancer stem cell marker ALDH1 in the detoxification of HNE and MDA, hypoxia induced increase of ROS generation may explain the implication of ALDH1 in this tumor biological context (Marchitti et al. 2008).

2 AIM AND MOTIVATION OF THIS WORK

The aim of this work is to investigate cellular regulatory mechanisms which possibly contribute to the aggressive biological behavior of Glioblastoma multiforme (GBM), which is still, despite decades of intensive research one of the most deadly oncologic pathologies.

Since it could be established that a distinct subset of GBM cells may have a neural stem cell origin, (Canoll & Goldman 2008; Dirks 2006; R. Chen et al. 2010; Galli 2004) a growing number of reports documentate the clinical implications of such stem-like cells.

Works that were conducted in our laboratory could identify Aldehyde dehydrogenase 1 (ALDH1) as a potential marker for this cellular subpopulation, which could already been used to isolate other tumorigenic stem-like cells in a large number of hematopoietic and other solid human tumors.

We have previously shown, that ALDH1 expression facilitates neurosphere formation in established glioblastoma cell lines and that inhibition of ALDH1 induces cellular differentiation and decreases clonogenic capacity.
The definite aims of this study are:

1. To the date, no other study has isolated human glioblastoma cells with fluorescence activated cell sorting (FACS) based on their ALDH1 expression and further tested these subpopulations for stem like behavior.

2. To investigate whether regulatory pathways are activated or altered upon exposure to hypoxia in cells that express high levels of ALDH1 and those which express no ALDH1. Consequently, this thesis aims to underline the direct involvement of ALDH1 in stem cell phenotype modulation.

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

Equipment	Supplier
Blotting chamber, semidry	Biorad, Munich
Gel chamber	Biorad, Munich
Flow Cytometer	Cyan Lx, Beckman Coulter, Fullerton, USA Cyan ADP, Beckman Coulter, Fullerton, USA
MoFlo Cell Sorter	Beckman Coulter, Fullerton
FACSAria	Becton Dickinson, Heidelberg
FACSCalibur	Becton Dickinson, Heidelberg
Microscopes	Axiovert Apotom Axiovert 200M, Carl Zeiss, Jena Zeiss LSM 510, confocal microscope, Carl Zeiss, Jena Leica SP 5, confocal microscope, Leica, Bensheim
Camera	Axiocam HRm, Carl Zeiss, Jena
Cellcounter	CASY1, Schäfer System, Reutlingen

Centrifuges	Eppendorf 5471R, Eppendorf, Hamburg Sigma 4K15, Sigma, Deisenhofen 5415D, Eppendorf, Hamburg
Heating block	Thermomixer 5436, Eppendorf, Hamburg
Incubator (38°C)	Memmert, Schwabach
Incubator (Cell Culture)	Heraeus, Munich
Laminar flow hood	Heraeus, Munich
Magnetic stirrer and heater	IKA-Labortechnik, Staufen
Photometer	Eppendorf, Hamburg
Pipettes	Multipipette plus, Eppendorf, Hamburg Pipetus akku, Hirschmann, Eberstadt Serological pipettes 5, 10, 25ml, Sarstedt Nümbrecht, Germany
NanoDrop spectrophotometer	Thermo Fisher Scientific, Wilmington, USA
Shaker	Polymax 1040, Heidolph, Kelheim
Waterbath	Gesellsch. f. Labortechnik, Burgwedel
Weighing machine	Sartorius, Göttingen
X-ray film developing machine	Konica Minolta, Unterföhring

3.1.2 Cell lines, working materials

Item	Supplier
LN18	E. van Meir, M.D., Lausanne, Switzerland
Accutase	PAA, Pasching, Austria
Ampicillin	Sigma, Taufkirchen, Germany
Bovine serum albumin (BSA)	Invitrogen, Karlsruhe, Germany
Dulbecco´s Modified Eagle Medium	Invitrogen, Karlsruhe, Germany
DMSO	Invitrogen, Karlsruhe, Germany
Ethanol	Sigma, Taufkirchen, Germany
Fetal calf serum (FCS)	Invitrogen, Karlsruhe, Germany
Gelatine	Sigma, Deisenhofen
Gentamycin	Invitrogen, Karlsruhe, Germany
L-Glutamin	Invitrogen, Karlsruhe, Germany
NaOH	Sigma, Deisenhofen
Paraformaldehyde	Sigma, Deisenhofen

Penicillin-Streptomycin	Invitrogen, Karlsruhe, Germany
Phosphate buffered saline (PBS)	Invitrogen, Karlsruhe, Germany
Rapamycin	Merck Biosciences, Darmstadt
Tris-hydrochloride (Tris-HCl)	Roth, Karlsruhe, Germany
Triton X-100	Biorad, Munich, Germany
Trypan Blue solution	Sigma, Taufkirchen, Germany
Trypsin/EDTA 0,5 %	Invitrogen, Karlsruhe, Germany
Vectashield Mounting Medium	Vector Laboratories, CA, USA

3.1.3 Buffers, reagents, solutions

Acetic acid	Roth, Karlsruhe, Germany
Aceton p.a.	Roth, Karlsruhe, Germany
30 % Acrylamid/Bisacrylamid	Roth, Karlsruhe, Germany
Ammoniumchlorid	Roth, Karlsruhe, Germany
Ammoniumpersulfat	Roth, Karlsruhe, Germany
Basic Fibroblast Growth Factor	Invitrogen, Karlsruhe, Germany

Biorad Protein Assay	Biorad, Munich, Germany	
Blocking buffer	5% skimmed milk powder in TTBS	
Dimethylsulfoxid	Roth, Karlsruhe, Germany	
Ethidium bromide	Sigma, Deisenhofen, Germany	
Formaldehyde	Roth, Karlsruhe, Germany	
Glycin	Sigma, Deisenhofen, Germany	
Isopropanol p.a.	Roth, Karlsruhe, Germany	
FACS staining buffer, pH 7.45	1x	PBS
	0.5% (w/v)	BSA
Lysis buffer, pH 7.4	150mM	NaCl
	50mM	HEPES
	1mM	EDTA
	10% (v/v)	Glycerol
	1% (v/v)	Triton-X-100
	10mM	Na4P2O7
	1 Tabl.	Protease inhibitors/100ml
	5ml	Tween20
Methanol p.a.	Roth, Karlsruhe, Germany	
Natriumacetate	Roth, Karlsruhe, Germany	
Natriumchloride	Roth, Karlsruhe, Germany	

N2 - supplement 100x	Invitrogen, Karlsruhe, Germany
5x SDS loading buffer	312,5 mM Tris-Cl pH 6,8 10% SDS 50% Glycerol 250mM DTT 0,05 % Bromphenol Blue ad 10ml H2O
10x SDS running buffer	250mM Tris 1,92M Glycin 1% SDS ad 1000ml H2O
Sample Buffer (SB)	10ml ddH2O 10µl 0.5mM Tris, pH 6.8 20µl 10% SDS 10µl 10% SDS 1.543 g DTT 0.1 g Bromophenol blue
Skimmed milk powder	Merck Bioscience, Darmstadt, Germany
Transferbuffer	Anode I 300mM Tris 20% methanol ad 500ml H2O Anode II 25mM Tris 20% methanol

	ad 500ml H2O
	Cathode 25mM Tris 20% methanol 40 mM amino-n-caprioicacid ad 500 ml H2O
10x Tris-buffer pH 7,6	60,5 g Tris-base 90g NaCl pH 7,6 ad 1000ml H2O
Triton X-100	Fluka, Neu-Ulm, Germany
Tween 20	Roth, Karlsruhe, Germany
Washing buffer	24,2g Tris 80g NaCl pH 7,6 ad 1000ml H2O

3.1.4 Gels

Buffer	**Composition**	
SDS-PAGE running gel 10%	7ml	ddH2O
	4.38 ml	1.5 M Tris, pH 8.8
	5.8 ml	30% Acryl 1% Bisacrylamide
	170µl	10% SDS
	8.8µl	Temed
	170µl	10% APS

SDS-PAGE stocking gel	6.2ml	ddH2O
	2.5ml	0.5 M Tris, pH 6.8
	1.2ml	30% Acryl 1% bis
	100µl	SDS
	5µl	Temed
	100µl	10% APS

3.1.5 Consumables

Amersham Hyperfilm ECL	GE Healthcare, Munich, Germany
Blotting paper	Sartorius, Göttingen, Germany
Cell culture plate, 6 well	Sarstedt, Nümbrecht, Germany
Cell culture plate 6, 10 cm	Sarstedt, Nümbrecht, Germany
Cell scraper	Sarstedt, Nümbrecht, Germany
Centrifuge tube 15ml, 50ml	Sarstedt, Nümbrecht, Germany
Cover glass	Schubert und Weiss, Munich, Germany
Cuvettes	Sarstedt, Nümbrecht, Germany
Cryo-tubes (2ml)	Sarstedt, Nümbrecht, Germany
Hyperfilm ECL	GE Healthcare, Munich, Germany
Microtiterplates 96-well	Sarstedt, Nümbrecht, Germany

Object slides	Menzel, Braunschweig, Germany
Syringe filter sterile, 0,2µm	Schubert und Weiss, München

3.1.6 Antibodies

Antibody	Dilution	Source
ALDH1	1:500	Abcam, Cambridge, USA
ßIII-Tubulin	1:100	Millipore, USA
Goat Anti-Mouse-IgG	1:10000	NEB, Frankfurt, Germany
Goat Anti-Rabbit-IgG	1:10000	NEB, Frankfurt, Germany

Software

FlowJo	Treestar, Ashland, USA
ImageJ	National Institute of Health (NIH), Bethesda, USA
AxioVision LE Rel. 4.5	Carl Zeiss, Jena
Microsoft Office	Microsoft, Redmond, USA

3.2 Methods

3.2.1 Cell line and cell culture

Human glioblastoma cell lines LN18 (Diserens et al. 1981) and primary cell lines T13, T30 and T40 were incubated under standard conditions (5% CO_2, 95% relative humidity and 37°C), using Dulbecco´s modified Eagle´s Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 2mM L-glutamine, 100 µg/ml streptomycin and 100 µg/ml penicillin (Invitrogen, Karlsruhe, Germany). Primary cell lines were obtained from tumor samples from patients, that were operated at the Dept. of Neurosurgery at the Technical University of Munich, Germany. Informed consent and ethics committee approval were obtained.

3.2.2 Neurosphere formation assay

To enable neurosphere formation, LN18 cells were cultivated under serum free medium conditions as described previously (Rasper et al. 2010). Cells were seeded in 96 well culture dishes, coated with 0,1% gelatine in a density of 5 x 10^4 cells per well. Dishes were cultured in a standard humidified incubator as described above.
Spheres were grown for 7 days. 4 random fields of view were photographed under a microscope to score diameters and total number of spheres counted in 4 fields of view (total 4 mm^2). Sphere diameters were measured at the longest perpendicular axes. The individual values were averaged. For serial passaging, spheroid bodies were dissociated by gentle tapping. Spheres were collected by centrifugation (1000 rpm) and mechanically dissociated, before resuspended in new 96 well dishes to be cultured under identical conditions. Resuspension was performed for up to 3 passages.

All microscopic analyzes and photographs were performed using ZEISS microscopes and cameras (Zeiss, Jena, Germany). Image analysis was performed with Image J software (NIH, Bethesda, USA).

3.2.3 Hypoxia

Cells were incubated under hypoxic conditions, using an air-tight chamber, that is connected to a vacuum pump and gas cylinders containing a mixture of 95% N_2 and 5% CO_2. Alternating oxygen evacuation and N_2 inflow gradually lowered O_2 concentration down to 1%. This oxygen concentration could be achieved and maintained after approximately 22 minutes. Upon completion, the whole chamber was transferred to a 37°C incubator. The hypoxic atmosphere was maintained for 24h for immortalized cells and 6h for primary cells. Subsequently, cell viability was determined by propidium iodide staining.

3.2.4 Aldefluor assay and analysis/isolation of $ALDH^{high}$ / $ALDH^{low}$ cells by FACS

For determination of ALDH activity we used the Aldefluor kit (StemCell Technologies, Vancouver, Canada) following the manufacturer´s recommendations for detection of tumor stem cells. In summary, $1x10^6$ cells were incubated in 1000µL ALDEFLUOR assay buffer containing the ALDH substrate (5µL per mL). Negative controls additionally contained 5µL of a 1.5mM DEAB stock, a specific ALDH inhibitor. Incubation was performed at 37°C in an incubator for 40 minutes. Subsequently, cells were centrifuged at 4°C for 5 minutes at 300g and pellets were resuspended in 500µL of 4°C ALDEFLUOR assay buffer. Cell viability was assessed using propidium iodide labeling.

For FACS analysis or sorting, cells were obtained using a MoFlo (Dako, Fort Collins, USA) or FACSAria III flow cytometer (BD Biosciences, San Diego, USA). Sorting gates were set according to the DEAB buffered control sample. Sorting pressure was set at 70psi at a sorting speed of 10.000 events per second (eps). Sorting mode was purity. At least $1x10^5$ events were recorded. Cells were sorted directly into prewarmed FCS. Side scatter and forward scatter was set for exclusion of cell

doublets. The purity of sorted cells was confirmed on a CyAn ADP (Dako, Fort Collins, USA). Data were analyzed with BD FACSDiva software V6.1.2 (BD Biosciences, San Diego, USA).

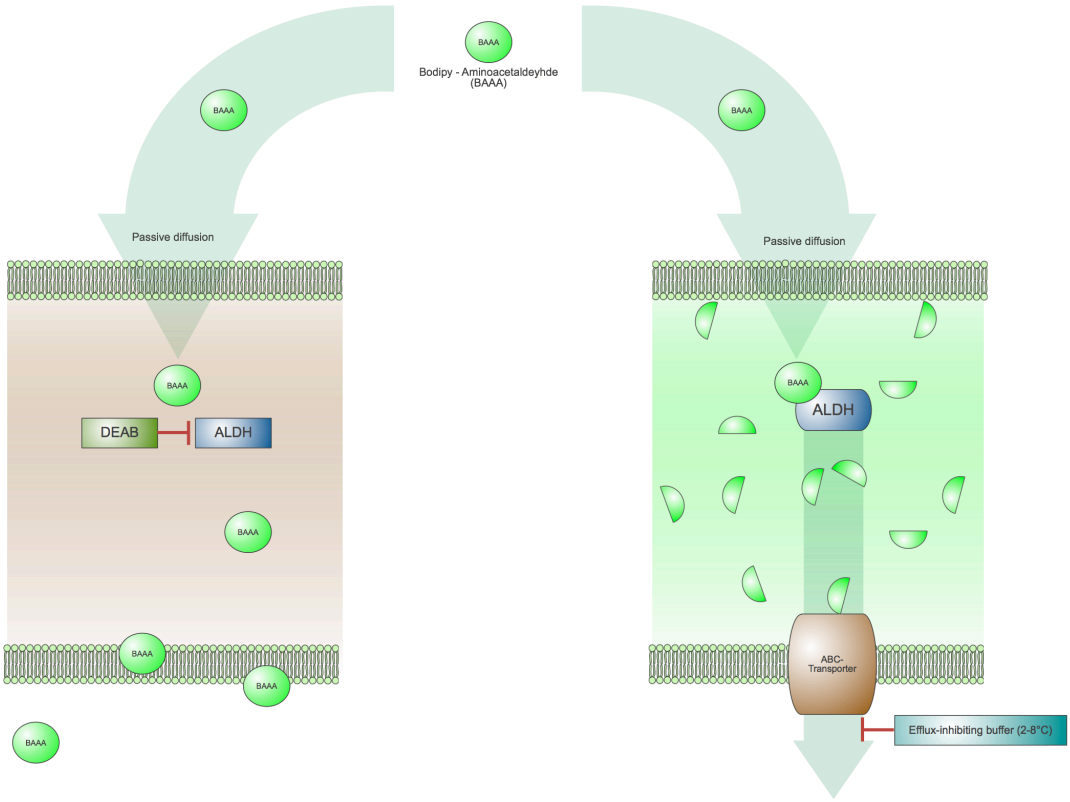

Figure 6: Aldefluor Assay (Stemcell Technologies, Inc., Vancouver, Canada)
The fluorescent substrate, BODIPY aminoacetaldehyde (BAAA) diffuses freely into viable cells by passive diffusion and gets converted by intracellular ALDHs into a negatively charged carboxylate ion (BODIPY aminoacetate, BAA^-), that is subsequently trapped intracellularly. Thus, cells that express high levels of ALDH can be identified by higher fluorescent intensity in flow cytometry.

3.2.5 Protein isolation and Western blot

Cells were rinsed with phosphate buffered saline at 4°C and lysed in 300 μL of lysis buffer (CST Technologies, Port Washington, USA) -/+ PMSF. After dissociation, cell lysates were centrifuged at 4°C for 15 minutes at 10000 rpm. Equal amounts of proteins were separated by 10% SDS-PAGE and transferred to a nitrocellulose

membrane. Membranes were incubated at 4°C over night with monoclonal primary antibodies, continued by incubation with a HRP-conjugated anti-mouse secondary antibody (1:5000, BD Biosciences) at room temperature for 2 hours. Chemiluminescence immune reaction was visualized using high-performance films (GE Healthcare, Buckinghamshire, Great Britain). Loading controls were performed with an alpha-tubulin immunoblot. Antibody dilutions: anti-ALDH1 (1:500) (BD Biosciences, San Diego, USA), anti-HIF-1α (1:1000) (CST, Port Washington, USA), anti-tubulin (1:10.000) (Sigma Aldrich, Hamburg, Germany). Quantification of band intensities was performed with Image J densitometry software.

3.2.6 Immunofluorescence

Cells were fixed on glass chamber slides using acetone and methanol. ALDH1 expression in glioblastoma cells were analyzed by means of immunofluorescence with primary anti-ALDH1 antibodies. Incubation was performed at 4°C over night and unspecific binding sites were blocked with normal goat serum (20 %). Incubation with a fluorescent anti-mouse secondary antibody (1:100) was performed at room temperature for 1 hour. The immunoreaction was visualized under a ZEISS Microscope (Zeiss, Jena, Germany).

All experiments were performed in triplicate.

4 RESULTS

4.1 Neurosphere formation in LN18 cells is increased in ALDH^{high} and absent in ALDH^{low} populations.

For detailed investigation and characterization of LN18 cells with high or low ALDH1 expression (further referred as ALDH^{high} or ALDH^{low}, respectively), FACS sorting was conducted after cells were labeled with the fluorescent Aldefluor substrate and sorting margins were set corresponding to ALDH1 inhibited control samples. Sorted cells of both fractions were subsequently processed for immunofluorescence, Western blot analysis, asymmetric division- and neurosphere formation assays. In the initial FACS analysis, we found 5.9% of cells with high ALDH1 enzyme activity and isolated them accordingly. ALDH1 activity, detected by Western analysis, revealed a 10 fold higher expression in the ALDH^{high} fraction in comparison to ALDH^{low} cells (Fig. 7). Strong differences in ALDH1 expression could also be confirmed in immunofluorescence analysis of ALDH^{high} and ALDH^{low} samples, using anti-ALDH1 antibodies (Fig. 9)

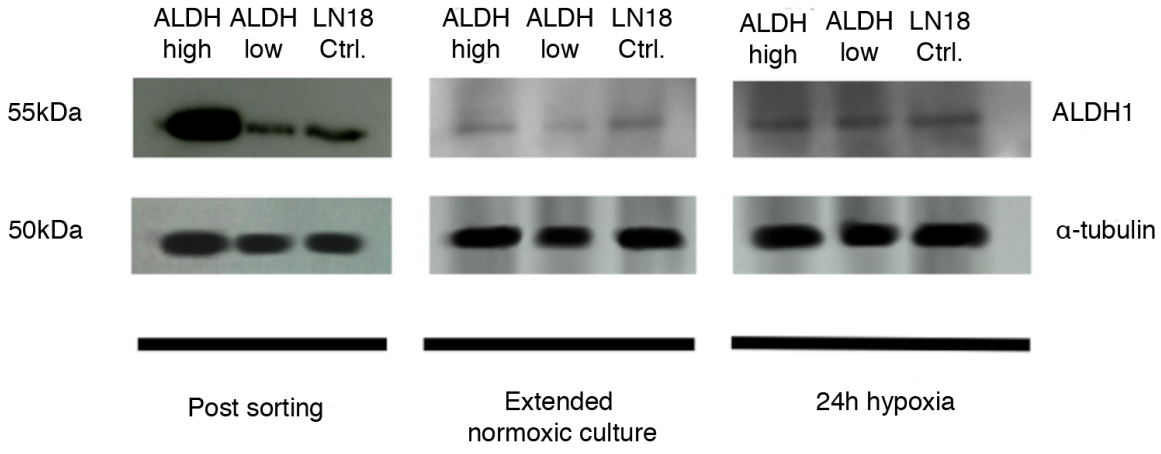

Figure 7: Western Blot analysis of ALDH1 expression in LN18 human glioblastoma cells.

Post sorting blots of ALDH^{high} and ALDH^{low} populations confirm proper isolation. Upon extended culture, ALDH1 expression is reduced in ALDH^{high} populations and remains depleted in the ALDH^{low} fraction. In hypoxic settings, all fractions show increased ALDH1 protein expression levels.

We detected stable neurosphere formation in ALDH^{high} cells (Fig. 10). Sphere formation was visible after 4-5 days of culture under serum free medium conditions and serial transfer was performed for 3 passages. Importantly, we observed a complete absence of neurospheres in the ALDH^{low} cell fraction under the same conditions. These findings show that LN18 cells, labeled by Aldefluor could be sorted, based on their ALDH activity and that expression levels correlate with tumor sphere formation.

4.2 LN18 cells demonstrate asymmetric division of ALDH^{high} cells under extended normoxic culture conditions

LN18 cells, that were previously sorted into ALDH^{high} and ALDH^{low} populations were tested for asymmetric division. Thus both fractions were exposed to extended standard cultures for 14 days. Re-analysis of ALDH^{high} cells with FACS after this period revealed, that the ALDH^{high} fraction gave rise to ALDH^{high} and ALDH^{low} cells. (Fig. 8).

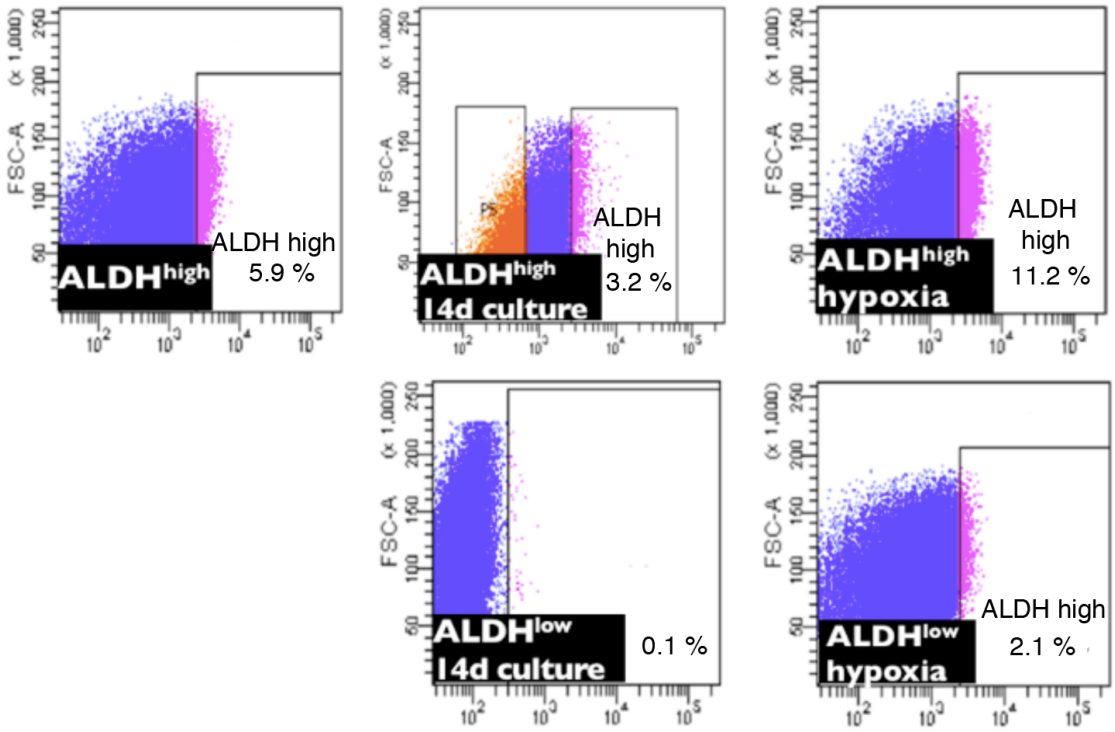

Figure 8: FACS Analysis of human LN18 glioblastoma cells and identification of ALDH^{high} and ALDH^{low} fractions. FACS isolation revealed 5.9% ALDH^{high} cells in native LN18 cells. Percentage of ALDH^{high} cells is reduced to almost the half after self-renewing division after 14 days. Subsequent exposure to hypoxia increased the fraction of ALDH^{high} cells more than threefold in formerly ALDH^{high} cells and induced the ALDH^{high} phenotype in formerly ALDH^{low} cells. Control samples were inhibited with DEAB.

To determine the quantity of asymmetric cell division, ALDH1 protein expression levels of the initially pure ALDH^{high} fraction were examined with Western blot and Immunofluorescence analysis. As expected, in both methods, cells showed less ALDH1 expression and reduced immunofluorescence intensity after this period (Fig. 7,9).

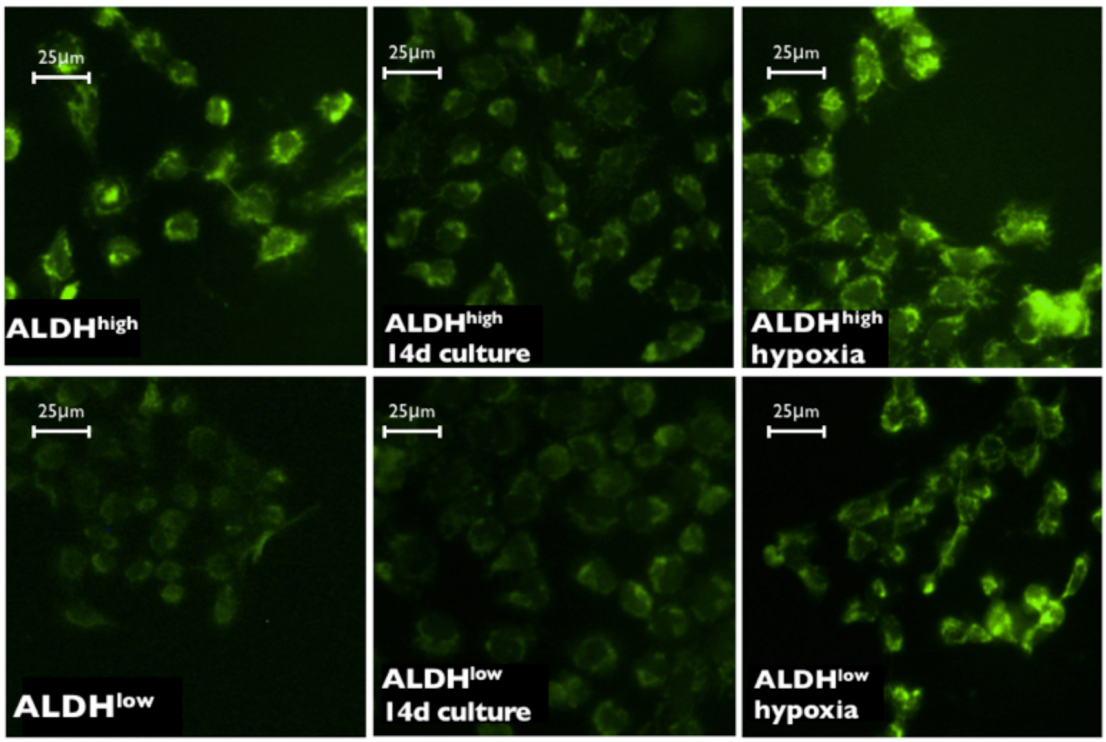

Figure 9: ALDH1 Immunofluorescence analysis of ALDH^{high} and ALDH^{low} human LN18 glioblastoma cells. Fluorescence intensity is reduced in ALDH^{high} populations after 14d self renewing culture and increased markedly in both ALDH^{high} and ALDH^{low} populations upon exposure to hypoxic conditions. (Size-bar = 25 µm)

Appropriately, neurosphere formation assays of ALDH^{high} cells exhibited smaller mean diameter measures compared to freshly sorted ALDH^{high} cells (Fig. 10).

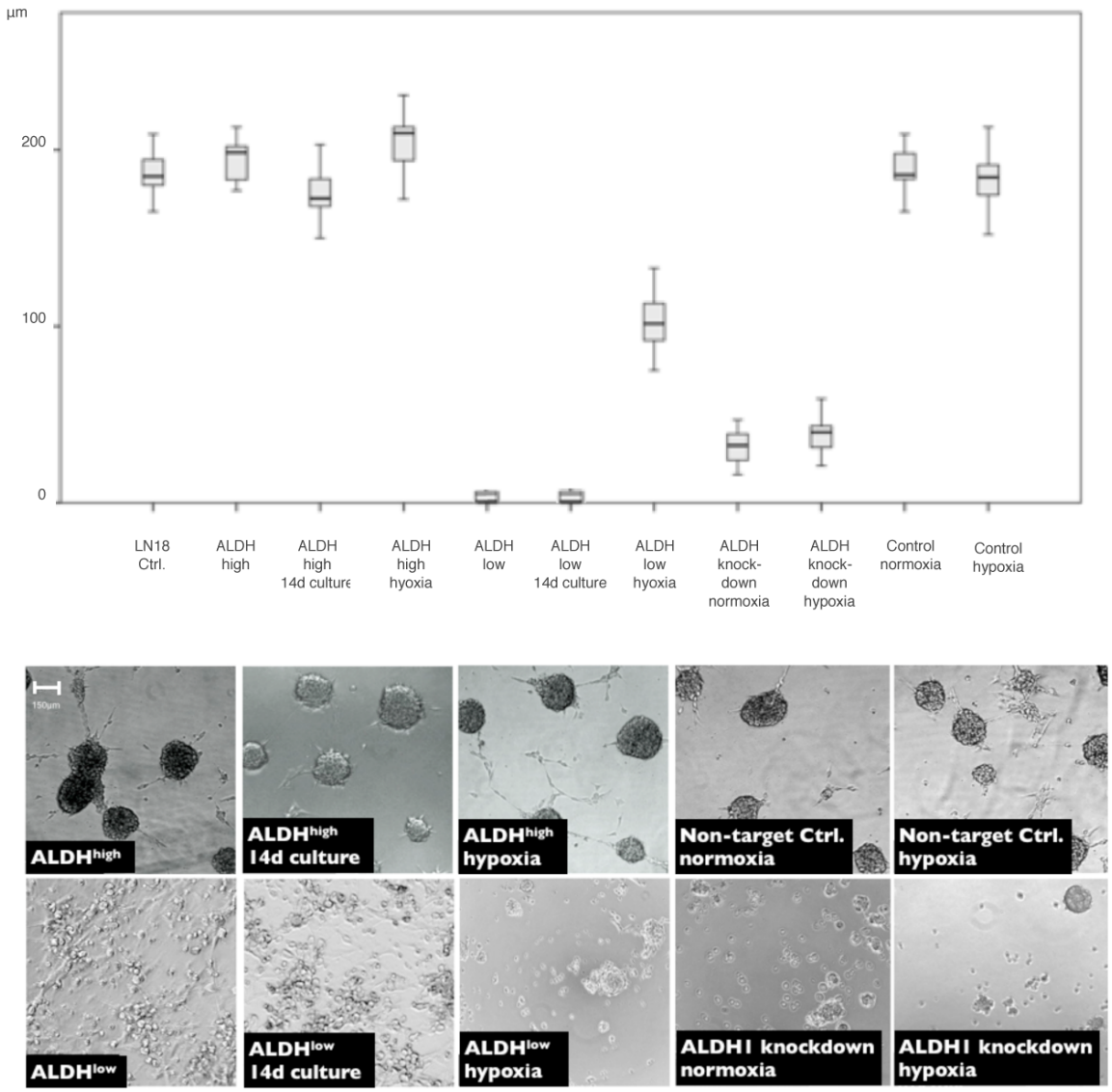

Figure 10: Neurospheres, generated from human LN18 glioblastoma cells and of ALDH^{high} and ALDH^{low} populations.

ALDH^{high} and ALDH^{low} cells established larger and more neurospheres after hypoxic culture. No neurosphere formation could be detected in FACS isolated ALDH^{low} cells. ALDH1 shRNA cells give rise to small neurospheres. (Size-bar = 150 µm)

4.3 Remaining absence of asymmetric division in ALDH^{low} cells

Under identical conditions, the ALDH^{low} fraction showed no evidence of asymmetric division, since no occurrence of ALDH^{high} cells could be identified in this population, when cultured under standard conditions for 14 days (Fig. 8).
Focusing on the functional stem cell phenotype, we further examined this ALDH^{low} population in neurosphere formation assays. After an observation period of 10 days, we could not identify neurosphere generation, suggesting that upon cell division, ALDH^{low} cells were not able to generate cells with neurosphere forming capacity (Fig. 10).
Finally, ALDH1 expression was measured and quantified with immunofluorescence and Western blot, indicating an equally low ALDH1 expression over time, as seen by both methods (Fig. 7, 9).

4.4 Hypoxia increases ALDH1 expression and neurosphere formation in ALDH^{high} cells

Next, we studied whether hypoxia might influence the ALDH1 expression in glioblastoma cells and to test functional stem cell characteristics when cells are exposed to a low-oxygen atmosphere.
To resemble orthotopic hypoxic conditions in a brain tumors microenvironment, we subsequently exposed the cells, which previously underwent extended culture under normoxic conditions to hypoxia (1% O_2) for 24 hours in hypoxia chambers. Using the same test panel as before, we cultured the hypoxia treated ALDH^{high} fraction in serum-free medium culture conditions. Analysis of neurosphere generation after hypoxic culture showed more frequent sphere formation and enlarged mean diameters in comparison to freshly sorted ALDH^{high} cells and compared to cells that underwent extended culture for testing of asymmetric division (Fig. 10).
In FACS-analysis, the proportion of post-hypoxic high ALDH1 expressing cells rose markedly to 11.2%, corroborating a hypoxic influence on ALDH1 expression (Fig. 8).

4.5 Hypoxia induces ALDH1 expression in formerly negative LN18 ALDH^{low} cells

The formerly neurosphere-negative ALDH^{low} fraction gave rise to neurospheres in serum-free medium conditions after exposure to hypoxia for 24h, although spheres were smaller in diameter (mean diameter 102 µm) and less frequent compared to ALDH^{high} neurospheres (Fig. 10). Reanalyzing post hypoxic ALDH^{low} cells with FACS demonstrated a rise from 0.1% to 2.1% of ALDH^{high} cells (Fig. 8).

Immunofluorescence and Western blot showed highly increased protein expression and fluorescence intensity compared to the pre-hypoxic state. Protein immunoblot detected a 2,5-fold higher band intensity in hypoxic ALDH^{low} populations compared to the immunoblot performed after extended normoxia culture (Fig. 7). In summary, these findings in the ALDH^{low} fraction indicate, that ALDH1 expression and functional stem cell behavior by means of neurosphere formation is inducible by hypoxia.

4.6 HIF-1α and ALDH1 heterogeneously coexpressed upon hypoxia culture in primary GBM cell lines

In addition to established GBM cell lines, we used Western immunoblots to test three primary GBM cell lines for their ALDH1 expression under normoxic and hypoxic conditions. In two of these primary cell lines, we found the expression of ALDH1 strongly or moderately enhanced upon hypoxic culture, whereas ALDH1 protein expression were not detectable in another primary cell line, unrelated to culture oxygen levels (Fig. 11).

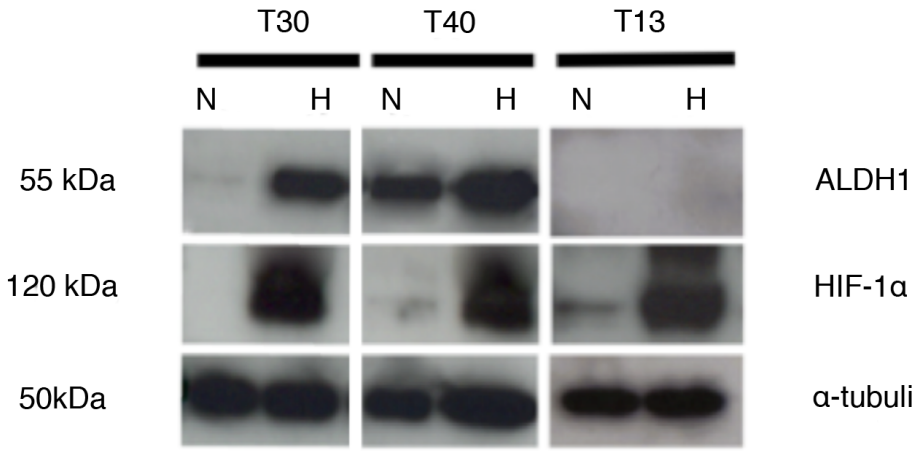

Figure 11: Western Blot analysis of ALDH1 and HIF-1α expression in primary GBM cell lines. T30 and T40 primary GBM cells exhibit increased ALDH1expression upon exposure to hypoxia, whereas T13 cells lack ALDH expression.

4.7 ALDH1 gene knockdown with shRNA in LN18 cells confirms implications of ALDH1

In order to confirm the direct contributions of the ALDH1 enzyme to a stem cell phenotype, we explored the possibility whether LN18 shALDH1 could serve as a control model to our previously tested cells and cultured them under serum-free medium conditions.

Consistent with our hypothesis, only a small number of neurospheres with a mean diameter of 28 µm could be detected in ALDH1 knockdown cells that were cultured under normoxic standard conditions. To investigate a possible hypoxic influence on sphere formation capacity, we incubated LN18 ALDH1 knockdown cells 24 hours under hypoxic conditions (1% O_2) and found mean diameters only slightly increased to 34 µm (Fig. 10).

Both, under normoxia and hypoxia conditions, neurospheres closely resembled untreated native LN18 cells by giving rise to spheres with 184µm in median size and almost identical in total number of spheres (Fig. 10).

5 DISCUSSION

5.1 Cancer Initiating Cells in Glioblastoma multiforme

The diagnosis of glioblastoma predicts a fatal outcome, with the vast majority of patients not surviving the first two years after initial diagnosis. Tumor recurrence occurs in almost all cases, despite macroscopic complete resection and radiochemotherapy (Nishikawa 2010). Advances in identification and isolation of tumor-initiating cells in human GBM samples suggested that this distinct subset of tumor cells might mediate tumor regrowth due to their resistance to current therapeutic regimes (Cheng et al. 2010).

The identification of these cell subsets in solid tumors brought critical and fundamental changes in the understanding of tumor biology, development of novel cancer therapeutics, early detection and prognosis (R. Chen et al. 2010). Treatment modalities that target rapidly dividing and differentiated tumor cells, may leave their stem cell counterparts behind and thus preparing the basis for tumor relapse, metastasis and fatal outcome of cancer diseases (Vescovi et al. 2006). In many facets, cancer growth and progression resemble the rapid expansion of biological matter that takes place in embryological development. Taken together with the frequent recapitulation of fetal and embryological signaling, that could be observed in putative cancer stem cells, it is tempting to speculate that carcinogenesis might be originated by aberrated immature cells or stem cells. The data presented in this thesis provide a strong argument for the role of microenvironmental influences, that may lead to reprogramming events in differentiated brain tumor cells towards a tumor stem cell phenotype.

In this thesis it could be demonstrated, that the enzymatic stem cell marker ALDH1 differentiates between neurosphere-forming and non-neurosphere forming populations. In addition we were able to show, that only cells with high ALDH1 expression ($ALDH^{high}$) divide in an asymmetric stem-cell like manner by restoring $ALDH^{high}$ and $ALDH^{low}$ populations within days. These findings provide support to the

hypothesis, that ALDH^{low} populations contain primarily post mitotic differentiated cells, while ALDH^{high} cells harbour stem or progenitor cells that are capable of self-renewal.

We have also demonstrated that ALDH1 is upregulated upon exposure to hypoxia. Importantly, this finding have been equally observed in formerly FACS isolated ALDH^{low} cells, indicating that low oxygen levels are able to induce ALDH1 expression. These findings underline the role of microenvironmental influences, that may lead to reprogramming of differentiated brain tumor cells towards a stem-like tumor cell phenotype.

Furthermore, we could show, that ALDH1 expression in a sample collection of primary GBM cells is more variable, indicating either the presence of non- or low vs. high ALDH1 expressing GBM subtypes or variable levels of ALDH1 expression within a single tumor, that might explain the absence of ALDH1 expression in one of the three tested primary GBM cell lines. The latter finding is consistent with our previous report, in which we (Rasper et al. 2010) reported that cells with high ALDH1 expression can be found much more frequently in perivascular niches in GBM specimens. Therefore the presence of fluctuating ALDH^{high} quantities among different primary cell lines could possibly be explained by the inherent variations in intraoperative neurosurgical sampling.

Finally, ALDH1 knockdown experiments confirmed the crucial implications of the ALDH enzyme as a marker and stem cell regulator in brain tumor cells.

5.2 Implications of hypoxia on stem cell regulation

For more than a decade, hypoxia is generally considered to play a key role in tumor progression by modulating core tumor pathways associated with angiogenesis, adapted metabolism, invasiveness and survival (Bertout et al. 2008). In agreement with that, stem-like tumor cells could be isolated from distinct anatomical niches, containing the same hypoxic microenvironment of physiologic stem cell niches.

Additionally to molecular cues that might be driven by hypoxic conditions (Ito et al. 2006), the low perfusion of stem cell niches might shield stem-like tumor cells from chemotherapeutic agents and immune responses (Moore & Lemischka 2006) by induction of multidrug resistance proteins (Comerford et al. 2002).

Studies in embryology and perinatal neurogenesis revealed the regulatory implications of low oxygen conditions for stem cells. To date it is well established, that cell proliferation, survival and differentiation of stem and progenitor cells are strongly linked with hypoxia related signalling (Mazumdar et al. 2009; Vieira et al. 2011) (Fig. 12).

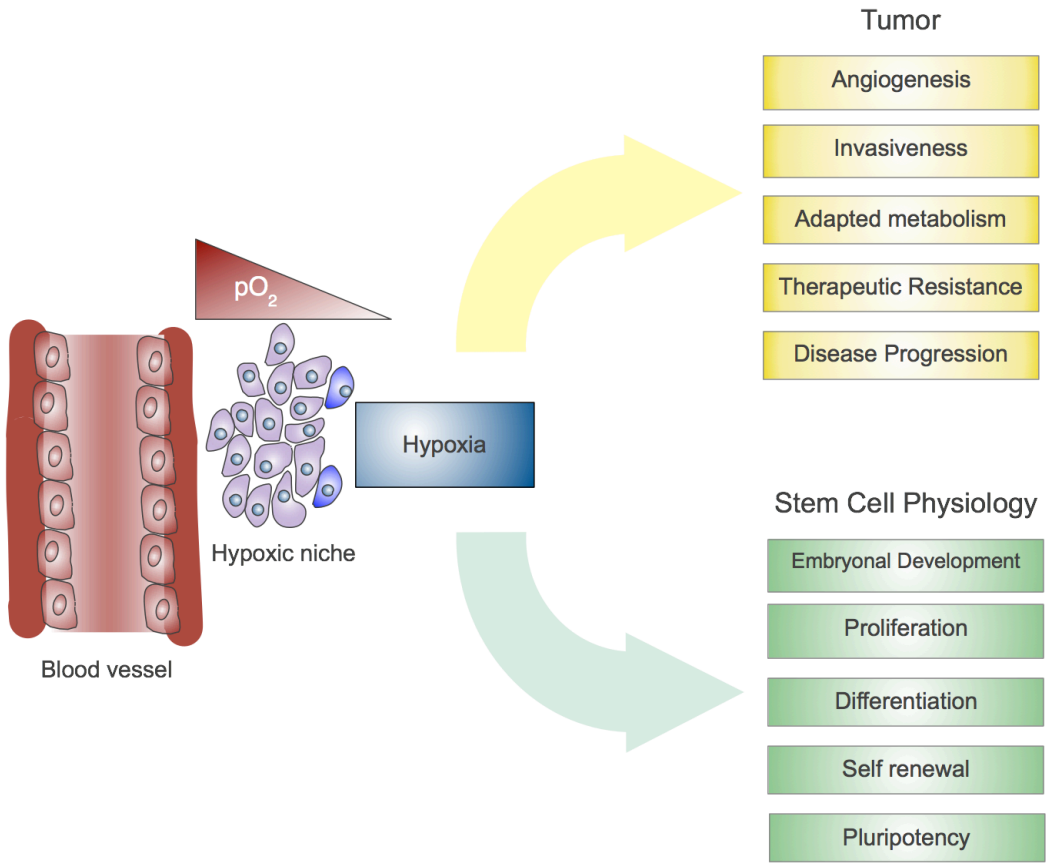

Figure 12: The hypoxic niche in tumor and stem cell physiology
A hypoxic environment is believed to stimulate vessel sprouting and metabolic adaption, resulting in increased invasiveness, disease progression and therapeutic resistance. In stem cell physiology hypoxia drives proliferation, self renewal, pluripotentcy and influences differentiation and embryonal development.

In fetal and embryo development, placental oxygen is physiologically limited and development occurs in a hypoxic environment (Ottosen et al. 2006). Studies have shown, that low oxygen levels are decisive for brain development, vessel formation and neural fold closure (Panchision 2009). Furthermore, investigations on human embryonic stem cells (hESC) revealed the ability of these cells to maintain their pluripotent differentiation and self-renewal capacity when cultured in low-oxygen atmosphere (Ezashi et al. 2005).

In line with these findings, Yoshida et al. reported enhanced generation of induced pluripotent stem cells under hypoxia (Yoshida et al. 2009).

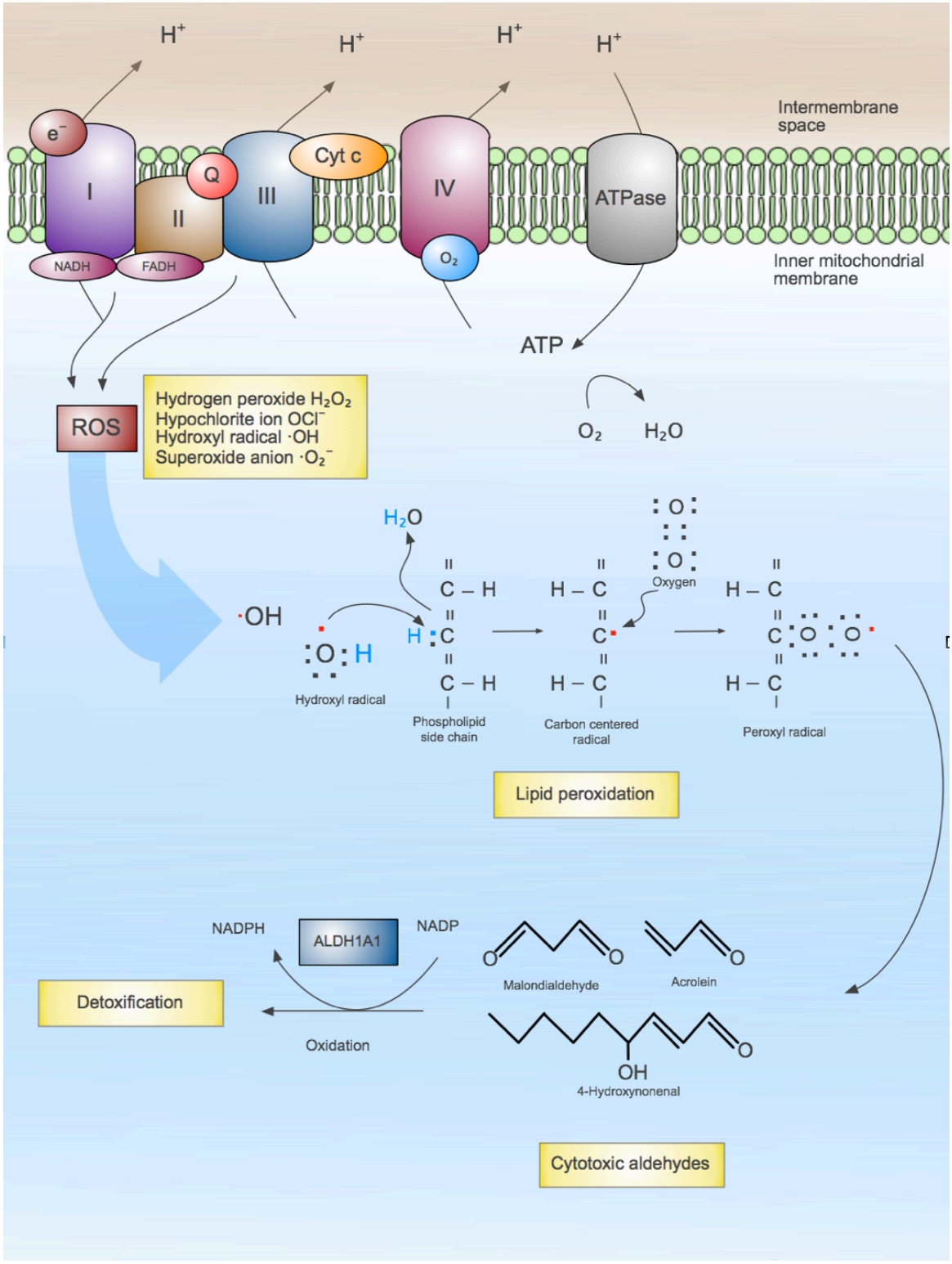

Figure 13: Reactive Oxygen Species (ROS) production in cellular respiration and the role of ALDH1

Similar observations were reported in the field of adult stem cells. Hematopoietic stem cells and neural precursor cells show enhanced survival, clonogenic growth and proliferation under hypoxic culture conditions (Studer et al. 2000).

In this study, we have demonstrated that ALDH1 expression in human glioblastoma cells is upregulated in the fraction of stem-like cells under low oxygen conditions. The enzymatic function of ALDH1 and its potent detoxification capacities may explain the underlying mechanisms, that may prove to be essential for stem cell survival in particular under hypoxic conditions.

As shown in a recent study by Jezek, during cellular hypoxia, the generation of mitochondrial reactive oxygen species (ROS) is paradoxically increased and thus ROS might serve as oxygen sensors inside the signal cascade of hypoxic responses (Jezek & Hlavatá 2005). Highly reactive hydroxyl radicals react with polyunsaturated fatty acids in cellular membranes, leading to lipid peroxidation (LPO) derived products, such as 4-hydroxynonenal (HNE), maliodialdehyde (MDA) or acrolein (K. Uchida 2003; Stevens & Maier 2008).
These highly reactive aldehydes show numerous cytotoxic potentials. ALDH1 is known to possess a high catalytic efficiency for acrolein, HNE and MDA. In mouse models, ALDH1a1 knockdown by siRNA made Hepa-1c1c7 cells significantly more sensitive to acrolein-induced cell death (Makia et al. 2011). In a study by Lassen and colleagues, investigations of several Aldh1a1 knock down models showed, that ALDH1 possesed a key role in protecting the mouse eye lens and cornea by detoxification of aldehydes and hence prevents cataract (Lassen et al. 2008).

In this context, it is interesting and comes along with potential therapeutic implications, that ALDH1 expression could be demonstrated to reduce chemotherapeutic effectivity of oxazaphosphorines, such as cyclophosphamine and ifosfamide by oxidation of aldehyde metabolites of these drugs (Sládek 1999; Ross et al. 2000). In this respect, ALDH1 level could be associated with prediction of therapeutic response and patient outcome (Sládek 2002) (Fig. 13).

ALDH1 knockdown in our cell line resulted in heavily reduced neurosphere generation, possibly suggesting an insufficient detoxification of LPO derived products. ALDH1 function in glioblastoma stem cells might therefore fulfill two purposes. First, serving as a detoxification system that prevents cellular damage by metabolisation of ROS and secondly by maintaining these ROS on levels which are low enough to function as signalling molecules, that finally stabilize stemness and prevents differentiation.The influence of ROS on cellular differentiation is supported by a number of recent studies on differentiation of neural stem and precursor cells. A study by Tsatmali et al. revealed, that embryonic rat cortical precursor cells show increased ROS production upon induction of differentiation with FGF2. The authors also observed positive correlations between ROS generation and the neuronal marker ß-III tubulin and higher levels of mitochondrial respiratory chain compounds (Tsatmali et al. 2005).

5.3 Hypoxia crosstalk with stem cell regulating pathways

Besides the implications of hypoxia on cellular metabolism and reactive oxygen species production, it is becoming more evident, that hypoxia inducible factors (HIFs) affect tumor growth by promotion of stem cell identity and inhibition of differentiation (Keith & Simon 2007) by crosstalk with other oncogenic pathways.

5.3.1 Notch

The Notch signaling pathway provides interesting insights into HIF mediated stem cell regulation, even though Notch effects are context dependent (Bray 2006). Overexpression of Notch, for instance, results rather in terminal differentiation of neural stem cells. Still, the vast majority of studies suggest that Notch-response-elements (NREs) preferably inhibit stem cell differentiation. In a study by Gustafsson et al. it was demonstrated, that hypoxic culture conditions can initiate the transcription of genes involved in increased survival of neuronal stem cells (Gustafsson et al. 2005) and inhibition of stem cell differentiation, upon HIF-1α

binding to the intracellular domain of the Notch receptor. Chromatin immunoprecipitation experiments proved that HIF-1α was physically bound to a DNA-binding complex containing the Notch intracellular domain (Keith & Simon 2007). This could also been shown for myogenic cells, but HIF-2α mediated in A-498 renal carcinoma cells in the same Notch-dependent manner. The underlying mechanism for binding of HIF-1α to the intracellular Notch domain remain poorly understood and it is not yet thoroughly clear, if other target genes, besides Hes-1 and Hey-2, members of the basic helix-loop helix transcription factor family, are activated following binding of HIF-1α to the intracellular Notch receptor (Gustafsson et al. 2005) (Fig. 14).

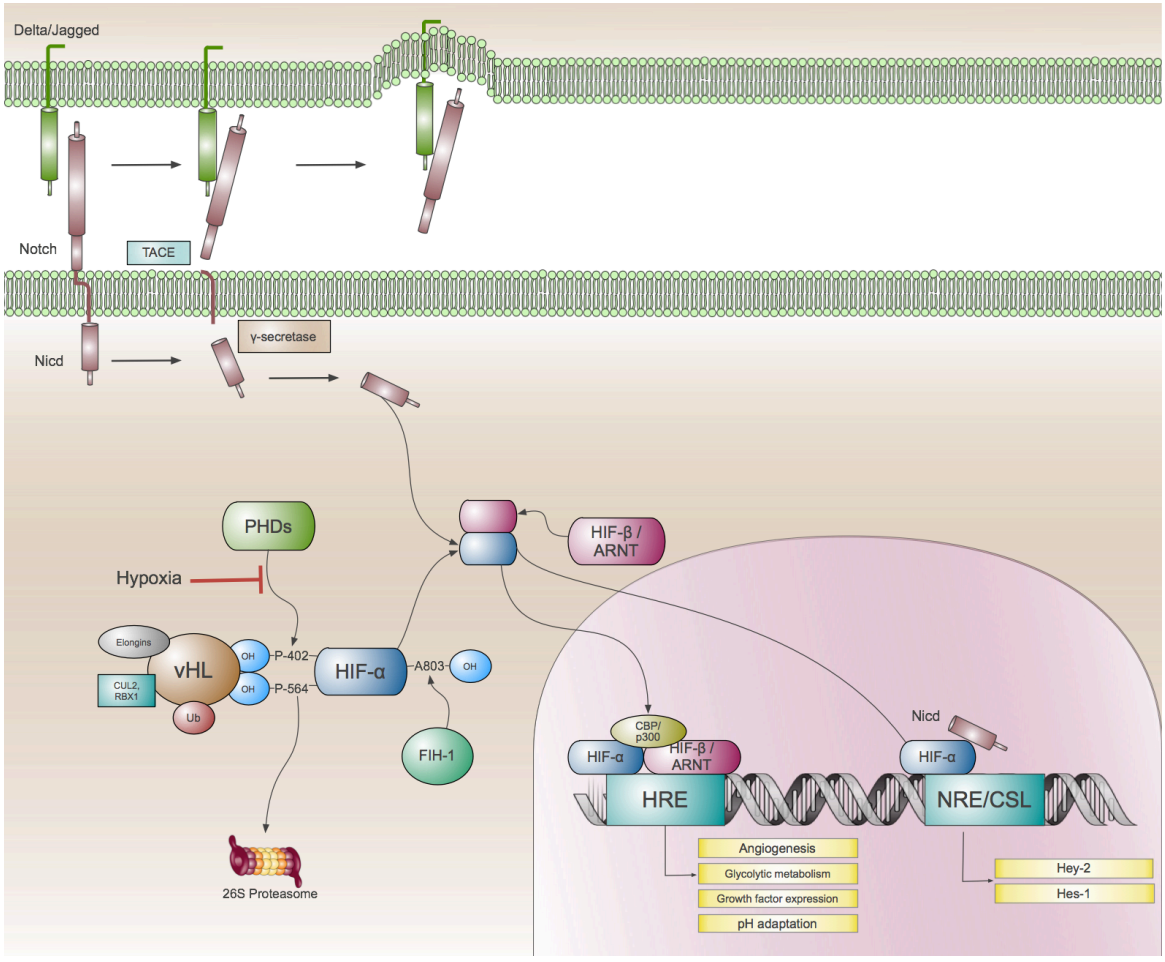

Figure 14: The Notch pathway (modified from U. Lendahl et al., Nat. Rev. Genetics 2009)

However, these gene are known to suppress the transcription of the achete scute gene family, which mediate neuronal differentiation (Ishibashi 2004). The role of Notch signaling in self-renewing organs has been investigated in great detail, for example in the homeostasis of the mammalian intestine. Upregulation of a dominant form of the intracellular Notch receptor in mice inhibited differentiation of crypt cells (Fre et al. 2005). Further on, as revealed by toxicology studies, inhibition of Notch signaling can occur as a side effect upon medication with y-secretase-inhibitors, that are developed for treatment of Alzheimer´s disease. Rodents, treated with this drug exhibited goblet cell metaplasia within the intestine, indicating a lack of Notch dependent regulation of crypt cell differentiation (van Es et al. 2005).

Another system with obvious critical self-renewing components is the hematopoietic system. Gain-of-function studies of a dominant active form of the intracellular Notch receptor in hematopoietic cells resulted in inhibition of differentiation (Kumano et al. 2001) or in increased self-renewal of hematopoietic stem cells of bone marrow progenitor cells in another study (Stier et al. 2002). Aberrant Notch signaling is also linked to T cell malignancies. Weng and coworkers described a gain-of-function mutation in the Notch receptor in 54 of 96 patients suffering acute lymphoblastic leukemia (ALL) (Weng et al. 2004; Mazumdar et al. 2009).

5.3.2 Oct-4

Another gene, that could be upregulated when cells are exposed to low oxygen concentration is Oct4. This gene occupies an important and central role in the understanding of development regulation in human embryonic stem cells (hESCs) (Boyer et al. 2005). During preimplantational development, Oct4 maintains embryonic stem cells in a pluripotent state (Hochedlinger et al. 2005). In consistence with our findings, that hypoxia induced neurosphere building in LN18 glioblastoma cells, Ezashi reported that low oxygen gradients significantly increased the rate of embryoid bodies and Oct4 expression detectable in hypoxic cultures of human embryonic stem cells (Ezashi et al. 2005).

Among transcription factors, that are required to induce pluripotent self-renewing stem cells, Oct4 demonstrated to play an essential role. SOX2, KLF4, NANONG and Lin28 are other factors that are currently discussed, still Oct4 proved to be crucial for iPS induction in most studies (Utikal et al. 2009; Bunaciu & Yen 2011). The Oct4 gene and Oct4 protein were found to be expressed in adult stem cells and human tumor cells, whereas no expression could be detected as soon as these cells were induced to differentiate (Tai et al. 2005). Along with this findings, Oct4 was found to be abundant expressed in testicular germ cell tumors (Gidekel et al. 2003). Overexpression of Oct4 also resulted in premalignant dysplastic lesions in epithelial tissues, in which Oct4 plays a physiological role in self renewing of these tissues (Hochedlinger et al. 2005).

As shown by Covello and colleagues, HIF-2α could be identified as a direct upstream regulator of Oct-4, whereas Oct-4 is apparently independent of HIF-1α activation (Covello 2006). In this study, hypoxic tumor cells remained in an undifferentiated and malignant state, as long as Oct4 expression was observable. In a HIF-2α knock-in rodent model, in which HIF-1α was replaced by HIF-2α, embryonic teratomas exhibited markedly increased Oct-4 and Nanog expression. Further on, they showed that expanded HIF-2α expression was sufficient to up-regulate Oct4, Tgf-ß and VEGF in tumor cells and in the context of embryonic development (Covello et al. 2005). These findings could be confirmed in more recent studies, suggesting that HIF-2α might serve as possible specific target, since hypoxia enhanced HIF-2α expression in glioma stem cells without affecting non-stem GBM cancer cells (Z. Huang et al. 2010).

The particular role of Oct4 in GBM has become clear in a number of recent studies, in which downstream targets of Oct4 could be detected more frequently in high-grade astrocytic tumors compared to lower grade gliomas (LGG) (Ben-Porath et al. 2008).

5.4 Comparison of ALDH1 with surface markers, advantages and limitations

A particular challenge that expands beyond the implications of hypoxia on stem cell signalling and associated pathways lies in the identification of reliable markers for stem-like cells. This has proven to be extremely difficult, since it is so far still unclear, what stem cells and stem-like cancer cells have in common and thus if marker for stem cells may likewise serve for their malignant counterparts (Ben-Porath et al. 2008).
It has become more clear however, that surface located stem cell marker contain inherent pitfalls. In this context, it might be assumed, that preparation methods and laboratory handling of cells may induce stress response in cells, which result in changes on the cell surface.

For identification of stem-like cells in gliomas, a number of cell markers were proposed in the last years. A great number of published work in this field has been performed on the base of CD133 expression. Nevertheless, several studies showed that CD133- cells likewise contain tumorigenic potential upon transplantation into immunodeficient mice (J. Wang et al. 2008; Ogden et al. 2008; Shmelkov et al. 2008).
Another marker, A2B5, a cell surface ganglioside, that is expressed on glial and neuronal progenitor cells (Nunes et al. 2003) and also on neural stem cells in the subventricular zone. $A2B5^{+}/CD133^{+}$ and $A2B5^{+}/CD133^{-}$ implantation experiments revealed, that both fractions can initiate tumor growth. However, it remains to be determined, if A2B5 can prove to be able to identify stem-like glioma cells reliably, given the untypical high number of 33 - 90% A2B5 expressing cells in anaplastic astrocytoma and GBM (Ogden et al. 2008).

A method to identify stem-like tumor cells without any usage of intra- or extracellular markers has been proposed recently by Clément and co-workers by exploiting intrinsic autofluorescence properties and distinctive morphologies of cells obtained from tumor samples and glioma cell cultures (Clément et al. 2010).
Further on, cell labeling and staining methods, as commonly used for stem cell identification may produce artifacts in the investigated cells. The same is true to a certain extent for the Aldefluor assay, although the ALDH substrate does not enter

the nucleus nor does it bind to DNA such as dyes that are used for identification of side populations (Pearce et al. 2005).

One distinct advantage of the Aldefluor based labeling method is that cells require an intact membrane to retain the fluorescent substrate. Therefore, dead and apoptotic cells will not be included into counting or isolation of cells (Storms et al. 1999).

5.5 Limitations of this study

While the insights of our work into regulatory effects of ALDH1 expression are important, some limitations of the current study have to be named, as they also pave the way for future work in our field. One shortcoming of this study is, that investigations were performed using mainly an established cell line. The problem inherent with immortalized cell lines is that cloning will finally select specific cell types, while other cells, that might be crucial for cell-cell interaction or secretion of signaling molecules might not survive during dozens of passages.

Another drawback is that colocalization with other cancer stem cell markers had not been performed. Evaluation of ALDH1 expression in comparison to more established markers might thus have become more meticulous. A number of potential glioblastoma stem cell marker, have been proposed during the last years, including CD133 (Yin et al. 1997), A2B5 (Nunes et al. 2003), CD15 (Son et al. 2009), podoplanin (Mishima et al. 2006) and others (Clément et al. 2010).
Still, as all supposed cancer stem cell marker still lack final proof and acceptance, to the date, the gold standard for evaluation of new markers certainly remain functional assays, like the neurosphere formation assay, that we have conducted throughout all the study.

Finally, our study, like other studies which have used the Aldefluor assay (Ginestier et al. 2009; Jiang et al. 2009), is limited by the inherent lack of this assay to detect ALDH isoforms other than ALDH1A1. So far, it has not been proven, that only

ALDH1A1 is a marker for normal and cancer stem cells. ALDH3A1 has been described to protect the cornea from oxidative stress, derived from increased protein oxidation and the occurrence of HNE and MDA products (Lassen et al. 2008). As the metabolism of these products play an important role in ALDH1A1 mediated detoxification, other ALDH isoforms may also serve as a cancer stem cell marker and may be overseen in studies, that use only Aldefluor for cell identification and isolation.

5.6 Cancer stem cells as the root of all evil?

We observed enhanced neurosphere formation in LN18 cells that underwent hypoxic culture. Sphere mean diameter and total number were greater in cells with higher ALDH1 expression. Additionally, formerly ALDH negative cells, in which no neurosphere formation could be detected, showed induced sphere building after hypoxia treatment. Our findings are consistent with those reported by Mathieu et al. The authors hypothesized, whether hypoxia would be able and sufficient to induce hESC signatures in a variety of tumor cells. Studying various cancer cell lines from prostate, brain, kidney, cervic, lung, colon, liver and breast, they found iPSC inducers OCT4, NANOG, SOX2, KLF4, cMYC and mRNA-302 activated. In accordance with our work, hypoxia induced neurosphere formation in CD133 negative glioma cells (Mathieu et al. 2011). The finding of a positive correlation between Aldefluor positive tumor cells and sphere formation was observed by us and others. Charafe-Jauffret reported Aldefluor positive cells formed significantly more tumorspheres compared with Aldefluor negative cells (Charafe-Jauffret et al. 2009).

Bar and colleagues investigated the effects of hypoxia on glioma cell derived neurospheres from CD133 positive and freshly isolated glioblastoma cells. CD133 protein levels and Notch related genes were induced by growth in low oxygen. More importantly, using a lentiviral construct encoding an oxygen stable double mutant of HIF-1α, they found CD133 levels markedly increased under normoxic conditions. Further on, hypoxia also increased the percentage of GBM side-population cells (Bar et al. 2010). These results compare favourably with those reported by Seidel et al.

GBM cells, that expressed the distinct side population signatures ASPHD2 and MAML3, could be detected in perivascular and perinecrotic hypoxic areas, as revealed by immunohistochemistry in GBM specimens. In vitro analysis following hypoxic incubation likewise demonstrated increases of CD133 positive and side population signature positive cells. Sphere forming capacity was enhanced under hypoxia and markedly decreased in HIF-1α and HIF-2α knockdown experiments (Seidel et al. 2010).

Another study used a larger set of putative GBM stem cell markers in addition to CD133, such as CXCR4 (CD184), CD44low and A2B5 and showed that low oxygen culture promotes self-renewal, preceded by upregulation of HIF-1α. Hypoxic cultured marker positive cells retained in an undifferentiated state, whereas cells in normal oxygen concentrations did not (Soeda et al. 2009). Induction of a more stem-like phenotype after hypoxia was also described by Heddleston et al. In contrast to previous studies, stem cell factors such as OCT4, NANOG and c-MYC were upregulated in cancer stem cells exclusively under the modulation of HIF-2α (Heddleston et al. 2009; Z. Li et al. 2009b). McCord and colleagues have taken a different view on hypoxia and exposed cells to 7% oxygen (McCord et al. 2009), in contrast to more severe states of hypoxia, that has been used in most other studies. The results of this work however are in line with previous published work, despite that only HIF-2α, but not HIF-1α was affected under 7% oxygen.

5.6.1 A different view on cancer stem cells

In contrast to this thesis and most of the studies, published in the field of cancer stem cell research, other studies take a more critical view and challenge the cancer stem cell hypothesis with severe questions, that need to be addressed in future studies. Morrison and colleagues questioned the model of a hierarchical tumor model, in which only a minority of cells have tumorigenic potential upon transplantation into immune deficient animals. By changing transplantation parameters, such as the mouse strain used for xenotransplantation, the time over which animals were followed up and the injection method, tumor initiating could be increased

dramatically. Further on, an analysis of over 50 surface marker revealed, that they could not differentiate between tumorigenic and non-tumorigenic cells (Quintana et al. 2008; Hill 2006).

The side-population (SP) assay is widely used to isolate CSCs. By the expression of ATP-binding cassette transporters, CSCs are thought to efficiently efflux a nuclear staining dye and thus appear as a non-fluorescent population in flow cytometry (Kondo et al. 2004). Some authors reported limitations of this assay however. Zheng and colleagues reported that C6 glioma cells were clonogenic, but not detectable in SP assays (Zheng et al. 2007). Another study found both SP and non-SP glioma cells to be tumorigenic in mice (Bleau et al. 2009).
It is yet unclear to which extent cancer stem cells share characteristics with normal stem cells. Multipotency, a defining feature of normal stem cells, is normally not present in malignant stem-like cells. Some authors, hence proposed to use rather terms such as „cancer- or tumor-initiating cells“ (O'Brien et al. 2007; Ricci-Vitiani et al. 2006). Moreover, the markers that are currently used to define and isolate CSCs are not uniquely expressed on cancer stem cells, because they are likewise frequently found on normal cells (Al-Hajj & Clarke 2004). To date, these markers are mostly used to isolate subpopulations, enriched with tumorigenic cells.

As questioned by other authors, it is doubted whether CSCs could remain genetically stable during the frequent mutations that accumulate in tumor cells in the course of tumor progression (Bielas & Loeb 2005).
In disagreement with numerous studies, whose investigators implanted CSCs cells in varying quantity in order to demonstrate, that a few CSC marker positive cells are sufficient to give rise to tumor formation (Wright et al. 2008), others reported that these results might base on artifacts, due to retained immunological host response. In such a scenario, a small number of cells may not arouse an immune reaction and therefore tumor formation can occur. When a medium amount of cells is injected, the immune system might recognize the foreign cells and thus could successfully prevent tumor formation. Finally, the authors suggest, large number of injected cells may provoke an immune system activation, but the abundance of tumor cells could give

rise to a tumor despite host defense (Hill & Perris 2007). Use of NOD-SCID-interleukin 2 receptor γ (IL2R γ null) mice, which are deficient of both T killer cells and mature other lymphocytes might provide a more reliable mice model, as suggested by Quintana et al. (Quintana et al. 2008).

6 SUMMARY

Increasing evidence supports the hypothesis that regulatory influences of the extracellular microenvironment may contribute to reprogramming of tumor-cells into stem-like behaving cells. As suggested in this study, the detoxification capacity of tumor cells with high ALDH1 activity may ensure genetic stability and a survival benefit to other cells. This might prove especially useful in the extracellular context of low oxygen concentrations, as it typically occurs in stem cell niches and tumor related hypoxia..

In the current study, we have shown human glioblastoma cells, isolated on the basis of their ALDH1 expression exhibit markedly higher neurosphere formation and asymmetric division capacity. Secondly, we observed that hypoxic culture conditions were able to induce high ALDH1 expression and neurosphere formation in formerly low ALDH expressing cells. Furthermore we could indicate, that ALDH1 expression is upregulated under hypoxia in primary human glioblastoma cells. Finally, the functional meaning of ALDH1 could be underlined by ALDH1 shRNA knockdown.

The results of our study indicate an intrinsic involvement of ALDH1 in survival and functional stem cell characteristics of human glioblastoma cells under normoxic and hypoxic conditions. However the signalling functions of ALDH1 mediated metabolites and their downstream targets awaits further clarification and future studies will have to show, whether ALDH1 expression in specimens of human glioblastoma can be related with clinical parameters, ultimately possibly identifying implications of ALDH1 mediated metabolism and signalling in relation to tumor relapse or drug- and radioresistance in GBM patients.

Adamson, C., Kanu, O. O., Mehta, A. I., Di, C., Lin, N., Mattox, A. K., & Bigner, D. D. (2009). Glioblastoma multiforme: a review of where we have been and where we are going. Expert opinion on investigational drugs, 18(8), 1061–1083.

Adibhatla, R. M., & Hatcher, J. F. (2008). Phospholipase A(2), reactive oxygen species, and lipid peroxidation in CNS pathologies. BMB reports, 41(8), 560–567.

Afify, A., Purnell, P., & Nguyen, L. (2009). Role of CD44s and CD44v6 on human breast cancer cell adhesion, migration, and invasion. Experimental and molecular pathology, 86(2), 95–100.

Aigner, S., Sthoeger, Z. M., Fogel, M., Weber, E., Zarn, J., Ruppert, M., Zeller, Y., et al. (1997). CD24, a mucin-type glycoprotein, is a ligand for P-selectin on human tumor cells. Blood, 89(9), 3385–3395.

Al-Hajj, M., & Clarke, M. F. (2004). Self-renewal and solid tumor stem cells. Oncogene, 23(43), 7274–7282.

Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J., & Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. Proceedings of the National Academy of Sciences of the United States of America, 100(7), 3983–3988.

Baeriswyl, V., & Christofori, G. (2009). The angiogenic switch in carcinogenesis. Seminars in cancer biology, 19(5), 329–337.

Balss, J., Meyer, J., Mueller, W., Korshunov, A., Hartmann, C., & Deimling, A. (2008). Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathologica, 116(6), 597–602.

Bar, E. E., Chaudhry, A., Lin, A., Fan, X., Schreck, K., Matsui, W., Piccirillo, S., et al. (2007). Cyclopamine-Mediated Hedgehog Pathway Inhibition Depletes Stem-Like Cancer Cells in Glioblastoma. Stem Cells, 25(10), 2524–2533.

Bar, E. E., Lin, A., Mahairaki, V., Matsui, W., & Eberhart, C. G. (2010). Hypoxia Increases the Expression of Stem-Cell Markers and Promotes Clonogenicity in Glioblastoma Neurospheres. The American Journal of Pathology, 177(3), 1491–1502. American Society for Investigative Pathology.

Bailey, P. & Cushing, HW. (1926). A classification of the tumors of the glioma group on a histogenetic basis with a correlated study of prognosis. Philadelphia : JB Lippincott

Ben-Porath, I., Thomson, M. W., Carey, V. J., Ge, R., Bell, G. W., Regev, A., & Weinberg, R. A. (2008). An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nature genetics, 40(5), 499–507.

Bertout, J. A., Patel, S. A., & Simon, M. C. (2008). The impact of O2 availability on human cancer. Nature Reviews Cancer, 8(12), 967–975.

Bielas, J. H., & Loeb, L. A. (2005). Mutator phenotype in cancer: timing and perspectives. Environmental and molecular mutagenesis, 45(2-3), 206–213.

Black, W., & Vasiliou, V. (2009). The aldehyde dehydrogenase gene superfamily resource center. Human genomics, 4(2), 136–142.

Bleau, A.-M., Hambardzumyan, D., Ozawa, T., Fomchenko, E. I., Huse, J. T., Brennan, C. W., & Holland, E. C. (2009). PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell Stem Cell, 4(3), 226–235.

Bock, H. C., Puchner, M. J. A., Lohmann, F., Schütze, M., Koll, S., Ketter, R., Buchalla, R., et al. (2010). First-line treatment of malignant glioma with carmustine implants followed by concomitant radiochemotherapy: a multicenter experience. Neurosurgical Review, 33(4), 441–449.

Bonnet, D., & Dick, J. E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nature medicine, 3(7), 730–737.

Boonyaratanakornkit, J. B., Yue, L., Strachan, L. R., Scalapino, K. J., LeBoit, P. E., Lu, Y., Leong, S. P., et al. (2010). Selection of Tumorigenic Melanoma Cells Using ALDH. Journal of Investigative Dermatology, 130(12), 2799–2808. Nature Publishing Group.

Bourguignon, L. Y. W., Xia, W., & Wong, G. (2009). Hyaluronan-mediated CD44 interaction with p300 and SIRT1 regulates beta-catenin signaling and NFkappaB-specific transcription activity leading to MDR1 and Bcl-xL gene expression and chemoresistance in breast tumor cells. The Journal of biological chemistry, 284(5), 2657–2671.

Boyer, L. A., Lee, T. I., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., Guenther, M. G., et al. (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. Cell, 122(6), 947–956.

Bramanti, V., Tomassoni, D., Avitabile, M., Amenta, F., & Avola, R. (2010). Biomarkers of glial cell proliferation and differentiation in culture. Frontiers in bioscience (Scholar edition), 2, 558–570.

Bray, S. J. (2006). Notch signalling: a simple pathway becomes complex. Nature reviews. Molecular cell biology, 7(9), 678–689.

Brocker, C., Cantore, M., Failli, P., & Vasiliou, V. (2011). Aldehyde dehydrogenase 7A1 (ALDH7A1) attenuates reactive aldehyde and oxidative stress induced cytotoxicity. Chemico-Biological Interactions, 191(1-3), 269–277.

Bucci, M. K., Maity, A., Janss, A. J., Belasco, J. B., Fisher, M. J., Tochner, Z. A., Rorke, L., et al. (2004). Near complete surgical resection predicts a favorable outcome in pediatric patients with nonbrainstem, malignant gliomas: results from a single center in the magnetic resonance imaging era. Cancer, 101(4), 817–824.

Bunaciu, R. P., & Yen, A. (2011). Activation of the Aryl Hydrocarbon Receptor AhR Promotes Retinoic Acid-Induced Differentiation of Myeloblastic Leukemia Cells by Restricting Expression of the Stem Cell Transcription Factor Oct4. Cancer research, 71(6), 2371–2380.

Cairncross, J. G., Ueki, K., Zlatescu, M. C., Lisle, D. K., Finkelstein, D. M., Hammond, R. R., Silver, J. S., et al. (1998). Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. Journal of the

National Cancer Institute, 90(19), 1473–1479.

Cairns, R. A., Harris, I. S., & Mak, T. W. (2011). Regulation of cancer cell metabolism. Nature Reviews Cancer, 11(2), 85–95.

Canoll, P., & Goldman, J. E. (2008). The interface between glial progenitors and gliomas. Acta Neuropathologica, 116(5), 465–477.

Capper, D., Weissert, S., Balss, J., Habel, A., Meyer, J., Jäger, D., Ackermann, U., et al. (2010). Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. Brain pathology (Zurich, Switzerland), 20(1), 245–254.

Challen, G. A., & Little, M. H. (2006). A side order of stem cells: the SP phenotype. Stem Cells, 24(1), 3–12.

Chang, B., Liu, G., Xue, F., Rosen, D. G., Xiao, L., Wang, X., & Liu, J. (2009). ALDH1 expression correlates with favorable prognosis in ovarian cancers. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc, 22(6), 817–823.

Charafe-Jauffret, E., Ginestier, C., Iovino, F., Wicinski, J., Cervera, N., Finetti, P., Hur, M. H., et al. (2009). Breast Cancer Cell Lines Contain Functional Cancer Stem Cells with Metastatic Capacity and a Distinct Molecular Signature. Cancer research, 69(4), 1302–1313.

Chen, R., Nishimura, M. C., Bumbaca, S. M., Kharbanda, S., Forrest, W. F., Kasman, I. M., Greve, J. M., et al. (2010). A Hierarchy of Self-Renewing Tumor-Initiating Cell Types in Glioblastoma. Cancer Cell, 17(4), 362–375. Elsevier Ltd.

Chen, Y.-C., Hsu, H.-S., Chen, Y.-W., Tsai, T.-H., How, C.-K., Wang, C.-Y., Hung, S.-C., et al. (2008). Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. PLoS ONE, 3(7), e2637.

Cheng, L., Bao, S., & Rich, J. N. (2010). Potential therapeutic implications of cancer stem cells in glioblastoma. Biochemical Pharmacology, 1–12. Elsevier Inc.

Cheung, A. M. S., Wan, T. S. K., Leung, J. C. K., Chan, L. Y. Y., Huang, H., Kwong, Y. L., Liang, R., et al. (2007). Aldehyde dehydrogenase activity in leukemic blasts defines a subgroup of acute myeloid leukemia with adverse prognosis and superior NOD/SCID engrafting potential. Leukemia, 21(7), 1423–1430.

Chigurupati, S., Venkataraman, R., Barrera, D., Naganathan, A., Madan, M., Paul, L., Pattisapu, J. V., et al. (2010). Receptor Channel TRPC6 Is a Key Mediator of Notch-Driven Glioblastoma Growth and Invasiveness. Cancer research, 70(1), 418–427.

Cho, Y. M., Kwon, S., Pak, Y. K., Seol, H. W., Choi, Y. M., Park, D. J., Park, K. S., et al. (2006). Dynamic changes in mitochondrial biogenesis and antioxidant enzymes during the spontaneous differentiation of human embryonic stem cells. Biochemical and Biophysical Research Communications, 348(4), 1472–1478.

Chu, P., Clanton, D. J., Snipas, T. S., Lee, J., Mitchell, E., Nguyen, M.-L., Hare, E., et al. (2009). Characterization of a subpopulation of colon cancer cells with stem cell-like properties. International Journal of Cancer, 124(6), 1312–1321.

Clay, M. R., Tabor, M., Owen, J. H., Carey, T. E., Bradford, C. R., Wolf, G. T., Wicha, M. S., et al. (2010). Single-marker identification of head and neck squamous cell carcinoma cancer stem cells with aldehyde dehydrogenase. Head & Neck, 32(9), 1195–1201.

Clément, V., Marino, D., Cudalbu, C., Hamou, M.-F., Mlynarik, V., de Tribolet, N., Dietrich, P.-Y., et al. (2010). Marker-independent identification of glioma-initiating cells. Nature Publishing Group, 7(3), 224–228. Nature Publishing Group.

Clément, V., Sanchez, P., de Tribolet, N., Radovanovic, I., & Ruiz i Altaba, A. (2007). HEDGEHOG-GLI1 Signaling Regulates Human Glioma Growth, Cancer Stem Cell Self-Renewal, and Tumorigenicity. Current Biology, 17(2), 165–172.

Coffey, R. J., Lunsford, L. D., & Taylor, F. H. (1988). Survival after stereotactic biopsy of malignant gliomas. Neurosurgery, 22(3), 465–473.

Collins, A. T., Berry, P. A., Hyde, C., Stower, M. J., & Maitland, N. J. (2005). Prospective identification of tumorigenic prostate cancer stem cells. Cancer research, 65(23), 10946–10951.

Comerford, K. M., Wallace, T. J., Karhausen, J., Louis, N. A., Montalto, M. C., & Colgan, S. P. (2002). Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene. Cancer research, 62(12), 3387–3394.

Covello, K. L. (2006). HIF-2 regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes & Development, 20(5), 557–570.

Covello, K. L., Simon, M. C., & Keith, B. (2005). Targeted replacement of hypoxia-inducible factor-1alpha by a hypoxia-inducible factor-2alpha knock-in allele promotes tumor growth. Cancer research, 65(6), 2277–2286.

Cox, C. V., Evely, R. S., Oakhill, A., Pamphilon, D. H., Goulden, N. J., & Blair, A. (2004). Characterization of acute lymphoblastic leukemia progenitor cells. Blood, 104(9), 2919–2925.

Dalerba, P., Dylla, S. J., Park, I.-K., Liu, R., Wang, X., Cho, R. W., Hoey, T., et al. (2007). Phenotypic characterization of human colorectal cancer stem cells. Proceedings of the National Academy of Sciences of the United States of America, 104(24), 10158–10163.

Danet, G. H., Pan, Y., Luongo, J. L., Bonnet, D. A., & Simon, M. C. (2003). Expansion of human SCID-repopulating cells under hypoxic conditions. Journal of Clinical Investigation, 112(1), 126–135.

Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., Driggers, E. M., Fantin, V. R., et al. (2011). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature, 465(7300), 966–966. Nature Publishing Group.

Dean, M., Fojo, T., & Bates, S. (2005). Tumour stem cells and drug resistance. Nature Reviews Cancer, 5(4), 275–284.

Delamarre, E., Taboubi, S., Mathieu, S., Bérenguer, C., Rigot, V., Lissitzky, J.-C., Figarella-

Branger, D., et al. (2009). Expression of integrin alpha6beta1 enhances tumorigenesis in glioma cells. The American Journal of Pathology, 175(2), 844–855.

Devaux, B. C., O'Fallon, J. R., & Kelly, P. J. (1993). Resection, biopsy, and survival in malignant glial neoplasms. A retrospective study of clinical parameters, therapy, and outcome. Journal of neurosurgery, 78(5), 767–775.

Diaz, L. K., Zhou, X., Wright, E. T., Cristofanilli, M., Smith, T., Yang, Y., Sneige, N., et al. (2005). CD44 expression is associated with increased survival in node-negative invasive breast carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research, 11(9), 3309–3314.

Dietrich, J., Diamond, E. L., & Kesari, S. (2010). Glioma stem cell signaling: therapeutic opportunities and challenges. Expert Review of Anticancer Therapy, 10(5), 709–722.

Dirks, P. B. (2006). Cancer: stem cells and brain tumours. Nature, 444(7120), 687–688.

Diserens, A. C., de Tribolet, N., Martin-Achard, A., Gaide, A. C., Schnegg, J. F., & Carrel, S. (1981). Characterization of an established human malignant glioma cell line: LN-18. Acta Neuropathologica, 53(1), 21–28.

Dolecek, T. A., Propp, J. M., Stroup, N. E., & Kruchko, C. (2012). CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. Neuro-Oncology, 14 Suppl 5, v1–49.

Draheim, K. M., & Lyle, S. (2011). Epithelial stem cells. Methods in molecular biology (Clifton, N.J.), 750, 261–274.

Du, Z., Jia, D., Liu, S., Wang, F., Li, G., Zhang, Y., Cao, X., et al. (2009). Oct4 is expressed in human gliomas and promotes colony formation in glioma cells. Glia, 57(7), 724–733.

Dubbink, H. J., Taal, W., van Marion, R., Kros, J. M., van Heuvel, I., Bromberg, J. E., Zonnenberg, B. A., et al. (2009). IDH1 mutations in low-grade astrocytomas predict survival but not response to temozolomide. Neurology, 73(21), 1792–1795.

Eramo, A., Lotti, F., Sette, G., Pilozzi, E., Biffoni, M., Di Virgilio, A., Conticello, C., et al. (2008). Identification and expansion of the tumorigenic lung cancer stem cell population. Cell death and differentiation, 15(3), 504–514.

Esteller, M., Garcia-Foncillas, J., Andion, E., Goodman, S. N., Hidalgo, O. F., Vanaclocha, V., Baylin, S. B., et al. (2000). Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. The New England journal of medicine, 343(19), 1350–1354.

Evans, S. M. (2004a). Comparative Measurements of Hypoxia in Human Brain Tumors Using Needle Electrodes and EF5 Binding. Cancer research, 64(5), 1886–1892.

Evans, S. M. (2004b). Hypoxia Is Important in the Biology and Aggression of Human Glial Brain Tumors. Clinical Cancer Research, 10(24), 8177–8184.

Ezashi, T., Das, P., & Roberts, R. M. (2005). Low O2 tensions and the prevention of differentiation of hES cells. Proceedings of the National Academy of Sciences of the

United States of America, 102(13), 4783–4788.

Fadul, C., Wood, J., Thaler, H., Galicich, J., Patterson, R. H., & Posner, J. B. (1988). Morbidity and mortality of craniotomy for excision of supratentorial gliomas. Neurology, 38(9), 1374–1379.

Fan, X., Khaki, L., Zhu, T. S., Soules, M. E., Talsma, C. E., Gul, N., Koh, C., et al. (2009). Notch Pathway Blockade Depletes CD133-Positive Glioblastoma Cells and Inhibits Growth of Tumor Neurospheres and Xenografts. Stem Cells, N/A–N/A.

Fang, X., Zheng, P., Tang, J., & Liu, Y. (2010). CD24: from A to Z. Cellular & molecular immunology, 7(2), 100–103.

Felsberg, J., Erkwoh, A., Sabel, M. C., Kirsch, L., Fimmers, R., Blaschke, B., Schlegel, U., et al. (2004). Oligodendroglial tumors: refinement of candidate regions on chromosome arm 1p and correlation of 1p/19q status with survival. Brain pathology (Zurich, Switzerland), 14(2), 121–130.

Fre, S., Huyghe, M., Mourikis, P., Robine, S., Louvard, D., & Artavanis-Tsakonas, S. (2005). Notch signals control the fate of immature progenitor cells in the intestine. Nature, 435(7044), 964–968.

Freije, W. A., Castro-Vargas, F. E., Fang, Z., Horvath, S., Cloughesy, T., Liau, L. M., Mischel, P. S., et al. (2004). Gene expression profiling of gliomas strongly predicts survival. Cancer research, 64(18), 6503–6510.

Galli, R. (2004). Isolation and Characterization of Tumorigenic, Stem-like Neural Precursors from Human Glioblastoma. Cancer research, 64(19), 7011–7021.

Gerson, S. L. (2004). MGMT: its role in cancer aetiology and cancer therapeutics. Nature Reviews Cancer, 4(4), 296–307.

Gidekel, S., Pizov, G., Bergman, Y., & Pikarsky, E. (2003). Oct-3/4 is a dose-dependent oncogenic fate determinant. Cancer Cell, 4(5), 361–370.

Ginestier, C., Hur, M. H., Charafe-Jauffret, E., Monville, F., Dutcher, J., Brown, M., Jacquemier, J., et al. (2007). ALDH1 Is a Marker of Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome. Cell Stem Cell, 1(5), 555–567.

Ginestier, C., Wicinski, J., Cervera, N., Monville, F., Finetti, P., Bertucci, F., Wicha, M. S., et al. (2009). Retinoid signaling regulates breast cancer stem cell differentiation. Cell Cycle, 8(20), 3297–3302.

Glantz, M. J., Burger, P. C., Herndon, J. E., Friedman, A. H., Cairncross, J. G., Vick, N. A., & Schold, S. C. (1991). Influence of the type of surgery on the histologic diagnosis in patients with anaplastic gliomas. Neurology, 41(11), 1741–1744.

Glinsky, G. V. (2007). Stem cell origin of death-from-cancer phenotypes of human prostate and breast cancers. Stem cell reviews, 3(1), 79–93.

Gong, C., Yao, H., Liu, Q., Chen, J., Shi, J., Su, F., & Song, E. (2010). Markers of Tumor-Initiating Cells Predict Chemoresistance in Breast Cancer. (A. Swarbrick, Ed.)PLoS ONE, 5(12), e15630.

Goodell, M. A., Brose, K., Paradis, G., Conner, A. S., & Mulligan, R. C. (1996). Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. The Journal of experimental medicine, 183(4), 1797–1806.

Griffin, C. A., Burger, P., Morsberger, L., Yonescu, R., Swierczynski, S., Weingart, J. D., & Murphy, K. M. (2006). Identification of der(1;19)(q10;p10) in five oligodendrogliomas suggests mechanism of concurrent 1p and 19q loss. Journal of neuropathology and experimental neurology, 65(10), 988–994.

Grunewald, M., Avraham, I., Dor, Y., Bachar-Lustig, E., Itin, A., Jung, S., Yung, S., et al. (2006). VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell, 124(1), 175–189.

Gudas, L. J., & Wagner, J. A. (2011). Retinoids regulate stem cell differentiation. Journal of Cellular Physiology, 226(2), 322–330.

Gustafsson, M. V., Zheng, X., Pereira, T., Gradin, K., Jin, S., Lundkvist, J., Ruas, J. L., et al. (2005). Hypoxia requires notch signaling to maintain the undifferentiated cell state. Developmental cell, 9(5), 617–628.

Guyton, A.C., Hall, J.E. (2006). Textbook of Medical Physiology. Philadelphia: Elsevier Saunders

Halatsch, M.-E., Schmidt, U., Behnke-Mursch, J., Unterberg, A., & Wirtz, C. R. (2006). Epidermal growth factor receptor inhibition for the treatment of glioblastoma multiforme and other malignant brain tumours. Cancer treatment reviews, 32(2), 74–89.

Hamori, E., Arndt-Jovin, D. J., Grimwade, B. G., & Jovin, T. M. (1980). Selection of viable cells with known DNA content. Cytometry Part B: Clinical Cytometry, 1(2), 132–135.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of Cancer: The Next Generation. Cell, 144(5), 646–674. Elsevier Inc.

Harris, A. L. (2002). HYPOXIA — A KEY REGULATORY FACTOR IN TUMOUR GROWTH. Nature Reviews Cancer, 2(1), 38–47.

Hart, M. G., Grant, R., & Metcalfe, S. E. (1996). Biopsy versus resection for high grade glioma. (M. G. Hart, Ed.). Chichester, UK: John Wiley & Sons, Ltd.

Hartmann, C., Meyer, J., Balss, J., Capper, D., Mueller, W., Christians, A., Felsberg, J., et al. (2009). Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathologica, 118(4), 469–474.

Heath, V. L., & Bicknell, R. (2009). Anticancer strategies involving the vasculature. Nature Publishing Group, 6(7), 395–404.

Heddleston, J. M., Li, Z., McLendon, R. E., Hjelmeland, A. B., & Rich, J. N. (2009). The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. Cell Cycle, 8(20), 3274–3284.

Hegi, M. E., Diserens, A.-C., Gorlia, T., Hamou, M.-F., de Tribolet, N., Weller, M., Kros, J.

M., et al. (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. The New England journal of medicine, 352(10), 997–1003.

Hegi, M. E., Liu, L., Herman, J. G., Stupp, R., Wick, W., Weller, M., Mehta, M. P., et al. (2008). Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. Journal of Clinical Oncology, 26(25), 4189–4199.

Hill, R. P. (2006). Identifying cancer stem cells in solid tumors: case not proven. Cancer research, 66(4), 1891–5– discussion 1890.

Hill, R. P., & Perris, R. (2007). “Destemming” cancer stem cells. JNCI Journal of the National Cancer Institute, 99(19), 1435–1440.

Hirschmann-Jax, C., Foster, A. E., Wulf, G. G., Nuchtern, J. G., Jax, T. W., Gobel, U., Goodell, M. A., et al. (2004). A distinct “side population” of cells with high drug efflux capacity in human tumor cells. Proceedings of the National Academy of Sciences of the United States of America, 101(39), 14228–14233.

Hochedlinger, K., Yamada, Y., Beard, C., & Jaenisch, R. (2005). Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. Cell, 121(3), 465–477.

Holmes, D. I. R., & Zachary, I. C. (2008). Vascular endothelial growth factor regulates stanniocalcin-1 expression via neuropilin-1-dependent regulation of KDR and synergism with fibroblast growth factor-2. Cellular signalling, 20(3), 569–579.

Huang, E. H., Hynes, M. J., Zhang, T., Ginestier, C., Dontu, G., Appelman, H., Fields, J. Z., et al. (2009). Aldehyde Dehydrogenase 1 Is a Marker for Normal and Malignant Human Colonic Stem Cells (SC) and Tracks SC Overpopulation during Colon Tumorigenesis. Cancer research, 69(8), 3382–3389.

Huang, Z., Cheng, L., Guryanova, O. A., Wu, Q., & Bao, S. (2010). Cancer stem cells in glioblastoma—molecular signaling and therapeutic targeting. Protein & Cell, 1(7), 638–655.

Hurt, E. M., Kawasaki, B. T., Klarmann, G. J., Thomas, S. B., & Farrar, W. L. (2008). CD44+ CD24(-) prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis. British Journal of Cancer, 98(4), 756–765.

Ichimura, K., Pearson, D. M., Kocialkowski, S., Bäcklund, L. M., Chan, R., Jones, D. T. W., & Collins, V. P. (2009). IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro-Oncology, 11(4), 341–347.

Ihrie, R. A., & Alvarez-Buylla, A. (2011). Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. Neuron, 70(4), 674–686.

Iliopoulos, O., Levy, A. P., Jiang, C., Kaelin, W. G., & Goldberg, M. A. (1996). Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. Proceedings of the National Academy of Sciences of the United States of America, 93(20), 10595–10599.

Intergroup Radiation Therapy Oncology Group Trial 9402, Cairncross, G., Berkey, B.,

Shaw, E., Jenkins, R., Scheithauer, B., Brachman, D., et al. (2006). Phase III trial of chemotherapy plus radiotherapy compared with radiotherapy alone for pure and mixed anaplastic oligodendroglioma: Intergroup Radiation Therapy Oncology Group Trial 9402. Journal of Clinical Oncology, 24(18), 2707–2714.

Ishibashi, M. (2004). Molecular mechanisms for morphogenesis of the central nervous system in mammals. Anatomical science international, 79(4), 226–234.

Ito, K., Hirao, A., Arai, F., Takubo, K., Matsuoka, S., Miyamoto, K., Ohmura, M., et al. (2006). Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nature medicine, 12(4), 446–451.

Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., et al. (2001). HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science, 292(5516), 464–468.

Iyer, N. V., Kotch, L. E., Agani, F., Leung, S. W., Laughner, E., Wenger, R. H., Gassmann, M., et al. (1998). Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes & Development, 12(2), 149–162.

Jaiswal, S., Traver, D., Miyamoto, T., Akashi, K., Lagasse, E., & Weissman, I. L. (2003). Expression of BCR/ABL and BCL-2 in myeloid progenitors leads to myeloid leukemias. Proceedings of the National Academy of Sciences of the United States of America, 100(17), 10002–10007.

Jemal, A., Siegel, R., Xu, J., & Ward, E. (2010). Cancer Statistics, 2010. CA: A Cancer Journal for Clinicians, 60(5), 277–300.

Jenkins, R. B., Blair, H., Ballman, K. V., Giannini, C., Arusell, R. M., Law, M., Flynn, H., et al. (2006). A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. Cancer research, 66(20), 9852–9861.

Jensen, J. B., & Parmar, M. (2006). Strengths and limitations of the neurosphere culture system. Molecular neurobiology, 34(3), 153–161.

Jensen, R. L. (2009). Brain tumor hypoxia: tumorigenesis, angiogenesis, imaging, pseudoprogression, and as a therapeutic target. Journal of neuro-oncology, 92(3), 317–335.

Jeuken, J. W. M., Deimling, von, A., & Wesseling, P. (2004). Molecular pathogenesis of oligodendroglial tumors. Journal of neuro-oncology, 70(2), 161–181.

Jezek, P., & Hlavatá, L. (2005). Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. International Journal of Biochemistry and Cell Biology, 37(12), 2478–2503.

Jiang, F., Qiu, Q., Khanna, A., Todd, N. W., Deepak, J., Xing, L., Wang, H., et al. (2009). Aldehyde Dehydrogenase 1 Is a Tumor Stem Cell-Associated Marker in Lung Cancer. Molecular Cancer Research, 7(3), 330–338.

Jones, D. T. W., Kocialkowski, S., Liu, L., Pearson, D. M., Ichimura, K., & Collins, V. P. (2009). Oncogenic RAF1 rearrangement and a novel BRAF mutation as alternatives

to KIAA1549:BRAF fusion in activating the MAPK pathway in pilocytic astrocytoma. Oncogene, 28(20), 2119–2123.

Jordan, C. T., Guzman, M. L., & Noble, M. (2006). Cancer stem cells. The New England journal of medicine, 355(12), 1253–1261.

Kai, K., Arima, Y., Kamiya, T., & Saya, H. (2010). Breast cancer stem cells. Breast cancer (Tokyo, Japan), 17(2), 80–85.

Kallio, P. J., Wilson, W. J., O'Brien, S., Makino, Y., & Poellinger, L. (1999). Regulation of the hypoxia-inducible transcription factor 1alpha by the ubiquitin-proteasome pathway. The Journal of biological chemistry, 274(10), 6519–6525.

Kashyap, V., Gudas, L. J., Brenet, F., Funk, P., Viale, A., & Scandura, J. M. (2011). Epigenomic reorganization of the clustered Hox genes in embryonic stem cells induced by retinoic acid. The Journal of biological chemistry, 286(5), 3250–3260.

Keime-Guibert, F., Chinot, O., Taillandier, L., Cartalat-Carel, S., Frenay, M., Kantor, G., Guillamo, J.-S., et al. (2007). Radiotherapy for glioblastoma in the elderly. The New England journal of medicine, 356(15), 1527–1535.

Keith, B., & Simon, M. C. (2007). Hypoxia-Inducible Factors, Stem Cells, and Cancer. Cell, 129(3), 465–472.

KERNOHAN, J. W., & MABON, R. F. (1949). A simplified classification of the gliomas. Proceedings of the staff meetings. Mayo Clinic, 24(3), 71–75.

Klarmann, G. J., Hurt, E. M., Mathews, L. A., Zhang, X., Duhagon, M. A., Mistree, T., Thomas, S. B., et al. (2009). Invasive prostate cancer cells are tumor initiating cells that have a stem cell-like genomic signature. Clinical & experimental metastasis, 26(5), 433–446.

Kleihues, P., & Ohgaki, H. (1999). Primary and secondary glioblastomas: from concept to clinical diagnosis. Neuro-Oncology, 1(1), 44–51.

Kondo, T., Setoguchi, T., & Taga, T. (2004). Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proceedings of the National Academy of Sciences of the United States of America, 101(3), 781–786.

Korshunov, A., Meyer, J., Capper, D., Christians, A., Remke, M., Witt, H., Pfister, S., et al. (2009). Combined molecular analysis of BRAF and IDH1 distinguishes pilocytic astrocytoma from diffuse astrocytoma. Acta Neuropathologica, 118(3), 401–405.

Kreth, F. W., Warnke, P. C., Scheremet, R., & Ostertag, C. B. (1993). Surgical resection and radiation therapy versus biopsy and radiation therapy in the treatment of glioblastoma multiforme. Journal of neurosurgery, 78(5), 762–766.

Krex, D., Klink, B., Hartmann, C., Deimling, von, A., Pietsch, T., Simon, M., Sabel, M., et al. (2007). Long-term survival with glioblastoma multiforme. Brain, 130(10), 2596–2606.

Kumano, K., Chiba, S., Shimizu, K., Yamagata, T., Hosoya, N., Saito, T., Takahashi, T., et al. (2001). Notch1 inhibits differentiation of hematopoietic cells by sustaining GATA-2 expression. Blood, 98(12), 3283–3289.

Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., et al. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature, 367(6464), 645–648.

Lassen, N., Black, W. J., Estey, T., & Vasiliou, V. (2008). The role of corneal crystallins in the cellular defense mechanisms against oxidative stress. Seminars in cell & developmental biology, 19(2), 100–112.

Laws, E. R., Parney, I. F., Huang, W., Anderson, F., Morris, A. M., Asher, A., Lillehei, K. O., et al. (2003). Survival following surgery and prognostic factors for recently diagnosed malignant glioma: data from the Glioma Outcomes Project. Journal of neurosurgery, 99(3), 467–473.

Lee, Y., Scheck, A. C., Cloughesy, T. F., Lai, A., Dong, J., Farooqi, H. K., Liau, L. M., et al. (2008). Gene expression analysis of glioblastomas identifies the major molecular basis for the prognostic benefit of younger age. BMC Medical Genomics, 1(1), 52.

Li, A., Walling, J., Ahn, S., Kotliarov, Y., Su, Q., Quezado, M., Oberholtzer, J. C., et al. (2009a). Unsupervised Analysis of Transcriptomic Profiles Reveals Six Glioma Subtypes. Cancer research, 69(5), 2091–2099.

Li, C., Heidt, D. G., Dalerba, P., Burant, C. F., Zhang, L., Adsay, V., Wicha, M., et al. (2007). Identification of pancreatic cancer stem cells. Cancer research, 67(3), 1030–1037.

Li, X., Lewis, M. T., Huang, J., Gutierrez, C., Osborne, C. K., Wu, M. F., Hilsenbeck, S. G., et al. (2008). Intrinsic Resistance of Tumorigenic Breast Cancer Cells to Chemotherapy. JNCI Journal of the National Cancer Institute, 100(9), 672–679.

Li, Z., Bao, S., Wu, Q., Wang, H., Eyler, C., Sathornsumetee, S., Shi, Q., et al. (2009b). Hypoxia-Inducible Factors Regulate Tumorigenic Capacity of Glioma Stem Cells. Cancer Cell, 15(6), 501–513.

Lo, H.-W., Zhu, H., Cao, X., Aldrich, A., & Ali-Osman, F. (2009). A novel splice variant of GLI1 that promotes glioblastoma cell migration and invasion. Cancer research, 69(17), 6790–6798.

Lopez, J. I., Camenisch, T. D., Stevens, M. V., Sands, B. J., McDonald, J., & Schroeder, J. A. (2005). CD44 attenuates metastatic invasion during breast cancer progression. Cancer research, 65(15), 6755–6763.

Louis, D. N., Ohgaki, H., Wiestler, O. D., Cavenee, W. K., Burger, P. C., Jouvet, A., Scheithauer, B. W., et al. (2007). The 2007 WHO Classification of Tumours of the Central Nervous System. Acta Neuropathologica, 114(2), 97–109.

Ma, S., Chan, K. W., Lee, T. K. W., Tang, K. H., Wo, J. Y. H., Zheng, B. J., & Guan, X. Y. (2008a). Aldehyde Dehydrogenase Discriminates the CD133 Liver Cancer Stem Cell Populations. Molecular Cancer Research, 6(7), 1146–1153.

Ma, S., Lee, T. K., Zheng, B. J., Chan, K. W., & Guan, X. Y. (2008b). CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene, 27(12), 1749–1758.

Mack, B., & Gires, O. (2008). CD44s and CD44v6 expression in head and neck epithelia. PLoS ONE, 3(10), e3360.

Makia, N. L., Bojang, P., Falkner, K. C., Conklin, D. J., & Prough, R. A. (2011). Murine hepatic aldehyde dehydrogenase 1a1 is a major contributor to oxidation of aldehydes formed by lipid peroxidation. Chemico-Biological Interactions, 191(1-3), 278–287.

Mao, X.-G., Zhang, X., Xue, X.-Y., Guo, G., Wang, P., Zhang, W., Fei, Z., et al. (2009). Brain Tumor Stem-Like Cells Identified by Neural Stem Cell Marker CD15. Translational oncology, 2(4), 247–257.

Marcato, P., Dean, C. A., Giacomantonio, C. A., & Lee, P. W. K. (2011). Aldehyde dehydrogenase: Its role as a cancer stem cell marker comes down to the specific isoform. Cell Cycle, 10(9), 1378–1384.

Marchitti, S. A., Brocker, C., Stagos, D., & Vasiliou, V. (2008). Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. Expert opinion on drug metabolism & toxicology, 4(6), 697–720.

Marhaba, R., & Zöller, M. (2004). CD44 in cancer progression: adhesion, migration and growth regulation. Journal of molecular histology, 35(3), 211–231.

Mathieu, J., Zhang, Z., Zhou, W., Wang, A. J., Heddleston, J. M., Pinna, C. M. A., Hubaud, A., et al. (2011). HIF induces human embryonic stem cell markers in cancer cells. Cancer research, 71(13), 4640–4652.

Mathur, D., Bost, A., Driver, I., & Ohlstein, B. (2010). A transient niche regulates the specification of Drosophila intestinal stem cells. Science, 327(5962), 210–213.

Mazumdar, J., Dondeti, V., & Simon, M. C. (2009). Hypoxia-inducible factors in stem cells and cancer. Journal of Cellular and Molecular Medicine, 13(11-12), 4319–4328.

McCord, A. M., Jamal, M., Shankavarum, U. T., Lang, F. F., Camphausen, K., & Tofilon, P. J. (2009). Physiologic Oxygen Concentration Enhances the Stem-Like Properties of CD133+ Human Glioblastoma Cells In vitro. Molecular Cancer Research, 7(4), 489–497.

McLendon, R., Friedman, A., Bigner, D., Van Meir, E. G., Brat, D. J., M Mastrogianakis, G., Olson, J. J., et al. (2008). Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature, 455(7216), 1061–1068.

Miller, C. R., & Perry, A. (2007). Glioblastoma. Archives of pathology & laboratory medicine, 131(3), 397–406.

Miller, C. R., Dunham, C. P., Scheithauer, B. W., & Perry, A. (2006). Significance of Necrosis in Grading of Oligodendroglial Neoplasms: A Clinicopathologic and Genetic Study of Newly Diagnosed High-Grade Gliomas. Journal of Clinical Oncology, 24(34), 5419–5426.

Mishima, K., Kato, Y., Kaneko, M. K., Nishikawa, R., Hirose, T., & Matsutani, M. (2006). Increased expression of podoplanin in malignant astrocytic tumors as a novel molecular marker of malignant progression. Acta Neuropathologica, 111(5), 483–

488.

Moore, K. A., & Lemischka, I. R. (2006). Stem cells and their niches. Science, 311(5769), 1880–1885.

Moreb, J. S., Zucali, J. R., Ostmark, B., & Benson, N. A. (2007). Heterogeneity of aldehyde dehydrogenase expression in lung cancer cell lines is revealed by Aldefluor flow cytometry-based assay. Cytometry Part B: Clinical Cytometry, 72B(4), 281–289.

Nigam, S., & Schewe, T. (2000). Phospholipase A(2)s and lipid peroxidation. Biochimica et biophysica acta, 1488(1-2), 167–181.

Nishikawa, R. (2010). Standard therapy for glioblastoma--a review of where we are. Neurologia medico-chirurgica, 50(9), 713–719.

Nobusawa, S., Watanabe, T., Kleihues, P., & Ohgaki, H. (2009). IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clinical cancer research : an official journal of the American Association for Cancer Research, 15(19), 6002–6007.

Noy, N. (2010). Between death and survival: retinoic acid in regulation of apoptosis. Annual review of nutrition, 30, 201–217.

Nunes, M. C., Roy, N. S., Keyoung, H. M., Goodman, R. R., McKhann, G., Jiang, L., Kang, J., et al. (2003). Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. Nature medicine, 9(4), 439–447.

O'Brien, C. A., Pollett, A., Gallinger, S., & Dick, J. E. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature, 445(7123), 106–110.

Ohgaki, H., & Kleihues, P. (2007). Genetic pathways to primary and secondary glioblastoma. The American Journal of Pathology, 170(5), 1445–1453.

Orkin, S. H., & Zon, L. I. (2008). Hematopoiesis: an evolving paradigm for stem cell biology. Cell, 132(4), 631–644.

Ottosen, L. D. M., Hindkaer, J., Husth, M., Petersen, D. E., Kirk, J., & Ingerslev, H. J. (2006). Observations on intrauterine oxygen tension measured by fibre-optic microsensors. Reproductive biomedicine online, 13(3), 380–385.

Panchision, D. M. (2009). The role of oxygen in regulating neural stem cells in development and disease. Journal of Cellular Physiology, 220(3), 562–568.

Park, S.-K., Dadak, A. M., Haase, V. H., Fontana, L., Giaccia, A. J., & Johnson, R. S. (2003). Hypoxia-induced gene expression occurs solely through the action of hypoxia-inducible factor 1alpha (HIF-1alpha): role of cytoplasmic trapping of HIF-2alpha. Molecular and cellular biology, 23(14), 4959–4971.

Parmar, K., Mauch, P., Vergilio, J.-A., Sackstein, R., & Down, J. D. (2007). Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proceedings of the National Academy of Sciences of the United States of America, 104(13), 5431–5436.

Parsons, D. W., Jones, S., Zhang, X., Lin, J. C. H., Leary, R. J., Angenendt, P., Mankoo, P., et al. (2008). An Integrated Genomic Analysis of Human Glioblastoma Multiforme. Science, 321(5897), 1807–1812.

Patrawala, L., Calhoun, T., Schneider-Broussard, R., Li, H., Bhatia, B., Tang, S., Reilly, J. G., et al. (2006). Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene, 25(12), 1696–1708.

Pearce, D. J., Taussig, D., Simpson, C., Allen, K., Rohatiner, A. Z., Lister, T. A., & Bonnet, D. (2005). Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukemia samples. Stem Cells, 23(6), 752–760.

Pelloski, C. E., Ballman, K. V., Furth, A. F., Zhang, L., Lin, E., Sulman, E. P., Bhat, K., et al. (2007). Epidermal growth factor receptor variant III status defines clinically distinct subtypes of glioblastoma. Journal of Clinical Oncology, 25(16), 2288–2294.

Penzes, P., Wang, X., & Napoli, J. L. (1997). Enzymatic characteristics of retinal dehydrogenase type I expressed in Escherichia coli. Biochimica et biophysica acta, 1342(2), 175–181.

Pfister, S., Janzarik, W. G., Remke, M., Ernst, A., Werft, W., Becker, N., Toedt, G., et al. (2008). BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. Journal of Clinical Investigation, 118(5), 1739–1749.

Phillips, H. S., Kharbanda, S., Chen, R., Forrest, W. F., Soriano, R. H., Wu, T. D., Misra, A., et al. (2006). Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell, 9(3), 157–173.

Piccirillo, S. G. M., Binda, E., Fiocco, R., Vescovi, A. L., & Shah, K. (2009). Brain cancer stem cells. Journal of Molecular Medicine, 87(11), 1087–1095.

Pichlmeier, U., Bink, A., Schackert, G., Stummer, W., ALA-Glioma Study Group. (2008). Resection and survival in glioblastoma multiforme: an RTOG recursive partitioning analysis of ALA study patients. Neuro-Oncology, 10(6), 1025–1034.

Pirruccello, S. J., & LeBien, T. W. (1986). The human B cell-associated antigen CD24 is a single chain sialoglycoprotein. Journal of immunology (Baltimore, Md. : 1950), 136(10), 3779–3784.

Pistollato, F., Chen, H.-L., Schwartz, P. H., Basso, G., & Panchision, D. M. (2007). Oxygen tension controls the expansion of human CNS precursors and the generation of astrocytes and oligodendrocytes. Molecular and cellular neurosciences, 35(3), 424–435.

Preusser, M., Gelpi, E., Rottenfusser, A., Dieckmann, K., Widhalm, G., Dietrich, W., Bertalanffy, A., et al. (2008). Epithelial Growth Factor Receptor Inhibitors for treatment of recurrent or progressive high grade glioma: an exploratory study. Journal of neuro-oncology, 89(2), 211–218.

Quigley, M. R., & Maroon, J. C. (1991). The relationship between survival and the extent of

the resection in patients with supratentorial malignant gliomas. Neurosurgery, 29(3), 385–8– discussion 388–9.

Quintana, E., Shackleton, M., Sabel, M. S., Fullen, D. R., Johnson, T. M., & Morrison, S. J. (2008). Efficient tumour formation by single human melanoma cells. Nature, 456(7222), 593–598.

Rafii, S., & Lyden, D. (2008). Cancer. A few to flip the angiogenic switch. Science, 319(5860), 163–164.

Raica, M., Cimpean, A. M., & Ribatti, D. (2009). Angiogenesis in pre-malignant conditions. European journal of cancer (Oxford, England : 1990), 45(11), 1924–1934.

Ramírez-Bergeron, D. L., & Simon, M. C. (2001). Hypoxia-inducible factor and the development of stem cells of the cardiovascular system. Stem Cells, 19(4), 279–286.

Rasheed, Z. A., Yang, J., Wang, Q., Kowalski, J., Freed, I., Murter, C., Hong, S. M., et al. (2010). Prognostic Significance of Tumorigenic Cells With Mesenchymal Features in Pancreatic Adenocarcinoma. JNCI Journal of the National Cancer Institute, 102(5), 340–351.

Rasper, M., Schafer, A., Piontek, G., Teufel, J., Brockhoff, G., Ringel, F., Heindl, S., et al. (2010). Aldehyde dehydrogenase 1 positive glioblastoma cells show brain tumor stem cell capacity. Neuro-Oncology, 12(10), 1024–1033.

Reya, T., Morrison, S. J., Clarke, M. F., & Weissman, I. L. (2001). Stem cells, cancer, and cancer stem cells. Nature, 414(6859), 105–111.

Reynolds, B. A., & Rietze, R. L. (2005). Neural stem cells and neurospheres--re-evaluating the relationship. Nature Methods, 2(5), 333–336.

Ricci-Vitiani, L., Lombardi, D. G., Pilozzi, E., Biffoni, M., Todaro, M., Peschle, C., & De Maria, R. (2006). Identification and expansion of human colon-cancer-initiating cells. Nature, 445(7123), 111–115.

Ricci-Vitiani, L., Pallini, R., Biffoni, M., Todaro, M., Invernici, G., Cenci, T., Maira, G., et al. (2010). Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature, 468(7325), 824–828.

RINGERTZ, N. (1950). Grading of gliomas. Acta pathologica et microbiologica Scandinavica, 27(1), 51–64.

Roa, W., Brasher, P. M. A., Bauman, G., Anthes, M., Bruera, E., Chan, A., Fisher, B., et al. (2004). Abbreviated course of radiation therapy in older patients with glioblastoma multiforme: a prospective randomized clinical trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 22(9), 1583–1588.

Rong, Y., Durden, D. L., Van Meir, E. G., & Brat, D. J. (2006). “Pseudopalisading” necrosis in glioblastoma: a familiar morphologic feature that links vascular pathology, hypoxia, and angiogenesis. Journal of neuropathology and experimental neurology, 65(6), 529–539.

Salcman, M. (1988). Surgical resection of malignant brain tumors: who benefits? Oncology

(Williston Park, N.Y.), 2(8), 47–56– 59–60– 63.

Salvati, M., D'elia, A., Frati, A., Brogna, C., Santoro, A., & Delfini, R. (2011). Safety and feasibility of the adjunct of local chemotherapy with biodegradable carmustine (BCNU) wafers to the standard multimodal approach to high grade gliomas at first diagnosis. Journal of neurosurgical sciences, 55(1), 1–6.

Sanai, N., Polley, M.-Y., McDermott, M. W., Parsa, A. T., & Berger, M. S. (2011). An extent of resection threshold for newly diagnosed glioblastomas. Journal of neurosurgery, 115(1), 3–8.

Sanson, M., Marie, Y., Paris, S., Idbaih, A., Laffaire, J., Ducray, F., Hallani, El, S., et al. (2009). Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. Journal of Clinical Oncology, 27(25), 4150–4154.

Santilli, G., Lamorte, G., Carlessi, L., Ferrari, D., Rota Nodari, L., Binda, E., Delia, D., et al. (2010). Mild hypoxia enhances proliferation and multipotency of human neural stem cells. PLoS ONE, 5(1), e8575.

Sarangi, A., Valadez, J. G., Rush, S., Abel, T. W., Thompson, R. C., & Cooper, M. K. (2009). Targeted inhibition of the Hedgehog pathway in established malignant glioma xenografts enhances survival. Oncogene, 28(39), 3468–3476. Nature Publishing Group.

Sathornsumetee, S., Cao, Y., Marcello, J. E., Herndon, J. E., McLendon, R. E., Desjardins, A., Friedman, H. S., et al. (2008). Tumor Angiogenic and Hypoxic Profiles Predict Radiographic Response and Survival in Malignant Astrocytoma Patients Treated With Bevacizumab and Irinotecan. Journal of Clinical Oncology, 26(2), 271–278.

Satoh, Y., Matsumura, I., Tanaka, H., Ezoe, S., Sugahara, H., Mizuki, M., Shibayama, H., et al. (2004). Roles for c-Myc in self-renewal of hematopoietic stem cells. The Journal of biological chemistry, 279(24), 24986–24993.

Sánchez-García, I., Vicente-Dueñas, C., & Cobaleda, C. (2007). The theoretical basis of cancer-stem-cell-based therapeutics of cancer: can it be put into practice? BioEssays : news and reviews in molecular, cellular and developmental biology, 29(12), 1269–1280.

Schofield, R. (1978). The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood cells, 4(1-2), 7–25.

Seidel, S., Garvalov, B. K., Wirta, V., Stechow, von, L., Schanzer, A., Meletis, K., Wolter, M., et al. (2010). A hypoxic niche regulates glioblastoma stem cells through hypoxia inducible factor 2. Brain, 133(4), 983–995.

Seigel, G. M., Campbell, L. M., Narayan, M., & Gonzalez-Fernandez, F. (2005). Cancer stem cell characteristics in retinoblastoma. Molecular vision, 11, 729–737.

Semenza, G. L. (2009). Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene, 29(5), 625–634. Nature Publishing Group.

Shinojima, N., Tada, K., Shiraishi, S., Kamiryo, T., Kochi, M., Nakamura, H., Makino, K., et al. (2003). Prognostic value of epidermal growth factor receptor in patients with

glioblastoma multiforme. Cancer research, 63(20), 6962–6970.

Shirahata, M., Iwao-Koizumi, K., Saito, S., Ueno, N., Oda, M., Hashimoto, N., Takahashi, J. A., et al. (2007). Gene expression-based molecular diagnostic system for malignant gliomas is superior to histological diagnosis. Clinical cancer research : an official journal of the American Association for Cancer Research, 13(24), 7341–7356.

Shmelkov, S. V., Butler, J. M., Hooper, A. T., Hormigo, A., Kushner, J., Milde, T., St Clair, R., et al. (2008). CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. Journal of Clinical Investigation, 118(6), 2111–2120.

Silván, U., Díez-Torre, A., Arluzea, J., Andrade, R., Silió, M., & Aréchaga, J. (2009). Hypoxia and pluripotency in embryonic and embryonal carcinoma stem cell biology. Differentiation; research in biological diversity, 78(2-3), 159–168.

Singh, S. K., Hawkins, C., Clarke, I. D., Squire, J. A., Bayani, J., Hide, T., Henkelman, R. M., et al. (2004). Identification of human brain tumour initiating cells. Nature, 432(7015), 396–401.

Sládek, N. E. (1999). Aldehyde dehydrogenase-mediated cellular relative insensitivity to the oxazaphosphorines. Current pharmaceutical design, 5(8), 607–625.

Sládek, N. E. (2002). Leukemic cell insensitivity to cyclophosphamide and other oxazaphosphorines mediated by aldehyde dehydrogenase(s). Cancer treatment and research, 112, 161–175.

Soeda, A., Park, M., Lee, D., Mintz, A., Androutsellis-Theotokis, A., McKay, R. D., Engh, J., et al. (2009). Hypoxia promotes expansion of the CD133-positive glioma stem cells through activation of HIF-1α. Oncogene, 28(45), 3949–3959. Nature Publishing Group.

Son, M. J., Woolard, K., Nam, D.-H., Lee, J., & Fine, H. A. (2009). SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell, 4(5), 440–452.

Stevens, J. F., & Maier, C. S. (2008). Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. Molecular nutrition & food research, 52(1), 7–25.

Stier, S., Cheng, T., Dombkowski, D., Carlesso, N., & Scadden, D. T. (2002). Notch1 activation increases hematopoietic stem cell self-renewal in vivo and favors lymphoid over myeloid lineage outcome. Blood, 99(7), 2369–2378.

Storms, R. W., Trujillo, A. P., Springer, J. B., Shah, L., Colvin, O. M., Ludeman, S. M., & Smith, C. (1999). Isolation of primitive human hematopoietic progenitors on the basis of aldehyde dehydrogenase activity. Proceedings of the National Academy of Sciences of the United States of America, 96(16), 9118–9123.

Studer, L., Csete, M., Lee, S. H., Kabbani, N., Walikonis, J., Wold, B., & McKay, R. (2000). Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen. Journal of Neuroscience, 20(19), 7377–7383.

Stummer, W., Pichlmeier, U., Meinel, T., Wiestler, O. D., Zanella, F., Reulen, H.-J., ALA-Glioma Study Group. (2006). Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. The lancet oncology, 7(5), 392–401.

Stupp, R., Hegi, M. E., Mason, W. P., van den Bent, M. J., Taphoorn, M. J. B., Janzer, R. C., Ludwin, S. K., et al. (2009). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. The lancet oncology, 10(5), 459–466.

Stupp, R., Mason, W. P., van den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J. B., Belanger, K., et al. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. The New England journal of medicine, 352(10), 987–996.

Su, Y., Qiu, Q., Zhang, X., Jiang, Z., Leng, Q., Liu, Z., Stass, S. A., et al. (2010). Aldehyde Dehydrogenase 1 A1-Positive Cell Population Is Enriched in Tumor-Initiating Cells and Associated with Progression of Bladder Cancer. Cancer Epidemiology Biomarkers & Prevention, 19(2), 327–337.

Sugawa, N., Ekstrand, A. J., James, C. D., & Collins, V. P. (1990). Identical splicing of aberrant epidermal growth factor receptor transcripts from amplified rearranged genes in human glioblastomas. Proceedings of the National Academy of Sciences of the United States of America, 87(21), 8602–8606.

Tai, M.-H., Chang, C.-C., Kiupel, M., Webster, J. D., Olson, L. K., & Trosko, J. E. (2005). Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. Carcinogenesis, 26(2), 495–502.

Todaro, M., Francipane, M. G., Medema, J. P., & Stassi, G. (2010a). Colon cancer stem cells: promise of targeted therapy. Gastroenterology (Vol. 138, pp. 2151–2162).

Todaro, M., Iovino, F., Eterno, V., Cammareri, P., Gambara, G., Espina, V., Gulotta, G., et al. (2010b). Tumorigenic and metastatic activity of human thyroid cancer stem cells. Cancer research, 70(21), 8874–8885.

Toedt, G., Barbus, S., Wolter, M., Felsberg, J., Tews, B., Blond, F., Sabel, M. C., et al. (2011). Molecular signatures classify astrocytic gliomas by IDH1 mutation status. International Journal of Cancer, 128(5), 1095–1103.

Tsatmali, M., Walcott, E. C., & Crossin, K. L. (2005). Newborn neurons acquire high levels of reactive oxygen species and increased mitochondrial proteins upon differentiation from progenitors. Brain research, 1040(1-2), 137–150.

Uchida, K. (2003). 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Progress in Lipid Research, 42(4), 318–343.

Uchida, N., Buck, D. W., He, D., Reitsma, M. J., Masek, M., Phan, T. V., Tsukamoto, A. S., et al. (2000). Direct isolation of human central nervous system stem cells. Proceedings of the National Academy of Sciences of the United States of America, 97(26), 14720–14725.

Ueshima, Y., Matsuda, Y., Tsutsumi, M., & Takada, A. (1993). Role of the aldehyde dehydrogenase-1 isozyme in the metabolism of acetaldehyde. Alcohol and alcoholism (Oxford, Oxfordshire). Supplement, 1B, 15–19.

Utikal, J., Maherali, N., Kulalert, W., & Hochedlinger, K. (2009). Sox2 is dispensable for the reprogramming of melanocytes and melanoma cells into induced pluripotent stem cells. Journal of cell science, 122(Pt 19), 3502–3510.

van den Bent, M. J., Carpentier, A. F., Brandes, A. A., Sanson, M., Taphoorn, M. J. B., Bernsen, H. J. J. A., Frenay, M., et al. (2006). Adjuvant procarbazine, lomustine, and vincristine improves progression-free survival but not overall survival in newly diagnosed anaplastic oligodendrogliomas and oligoastrocytomas: a randomized European Organisation for Research and Treatment of Cancer phase III trial. Journal of Clinical Oncology, 24(18), 2715–2722.

van den Bent, M. J., Dubbink, H. J., Marie, Y., Brandes, A. A., Taphoorn, M. J. B., Wesseling, P., Frenay, M., et al. (2010). IDH1 and IDH2 mutations are prognostic but not predictive for outcome in anaplastic oligodendroglial tumors: a report of the European Organization for Research and Treatment of Cancer Brain Tumor Group. Clinical cancer research : an official journal of the American Association for Cancer Research, 16(5), 1597–1604.

van den Bent, M. J., Dubbink, H. J., Sanson, M., van der Lee-Haarloo, C. R., Hegi, M., Jeuken, J. W. M., Ibdaih, A., et al. (2009). MGMT promoter methylation is prognostic but not predictive for outcome to adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors: a report from EORTC Brain Tumor Group Study 26951. Journal of Clinical Oncology, 27(35), 5881–5886.

van den Hoogen, C., van der Horst, G., Cheung, H., Buijs, J. T., Lippitt, J. M., Guzman-Ramirez, N., Hamdy, F. C., et al. (2010). High Aldehyde Dehydrogenase Activity Identifies Tumor-Initiating and Metastasis-Initiating Cells in Human Prostate Cancer. Cancer research, 70(12), 5163–5173.

van Es, J. H., van Gijn, M. E., Riccio, O., van den Born, M., Vooijs, M., Begthel, H., Cozijnsen, M., et al. (2005). Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. Nature, 435(7044), 959–963.

Vaupel, P., & Hockel, M. (2000). Blood supply, oxygenation status and metabolic micromilieu of breast cancers: characterization and therapeutic relevance. International journal of oncology, 17(5), 869–879.

Verhaak, R. G. W., Hoadley, K. A., Purdom, E., Wang, V., Qi, Y., Wilkerson, M. D., Miller, C. R., et al. (2010). Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell, 17(1), 98–110.

Vermeulen, L., Todaro, M., de Sousa Mello, F., Sprick, M. R., Kemper, K., Perez Alea, M., Richel, D. J., et al. (2008). Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. Proceedings of the National Academy of Sciences of the United States of America, 105(36), 13427–13432.

Vescovi, A. L., Galli, R., & Reynolds, B. A. (2006). Brain tumour stem cells. Nature

Reviews Cancer, 6(6), 425–436.

Vieira, H. L. A., Alves, P. M., & Vercelli, A. (2011). Modulation of neuronal stem cell differentiation by hypoxia and reactive oxygen species. Progress in Neurobiology, 93(3), 444–455. Elsevier Ltd.

Virchow, R. (1863). Die Krankhaften Geschwulste. Berlin: August Hirschwald

Voog, J., D'Alterio, C., & Jones, D. L. (2008). Multipotent somatic stem cells contribute to the stem cell niche in the Drosophila testis. Nature, 454(7208), 1132–1136.

Walcott, J. C., & Provis, J. M. (2003). Müller cells express the neuronal progenitor cell marker nestin in both differentiated and undifferentiated human foetal retina. Clinical & experimental ophthalmology, 31(3), 246–249.

Wang, J., Sakariassen, P. Ø., Tsinkalovsky, O., Immervoll, H., Bøe, S. O., Svendsen, A., Prestegarden, L., et al. (2008). CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. International Journal of Cancer, 122(4), 761–768.

Wang, L., Park, P., Zhang, H., La Marca, F., & Lin, C.-Y. (2010). Prospective identification of tumorigenic osteosarcoma cancer stem cells in OS99-1 cells based on high aldehyde dehydrogenase activity. International Journal of Cancer, 128(2), 294–303.

Wang, X., Penzes, P., & Napoli, J. L. (1996). Cloning of a cDNA encoding an aldehyde dehydrogenase and its expression in Escherichia coli. Recognition of retinal as substrate. The Journal of biological chemistry, 271(27), 16288–16293.

Watanabe, T., Nobusawa, S., Kleihues, P., & Ohgaki, H. (2009). IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. The American Journal of Pathology, 174(4), 1149–1153.

Weigmann, A., Corbeil, D., Hellwig, A., & Huttner, W. B. (1997). Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. Proceedings of the National Academy of Sciences of the United States of America, 94(23), 12425–12430.

Weller, M., Felsberg, J., Hartmann, C., Berger, H., Steinbach, J. P., Schramm, J., Westphal, M., et al. (2009). Molecular Predictors of Progression-Free and Overall Survival in Patients With Newly Diagnosed Glioblastoma: A Prospective Translational Study of the German Glioma Network. Journal of Clinical Oncology, 27(34), 5743–5750.

Weller, M., Müller, B., Koch, R., Bamberg, M., Krauseneck, P., Neuro-Oncology Working Group of the German Cancer Society. (2003). Neuro-Oncology Working Group 01 trial of nimustine plus teniposide versus nimustine plus cytarabine chemotherapy in addition to involved-field radiotherapy in the first-line treatment of malignant glioma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 21(17), 3276–3284.

Weng, A. P., Ferrando, A. A., Lee, W., Morris, J. P., Silverman, L. B., Sanchez-Irizarry, C., Blacklow, S. C., et al. (2004). Activating mutations of NOTCH1 in human T cell

acute lymphoblastic leukemia. Science, 306(5694), 269–271.

Westphal, M., Ram, Z., Riddle, V., Hilt, D., Bortey, E., On behalf of the Executive Committee of the Gliadel® Study Group. (2006). Gliadel® wafer in initial surgery for malignant glioma: long-term follow-up of a multicenter controlled trial. Acta Neurochirurgica, 148(3), 269–275.

Wick, W., Hartmann, C., Engel, C., Stoffels, M., Felsberg, J., Stockhammer, F., Sabel, M. C., et al. (2009). NOA-04 Randomized Phase III Trial of Sequential Radiochemotherapy of Anaplastic Glioma With Procarbazine, Lomustine, and Vincristine or Temozolomide. Journal of Clinical Oncology, 27(35), 5874–5880.

Wood, J. R., Green, S. B., & Shapiro, W. R. (1988). The prognostic importance of tumor size in malignant gliomas: a computed tomographic scan study by the Brain Tumor Cooperative Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 6(2), 338–343.

Wright, M. H., Calcagno, A., Salcido, C. D., Carlson, M. D., Ambudkar, S. V., & Varticovski, L. (2008). Brca1 breast tumors contain distinct CD44+/CD24- and CD133+ cells with cancer stem cell characteristics. Breast Cancer Research, 10(1), R10.

Wu, C., & Alman, B. A. (2008). Side population cells in human cancers. Cancer letters, 268(1), 1–9.

Wulf, G. G., Wang, R. Y., Kuehnle, I., Weidner, D., Marini, F., Brenner, M. K., Andreeff, M., et al. (2001). A leukemic stem cell with intrinsic drug efflux capacity in acute myeloid leukemia. Blood, 98(4), 1166–1173.

Yan, H., Parsons, D. W., Jin, G., McLendon, R., Rasheed, B. A., Yuan, W., Kos, I., et al. (2009). IDH1 and IDH2 mutations in gliomas. The New England journal of medicine, 360(8), 765–773.

Yin, A. H., Miraglia, S., Zanjani, E. D., Almeida-Porada, G., Ogawa, M., Leary, A. G., Olweus, J., et al. (1997). AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood, 90(12), 5002–5012.

Yip, S., Iafrate, A. J., & Louis, D. N. (2008). Molecular diagnostic testing in malignant gliomas: a practical update on predictive markers. Journal of neuropathology and experimental neurology, 67(1), 1–15.

Yoshida, Y., Takahashi, K., Okita, K., Ichisaka, T., & Yamanaka, S. (2009). Hypoxia Enhances the Generation of Induced Pluripotent Stem Cells. Cell Stem Cell, 5(3), 237–241.

Yu, J., Xie, R., Tan, L., Xu, W., Zeng, S., Chen, J., Tang, M., et al. (2002). Expression of the full-length and 3'-spliced cry1Ab gene in the 135-kDa crystal protein minus derivative of Bacillus thuringiensis subsp. kyushuensis. Current microbiology, 45(2), 133–138.

Zhang, D. X., & Gutterman, D. D. (2007). Mitochondrial reactive oxygen species-mediated signaling in endothelial cells. AJP: Heart and Circulatory Physiology, 292(5), H2023–H2031.

Zhao, D., McCaffery, P., Ivins, K. J., Neve, R. L., Hogan, P., Chin, W. W., & Dräger, U. C. (1996). Molecular identification of a major retinoic-acid-synthesizing enzyme, a retinaldehyde-specific dehydrogenase. European journal of biochemistry / FEBS, 240(1), 15–22.

Zheng, X., Shen, G., Yang, X., & Liu, W. (2007). Most C6 cells are cancer stem cells: evidence from clonal and population analyses. Cancer research, 67(8), 3691–3697.

Zhou, S., Schuetz, J. D., Bunting, K. D., Colapietro, A. M., Sampath, J., Morris, J. J., Lagutina, I., et al. (2001). The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nature medicine, 7(9), 1028–1034.

8 INDEX OF FIGURES AND TABLES

8.1 Figures

8.2. Tables

9 ABBREVIATIONS

2HG	2-Hydroxyglutarate
ADP	Adenosine Diphosphate
ARNT	Aryl Hydrocarbon Receptor Nuclear Transporter
ATP	Adenosine Triphosphate
BAAA	BODIPY aminoacetaldehyde
CNS	Central Nervous System
DEAB	Diethylamino-Benzaldehyde
EPO	Erythropoietin
ETC	Electron Transport Chain
FGF	Fibroblast Growth Factor
FIH-1	HIF-1 Inhibiting Factor
GBM	Glioblastoma multiforme
hESCs	Human Embryonic Stem Cells
HNE	4-Hydroxynonenal
HRE	Hypoxia Responsive Elements
IDH	Isocitrate Dehydrogenase
KPS	Karnofsky Performance Score
LGG	Lower Grade Glioma
MDA	Maliodialdeheyde
MMP	Matrix Metalloproteinases
NADH	Nicotinamide Adenine Dinucleotide
Nicd	Notch Intracellular Domain
NRE	Notch-Response-Elements
ODDDs	Oxygen-Dependent Degradation Domains
OS	Overall Survival
p300/CBP	E1A binding protein p300/CREB-binding protein

PDGF	Platelet Derived Growth Factor
PFS	Progression Free Survival
PHD	Prolyl Hydroxylase Domain
Pol II	DNA polymerase II
RAR	Retinoic Acid Receptor
RARE	Retinoic Acid Response Element
RXR	Retinoid X Receptor
SGZ	Subgranular Zone
SVZ	Subventricular Zone
TCGA	Cancer Genome Atlas
TMZ	Temozolomide
VEGF-A	Vascular Endothelial Growth Factor-A
VEGFR	Vascular Endothelial Growth Factor Receptor
vHL	von Hippel-Lindau
WBRT	Whole Brain Radiation Therapy
WHO	World Health Organization

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Kongressbeiträge:

A User-Centered and Workflow-Aware Unified Display for the Operating Room. MICCAI, Nice, 2012

Multimodality Imaging in Cerebral Gliomas. 1H MR Spectroscopy and [18F]-FET-PET Imaging of tumor heterogeneity with neuropathological correlation. German Society for Neuroradiolgoy (DGNR), Cologne, 2011

Aldehyde dehydrogenase Isoform 1 (ALDH1) expression in glioblastoma stem cells. German Society for Neuroanatomy and Neuropathology (DGNN), Tuebingen, 2011

Operation-Microscope-Mounted Touch Display for Intraoperative Imaging Visualization. German Society for Neurosurgery (DGNC), Annual Meeting, Hamburg, 2010

In-vivo Tomographic Optical Molecular Imaging of Brain Tumors in Mice. World Molecular Imaging Congress (WMIC), Montreal, 2009