Technische Universität München
TUM School of Medicine and Health

Heat Shock Protein 70 as a Biomarker in Gliomas

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Vollständiger Abdruck der von der TUM School of Medicine and Health der Technischen Universität München zur Erlangung eines

Doktors der Zahnheilkunde (Dr. med. dent.)

genehmigten Dissertation.

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Prüfende der Dissertation:

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Die Dissertation wurde am 11.03.2024 bei der Technischen Universität München eingereicht und durch die TUM School of Medicine and Health am 10.10.2024 angenommen.

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1 List of Abbreviations

APC	Antigen presenting cells
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computer tomography
DNA	Deoxyribonucleic acid
EEG	Electroencephalogram
GBM	Glioblastoma multiforme
HIF	Hypoxia-inducible factor
HR	Hazard ratio
IARC	International Agency for Research on Cancer
LOH	Loss of heterozygosity
MGMT	Methylguanine DNA methyltransferase
MRI	Magnetic resonance imaging
mRNA	messenger ribonucleic acid
NOS	Not otherwise specified
PA	Pilocytic astrocytoma
PET	Positron emission tomography
RCT	Radiochemotherapy
RF	Risk factors
RT	Radiotherapy
TKD	Hsp70-peptide TKD (TKDNNLLKRFELSG)
TMZ	Temozolomide
Hsc	Constitutively expressed HSP
HSP	Heat shock protein family
WHO	World Health Organization
w&w	watch and wait

2 Introduction

2.1 Heat shock proteins (HSPs)

The heat shock response was firstly described by Ritossa in the 1960s. Comparing gene expression patterns in giant cells of the salivary glands of Drosophila after exposure to elevated temperatures, it was found that "chromosomal puffing" occurred which is indicative for an elevated mRNA synthesis of a certain class of proteins. (Tissiéres et al. 1974; Ritossa 1996; Ritossa 1962) Because of the heat inducibility, these proteins were termed heat shock proteins (HSPs). After exogenous or endogenous stress their synthesis is highly induced whereas the synthesis of other proteins is reduced. It is known that even under physiological conditions cytosolic HSPs are relevant for synthesis, folding and transport of nascent polypeptides. (Hartl und Hayer-Hartl 2002; Csermely 2001)

The different families of HSPs differ in their molecular weights ranging from a size of 30kDa up to 110kDa. They are highly conserved and ubiquitously distributed in all organisms, from archaea and bacteria up to eukaryotes. (Günther und Walter 1994) According to their molecular weight HSPs they are divided into different families: the so-called small heat shock proteins (HSP20), the HSP40-, the HSP60- (or chaperonin), the HSP70-, the HSP90- and the HSP110-family. (Morimoto 1998; Parsell et al. 1994)

New Nomenclature	Classification by molecular weight
HSPH	HSP110
HSPC	HSP90
HSPA	HSP70
DNAJ	HSP40
HSPB	Small HSP
HSPD/E	HSP60/HSP10
CCT	TRiC

Table 1: Nomenclature of HSPs (Kampinga et al. 2009)

The various families vastly differ regarding their structure and expression. Inside of cells HSPs are present in many different compartments, e.g. in the cytoplasm (e.g. Hsp70, Hsc70), endoplasmic reticulum (e.g. Grp78), lysosomes (e.g. Hsp70), mitochondria (e.g. HSP60, Grp75) and in the outer membrane (e.g. Hsp70). (Todryk et al. 2003; Kiang 1998; Multhoff et al. 1995b)

Among the heat shock protein families, the HSP70-family belongs to the most abundant and stress inducible members. Its members are found in nearly all organisms, subcellular compartments (Kampinga et al. 2009), membranes (Multhoff et al. 1995b) and even in the extracellular space. (Feige und Polla 1994; Pockley und Multhoff 2008; Vega et al. 2008)

2.1.1 Cytoplasmic HSP70

The most important representatives of the HSP70-family are the stress inducible 72kDa Hsp70 and the constitutively expressed Hsc70 with a molecular weight of 73kDa. The amino acid sequence of Hsp70 and Hsc70 shows a sequence homology of more than 80%. (Günther und Walter 1994) The aforementioned Hsp70 is encoded by two highly homologous genes, HSPA1A and HSPA1B, the products of these genes only differ by 2 amino acids and are believed to be fully interchangeable proteins. Hsc70, encoded by gene HSPA8, is expressed in all cells constitutively and can make up 1% of all cytoplasmic proteins under physiological conditions. (Feige und Polla 1994; Kampinga et al. 2009) It is only weakly inducible (about 3- to 6-fold). In contrast, Hsp70 is only weakly expressed under physiological conditions, but can be strongly induced by various stress stimuli (about 20-fold).

2.1.1.1 Functions

Cytoplasmic Hsp70 together with its co-chaperones is responsible for the correct folding of proteins but also assists protein degradation. (Mathew et al. 1998) The co-chaperones such as Hsp40, Hsp90, Hip and Hop can determine the function of Hsp70. (Hartl und Hayer-Hartl 2002; Höhfeld und Jentsch 1997; Höhfeld et al. 2001; Meacham et al. 2001) Furthermore, Hsp70 is essential for the transport of other proteins into mitochondria. (Stuart et al. 1994) Also, it has been shown that HSP70 plays a role in the regulation of the cell cycle. (Helmbrecht et al. 2000) In a mouse model, increased Hsp70 levels led to

reduced radiation induced damage. The rate of apoptosis and the radiation induced arrest in the G2/M-phase was also found to be reduced. (Lee et al. 2001)

An increased Hsp70 expression is associated with an increased ability of tumor cells to protect themselves from stress induced damage by interfering with apoptotic pathways. (Gabai et al. 1998; Jäättelä 1999; Wei et al. 1995; Gehrmann et al. 2005)

2.1.2 Membrane-bound HSP70

Aside from the described functions of Hsp70 in the cytosol to maintain protein homeostasis, Hsp70 also has immunostimulatory effects when located on the cellular membrane or released into the extracellular space. It has been shown that extracellular Hsp70 in combination with immunogenic peptides can induce an MHC class I mediated CD8+ T cell response. Intracellularly, Hsp70 transports immunogenic peptides together with TAP molecules from the lysosomes or the proteasome to the ER, where the peptide is loaded on MHC molecules, which then present these peptides on the outer cellular membrane to the immune system. Depending on whether they are the body's own or foreign peptides, an immune response is either initiated or suppressed. Cell based immunotherapies use HSP70-peptide-complexes derived from tumors as a vaccine for the stimulation of T cells. A series of receptors like CD91, TLR2/4 were identified on APC, which are responsible for the binding and uptake of HSP-peptide-complexes. (Basu et al. 2000; Binder et al. 2001; Ishii et al. 1999)

In 1995, Multhoff et al. already proved the localization of HSP70 on the plasma membrane of tumor cells, but not on normal, healthy cells. (Multhoff et al. 1995b; Multhoff 2007) The expression of HSP70 on the membrane is associated with an increased sensitivity to lysis by natural killer (NK) cells . (Botzler et al. 1996; Multhoff et al. 1997; Multhoff 1999)

In immunodeficient mice, tumor growth was inhibited by Hsp70-peptide TKD-activated NK cells. (Moser et al. 2002) It is interesting to note in this context that T cells cannot be activated in their lysis capacity against HSP70 membrane-positive cells by Hsp70 protein or the Hsp70 peptide TKD. T cells also do not differentiate between HSP70-membrane positive or negative tumor cells. (Multhoff et al. 1995a)

It was also shown that the HSP70 expression on the cellular membrane of tumor cells could be increased by heat shock or membrane interacting substances (e.g. ET-18-OCH3).

(Botzler et al. 1999) Cells with an increased Hsp70 expression on the cell membrane are more susceptible to lysis by TKD-activated NK cells.

In conclusion, it can be stated that membrane bound or extracellularly located HSP70 has a stimulating effect on the immune system, but membrane-HSP70+ tumors have been linked to significantly decreased overall survival in tumor patients which implicates the expression of this molecule as a negative prognostic marker. (Pfister et al. 2007)

The group of Prof. Multhoff developed a novel monoclonal antibody (cmHsp70.1 mAb) recognizing membrane-bound Hsp70 on viable tumor cells. It was generated by immunization of mice with the TKD-peptide, the 14 amino acid long sequence of the C-terminal end of HSP70. The human TKD sequence only differs from the murine sequence in one amino acid (Zhang et al. 2007), therefore the cmHsp70.1 monoclonal antibody displays a cross-reactivity for human and murine Hsp70. A special characteristic of the cmHsp70.1 antibody is the specificity for Hsp70 with no cross-reactivity to the highly homologous Hsc70, thus specifically binding the stress-inducible form of the HSP70-family. This antibody is also able to detect Hsp70 on the cell surface of viable tumor cells, which indicates that a part of the C terminus is presented on the cell membrane of tumor cells. (Multhoff und Hightower 2011; Stangl et al. 2011)

2.1.3 Hsp70 in oncology

2.1.3.1 Chemotherapy

The discrepancy between protection and immune stimulation by Hsp70 is of crucial importance in the evaluation of therapies in the fight against cancer. On the one hand, the various forms of therapy aim to kill the degenerate cells; on the other hand, there is the possibility that the very choice of therapy induces Hsp70 in the cytoplasm, which in turn exerts its protective properties. High levels of Hsp70 in the cytoplasm contribute to anti-apoptotic mechanisms, as shown by many different research groups. (Wei et al. 1995; Jäättelä et al. 1998; Gabai et al. 1998) For example, in prostate carcinoma cells, high endogenous Hsp70 levels have been associated with increased drug resistance. (Roigas et al. 1998)

2.1.3.2 Radiotherapy

Another, physical therapy approach against a variety of tumors is gamma irradiation. Although many effects of irradiation remain unexplained to date, an effect on Hsp70 expression has been demonstrated in various cell systems. In animal cells, cytoplasmic Hsp70 was increased by nonlethal gamma irradiation. (Sierra-Rivera et al. 1993) In human fibroblasts, Hsp70 expression was increased by high-energy UV light. (Suzuki und Watanabe 1992) Overexpression of HSP70 in mouse cells resulted in a reduction in radiation-induced damage, particularly arrest of cells in G2/M phase and associated radiation-induced cell death. (Lee et al. 2001)

After irradiation, almost all cellular components are damaged: membranes, proteins, DNA, and low molecular weight substances such as water due to the formation of radicals. The irradiation dose used is limited by the damage to peripheral healthy cells. (Stone et al. 2003) In addition, irradiation itself can lead to tumor formation. (Travis 2002) To increase efficiency, gamma irradiation is often used in conjunction with chemotherapy. (Bartelink et al. 2002; Hennequin und Favaudon 2002)

2.2 Gliomas

Gliomas are the most common primary brain tumors in adults. The age-standardized incidence in Europe is 6 per 100,000 people per year. Men are more commonly affected than women, with a ratio of 6:4. About half of patients are diagnosed with the most malignant form, glioblastoma WHO-grade IV (GBM).

There is no known way of early detection. The WHO differentiates gliomas into WHO-grade I to IV. Prognosis depends primarily on molecular markers and secondarily on WHO grading. Only WHO-grade I gliomas are curable with local treatment alone. The standard therapeutic procedure is resection as completely as possible. Diffusely infiltrating gliomas WHO-grade II-IV require postoperative therapy in most cases. Due to their infiltrating growth, gliomas of WHO-grade II and higher are not curatively treatable (exception: pleomorphic xanthoastrocytoma (PXA) WHO-grade II). Metastases outside the CNS compartment are very rare and usually occur in late-stage disease. The main postoperative therapy is radiotherapy. Chemotherapy after initial diagnosis and in case of

recurrence includes cytostatics, antiangiogenic drugs and experimental approaches. (Hofer et al. 2020)

2.2.1 Definition and basic information

Primary brain tumors are neoplasms originating from cells of the brain or meninges, in contrast to secondary brain tumors such as brain metastases or malignant lymphomas (PCSNL) originating outside the CNS. Gliomas are the most common primary brain tumors in adults, with an incidence of approximately 50%. In the 2016 WHO classification, they are defined histomorphologically and molecularly and classified into WHO grades I to IV. (Louis et al. 2016a; Louis et al. 2016b)

Among WHO-grade I gliomas, pilocytic astrocytoma (PA) is the most common variant, which is biologically and clinically distinct from WHO-grade II-IV gliomas. PA is a typical childhood disease, occasionally occurring in adolescents and sporadically in adults.

WHO-grade II-III gliomas that diffusely infiltrate normal brain tissue are biologically and prognostically classified into three groups according to the presence or absence of somatic mutations in IDH1 or, less commonly, IDH2 genes and the presence or absence of LOH 1p/19q.

In the absence of molecular markers, e.g., insufficient material or indeterminate result, WHO-grade II and III gliomas with corresponding histomorphologic features are classified into two groups, “oligodendroglioma, NOS (not otherwise specified)” and “astrocytoma, NOS” respectively.

WHO grading (I-IV) is reported in every histopathological report but is overshadowed by molecular markers in prognostic relevance. Integrated diagnostics (histomorphology and molecular markers) are sufficient for daily clinical prognostic prediction, treatment planning and stratification of patients for therapeutic trials. (Hofer et al. 2020)

2.2.2 Epidemiology

In the routine evaluation of the cancer registry, the data of malignant tumors of the brain (ICD-10: C71) are usually published in combination with malignant neoplasms of the meninges (ICD-10: C70) and malignant neoplasms of the central nervous system (ICD-10:

C72). Annually, about 4,000 cases in men and about 3,200 cases in women are diagnosed in Germany. (Robert-Koch-Institut 2015) Thus, this group of neoplasms (ICD-10: C70-C72) ranks 16th in cancer rates among women and 15th among men. Approximately 95% of these cases are malignant tumors of the brain. The overall 5-year survival rate of the total group (C70-C72) is reported as 19% in men and 21% women, and the relative 5-year survival rate, taking into account mortality in the general population, is reported as 21% in men and 22% in women. The 10-year relative survival rate is 15% in men and 19% in women. (Robert-Koch-Institut 2015)

The number of new cases and deaths has increased slightly over the past decade (cases: men: +1.5%/year, women +1.3%/year; deaths: men: +1.5%/year, women: +0.6%/year on average). This increase is due to the changing composition of the population with an increase in people of older age.

The median age of onset is at 64 years in men and 67 years in women and therefore lies 6 years (men) and 2 years (women) below the median age of onset for cancer overall. The median age at death is 67 years in men and 70 years in women. Most cases of disease occur in the range of 70 to 74 years in both sexes. Approximately 4% of all cases occur in children and adolescents (0-19 years).

Based on the current cases and the 13th Coordinated Population Projection of the Federal Statistical Office (V1), it can be assumed that there will be an increase in cases of about 12% to 7,700 incidents in 2040 due to the shift in age structure of the population alone. (Hofer et al. 2020)

2.2.3 Pathogenesis

Studies in transgenic mice showed that gliomas arise from different progenitor cells, e.g. astrocytic, oligodendroglial or neural stem cells. Over the past 25 years, hundreds of molecular alterations of gliomas have been found in gliomas, some of which are particularly noteworthy and of practical importance. They can reprogram the epigenome and the transcriptome. Tumor growth is promoted by the pathologically altered metabolism. IDH mutations are currently known to be among the earliest genetic alterations in glioma development, but are not sufficient by themselves for tumorigenesis. (Hofer et al. 2020)

Physiologically, IDH enzymes catalyze isocitrate to α-ketoglutarate (α-KG). The tumorigenic potential of mutant IDH is associated with a metabolic shift in the glioma cells, converting α-KG to 2-hydroxyglutarate (2-HG), which in turn serves as an oncometabolite and initiates genome-wide histone and DNA methylation (Noushmehr et al. 2010). In addition, 2-HG is thought to indirectly induce proliferation of astrocytes via HIF (hypoxia-inducible factor).

The ATRX gene plays an important role in chromatin remodeling telomere length regulation. Genetic alterations appear to be involved in the progression of WHO-grade II-III astrocytomas and eventually secondary GBM, among others.

TERT mutations are accompanied by increased telomerase activity. The telomerase-based signaling pathway seems to be another important mechanism in gliomagenesis. (Hofer et al. 2020)

2.2.4 Risk factors

The risk of developing glioma is increased by the following factors:

- Certain genetic diseases (< 5%):
    - Neurofibromatosis type I: risk for pilocytic astrocytomas and gliomas in the region of the optic nerve; less commonly, for high-malignant gliomas or malignant peripheral nerve sheath tumors
    - Neurofibromatosis type II: risk for acoustic neuroma, other schwannomas, meningioma, ependymoma, less commonly astrocytoma
    - Tuberous Sclerosis: risk for subependymal giant cell astrocytomas, hamartomas
    - Lynch- and Li-Fraumendi syndrome: risk for GBM and other gliomas
    - Melanoma neural system tumor syndrome and Ollier/Maffucci syndrome: risk for gliomas
    - Turcot syndrome: coincidence of GI tract and CNS tumors
- Familial clustering (~ 5-10%):
    - Immediate relatives of patients with gliomas have a 2-fold increased risk of brain tumors, especially if the affected index patient developed the disease at a young

age. (Goodenberger und Jenkins 2012) Linkage studies found no definable risk variants in family clusters. (Ostrom et al. 2014; Ostrom et al. 2015)

- Ionizing radiation:
  - After therapeutic irradiation: Gliomas and meningiomas can occur as early as 7-9 years after irradiation. (Ohgaki 2009) Risk appears to be particularly high in children.

- Cell phones:
  - Whether cell phones pose a risk for tumor development has not been definitively proven. (Benson et al. 2013) In 2011, the IARC (International Agency for Research on Cancer) defined radiofrequency fields as possibly carcinogenic. (Baan et al. 2011)

2.2.5 Clinical Presentation

The symptoms and clinical presentation of gliomas can vary widely and depend primarily on the location of the tumor and the function of the affected brain areas. The main symptoms include signs of increased intracranial pressure, epileptic seizures, and focal neurological disorders. Behavioral changes, burnouts, dementia, and decline in performance are more subtle changes that are usually noticed after the fact or by those close to them. On average, the duration of symptoms between initial manifestation and initial diagnosis in highly malignant gliomas is approximately 3 months. Highly proliferative gliomas are usually associated with perifocal edema leading to acute intracranial symptoms such as headache, nausea, and vomiting. General symptoms are uncommon. (Hofer et al. 2020)

2.2.6 Diagnosis

2.2.6.1 Imaging

Examination	Recommendation
CT	Often the first available imaging with clinical symptoms

Examination	Recommendation
MRI with contrast medium	Method of choice, also in case of suspected glioma in CT
Biopsy/operation	Histological confirmation is mandatory (exceptions may be difficult-to-access brain stem lesions, especially in children)
Amino acid-PET	Occasionally indicated to determine biopsy site (hotspot) or for treatment planning
Staging	Staging examination to look for manifestation of non-CNS tumor is not part of standard examination

Table 2: Diagnostics in newly symptomatic patients

2.2.6.2 Liquor diagnostics

Differential diagnostic considerations of inflammatory processes, including brain abscesses, germ cell tumors, primary cerebral lymphoma, or brain metastases may require CSF diagnosis. Lumbar puncture is contraindicated if there is evidence of intracranial pressure, especially in infratentorial tumors.

2.2.6.3 EEG (electroencephalogram)

EEG is indicated for evaluation of epilepsy and in cases with antiepileptic treatment.

2.2.6.4 Neuropsychological examination

Neuropsychological examination should be included in early diagnosis. It essentially involves the testing and assessment of cognitive functional areas (including higher visual perception, attention, memory, language, number processing, executive functions). In addition to personal and external anamnestic information, standardized test procedures are used, the results of which are compared with age- and education-corrected norm data. Co-assessment of affect and fatigue is also important, based on a qualitative behavioral

description as well as standardized questionnaires. Sometimes, they also comment on possible confounding variables such as headache, medication side effects, or decreased effort. The findings collected are used to assess neurorehabilitation and vocational reintegration potential, driving ability, or work capacity. They also serve as a baseline condition for subsequent follow-up examinations. (Hofer et al. 2020)

2.2.7 Classification

According to the WHO classification of 2016, gliomas are divided in WHO-grade I-IV lesions. (Louis et al. 2016a; Louis et al. 2016b)

Tumor entity/variant	**WHO-grade**
Diffuse astrocytic and oligodendroglial tumors	
Diffuse astrocytoma (IDH-mutant, -wildtype or NOS)	II
Anaplastic astrocytoma (IDH-mutant, -wildtype or NOS)	III
Glioblastoma (IDH-wildtype)	IV
Giant cell glioblastoma	IV
Gliosarcoma	IV
Glioblastoma (IDH-mutant or NOS)	IV
Oligodendroglioma (IDH-mutant & 1p/19q-codeleted or NOS)	II
Anaplastic Oligodendroglioma (IDH-mutant & 1p/19q-codeleted or NOS)	III
Other astrocytic tumors	
Pilocytic astrocytoma	I
Neuronal and mixed neuronal-glial tumors	
Gangliocytoma	I

Table 3: Grading of selected CNS tumors according to the 2016 CNS WHO (Louis et al. 2016a; Louis et al. 2016b)

Among WHO-grade I gliomas, pilocytic astrocytoma (PA) is the most common. Biologically and clinically, it is distinctly different from WHO-grade II-IV gliomas. Pilocytic astrocytoma typically occurs in childhood, occasionally in adolescents and sporadically in adults.

WHO-grade II-III diffuse infiltrating gliomas are classified into three types depending on the presence or absence of somatic mutations in IDH1 or, less commonly, IDH2 genes and presence or absence of a LOH 1p/19q:

- Type I: IDH_{mut}, LOH 1p/19q: oligodendrogliomas with good prognosis (~30% of WHO-grade II-III gliomas)
- Type II: IDH_{mut}, intact 1p/19q: astrocytomas with intermediate prognosis (~50% of WHO-grade II-III gliomas)
- Type III: IDH_{wt}, intact 1p/19q: astrocytomas with poor prognosis (~20% of WHO-grade II-III gliomas); biologically and prognostically similar to WHO-grade IV GBM

WHO-grade IV glioblastomas (GBM) are predominantly IDH_{wt} and only in a minority of cases IDH_{mut}. The latter are referred to as secondary GBM arising from lower grade gliomas.

Tissue obtained from resections or biopsies (with the advantage of stereotactic biopsy, in which samples are taken along the entire stereotactic approach) forms the basis of pathologic analysis.

Despite increasingly precise molecular classification a residue of unclassifiable gliomas (not otherwise specified, NOS) remains that can be characterized histomorphologically. (Malzkorn und Reifenberger 2016) The term NOS is also used when necessary investigative methods are not available.

In cases of questionable histology (e.g. inappropriate for age, localization), DNA methylation-based classification can be used, which is offered by various pathology institutes but is not yet funded by health insurance companies. (Capper et al. 2018)

2.2.8 Prognostic factors

Patients with WHO-grade II oligodendroglioma have a mean life expectancy of more than 15 years, whereas patients with glioblastoma without methylation of the MGMT gene promoter have an expected overall survival of only 14 months under current standard therapy. In patients with GBM and methylation of the MGMT gene promoter, the mean survival time is approximately 23 months. (Stupp et al. 2005) Most patients relapse within one year of initial therapy. (Franceschi et al. 2012)

Tumor entity/variant	Mean survival
WHO-grade II	
Oligodendroglioma (IDH_{mut} & LOH 1p/19q)	> 10 years
Diffuse astrocytoma (IDH_{mut})	10 years
Diffuse astrocytoma (IDH_{wt})	< 3 years
WHO-grade III	
Anaplastic oligodendroglioma (IDH_{mut} & LOH 1p/19q)	10 years
Anaplastic astrocytoma (IDH_{mut})	6-8 years
Anaplastic astrocytoma (IDH_{wt})	1-4 years
WHO-grade IV	
Glioblastoma (MGMT methylated)	23 months
Glioblastoma (MGMT not methylated)	14 months

Table 4: Prognosis of gliomas (Stummer et al. 2008; Stupp et al. 2009; van den Bent et al. 2017b)

2.2.9 Therapy

In any therapeutic decision, it is important to evaluate all risks and benefits for the patient. In particular, age, general condition, and neurological status must be included in the therapeutic concept. One of the cornerstones of glioma therapy is resection that is as complete as possible but does not impair function, which can be curative for WHO-grade I gliomas. For diffuse WHO-grade II-IV gliomas, macroscopic total resection is often possible but is usually not curative due to the diffuse infiltrative character of the tumors.

The extent of the resection is a determining factor in prognosis. Postoperative radiotherapy (RT) increases survival, although the timing of RT may vary depending on risk factors and WHO-grade. The third therapeutic cornerstone is tumor drug therapy. Predictive markers include LOH 1p/19q status and MGMT gene promoter methylation.

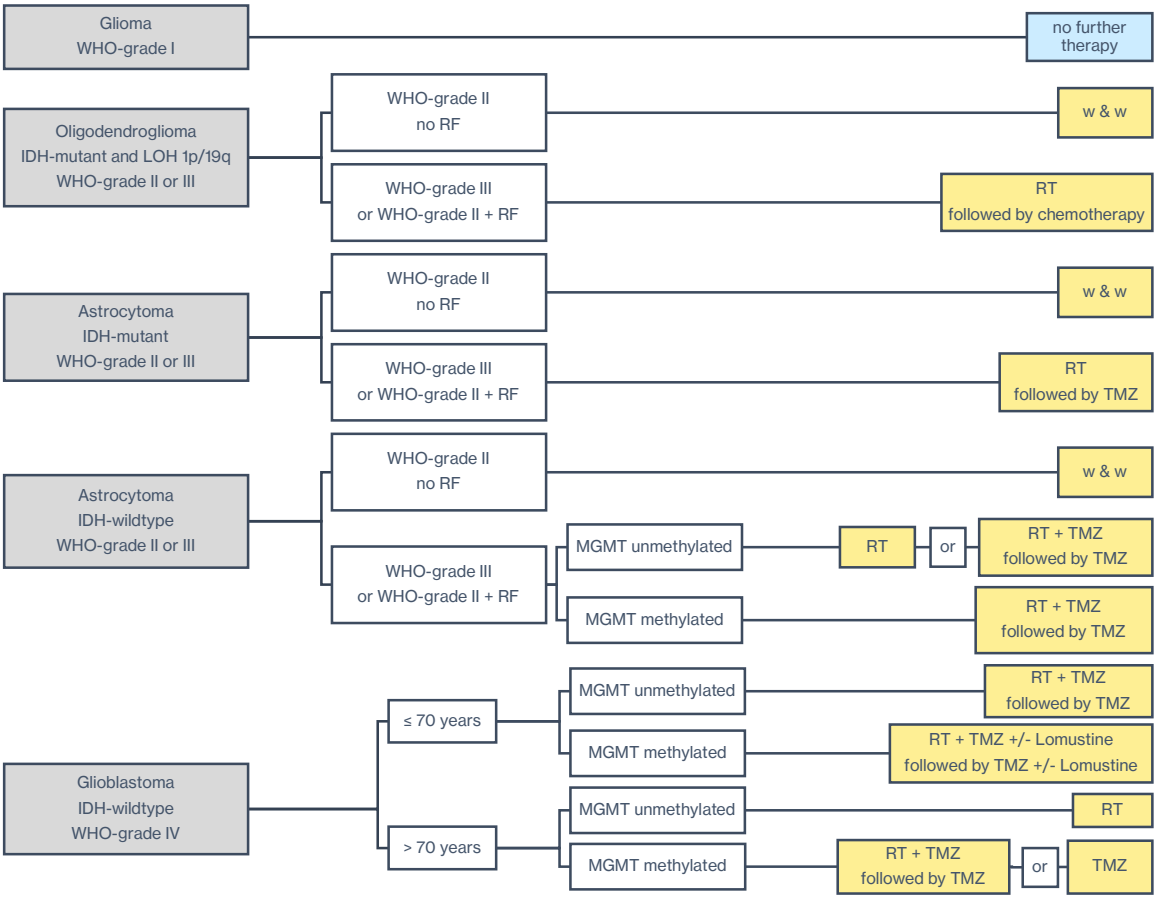

Figure 1: Algorithm of postoperative therapies in WHO-grade II-IV gliomas (Weller et al. 2017; Perry et al. 2017; Herrlinger et al. 2019)

2.2.9.1 Pilocytic astrocytoma, WHO-grade I

The treatment of choice is resection of the tumor. Incomplete resection is not an indication for postoperative RT. RT is recommended for symptomatic, inoperable tumors or for recurrences (54 Gy, 5 x 1.8-2 Gy/week). Treatment should be delivered as high-precision radiotherapy. (Hofer et al. 2020)

2.2.9.2 Diffuse oligodendroglioma, WHO-grade II

Resection of the tumor is the treatment of choice; inoperable tumors should be biopsied when possible.

Postoperative treatment for WHO-grade II gliomas is recommended for the following risk factors (Nabors et al. 2017; Pignatti et al. 2002):

- Age > 40 years

- Neurological deficits
- Uncontrollable seizures
- Tumors sized > 6 cm or exceeding midline
- Subtotal resection

The type of follow-up depends on molecular markers, see Figure 1. Over time, recurrences are the rule. From WHO-grade II, gliomas grow slowly but steadily and invasively; to date there is no curative treatment approach.

In the presence of the above risk factors, chemotherapy with PCV (procarbazine, CCNU, vincristine) regimen for 4-6 cycles following RT (54 Gy) significantly prolongs survival compared to RT alone (hazard ratio 0.59, median 5.5 years). Whether similar results can be achieved with temozolomide following RT is speculative. EORTC 22033-26033 trial showed prolonged progression-free survival after RT alone compared to temozolomide (TMZ) alone in WHO-grade II glioma, except for the subgroup with LOH 1p/19q, in which no difference was observed between RT and TMZ. (Baumert et al. 2016)

2.2.9.3 Diffuse astrocytoma, WHO-grade II

Therapy of choice is resection of the tumor; inoperable tumors should be biopsied when possible. After integrated diagnosis, evidence-based chemotherapy with the PCV regimen for 4-6 cycles after RT (54 Gy) is used for non-oligodendroglial gliomas with risk factors according to the RTOG 9802 trial. The EORTC 22033-26033 trial showed prolonged progression-free survival after RT alone compared with TMZ alone (hazard ratio 1.86). (Baumert et al. 2016)

2.2.9.4 Anaplastic oligodendroglioma, WHO-grade III

Resection of the tumor is the treatment of choice; inoperable tumors should be biopsied when possible. After integrated diagnosis, RT and chemotherapy are indicated. Combined chemotherapy with PCV regimen for 4-6 cycles after or before RT (60 Gy) significantly prolongs survival compared with RT alone (hazard ratio 0.59, median 7.4 years). (van den Bent et al. 2013; Cairncross et al. 2013)

The sequence of RT and chemotherapy has not been clarified in the studies mentioned (RTOG 9402, EORTC 26951). In RTOG 9402, the PCV regimen was used in 4 cycles before RT; in the EORTC trial, up to 6 cycles were used after RT.

2.2.9.5 Anaplastic astrocytoma, WHO-grade III

Therapy of choice is resection of the tumor; inoperable tumors should be biopsied when possible. According to the multiple armed CATNON trial, RT with 59.4 Gy followed by TMZ for 12 cycles in non-oligodendroglial gliomas significantly prolongs survival compared to RT alone (HR 0.57, 5-year-survival 56% vs. 44%). (van den Bent et al. 2017a)

Data from the NOA-04 trial published in 2009 suggest that initial postoperative therapy can be either chemotherapy with TMZ for 8 cycles, PCV for 4 cycles, or RT. (Wick et al. 2009) To date, available data suggest that RT followed by chemotherapy further prolongs overall survival compared with monotherapy.

IDH-wildtype astrocytomas behave biologically like glioblastomas or variants thereof and are treated accordingly. Postoperative RT alone (60 Gy) is an option in justified non-methylated MGMT cases. (Wick et al. 2009)

2.2.9.6 Glioblastoma, WHO-grade IV

In patients aged 70 years and younger, the first step is to resect the MRI contrast-enhanced tumor as much as possible. In case of inoperability, biopsy with integrated diagnosis should be performed. This is followed by combined radiochemotherapy (RCT) with TMZ for 6 cycles. (Stupp et al. 2005) The concomitant part includes RT with 66 Gy (33 x 2 Gy) and concurrent TMZ. In EORTC 22981-26981 trial, RCT resulted in a significant prolongation of overall survival compared to RT alone (HR 0.63, median 2.3 months). Toxicity mainly affected the hematopoietic system. Additional treatment with tumor-targeting fields (Optune®) resulted in prolonged progression-free (HR 0.62, median 3.1 months) and overall survival (HR 0.64, median 4.9 months) in the ITT population and can be considered independent of MGMT gene promoter methylation, according to the EF 14 study. (Stupp et al. 2017)

Intensification or prolongation of TMZ did not lead to better results. (Gilbert et al. 2013) In two randomized phase III trials, bevacizumab in addition to RCT prolonged progression-

and steroid-free survival but not overall survival. (Chinot et al. 2014; Gilbert et al. 2014) Rates of adverse events, including worsening cognitive function, were increased with bevacizumab. (Gilbert et al. 2014)

In the randomized phase III CeTeG trial conducted in newly diagnosed GBM patients with methylated MGMT gene promoter, the combination of CCNU (lomustine) in addition to standard therapy (TMZ and RT) increased median overall survival in the ITT population from 30.4 to 46.6 months with good tolerability. (Herrlinger et al. 2019)

In biologically elderly patients over 70 years of age in good general health, the recommendation of the drug treatment is based on the methylation of the MGMT gene promoter.

If the MGMT gene promoter is methylated, combined RCT with accelerated radiation (40 Gy over 15 fractions) with concomitant and adjuvant TMZ in patients ≥ 65 years of age results in prolonged overall survival compared to RT alone (HR 0.67, median 1.7 months) and also progression-free survival (HR 0.50, median 1.4 months). (Perry et al. 2017) Alternatively, if RCT is contraindicated, chemotherapy alone with TMZ 5/28 for 6 cycles (Malmström et al. 2012) or TMZ “one week on, one week off” for 6 months can be used. (Wick et al. 2012) RT remains an option in case of relapse or progression.

If the MGMT gene promoter is unmethylated, RT alone is hypofractionated with 34 Gy (3.4 Gy single dose) over 2 weeks or in standard fractions with 60 Gy (2 Gy single dose) over 6 weeks. (Wick et al. 2012; Roa et al. 2004; Malmström et al. 2012)

2.2.9.7 Treatment of relapses

To date, there is no standard procedure for recurrences in gliomas. Before treatment is initiated, pseudoprogression must be excluded radiologically. If MRI results are unclear, diagnostic measures such as amino acid PET or MR spectroscopy can be used. (Grosu et al. 2011)

Some patients profit from repeated surgical procedures. Recurrent resection for high-grade gliomas has been particularly successful when the contrast-enhanced lesion could be removed in toto. (Suchorska et al. 2016)

Repeated irradiation should be discussed depending on the timing, the dose of previous irradiation, and the current irradiation target. Of particular relevance is whether the recurrence is within or outside the old irradiation target. (Nieder et al. 2016)

In WHO-grade III gliomas with RT alone as first-line treatment the efficacy of systemic therapy is confirmed. Nitrosourea monotherapy, PCV chemotherapy, and TMZ appear to be roughly equivalent. Reexposure to TMZ is dependent on the time interval from prior therapy. It is likely to be more effective if the interval is longer than one year and the MGMT gene promoter is methylated. (Weller et al. 2015) In general, second-line chemotherapy is less effective than first-line chemotherapy.

In countries with approval of bevacizumab (CH), this antiangiogenetic antibody is a rapidly effective option in symptomatic patients, but without survival benefit in a randomized phase III trial. (Wick et al. 2017) Treatment with bevacizumab may also be considered for (symptomatic) radionecrosis. (Lubelski et al. 2013) Potentially applicable but overall less effective agents in recurrence include paclitaxel, irinotecan, carboplatin, or the combination of etoposide with platinum derivates.

Until now, glial tumor antigens were thought to be recognized by the peripheral immune system only after the gliomas had grown and triggered an inflammatory response that allowed passage through the blood-brain-barrier. There is now evidence that immune cells can enter CNS compartments through less complex barriers, such as the blood-liquor-barrier in the choroid plexus, without the need for inflammatory conditions. The choroid plexus may therefore play a selective role in immune cell transmission. (Shechter et al. 2013) Also to be considered are various immunosuppressive mechanisms induced by gliomas themselves. Despite the above obstacles, many hopes rest on various immunotherapeutic approaches. In a first randomized trial, nivolumab did not prolong overall survival compared with bevacizumab. (Reardon et al. 2017) Trial for first-line therapy in addition to standard therapy in GBM are not yet available, and active vaccination trials against specific tumor antigens of gliomas are under clinical investigation.

2.3 Scientific Issue and Objective

The aim of this dissertation is to examine biopsies of gliomas of different grades for their membranous expression of Hsp70 and to compare the results with tumor grade.

The main question is whether there is a correlation between membranous Hsp70 expression and glioma malignancy, as previous studies have found Hsp70 mRNA expression in low-grade glioma cell lines and human brain tissue samples (Hermisson et al. 2000), and grade-dependent expression glioblastoma cell lines has been discussed. (Beaman et al. 2014) In The Cancer Genome Atlas' data on Hsp70 expression, an association between Hsp70 expression and histologic grade of low-grade gliomas was found (Lee et al. 2015), which was later confirmed in an independent study for low-grade gliomas but not GBM. (Ceccarelli et al. 2016) As previously described, tumor cells exhibit the unique ability to translocate Hsp70 from the cytosol to the plasma membrane (Multhoff et al. 1995b; Gehrmann et al. 2008), implying the possibility of detecting the above-mentioned in vitro increase in mRNA expression also on the membrane of human tumor biopsies.

Prof. Multhoff's group has also shown that primary glioblastomas, which are usually IDH-wildtype, exhibit a unique overexpression of cytosolic, extracellular and also membrane-bound Hsp70 compared to secondary, or IDH-mutant, GBM. (Thorsteinsdottir et al. 2017) Therefore, the continuing question is whether various parameters, such as IDH status or MGMT promoter status, as well as patient age and recurrence, show a correlation with membrane expression of Hsp70.

Hopefully, this can contribute to further understanding of the role of Hsp70 in gliomas, as little is known so far about membrane expression of Hsp70 in human glioma tissue and this may be useful for future studies as well as potential verification as a diagnostic or prognostic marker or even as a monitoring parameter in tumor patients.

3 Material and Methods

3.1 Study Collective

Sixty-three adult patients aged 20 to 89 years old (Mean age: 55.05 ± 16.73 years) with diagnosed gliomas were recruited into the study and tumor tissue has been deposited in our tumor bank. The patient cohort consisted of oligodendrogliomas WHO-grade II (initial diagnosis/relapse n = 2/1), diffuse astrocytomas WHO-grade II (n = 5/3), anaplastic oligodendrogliomas WHO-grade III (n = 4/2), anaplastic astrocytomas WHO-grade III (n = 3/2) and primary glioblastomas WHO-grade IV (n = 36/5). The study was approved by the local ethical committee of the medical faculty of the Technical University Munich (TUM, #2403/09). Written informed consent has been obtained from all patients before the start of the therapy. Histological diagnosis and molecular diagnostics (MGMT gene promoter methylation, IDH1/IDH2 mutation) were performed by the Department of Neuropathology at the TUM. Glioblastomas were classified as primary GBM when they were IDH-wildtype (n = 41), as explained above. Tumor probes of 63 patients were collected for FACS analysis. All analyses were blinded for patient and tumor characteristics.

3.2 Flow Cytometry/FACS (Fluorescence Activated Cell Sorter)

Flow cytometry is a method to phenotype various cell characteristics by analyzing laser induced optical signals emitted by the fluorescence labeled antibodies that are bound to cells. The samples must be single-cell suspensions in a suitable buffer (usually PBS/10% FCS) to ensure the validity of the results of a single cell measurement.

Many innovations have made the first in the 1950s used flow cytometer the modern instrument it is today, being able to measure at rates of up to 10.000 cells per second or even more whilst detecting up to more than 30 parameters at the same time on one cell. Typically, a cytometer consists of three main components: fluidics, optics, and electronics. The fluidics system is transporting the sample from the sample tube to the flow cell, past the laser and finally either to collection tubes (in the case of cell sorters) or transferring to waste (in the case of cell scanners). The optical system includes excitation light sources, lenses and filters for the collection and movement of light in the instrument as well as the detection system generating the photocurrent. The electronics digitize and process the photocurrent from the optical systems detector, saving it for subsequent analysis.

As mentioned above, antibody-stained cells must be distributed in single-cell suspension before being taken up into the instrument and surrounded by the sheath fluid, forcing it into a single-file stream of cells. At the interrogation point, the laser light beam illuminates those single cells independently, scattering the light by striking different structures in the cell. The measured scatter can be correlated with relative cell size and structures, being termed forward angle scatter (FSC) and side angle scatter (SSC), depending on where the light is collected in relation to the laser beam's path. Meanwhile, the fluorophores associated with the cell are excited by the laser light, producing a fluorescence emission. The detector then passes all the gathered information on to the electronics.

The following parameters can be measured:

- Cell count
- Relative size (FSC), measured along the axis of the incoming light
- Granularity or complexity (SSC), measured 90° from the direction of the excitation light
- Fluorescence intensity (FL1, FL2, FL3, FL4) of the fluorochrome molecules (e.g. APC, PerCP, FITC, PE, etc.)

(How a Flow Cytometer Works | Thermo Fisher Scientific - DE 2020)

3.3 Procedure

Collected tumor probes were transferred to the laboratory and prepared for cell sampling. The tumor material was divided into 1mm^3 pieces, trypsinized for 8 minutes, and then pushed through a sterile mesh strainer (70µm). 5x10^5 cells per tube were treated for 30 minutes on ice with the following fluorescence-labeled antibodies after being washed with FACS buffer (PBS/10% FCS):

- tube 1: IgG1-FITC/APC (BD Biosciences)
- tube 2: CD45-APC (Thermo Fisher) and cmHsp70.1-FITC (multimmune GmbH)
- tube 3: pan-HLA class I-FITC (F5662, Sigma)

7AAD (BD Biosciences) was applied right before flow cytometric analysis after two washing procedures. On a FACSCalibur™ flow cytometer, only viable, 7AAD-negative

tumor cells that are CD45-negative (to exclude lymphocytes) were gated and examined (BD Biosciences). A positive control was provided by pan-HLA class I antibody staining, while a negative control was provided by isotype-matched control antibodies. For each measurement, at least 100,000 events were recorded. (Lobinger et al. 2021)

3.4 Statistics

All results are expressed as mean value ± standard deviation (SD). Statistical differences between data sets were assessed using Statistical Package for the Social Sciences (SPSS) software for Windows, version 25.0 (IBM). Data that were normally distributed according to the software were compared with Student's t-test; data that were not normally distributed were compared with the Mann-Whitney-Wilcoxon test (Mann-Whitney U test). Correlation between data sets was assessed with Spearman's rank correlation coefficient (Spearman's ρ). Differences between data sets were considered statistically significant at $p < 0.05$.

Percent Hsp70 is defined as the difference between the gated Hsp70-%-value and the isotype value.

MFI describes the mean fluorescence intensity on the surface of the cells.

Boxplots include both the median value (line) and the mean value (x).

4 Results

4.1 Age

4.1.1 Initial diagnosis

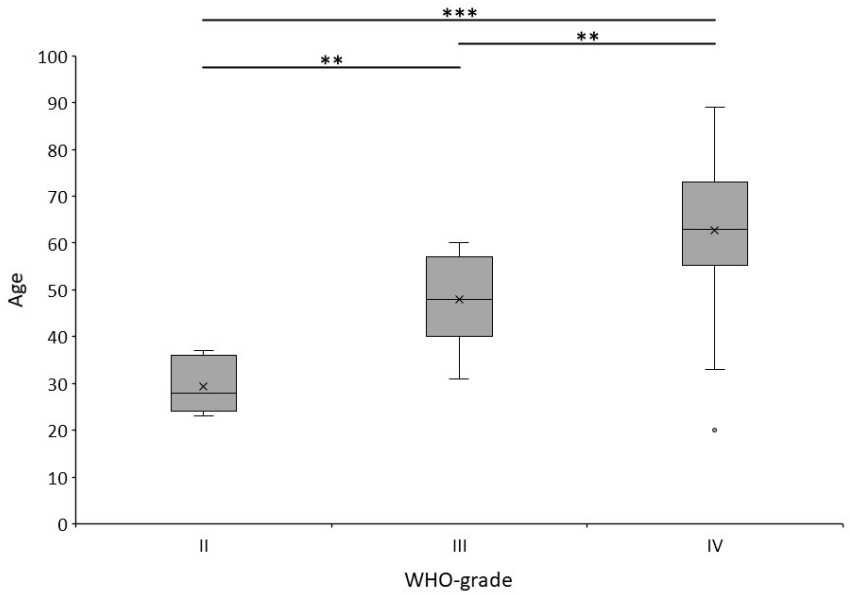

Figure 2: Age vs. WHO-grade in initially diagnosed patients

As shown in Figure 2, there was a highly significant age difference between patients with an initial diagnosis of WHO-grade II (n = 7) and patients with WHO-grade IV (n = 36) (WHO-grade II vs. WHO-grade IV: 29.43 ± 5.23 years vs. 62.75 ± 13.35 years, ***p ≤ 0.001).

There was also a very significant age difference between patients with an initial diagnosis of WHO-grade II (n = 7) and patients with WHO-grade III (n = 7) (WHO-grade II vs. WHO-grade III: 29.43 ± 5.23 years vs. 48.00 ± 9.40 years, **p ≤ 0.01).

Consistent with these results, a very significant age difference was found between patients with initial diagnosis of WHO-grade III (n = 7) and patients with WHO-grade IV (n = 36) (WHO-grade III vs. WHO-grade IV: 48.00 ± 9.40 years vs. 62.75 ± 13.35 years, **p ≤ 0.01).

4.1.2 Relapse

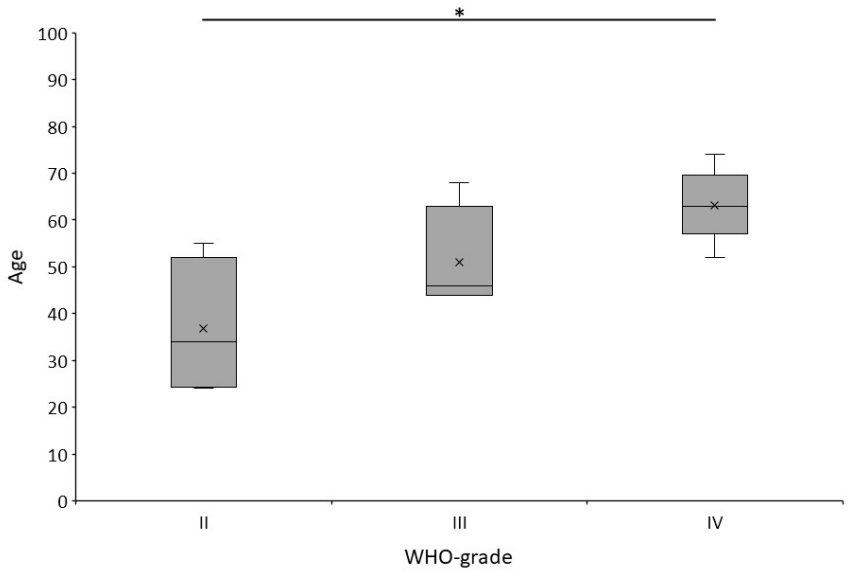

Figure 3: Age vs. WHO-grade in relapse patients

As shown in Figure 3, there was a significant age difference between patients with WHO-grade II recurrence (n = 4) and patients with WHO-grade IV recurrence (n = 5) (WHO-grade II vs. WHO-grade IV: 36.75 ± 12.97 years vs. 63.20 ± 7.03 years, *p ≤ 0.05).

Following the trend of Figure 2, there is an age difference between patients with WHO-grade II recurrence (n = 4) and patients with WHO-grade III recurrence (n = 4), but it is not statistically significant (WHO-grade II vs. WHO-grade III: 36.75 ± 12.97 years vs. 51.00 ± 9.95 years, n.s.).

The same applies to the difference between patients with WHO-grade III recurrence (n = 4) and patients with WHO-grade IV recurrence (n = 5) (WHO-grade III vs. WHO-grade IV: 51.00 ± 9.95 years vs. 63.20 ± 7.03 years, n.s.).

4.1.3 Initial diagnosis vs. Relapse

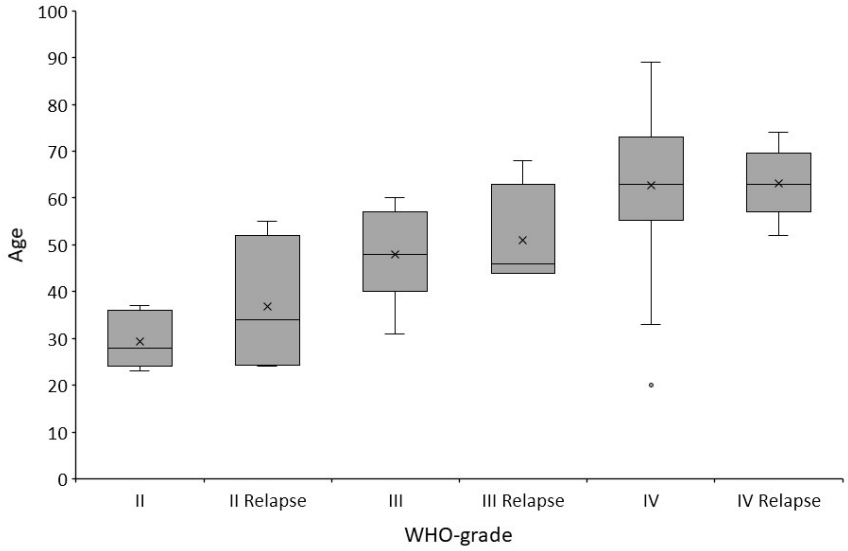

Figure 4: Age vs. WHO-grade in initially diagnosed vs. relapse patients

As shown in Figure 4, no large age difference was observed between patients with initial diagnosis and patients with relapse.

This was true for WHO-grade II patients (initial diagnosis/relapse n = 7/4) (initial diagnosis vs. relapse: 29.43 ± 5.23 years vs. 36.75 ± 12.97 years, n.s.).

Similar results were observed in WHO-grade III patients (initial diagnosis/relapse n = 7/4) (initial diagnosis vs. relapse: 48.00 ± 9.40 years vs. 51.00 ± 9.95 years, n.s.).

The same picture was seen in WHO-grade IV patients (initial diagnosis/relapse n = 36/5) (initial diagnosis vs. relapse: 62.75 ± 13.35 years vs. 63.20 ± 7.03 years, n.s.).

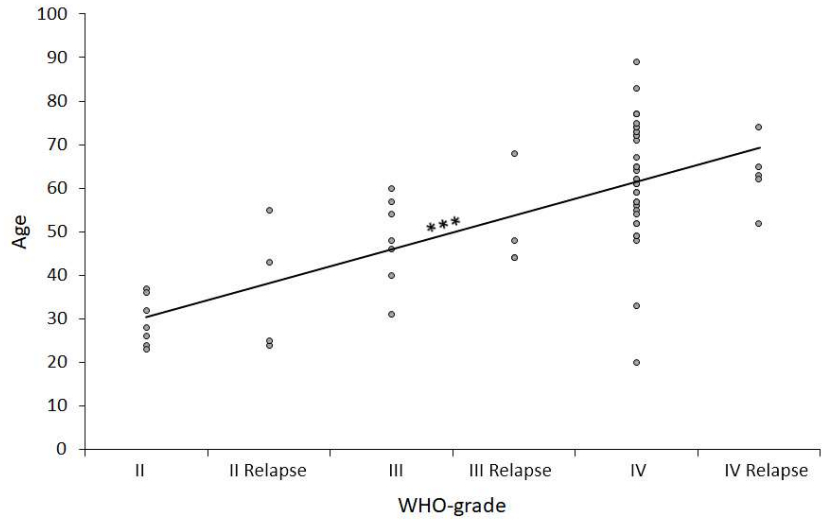

Figure 5: Linear correlation of Age vs. WHO-grade

Following the trend of Figure 2, Figure 3, and Figure 4, a highly significant linear correlation was found between increasing age and higher WHO-grade and their corresponding recurrences in all patients (n = 63), which is shown in Figure 5 (r = 0.647, ***p ≤ 0.001).

4.1.4 IDH-wildtype vs. -mutant

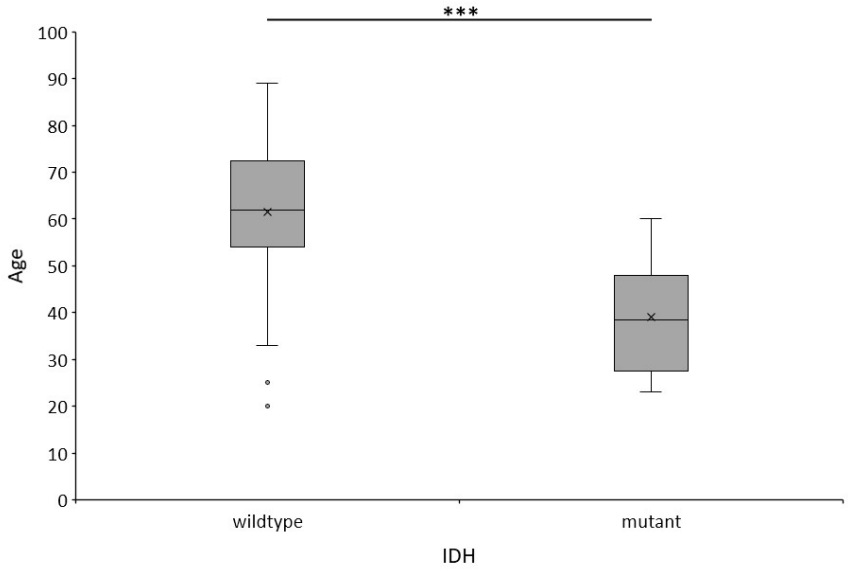

Figure 6: Age vs. IDH status

As shown in Figure 6, there was a highly significant age difference between IDH-wildtype patients (n = 45) and IDH-mutant patients (n = 18) (IDH-wildtype vs. IDH-mutant: 61.47 ± 13.72 years vs. 39.00 ± 11.54 years, ***p ≤ 0.001).

4.1.5 MGMT promoter unmethylated vs. methylated

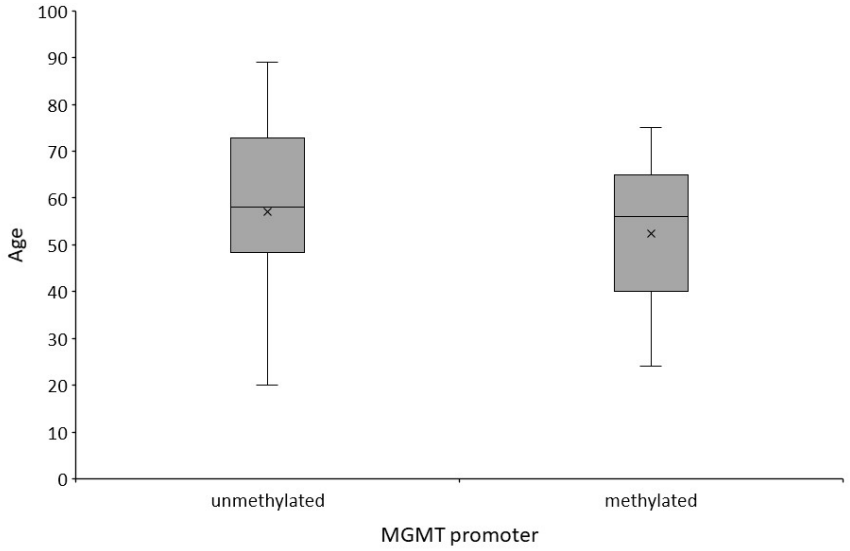

Figure 7: Age vs. MGMT promoter status

As shown in Figure 7, there was no big age difference between patients with unmethylated MGMT promoter (n = 36) and patients with methylated MGMT promoter (n = 27) (unmethylated vs. methylated: 57.08 ± 17.02 years vs. 52.33 ± 15.62 years, n.s.).

WHO-grade	Age median (range)	Total cases	MGMT promoter unmethylated (%)		MGMT promoter methylated (%)	
II	28 (23-37)	7	3	(42.9%)	4	(57.1%)
II Relapse	34 (24-55)	4	2	(50%)	2	(50%)
III	48 (31-60)	7	2	(28.6%)	5	(71.4%)
III Relapse	46 (44-68)	4	2	(50%)	2	(50%)
IV	63 (20-89)	36	23	(63.9%)	13	(36.1%)
IV Relapse	63 (52-74)	5	4	(80%)	1	(20%)

Table 5: MGMT promoter methylation in different subgroups

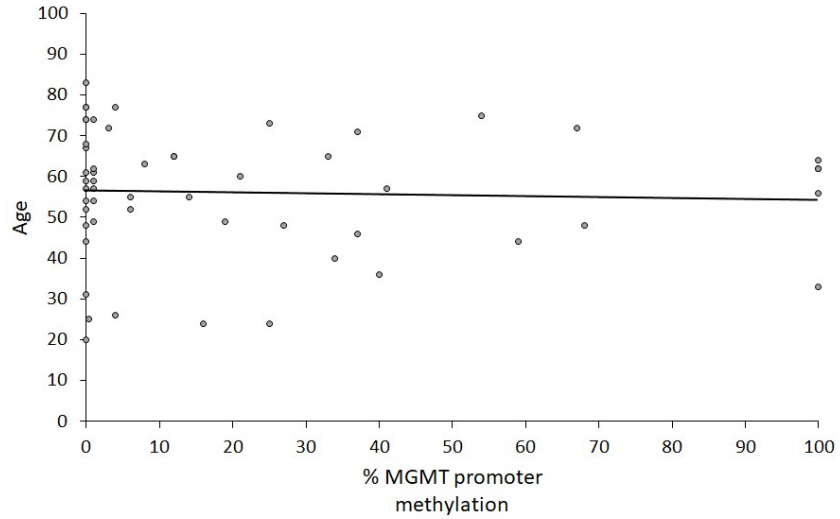

Figure 8: Linear correlation of Age vs. MGMT promoter methylation percentage

Figure 8 compares the linear correlation between age and the percentage value of MGMT promoter methylation (n = 54), there is no trend in either direction (r = -0.125, n.s.).

4.2 Hsp70 in %

4.2.1 Initial diagnosis

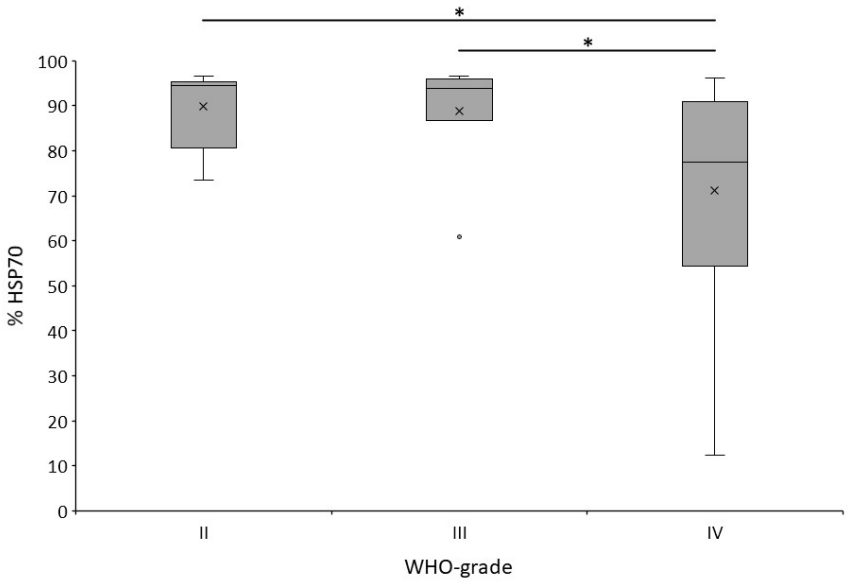

Figure 9: Hsp70 in % vs. WHO-grade in initially diagnosed patients

As shown in Figure 9, there was a significant difference between the percentages of membrane-bound Hsp70 of lower graded gliomas compared with WHO-grade IV gliomas.

At initial diagnosis, WHO-grade II tumors (n = 7) had significantly higher membrane-bound Hsp70 percentage than WHO-grade IV tumors (n = 36) (WHO-grade II vs. WHO-grade IV: 89.94 ± 8.39 % vs. 71.25 ± 21.78 %, *p ≤ 0.05).

The same was true when comparing first-diagnosed WHO-grade III tumors (n = 7) and WHO-grade IV tumors (n = 36) (WHO-grade III vs. WHO-grade IV: 88.84 ± 11.80 % vs. 71.25 ± 21.78 %, *p ≤ 0.05).

There was no significant difference between lower-grade WHO-grade II (n = 7) and WHO-grade III (n = 7) tumors (WHO-grade II vs. WHO-grade III: 89.94 ± 8.39 % vs. 88.84 ± 11.80 %, n.s.).

4.2.2 Relapse

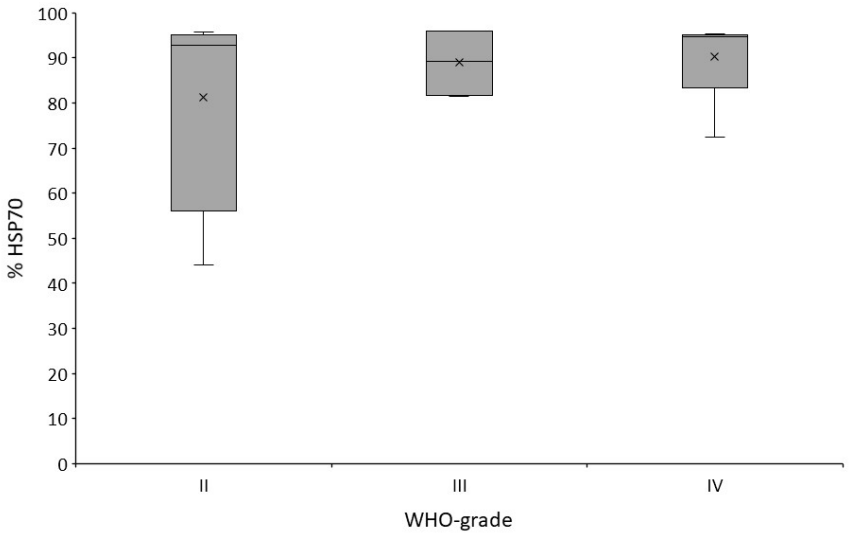

Figure 10: Hsp70 in % vs. WHO-grade in relapse patients

When comparing the membrane-bound Hsp70 percentages of relapse patients, no significant differences were observed.

Recurrent WHO-grade II tumors (n = 4) showed only slightly lower values than WHO-grade III tumors (n = 4), but with a much higher standard deviation (WHO-grade II vs. WHO-grade III: 81.31 ± 21.53 % vs. 88.93 ±6.97 %, n.s.).

The same was true for the comparison of recurrent WHO-grade II (n = 4) and WHO-grade IV (n = 5) tumors (WHO-grade II vs. WHO-grade IV: 81.31 ± 21.53 % vs. 90.30 ± 9.00 %, n.s.).

Comparison of recurrent WHO-grade III (n = 4) and WHO-grade IV (n = 5) tumors showed the same picture with very similar values (WHO-grade III vs. WHO-grade IV: 88.93 ±6.97 % vs. 90.30 ± 9.00 %, n.s.).

4.2.3 Initial diagnosis vs. Relapse

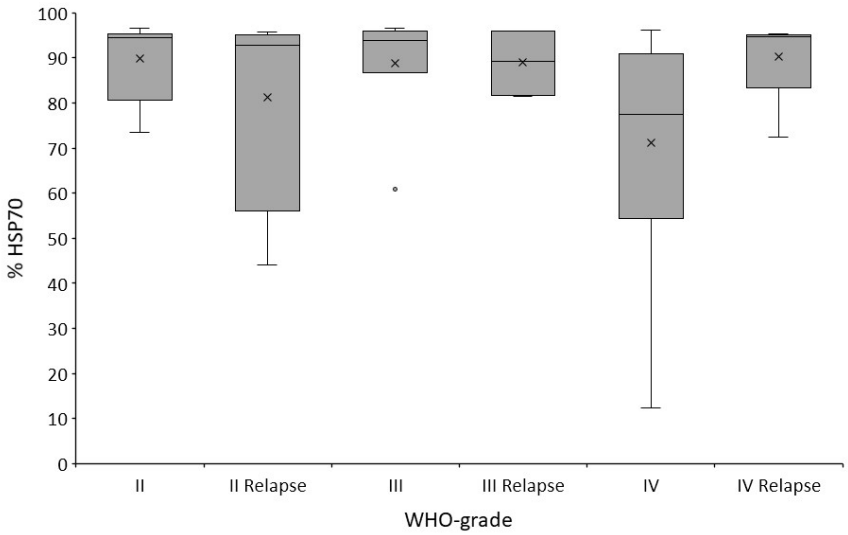

Figure 11: Hsp70 in % vs. WHO-grade in initially diagnosed vs. relapse patients

When comparing the mean percentages of first-diagnosed and relapsed patients with tumors of the same WHO-grade, no specific trend was observed.

First-diagnosed WHO-grade II tumors (n = 7) had slightly higher mean percentages than recurrent WHO-grade II tumors (n = 4), but the difference was not statistically significant due to the high standard deviation (initial diagnosis vs. relapse: 89.94 ± 8.39 % vs. 81.31 ± 21.53 %, n.s.).

First-diagnosed WHO-grade III tumors (n = 7) showed almost the same values as relapsed WHO-grade III tumors (n = 4) (initial diagnosis vs. relapse: 88.84 ± 11.80 % vs. 88.93 ±6.97 %, n.s.).

First-diagnosed WHO-grade IV tumors (n = 36) showed lower values than recurrent WHO-grade IV tumors (n = 5), almost reaching statistical significance with p = 0.056 (initial diagnosis vs. relapse: 71.25 ± 21.78 % vs. 90.30 ± 9.00 %, n.s.).

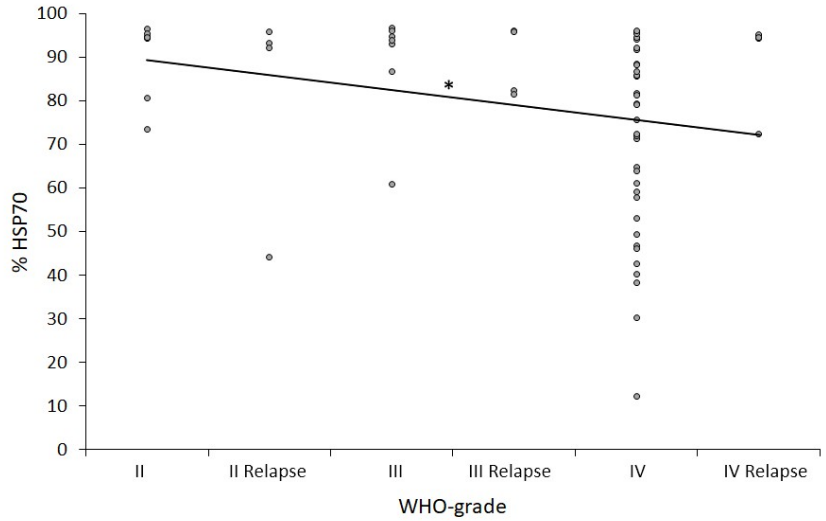

Figure 12: Linear correlation of Hsp70 in % vs. WHO-grade

Figure 12 shows that a significant linear correlation between increasing WHO-grade and decreasing membranous Hsp70 percentages for all patients (n = 63) was observed (r = -0.253, *p ≤ 0.05).

4.2.4 IDH-wildtype vs. -mutant

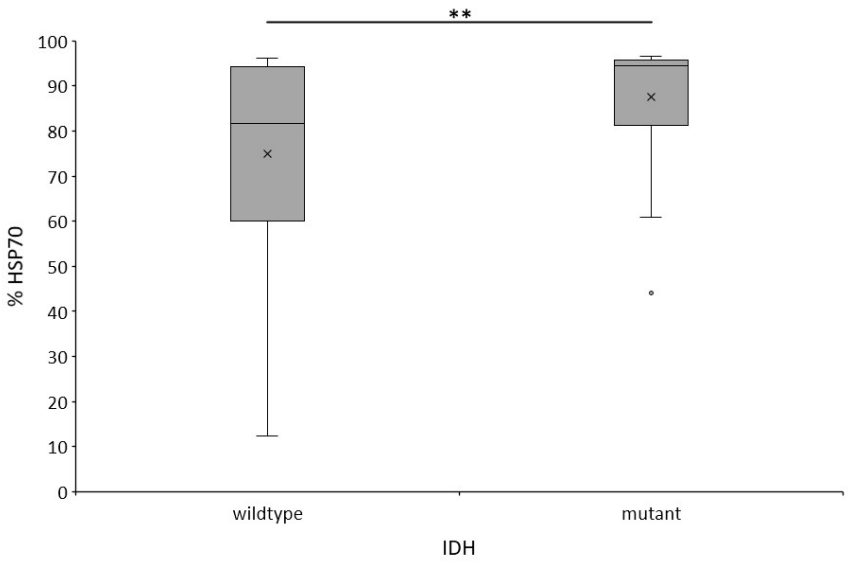

Figure 13: Hsp70 in % vs. IDH status

As shown in Figure 13, a very significant difference in membranous Hsp70 percentages between IDH-wildtype (n = 45) and IDH-mutant (n = 18) tumors was observed (IDH-wildtype vs. IDH-mutant: 74.97 ± 21.12 % vs. 87.51 ± 14.17 %, **p ≤ 0.01).

4.2.5 MGMT promoter unmethylated vs. methylated

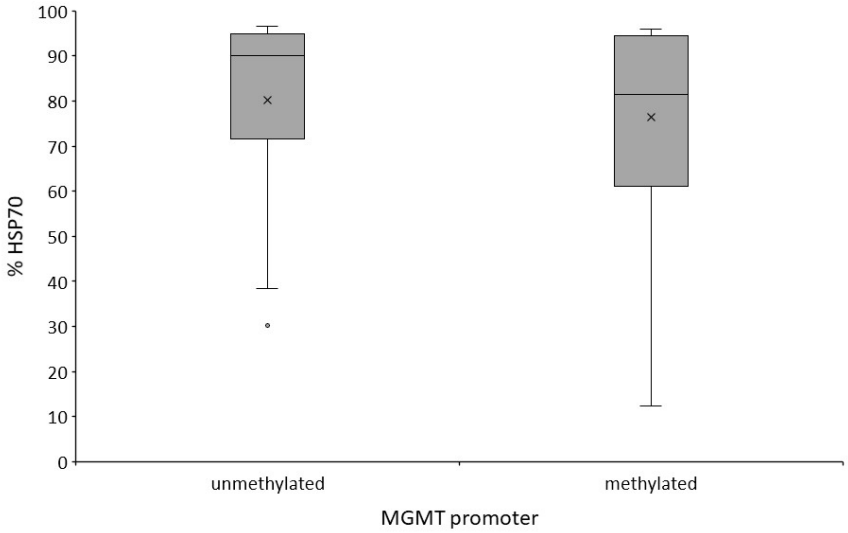

Figure 14: Hsp70 in % vs. MGMT promoter status

There was no significant difference between the percentages of tumors with unmethylated MGMT promoter (n = 36) compared with tumors with methylated MGMT promoter (n = 27) (unmethylated vs. methylated: 80.14 ± 19.71 % vs. 76.45 ± 20.66 %, n.s.).

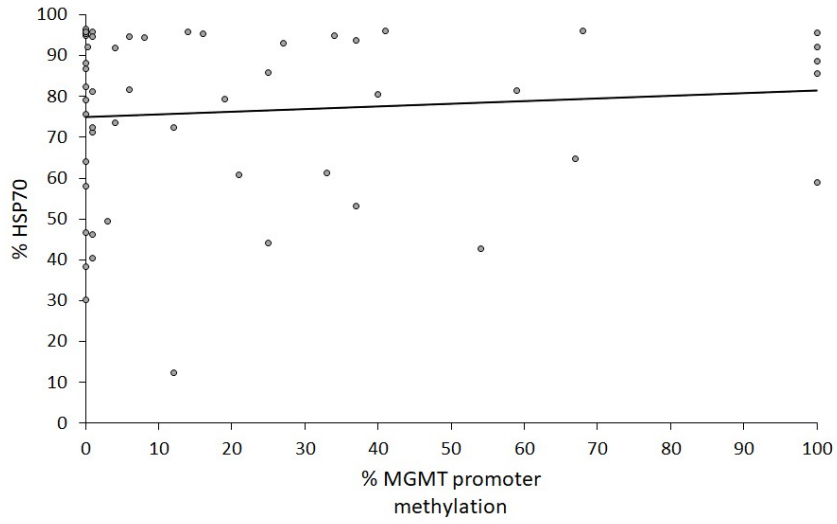

Figure 15: Linear correlation of Hsp70 in % vs. MGMT promoter methylation percentage

When the percentage of membrane-bound Hsp70 was compared with MGMT promoter methylation percentage (n = 54), no significant linear correlation was detected (r = 0.015, n.s.).

4.3 Hsp70 in MFI

4.3.1 Initial diagnosis

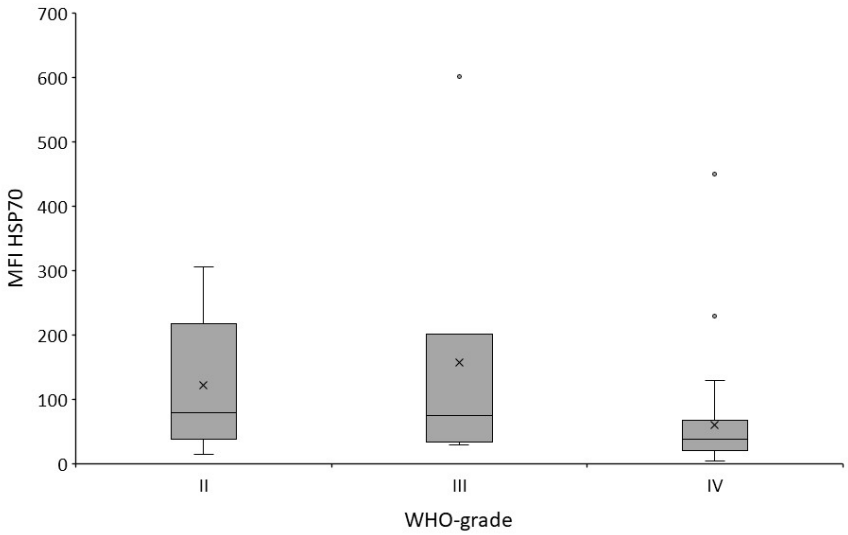

Figure 16: Hsp70 in MFI vs. WHO-grade in initially diagnosed patients

There was not much difference in mean fluorescence intensity between the lower-graded first-diagnosed tumors, but some difference with the highest-graded WHO-grade IV tumor.

The MFI value of the first-diagnosed WHO-grade II tumors (n = 7) was only slightly lower than that of the WHO-grade III tumors (n = 7), with both groups having large standard deviations (WHO-grade II vs. WHO-grade III: 121.50 ± 97.00 vs. 157.65 ± 189.74, n.s.).

The comparison between first-diagnosed WHO-grade II (n = 7) and WHO-grade IV (n = 36) tumors reached near statistical significance at p = 0.052 (WHO-grade II vs. WHO-grade IV: 121.50 ± 97.00 vs. 59.64 ± 78.67, n.s.).

There was also an almost statistically significant difference between the values of first-diagnosed WHO-grade III (n = 7) and WHO-grade IV (n = 26) tumors with p = 0.056 (WHO-grade III vs. WHO-grade IV: 157.65 ± 189.74 vs. 59.64 ± 78.67, n.s.).

4.3.2 Relapse

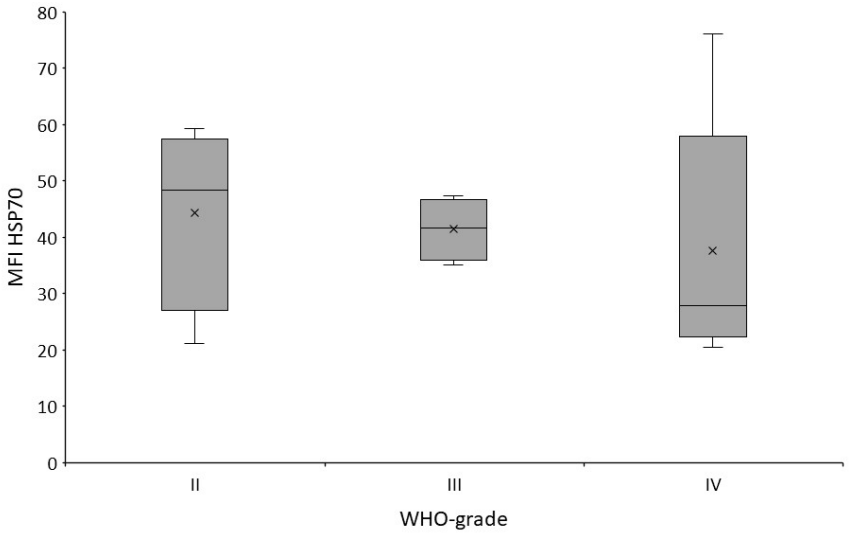

Figure 17: Hsp70 in MFI vs. WHO-grade in relapse patients

No significant difference was observed when comparing the differently graded tumor recurrences.

The mean fluorescence intensity of relapsed WHO-grade II (n = 4) and WHO-grade III (n = 4) tumors did not show much difference (WHO-grade II vs. WHO-grade III: 44.30 ± 14.36 vs. 41.45 ± 4.83, n.s.).

The same was true when comparing relapsed WHO-grade II (n = 4) and WHO-grade IV (n = 5) tumors (WHO-grade II vs. WHO-grade IV: 44.30 ± 14.36 vs. 37.64 ± 20.30, n.s.).

There was also no significant difference between the values of relapsed WHO-grade III (n = 4) and WHO-grade IV (n = 5) tumors (WHO-grade III vs. WHO-grade IV: 41.45 ± 4.83 vs. 37.64 ± 20.30, n.s.).

4.3.3 Initial diagnosis vs. Relapse

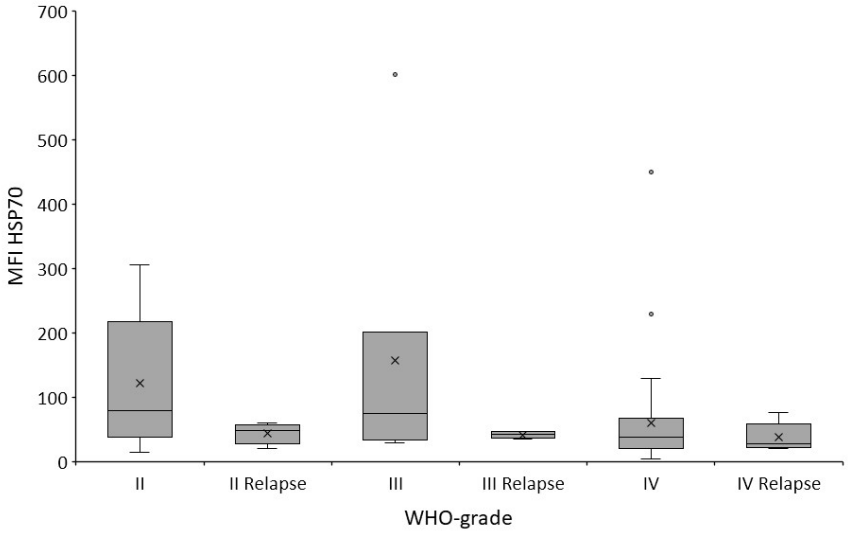

Figure 18: Hsp70 in MFI vs. WHO-grade in initially diagnosed vs. relapse patients

Comparison of the mean fluorescence intensity values of first-diagnosed and relapsed tumors of the same WHO-grade showed that the relapses had lower mean values than first-diagnosed tumors, but without statistical significance.

First-diagnosed WHO-grade II tumors (n = 7) had higher mean values than relapsed WHO-grade II tumors (n = 4), but they did not reach statistical significance due to the large standard deviations (initial diagnosis vs. relapse: 121.50 ± 97.00 vs. 44.30 ± 14.36, n.s.).

The same was true when comparing initially diagnosed WHO-grade III (n = 7) and relapsed WHO-grade III (n = 4) tumors (initial diagnosis vs. relapse: 157.65 ± 189.74 vs. 41.45 ± 4.83, n.s.).

Even a smaller difference was found when comparing initially diagnosed WHO-grade IV (n = 36) and relapsed WHO-grade IV (n = 5) tumors (initial diagnosis vs. relapse: 59.64 ± 78.67 vs. 37.64 ± 20.30, n.s.).

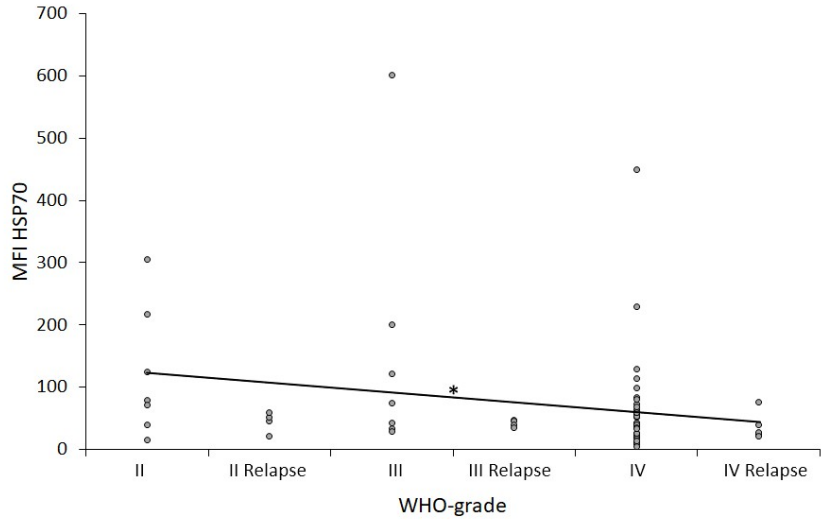

Figure 19: Linear correlation of Hsp70 in MFI vs. WHO-grade

Despite the large standard deviations, a statistically significant linear correlation was found between higher WHO-grade and lower MFI values over all patients (n = 63), shown in Figure 19 ($r = -0.304$, $^*p \leq 0.05$).

4.3.4 IDH-wildtype vs. -mutant

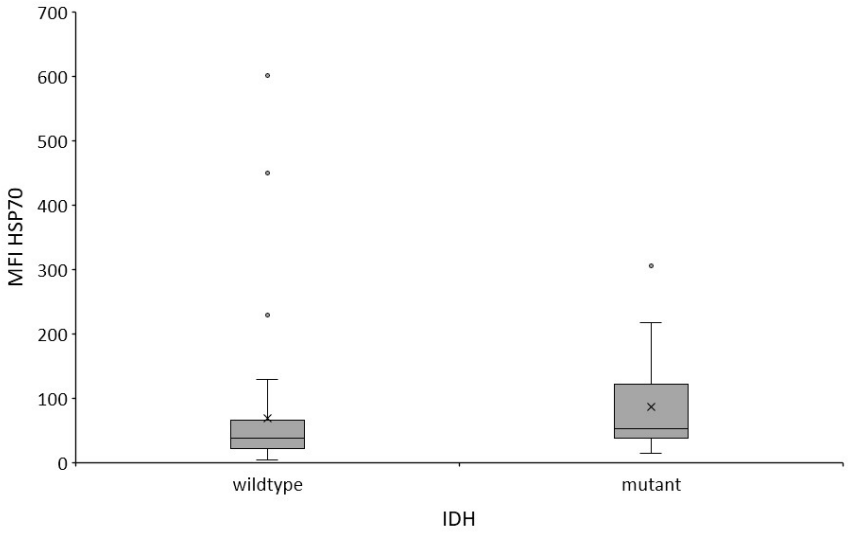

Figure 20: Hsp70 in MFI vs. IDH status

The difference between MFI values of IDH-wildtype (n = 45) and IDH-mutant (n = 18) tumors almost reached statistical significance with $p = 0.055$ (IDH-wildtype vs. IDH-mutant: 68.18 ± 107.32 vs. 86.90 ± 77.27, n.s.).

4.3.5 MGMT promoter unmethylated vs. methylated

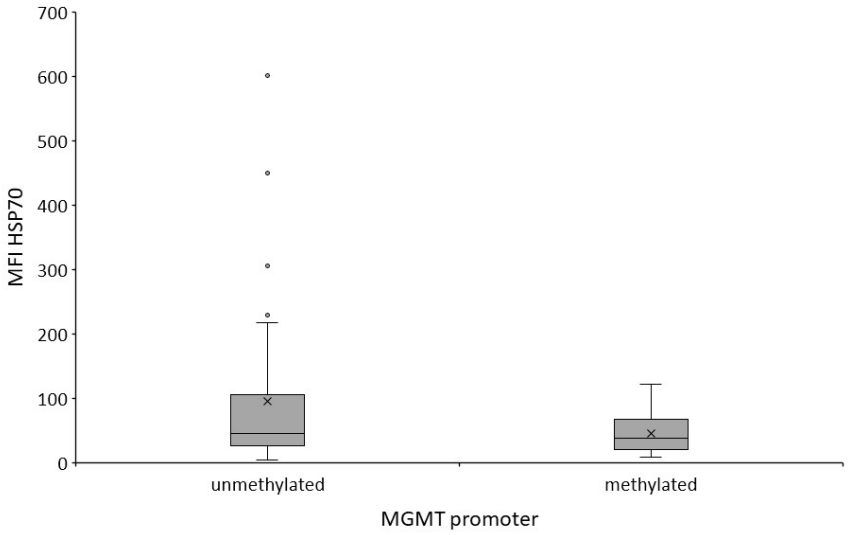

Figure 21: Hsp70 in MFI vs. MGMT promoter status

There was no statistically significant difference between MFI values of tumors with unmethylated MGMT promoter (n = 36) and those with methylated MGMT promoter (n = 27) (unmethylated vs. methylated: 94.76 ± 125.95 vs. 42.17 ± 29.31, n.s.).

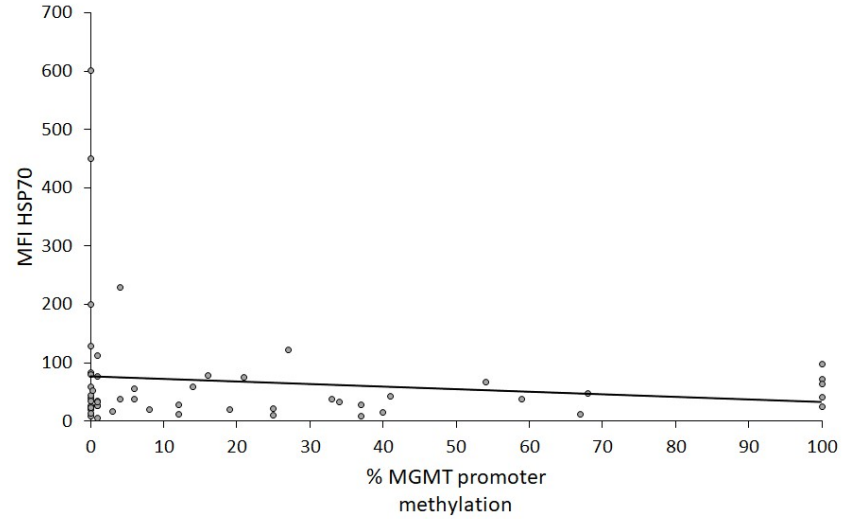

Figure 22: Linear correlation of Hsp70 in MFI vs. MGMT promoter methylation percentage

When the MFI value of membrane-bound Hsp70 was compared with the MGMT promoter methylation percentage (n = 54), no significant linear correlation was found (r = -0.073, n.s.).

4.4 Hsp70 in MFI vs. Hsp70 in %

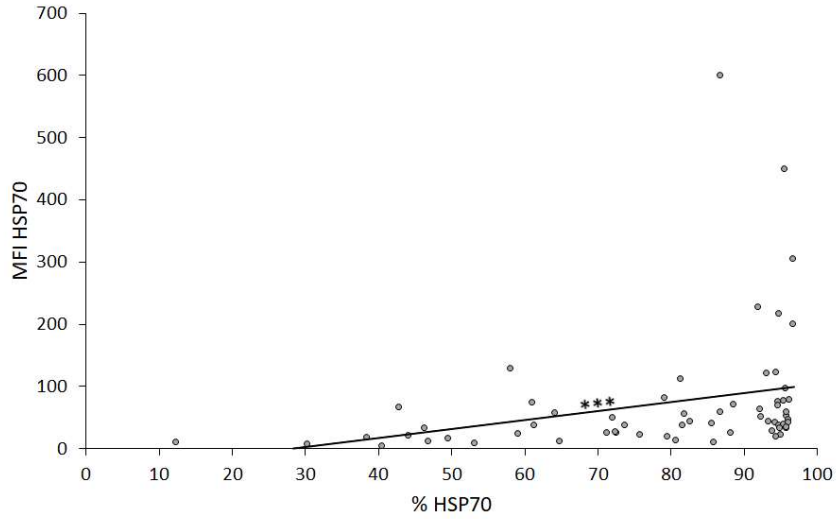

Figure 23: Linear correlation of Hsp70 in MFI vs. Hsp70 in %

Comparison of membrane-bound Hsp70 percentage with MFI values (n = 63) revealed a highly significant linear correlation (r = 0.503, ***p ≤ 0.001).

5 Discussion

Tumor cells isolated from 63 biopsies of patients with malignant gliomas were available for flow cytometry and further analysis. They were divided into different groups regarding the histopathological diagnosis and depending on primary occurrence or recurrence. Not included were 78 patients from the original set of 141 patients because either of lack of biopsy material, flow cytometry originally conducted with different settings or too little event counts in the defined gate for FACS analysis.

A general problem of the used methodic was the heterogeneity of the cell suspensions. Other studies examining membrane expression of heat shock proteins in tumors often used homogenous culture cell lines, the single cell suspensions acquired by processing primary human material contained other cells besides the tumor cells, e.g. leukocytes or endothelial cells. (Frelinger et al. 2010) Despite the use of leukocyte-specific antibodies (anti-CD45) and suitable negative controls, the differentiation between tumor and normal tissue was not always possible.

5.1 Age

The linear correlation of increasing age with more malignant histopathological grading (Figure 5) is in line with the statistically significant differences in mean age of the individual groups (Figure 2, Figure 3) and represents the observations in the literature of lower-graded gliomas occurring more frequently in younger patients, whereas higher-graded gliomas occur more frequently in older patients. (Davis et al. 1998; Fleury et al. 1997; Chakrabarti et al. 2005)

A slightly higher mean age in relapse groups compared to initially diagnosed groups also represents what is to be expected from the literature with frequent recurrence of the disease within one year after initial therapy in glioblastoma patients. (Franceschi et al. 2012)

5.2 IDH status

The mutation of the isocitrate dehydrogenase (IDH) enzymes was first identified in 2008 by Parsons et al. in 12% of glioblastoma patients (Parsons et al. 2008) and later postulated as defining a clinically relevant subtype of glioblastoma. (Verhaak et al. 2010) It has been assumed to be the initiating cause in the development of many gliomas. (Juratli et al. 2012b;

Watanabe et al. 2009) Furthermore, mutations in IDH1 and IDH2 genes have been linked to favorable outcome in these tumors by many authors (Juratli et al. 2012a; Yan et al. 2009; Parsons et al. 2008; Kizilbash et al. 2014; Hartmann et al. 2010; Combs et al. 2011; Houillier et al. 2010), with a median overall survival of 65 months for patients with IDH mutation and 20 months for patients with IDH-wildtype astrocytoma. (Yan et al. 2009)

The mutation of the IDH1 gene was found more frequently in younger patients (Parsons et al. 2008; Nobusawa et al. 2009; Sanson et al. 2009) and also most commonly in low-grade gliomas and secondary glioblastomas, but only in about 5% of primary glioblastomas. (Leu et al. 2013; Hartmann et al. 2009; Balss et al. 2008; Ichimura et al. 2009; Parsons et al. 2008; Watanabe et al. 2009; Yan et al. 2009)

These findings are reinforced in this study, as patients with IDH-mutant status are highly significantly younger than IDH-wildtype patients (Figure 6). Furthermore, most of the IDH-mutant cases in this study are lower graded gliomas, there were no appearances of IDH mutation in the highest graded WHO-grade IV glioblastoma or relapses thereof. Also, the other way around, most of the lower graded gliomas show IDH-mutation (81.82% of WHO-grade II and III gliomas, n = 22).

The question remains whether the correlation between age and IDH status is of actual significance considering the spectrum of gliomas WHO-grade II-IV or whether it is just a recursive sign of the fact that IDH mutation is less common in higher graded gliomas and, as mentioned before, the trend for older patients bearing higher-graded gliomas is known and described in the literature (Davis et al. 1998; Fleury et al. 1997; Chakrabarti et al. 2005) and is also represented in the results of this study. We could not find a significant difference in age between IDH-wildtype and IDH-mutant cases when each WHO-grade (II, II Relapse, III, III Relapse, IV, IV Relapse) was considered individually (not shown). However, due to the composition of the study collective, a comparison of the IDH statuses of the individual groups was not always possible as there is not one IDH-mutant case in the WHO-grade IV group, on the other hand over 80% of the lower graded gliomas are IDH-mutant.

As mentioned above, mutations in IDH genes have been linked to favorable outcome. Therefore, a correlation between IDH status and membrane-bound Hsp70 levels could potentially denote Hsp70 as prognostic marker on the surface of glioma cells. To date,

there are no publications known to the author comparing these two parameters, which makes it difficult to categorize the results of this study.

As it turns out, we were able to identify a very significant difference between the membranous Hsp70 levels (in %) of IDH-wildtype and IDH-mutant cases, considering all 63 patients (Figure 13), with higher Hsp70 percentage values in IDH-mutant cases. The examination of the mean fluorescence intensity values revealed a trend in the same direction, barely missing the significance threshold due to high standard deviations (Figure 20).

Considering each WHO-grade individually, there was no significant difference between the membranous Hsp70 levels (in %) of the two IDH statuses (not shown). However, as mentioned above, the composition of the study collective complicates the comparison of the IDH statuses of the individual groups. Hopefully, future studies can address this question with a more suited patient collective.

5.3 MGMT promoter methylation status

Since Hegi et al. (2005) linked the downregulation or silencing of MGMT to prolonged overall survival in glioblastoma patients (Hegi et al. 2005), it has newly been affirmed in a meta-analysis that there is a very promising significant association of MGMT promoter methylation with better overall and progression free survival. (Binabaj et al. 2018)

MGMT is a ubiquitously expressed protein in human tissue while being overexpressed in some gliomas. (Esteller et al. 1999) The protecting effect of MGMT is due to the encoded DNA-repair protein inhibiting the effect of alkylating chemotherapeutic agents as temozolomide (TMZ) by removing alkyl groups from guanine, where TMZ would normally target. Therefore, epigenetic silencing or downregulation of MGMT by promoter methylation may suppress this protecting mechanism and enhance the effectivity of chemotherapy with alkylating agents such as TMZ (Binabaj et al. 2018), and in turn also radiotherapy (Narayana et al. 2012), improving overall survival by up to 15 months. (Vredenburgh et al. 2012)

In our study, 27 of 63 cases (42.9%) were diagnosed with methylated MGMT promoter, 36 (57.1%) with unmethylated MGMT promoter, the median age was 57.0 years. This is a lower percentage of methylated MGMT promoter cases than in the literature, however,

the average patients' age also tended to be higher in reported cases: 59.1% methylated cases (n = 22), median age >80 years (Piccirilli et al. 2006), 57.5% (n = 64), median age 74 years (Gerstner et al. 2009), 50.6% (n = 83), median age 73.2 years. (Minniti et al. 2011)

Minniti et al. also mentioned a slight trend towards higher methylation percentages in elderly patients compared to younger patients (Minniti et al. 2011), referring to the 44.7% (n = 206) of methylated cases in the original study by Hegi et al. (Hegi et al. 2005) This is in contradiction with the findings of Leu et al., positively associating MGMT promoter methylation with IDH mutation (Leu et al. 2013) which, as mentioned above, was found more frequently in younger patients. (Parsons et al. 2008; Nobusawa et al. 2009; Sanson et al. 2009)

Therefore, a more in-depth look at the age and MGMT promoter status of our patients group seemed verified. However, there appeared to be no correlation between the patients' age when the biopsy was taken and the MGMT promoter methylation status (Figure 7). As most of the literature dealt with glioblastoma patients, the results were also assessed for the individual subgroups divided according to the WHO-grade (Table 5), delivering very divergent results for GBM, only 36.1% (n = 36) had a methylated MGMT promoter in a patient group with a median age of 63 years. With the additional information of methylation percentage in most cases (n = 57) provided, it was also interesting to test for correlation between age and methylation percentage. However, as Figure 8 shows, there is no linear correlation present. Concluding, there appears to be no trend towards more MGMT methylated cases in older or younger patients in our study.

Since the MGMT promoter status is considered, similar to the IDH status, a promising prognostic marker for better overall and progression free survival (Binabaj et al. 2018), a correlation with Hsp70 levels on the cell surface could possibly determine membranous Hsp70 as a prognostic marker itself. However, we could not detect any significant correlation, neither for percentage Hsp70 values (Figure 14) nor for MFI values (Figure 21). This was also true for the comparison with the exact MGMT promoter methylation percentage value in both cases (Figure 15, Figure 22).

As this is only a small excerpt of patients, the results cannot be transferred to every patient, more so future studies should try and assess larger quantities of patients regarding this topic.

6 Summary

Introduction: Hsp70 is a ubiquitously expressed protein with significance for protein folding, degradation, and cell cycle regulation. Various tumors express Hsp70 on the cell membrane, the amount in some cases correlating with the malignancy. Gliomas are the most common primary brain tumors in adults. They are differentiated into WHO-grade I to IV. Herein, we compared the Hsp70 levels on the cell surface of gliomas grade II-IV.

Methods: Tumor tissue was collected from sixty-three adult patients. The material was prepared for cell sampling. A novel monoclonal antibody (cmHsp70.1 mAb) was used in flow cytometry to determine the membrane-bound Hsp70.

Results: In our very limited patient population, initially diagnosed WHO-grade II (n = 7) and III (n = 7) tumors had significantly higher membrane-bound Hsp70 percentages than WHO-grade IV (n = 36) tumors. Tumors with mutated IDH (IDH-mutant) (n = 18) showed significantly higher membrane-bound Hsp70 percentages than IDH-wildtype (n = 45) tumors. Other comparisons did not yield significant differences.

Conclusion: This study could not support previous publications with the proposition of increased membrane-bound Hsp70 levels in tumors of higher malignancy. On the contrary, lower-graded gliomas showed higher levels of membrane-bound Hsp70. However, the validity of these results is uncertain since the sample size, especially of lower-graded gliomas, was very small.

7 Bibliography

Baan, Robert; Grosse, Yann; Lauby-Secretan, Béatrice; El Ghissassi, Fatiha; Bouvard, Véronique; Benbrahim-Tallaa, Lamia et al. (2011): Carcinogenicity of radiofrequency electromagnetic fields. In: *The Lancet Oncology* 12 (7), S. 624–626. DOI: 10.1016/S1470-2045(11)70147-4.

Balss, Jörg; Meyer, Jochen; Mueller, Wolf; Korshunov, Andrey; Hartmann, Christian; Deimling, Andreas von (2008): Analysis of the IDH1 codon 132 mutation in brain tumors. In: *Acta neuropathologica* 116 (6), S. 597–602. DOI: 10.1007/s00401-008-0455-2.

Bartelink, H.; Schellens, J.H.M; Verheij, M. (2002): The combined use of radiotherapy and chemotherapy in the treatment of solid tumours. In: *European journal of cancer (Oxford, England : 1990)* 38 (2), S. 216–222. DOI: 10.1016/s0959-8049(01)00363-x.

Basu, S.; Binder, R. J.; Suto, R.; Anderson, K. M.; Srivastava, P. K. (2000): Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. In: *International immunology* 12 (11), S. 1539–1546. DOI: 10.1093/intimm/12.11.1539.

Baumert, Brigitta G.; Hegi, Monika E.; van den Bent, Martin J.; Deimling, Andreas von; Gorlia, Thierry; Hoang-Xuan, Khê et al. (2016): Temozolomide chemotherapy versus radiotherapy in high-risk low-grade glioma (EORTC 22033-26033): a randomised, open-label, phase 3 intergroup study. In: *The Lancet Oncology* 17 (11), S. 1521–1532. DOI: 10.1016/S1470-2045(16)30313-8.

Beaman, Glenda Maria; Dennison, Sarah R.; Chatfield, Lee K.; Phoenix, David A. (2014): Reliability of HSP70 (HSPA) expression as a prognostic marker in glioma. In: *Molecular and cellular biochemistry* 393 (1-2), S. 301–307. DOI: 10.1007/s11010-014-2074-7.

Benson, Victoria S.; Pirie, Kirstin; Schüz, Joachim; Reeves, Gillian K.; Beral, Valerie; Green, Jane (2013): Mobile phone use and risk of brain neoplasms and other cancers: prospective study. In: *International journal of epidemiology* 42 (3), S. 792–802. DOI: 10.1093/ije/dyt072.

Binabaj, Maryam Moradi; Bahrami, Afsane; ShahidSales, Soodabeh; Joodi, Marjan; Joudi Mashhad, Mona; Hassanian, Seyed Mahdi et al. (2018): The prognostic value of MGMT promoter methylation in glioblastoma: A meta-analysis of clinical trials. In: *Journal of cellular physiology* 233 (1), S. 378–386. DOI: 10.1002/jcp.25896.

Binder, R. J.; Blachere, N. E.; Srivastava, P. K. (2001): Heat shock protein-chaperoned peptides but not free peptides introduced into the cytosol are presented efficiently by major histocompatibility complex I molecules. In: *The Journal of biological chemistry* 276 (20), S. 17163–17171. DOI: 10.1074/jbc.M011547200.

Botzler, C.; Issels, R.; Multhoff, G. (1996): Heat-shock protein 72 cell-surface expression on human lung carcinoma cells in associated with an increased sensitivity to lysis mediated by adherent natural killer cells. In: *Cancer immunology, immunotherapy : CII* 43 (4), S. 226–230. DOI: 10.1007/s002620050326.

Botzler, Claus; Ellwart, Joachim; Günther, Wolfgang; Eißner, Günther; Multhoff, Gabriele (1999): Synergistic effects of heat and ET-18-OCH3 on membrane expression of hsp70

and lysis of leukemic K562 cells. In: *Experimental hematology* 27 (3), S. 470–478. DOI: 10.1016/s0301-472x(98)00055-1.

Cairncross, Gregory; Wang, Meihua; Shaw, Edward; Jenkins, Robert; Brachman, David; Buckner, Jan et al. (2013): Phase III trial of chemoradiotherapy for anaplastic oligodendroglioma: long-term results of RTOG 9402. In: *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 31 (3), S. 337–343. DOI: 10.1200/JCO.2012.43.2674.

Capper, David; Jones, David T. W.; Sill, Martin; Hovestadt, Volker; Schrimpf, Daniel; Sturm, Dominik et al. (2018): DNA methylation-based classification of central nervous system tumours. In: *Nature* 555 (7697), S. 469–474. DOI: 10.1038/nature26000.

Ceccarelli, Michele; Barthel, Floris P.; Malta, Tathiane M.; Sabedot, Thais S.; Salama, Sofie R.; Murray, Bradley A. et al. (2016): Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. In: *Cell* 164 (3), S. 550–563. DOI: 10.1016/j.cell.2015.12.028.

Chakrabarti, Indro; Cockburn, Myles; Cozen, Wendy; Wang, Ya-Ping; Preston-Martin, Susan (2005): A population-based description of glioblastoma multiforme in Los Angeles County, 1974-1999. In: *Cancer* 104 (12), S. 2798–2806. DOI: 10.1002/cncr.21539.

Chinot, Olivier L.; Wick, Wolfgang; Mason, Warren; Henriksson, Roger; Saran, Frank; Nishikawa, Ryo et al. (2014): Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. In: *The New England journal of medicine* 370 (8), S. 709–722. DOI: 10.1056/NEJMoa1308345.

Combs, Stephanie E.; Rieken, Stefan; Wick, Wolfgang; Abdollahi, Amir; Deimling, Andreas von; Debus, Jürgen; Hartmann, Christian (2011): Prognostic significance of IDH-1 and MGMT in patients with glioblastoma: one step forward, and one step back? In: *Radiation oncology (London, England)* 6, S. 115. DOI: 10.1186/1748-717X-6-115.

Csermely, P. (2001): A nonconventional role of molecular chaperones: involvement in the cytoarchitecture. In: *News in physiological sciences : an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society* 16, S. 123–126. DOI: 10.1152/physiologyonline.2001.16.3.123.

Davis, F. G.; Freels, S.; Grutsch, J.; Barlas, S.; Brem, S. (1998): Survival rates in patients with primary malignant brain tumors stratified by patient age and tumor histological type: an analysis based on Surveillance, Epidemiology, and End Results (SEER) data, 1973-1991. In: *Journal of neurosurgery* 88 (1), S. 1–10. DOI: 10.3171/jns.1998.88.1.0001.

Esteller, M.; Hamilton, S. R.; Burger, P. C.; Baylin, S. B.; Herman, J. G. (1999): Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. In: *Cancer research* 59 (4), S. 793–797.

Feige, U.; Polla, B. S. (1994): Hsp70--a multi-gene, multi-structure, multi-function family with potential clinical applications. In: *Experientia* 50 (11-12), S. 979–986. DOI: 10.1007/bf01923452.

Fleury, Agns; Menegoz, Franois; Grosclaude, Pascale; Daures, Jean-Pierre; Henry-Amar, Michel; Raverdy, Nicole et al. (1997): Descriptive epidemiology of cerebral gliomas in

France. In: *Cancer* 79 (6), S. 1195–1202. DOI: 10.1002/(sici)1097-0142(19970315)79:6<1195::aid-cncr19>3.0.co;2-v.

Franceschi, Enrico; Stupp, Roger; van den Bent, Martin J.; van Herpen, Carla; Laigle Donadey, Florence; Gorlia, Thierry et al. (2012): EORTC 26083 phase I/II trial of dasatinib in combination with CCNU in patients with recurrent glioblastoma. In: *Neuro-oncology* 14 (12), S. 1503–1510. DOI: 10.1093/neuonc/nos256.

Frelinger, Jacob; Ottinger, Janet; Gouttefangeas, Cécile; Chan, Cliburn (2010): Modeling flow cytometry data for cancer vaccine immune monitoring. In: *Cancer immunology, immunotherapy : CII* 59 (9), S. 1435–1441. DOI: 10.1007/s00262-010-0883-4.

Gabai, Vladimir L.; Meriin, Anatoli B.; Yaglom, Julia A.; Volloch, Vladimir Z.; Sherman, Michael Y. (1998): Role of Hsp70 in regulation of stress-kinase JNK: implications in apoptosis and aging. In: *FEBS letters* 438 (1-2), S. 1–4. DOI: 10.1016/s0014-5793(98)01242-3.

Gehrmann, M.; Marienhagen, J.; Eichholtz-Wirth, H.; Fritz, E.; Ellwart, J.; Jäättelä, M. et al. (2005): Dual function of membrane-bound heat shock protein 70 (Hsp70), Bag-4, and Hsp40: protection against radiation-induced effects and target structure for natural killer cells. In: *Cell death and differentiation* 12 (1), S. 38–51. DOI: 10.1038/sj.cdd.4401510.

Gehrmann, Mathias; Liebisch, Gerhard; Schmitz, Gerd; Anderson, Robin; Steinem, Claudia; Maio, Antonio de et al. (2008): Tumor-specific Hsp70 plasma membrane localization is enabled by the glycosphingolipid Gb3. In: *PloS one* 3 (4), e1925. DOI: 10.1371/journal.pone.0001925.

Gerstner, E. R.; Yip, S.; Wang, D. L.; Louis, D. N.; Iafrate, A. J.; Batchelor, T. T. (2009): Mgmt methylation is a prognostic biomarker in elderly patients with newly diagnosed glioblastoma. In: *Neurology* 73 (18), S. 1509–1510. DOI: 10.1212/WNL.0b013e3181bf9907.

Gilbert, Mark R.; Dignam, James J.; Armstrong, Terri S.; Wefel, Jeffrey S.; Blumenthal, Deborah T.; Vogelbaum, Michael A. et al. (2014): A randomized trial of bevacizumab for newly diagnosed glioblastoma. In: *The New England journal of medicine* 370 (8), S. 699–708. DOI: 10.1056/NEJMoa1308573.

Gilbert, Mark R.; Wang, Meihua; Aldape, Kenneth D.; Stupp, Roger; Hegi, Monika E.; Jaeckle, Kurt A. et al. (2013): Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. In: *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 31 (32), S. 4085–4091. DOI: 10.1200/JCO.2013.49.6968.

Goodenberger, McKinsey L.; Jenkins, Robert B. (2012): Genetics of adult glioma. In: *Cancer genetics* 205 (12), S. 613–621. DOI: 10.1016/j.cancergen.2012.10.009.

Grosu, Anca-Ligia; Astner, Sabrina T.; Riedel, Eva; Nieder, Carsten; Wiedenmann, Nicole; Heinemann, Felix et al. (2011): An interindividual comparison of O-(2-18Ffluoroethyl)-L-tyrosine (FET)- and L-methyl-11Cmethionine (MET)-PET in patients with brain gliomas and metastases. In: *International journal of radiation oncology, biology, physics* 81 (4), S. 1049–1058. DOI: 10.1016/j.ijrobp.2010.07.002.

Günther, E.; Walter, L. (1994): Genetic aspects of the hsp70 multigene family in vertebrates. In: *Experientia* 50 (11-12), S. 987–1001. DOI: 10.1007/bf01923453.

Hartl, F. Ulrich; Hayer-Hartl, Manajit (2002): Molecular chaperones in the cytosol: from nascent chain to folded protein. In: *Science (New York, N.Y.)* 295 (5561), S. 1852–1858. DOI: 10.1126/science.1068408.

Hartmann, Christian; Hentschel, Bettina; Wick, Wolfgang; Capper, David; Felsberg, Jörg; Simon, Matthias et al. (2010): Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. In: *Acta neuropathologica* 120 (6), S. 707–718. DOI: 10.1007/s00401-010-0781-z.

Hartmann, Christian; Meyer, Jochen; Balss, Jörg; Capper, David; Mueller, Wolf; Christians, Arne 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. In: *Acta neuropathologica* 118 (4), S. 469–474. DOI: 10.1007/s00401-009-0561-9.

Hegi, Monika E.; Diserens, Annie-Claire; Gorlia, Thierry; Hamou, Marie-France; Tribolet, Nicolas de; Weller, Michael et al. (2005): MGMT gene silencing and benefit from temozolomide in glioblastoma. In: *The New England journal of medicine* 352 (10), S. 997–1003. DOI: 10.1056/NEJMoa043331.

Helmbrecht, K.; Zeise, E.; Rensing, L. (2000): Chaperones in cell cycle regulation and mitogenic signal transduction: a review. In: *Cell proliferation* 33 (6), S. 341–365. DOI: 10.1046/j.1365-2184.2000.00189.x.

Hennequin, C.; Favaudon, V. (2002): Biological basis for chemo-radiotherapy interactions. In: *European journal of cancer (Oxford, England : 1990)* 38 (2), S. 223–230. DOI: 10.1016/s0959-8049(01)00360-4.

Hermisson, M.; Strik, H.; Rieger, J.; Dichgans, J.; Meyermann, R.; Weller, M. (2000): Expression and functional activity of heat shock proteins in human glioblastoma multiforme. In: *Neurology* 54 (6), S. 1357–1365. DOI: 10.1212/wnl.54.6.1357.

Herrlinger, Ulrich; Tzaridis, Theophilos; Mack, Frederic; Steinbach, Joachim Peter; Schlegel, Uwe; Sabel, Michael et al. (2019): Lomustine-temozolomide combination therapy versus standard temozolomide therapy in patients with newly diagnosed glioblastoma with methylated MGMT promoter (CeTeG/NOA–09): a randomised, open-label, phase 3 trial. In: *The Lancet* 393 (10172), S. 678–688. DOI: 10.1016/S0140-6736(18)31791-4.

Hofer et al. (2020): Gliome im Erwachsenenalter. Hg. v. DGHO Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie e.V. Online verfügbar unter www.onkopedia.com, zuletzt geprüft am 23.03.2020.

Höhfeld, J.; Cyr, D. M.; Patterson, C. (2001): From the cradle to the grave: molecular chaperones that may choose between folding and degradation. In: *EMBO reports* 2 (10), S. 885–890. DOI: 10.1093/embo-reports/kve206.

Höhfeld, J.; Jentsch, S. (1997): GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1. In: *The EMBO journal* 16 (20), S. 6209–6216. DOI: 10.1093/emboj/16.20.6209.

Houillier, C.; Wang, X.; Kaloshi, G.; Mokhtari, K.; Guillevin, R.; Laffaire, J. et al. (2010): IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. In: *Neurology* 75 (17), S. 1560–1566. DOI: 10.1212/WNL.0b013e3181f96282.

How a Flow Cytometer Works | Thermo Fisher Scientific - DE (2020). Online verfügbar unter https://www.thermofisher.com/de/de/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/flow-cytometry-basics/flow-cytometry-fundamentals/how-flow-cytometer-works.html, zuletzt aktualisiert am 27.03.2020, zuletzt geprüft am 27.03.2020.

Ichimura, Koichi; Pearson, Danita M.; Kocialkowski, Sylvia; Bäcklund, L. Magnus; Chan, Raymond; Jones, David T. W.; Collins, V. Peter (2009): IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. In: *Neuro-oncology* 11 (4), S. 341–347. DOI: 10.1215/15228517-2009-025.

Ishii, T.; Udono, H.; Yamano, T.; Ohta, H.; Uenaka, A.; Ono, T. et al. (1999): Isolation of MHC class I-restricted tumor antigen peptide and its precursors associated with heat shock proteins hsp70, hsp90, and gp96. In: *Journal of immunology (Baltimore, Md. : 1950)* 162 (3), S. 1303–1309.

Jäättelä, M. (1999): Escaping cell death: survival proteins in cancer. In: *Experimental cell research* 248 (1), S. 30–43. DOI: 10.1006/excr.1999.4455.

Jäättelä, M.; Wissing, D.; Kokholm, K.; Kallunki, T.; Egeblad, M. (1998): Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases. In: *The EMBO journal* 17 (21), S. 6124–6134. DOI: 10.1093/emboj/17.21.6124.

Juratli, T. A.; Kirsch, M.; Geiger, K.; Klink, B.; Leipnitz, E.; Pinzer, T. et al. (2012a): The prognostic value of IDH mutations and MGMT promoter status in secondary high-grade gliomas. In: *Journal of neuro-oncology* 110 (3), S. 325–333. DOI: 10.1007/s11060-012-0977-2.

Juratli, Tareq A.; Kirsch, Matthias; Robel, Katja; Soucek, Silke; Geiger, Kathrin; Kummer, Rüdiger von et al. (2012b): IDH mutations as an early and consistent marker in low-grade astrocytomas WHO grade II and their consecutive secondary high-grade gliomas. In: *Journal of neuro-oncology* 108 (3), S. 403–410. DOI: 10.1007/s11060-012-0844-1.

Kampinga, Harm H.; Hageman, Jurre; Vos, Michel J.; Kubota, Hiroshi; Tanguay, Robert M.; Bruford, Elspeth A. et al. (2009): Guidelines for the nomenclature of the human heat shock proteins. In: *Cell Stress & Chaperones* 14 (1), S. 105–111. DOI: 10.1007/s12192-008-0068-7.

Kiang, J. (1998): Heat Shock Protein 70 kDa Molecular Biology, Biochemistry, and Physiology. In: *Pharmacology & therapeutics* 80 (2), S. 183–201. DOI: 10.1016/s0163-7258(98)00028-x.

Kizilbash, Sani H.; Giannini, Caterina; Voss, Jesse S.; Decker, Paul A.; Jenkins, Robert B.; Hardie, John et al. (2014): The impact of concurrent temozolomide with adjuvant radiation and IDH mutation status among patients with anaplastic astrocytoma. In: *Journal of neuro-oncology* 120 (1), S. 85–93. DOI: 10.1007/s11060-014-1520-4.

Lee, HoJoon; Palm, Jennifer; Grimes, Susan M.; Ji, Hanlee P. (2015): The Cancer Genome Atlas Clinical Explorer: a web and mobile interface for identifying clinical-genomic driver associations. In: *Genome medicine* 7, S. 112. DOI: 10.1186/s13073-015-0226-3.

Lee, Su-Jae; Choi, Sun-Ah; Lee, Kang-Hyun; Chung, Hee-Yong; Kim, Tae-Hwan; Cho, Chul-Koo; Lee, Yun-Sil (2001): Role of inducible heat shock protein 70 in radiation-induced cell death. In: *Cell Stress & Chaperones* 6 (3), S. 273. DOI: 10.1379/1466-1268(2001)006<0273:roihsp>2.0.co;2.

Leu, Severina; Felten, Stefanie von; Frank, Stephan; Vassella, Erik; Vajtai, Istvan; Taylor, Elisabeth et al. (2013): IDH/MGMT-driven molecular classification of low-grade glioma is a strong predictor for long-term survival. In: *Neuro-oncology* 15 (4), S. 469–479. DOI: 10.1093/neuonc/nos317.

Lobinger, Dominik; Gempt, Jens; Sievert, Wolfgang; Barz, Melanie; Schmitt, Sven; Nguyen, Huyen Thie et al. (2021): Potential Role of Hsp70 and Activated NK Cells for Prediction of Prognosis in Glioblastoma Patients. In: *Frontiers in molecular biosciences* 8, S. 669366. DOI: 10.3389/fmolb.2021.669366.

Louis, David N.; Ohgaki, Hiroko; Wiestler, Otmar D.; Cavenee, Webster K. (Hg.) (2016a): WHO classification of tumours of the central nervous system. International Agency for Research on Cancer; Deutsches Krebsforschungszentrum; Consensus and Editorial Meeting at the German Cancer Research Center. Revised 4th edition. Lyon: International Agency for Research on Cancer (World Health Organization classification of tumours).

Louis, David N.; Perry, Arie; Reifenberger, Guido; Deimling, Andreas von; Figarella-Branger, Dominique; Cavenee, Webster K. et al. (2016b): The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. In: *Acta neuropathologica* 131 (6), S. 803–820. DOI: 10.1007/s00401-016-1545-1.

Lubelski, Daniel; Abdullah, Kalil G.; Weil, Robert J.; Marko, Nicholas F. (2013): Bevacizumab for radiation necrosis following treatment of high grade glioma: a systematic review of the literature. In: *Journal of neuro-oncology* 115 (3), S. 317–322. DOI: 10.1007/s11060-013-1233-0.

Malmström, Annika; Grønberg, Bjørn Henning; Marosi, Christine; Stupp, Roger; Frappaz, Didier; Schultz, Henrik et al. (2012): Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. In: *The Lancet Oncology* 13 (9), S. 916–926. DOI: 10.1016/S1470-2045(12)70265-6.

Malzkorn, Bastian; Reifenberger, Guido (2016): Practical implications of integrated glioma classification according to the World Health Organization classification of tumors of the central nervous system 2016. In: *Current opinion in oncology* 28 (6), S. 494–501. DOI: 10.1097/CCO.0000000000000327.

Mathew, A.; Mathur, S. K.; Morimoto, R. I. (1998): Heat shock response and protein degradation: regulation of HSF2 by the ubiquitin-proteasome pathway. In: *Molecular and cellular biology* 18 (9), S. 5091–5098. DOI: 10.1128/mcb.18.9.5091.

Meacham, G. C.; Patterson, C.; Zhang, W.; Younger, J. M.; Cyr, D. M. (2001): The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. In: *Nature cell biology* 3 (1), S. 100–105. DOI: 10.1038/35050509.

Minniti, Giuseppe; Salvati, M.; Arcella, A.; Buttarelli, F.; D'Elia, A.; Lanzetta, G. et al. (2011): Correlation between O6-methylguanine-DNA methyltransferase and survival in elderly patients with glioblastoma treated with radiotherapy plus concomitant and adjuvant temozolomide. In: *Journal of neuro-oncology* 102 (2), S. 311–316. DOI: 10.1007/s11060-010-0324-4.

Morimoto, R. I. (1998): Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. In: *Genes & development* 12 (24), S. 3788–3796. DOI: 10.1101/gad.12.24.3788.

Moser, Christian; Schmidbauer, Christin; Gürtler, Ulrich; Gross, Catharina; Gehrmann, Mathias; Thonigs, Gerald et al. (2002): Inhibition of tumor growth in mice with severe combined immunodeficiency is mediated by heat shock protein 70 (Hsp70)-peptide–activated, CD94 positive natural killer cells. In: *Cell Stress & Chaperones* 7 (4), S. 365. DOI: 10.1379/1466-1268(2002)007<0365:IOTGIM>2.0.CO;2.

Multhoff, G. (1999): Heat shock protein 70 (Hsp70) stimulates proliferation and cytolytic activity of natural killer cells. In: *Experimental hematology* 27 (11), S. 1627–1636. DOI: 10.1016/s0301-472x(99)00104-6.

Multhoff, G.; Botzler, C.; Jennen, L.; Schmidt, J.; Ellwart, J.; Issels, R. (1997): Heat shock protein 72 on tumor cells: a recognition structure for natural killer cells. In: *Journal of immunology (Baltimore, Md. : 1950)* 158 (9), S. 4341–4350.

Multhoff, G.; Botzler, C.; Wiesnet, M.; Eissner, G.; Issels, R. (1995a): CD3- large granular lymphocytes recognize a heat-inducible immunogenic determinant associated with the 72-kD heat shock protein on human sarcoma cells. In: *Blood* 86 (4), S. 1374–1382.

Multhoff, G.; Botzler, C.; Wiesnet, M.; Müller, E.; Meier, T.; Wilmanns, W.; Issels, R. D. (1995b): A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor cells, but not on normal cells. In: *International journal of cancer* 61 (2), S. 272–279. DOI: 10.1002/ijc.2910610222.

Multhoff, Gabriele (2007): Heat shock protein 70 (Hsp70): membrane location, export and immunological relevance. In: *Methods (San Diego, Calif.)* 43 (3), S. 229–237. DOI: 10.1016/j.ymeth.2007.06.006.

Multhoff, Gabriele; Hightower, Lawrence E. (2011): Distinguishing integral and receptor-bound heat shock protein 70 (Hsp70) on the cell surface by Hsp70-specific antibodies. In: *Cell Stress & Chaperones* 16 (3), S. 251–255. DOI: 10.1007/s12192-010-0247-1.

Nabors, Louis Burt; Portnow, Jana; Ammirati, Mario; Baehring, Joachim; Brem, Henry; Butowski, Nicholas et al. (2017): NCCN Guidelines Insights: Central Nervous System Cancers, Version 1.2017. In: *Journal of the National Comprehensive Cancer Network : JNCCN* 15 (11), S. 1331–1345. DOI: 10.6004/jnccn.2017.0166.

Narayana, Ashwatha; Gruber, Deborah; Kunnakkat, Saroj; Golfinos, John G.; Parker, Erik; Raza, Shahzad et al. (2012): A clinical trial of bevacizumab, temozolomide, and radiation for newly diagnosed glioblastoma. In: *Journal of neurosurgery* 116 (2), S. 341–345. DOI: 10.3171/2011.9.JNS11656.

Nieder, Carsten; Andratschke, Nicolaus H.; Grosu, Anca L. (2016): Re-irradiation for Recurrent Primary Brain Tumors. In: *Anticancer research* 36 (10), S. 4985–4995. DOI: 10.21873/anticanres.11067.

Nobusawa, Sumihito; Watanabe, Takuya; Kleihues, Paul; Ohgaki, Hiroko (2009): IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. In: *Clinical cancer research : an official journal of the American Association for Cancer Research* 15 (19), S. 6002–6007. DOI: 10.1158/1078-0432.CCR-09-0715.

Noushmehr, Houtan; Weisenberger, Daniel J.; Diefes, Kristin; Phillips, Heidi S.; Pujara, Kanan; Berman, Benjamin P. et al. (2010): Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. In: *Cancer cell* 17 (5), S. 510–522. DOI: 10.1016/j.ccr.2010.03.017.

Ohgaki, Hiroko (2009): Epidemiology of brain tumors. In: *Methods in molecular biology (Clifton, N.J.)* 472, S. 323–342. DOI: 10.1007/978-1-60327-492-0_14.

Ostrom, Quinn T.; Bauchet, Luc; Davis, Faith G.; Deltour, Isabelle; Fisher, James L.; Langer, Chelsea Eastman et al. (2014): The epidemiology of glioma in adults: a "state of the science" review. In: *Neuro-oncology* 16 (7), S. 896–913. DOI: 10.1093/neuonc/nou087.

Ostrom, Quinn T.; Gittleman, Haley; Fulop, Jordonna; Liu, Max; Blanda, Rachel; Kromer, Courtney et al. (2015): CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. In: *Neuro-oncology* 17 Suppl 4, iv1-iv62. DOI: 10.1093/neuonc/nov189.

Parsell, D. A.; Kowal, A. S.; Singer, M. A.; Lindquist, S. (1994): Protein disaggregation mediated by heat-shock protein Hsp104. In: *Nature* 372 (6505), S. 475–478. DOI: 10.1038/372475a0.

Parsons, D. Williams; Jones, Siân; Zhang, Xiaosong; Lin, Jimmy Cheng-Ho; Leary, Rebecca J.; Angenendt, Philipp et al. (2008): An integrated genomic analysis of human glioblastoma multiforme. In: *Science (New York, N.Y.)* 321 (5897), S. 1807–1812. DOI: 10.1126/science.1164382.

Perry, James R.; Laperriere, Normand; O'Callaghan, Christopher J.; Brandes, Alba A.; Menten, Johan; Phillips, Claire et al. (2017): Short-Course Radiation plus Temozolomide in Elderly Patients with Glioblastoma. In: *The New England journal of medicine* 376 (11), S. 1027–1037. DOI: 10.1056/NEJMoa1611977.

Pfister, Karin; Radons, Jürgen; Busch, Raymonde; Tidball, James G.; Pfeifer, Michael; Freitag, Lutz et al. (2007): Patient survival by Hsp70 membrane phenotype: association with different routes of metastasis. In: *Cancer* 110 (4), S. 926–935. DOI: 10.1002/cncr.22864.

Piccirilli, Manolo; Bistazzoni, Simona; Gagliardi, Franco Maria; Landi, Alessandro; Santoro, Antonio; Giangaspero, Felice; Salvati, Maurizio (2006): Treatment of glioblastoma multiforme in elderly patients. Clinico-therapeutic remarks in 22 patients older than 80 years. In: *Tumori* 92 (2), S. 98–103.

Pignatti, Francesco; van den Bent, Martin; Curran, Desmond; Debruyne, Channa; Sylvester, Richard; Therasse, Patrick et al. (2002): Prognostic factors for survival in adult patients with cerebral low-grade glioma. In: *Journal of clinical oncology : official journal of*

the American Society of Clinical Oncology 20 (8), S. 2076–2084. DOI: 10.1200/JCO.2002.08.121.

Pockley, A. Graham; Multhoff, Gabriele (2008): Cell stress proteins in extracellular fluids: friend or foe? In: *Novartis Foundation symposium* 291, 86-95; discussion 96-100, 137-40. DOI: 10.1002/9780470754030.ch7.

Reardon, D. A.; Omuro, A.; Brandes, A. A.; Rieger, J.; Wick, A.; Sepulveda, J. et al. (2017): OS10.3 Randomized Phase 3 Study Evaluating the Efficacy and Safety of Nivolumab vs Bevacizumab in Patients With Recurrent Glioblastoma: CheckMate 143. In: *Neuro-oncology* 19 (suppl_3), iii21-iii21. DOI: 10.1093/neuonc/nox036.071.

Ritossa, F. (1962): A new puffing pattern induced by temperature shock and DNP in drosophila. In: *Experientia* 18 (12), S. 571–573. DOI: 10.1007/BF02172188.

Ritossa, Ferruccio (1996): Discovery of the heat shock response. In: *Cell Stress Chaper* 1 (2), S. 97. DOI: 10.1379/1466-1268(1996)001<0097:DOTHSR>2.3.CO;2.

Roa, W.; Brasher, P. M. A.; Bauman, G.; Anthes, M.; Bruera, E.; Chan, A. et al. (2004): Abbreviated course of radiation therapy in older patients with glioblastoma multiforme: a prospective randomized clinical trial. In: *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 22 (9), S. 1583–1588. DOI: 10.1200/JCO.2004.06.082.

Robert-Koch-Institut (2015): Krebs in Deutschland 2011/2012. Gesundheitsberichterstattung des Bundes. 10 Ausg. 2015.

Roigas, Jan; Wallen, Erik S.; Loening, Stefan A.; Moseley, Pope L. (1998): Effects of combined treatment of chemotherapeutics and hyperthermia on survival and the regulation of heat shock proteins in dunning R3327 prostate carcinoma cells. In: *The Prostate* 34 (3), S. 195–202. DOI: 10.1002/(sici)1097-0045(19980215)34:3<195::aid-pros7>3.0.co;2-h.

Sanson, Marc; Marie, Yannick; Paris, Sophie; Idbaih, Ahmed; Laffaire, Julien; Ducray, François et al. (2009): Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. In: *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 27 (25), S. 4150–4154. DOI: 10.1200/JCO.2009.21.9832.

Shechter, Ravid; London, Anat; Schwartz, Michal (2013): Orchestrated leukocyte recruitment to immune-privileged sites: absolute barriers versus educational gates. In: *Nature reviews. Immunology* 13 (3), S. 206–218. DOI: 10.1038/nri3391.

Shirahata, Mitsuaki; Ono, Takahiro; Stichel, Damian; Schrimpf, Daniel; Reuss, David E.; Sahm, Felix et al. (2018): Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. In: *Acta neuropathologica* 136 (1), S. 153–166. DOI: 10.1007/s00401-018-1849-4.

Sierra-Rivera, E.; Voorhees, G. J.; Freeman, M. L. (1993): Gamma irradiation increases hsp-70 in Chinese hamster ovary cells. In: *Radiation research* 135 (1), S. 40–45. DOI: 10.2307/3578394.

Stangl, Stefan; Gehrmann, Mathias; Dressel, Ralf; Alves, Frauke; Dullin, Christian; Themelis, George et al. (2011): In vivo imaging of CT26 mouse tumours by using

cmHsp70.1 monoclonal antibody. In: *Journal of cellular and molecular medicine* 15 (4), S. 874–887. DOI: 10.1111/j.1582-4934.2010.01067.x.

Stone, Helen B.; Coleman, C. Norman; Anscher, Mitchell S.; McBride, William H. (2003): Effects of radiation on normal tissue: consequences and mechanisms. In: *The Lancet Oncology* 4 (9), S. 529–536. DOI: 10.1016/s1470-2045(03)01191-4.

Stuart, R. A.; Cyr, D. M.; Neupert, W. (1994): Hsp70 in mitochondrial biogenesis: from chaperoning nascent polypeptide chains to facilitation of protein degradation. In: *Experientia* 50 (11-12), S. 1002–1011. DOI: 10.1007/bf01923454.

Stummer, Walter; Reulen, Hanns-Jürgen; Meinel, Thomas; Pichlmeier, Uwe; Schumacher, Wiebke; Tonn, Jörg-Christian et al. (2008): Extent of resection and survival in glioblastoma multiforme: identification of and adjustment for bias. In: *Neurosurgery* 62 (3), 564-76; discussion 564-76. DOI: 10.1227/01.neu.0000317304.31579.17.

Stupp, Roger; Hegi, Monika E.; Mason, Warren P.; van den Bent, Martin J.; Taphoorn, Martin J. B.; Janzer, Robert C. 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. In: *The Lancet Oncology* 10 (5), S. 459–466. DOI: 10.1016/S1470-2045(09)70025-7.

Stupp, Roger; Mason, Warren P.; van den Bent, Martin J.; Weller, Michael; Fisher, Barbara; Taphoorn, Martin J. B. et al. (2005): Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. In: *The New England journal of medicine* 352 (10), S. 987–996. DOI: 10.1056/NEJMoa043330.

Stupp, Roger; Taillibert, Sophie; Kanner, Andrew; Read, William; Steinberg, David; Lhermitte, Benoit et al. (2017): Effect of Tumor-Treating Fields Plus Maintenance Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With Glioblastoma: A Randomized Clinical Trial. In: *JAMA* 318 (23), S. 2306–2316. DOI: 10.1001/jama.2017.18718.

Suchorska, Bogdana; Weller, Michael; Tabatabai, Ghazaleh; Senft, Christian; Hau, Peter; Sabel, Michael C. et al. (2016): Complete resection of contrast-enhancing tumor volume is associated with improved survival in recurrent glioblastoma-results from the DIRECTOR trial. In: *Neuro-oncology* 18 (4), S. 549–556. DOI: 10.1093/neuonc/nov326.

Suzuki, Keiji; Watanabe, Masami (1992): Augmented expression of HSP72 protein in normal human fibroblasts irradiated with ultraviolet light. In: *Biochemical and Biophysical Research Communications* 186 (3), S. 1257–1264. DOI: 10.1016/S0006-291X(05)81541-4.

Thorsteinsdottir, Jun; Stangl, Stefan; Fu, Peng; Guo, Ketai; Albrecht, Valerie; Eigenbrod, Sabina et al. (2017): Overexpression of cytosolic, plasma membrane bound and extracellular heat shock protein 70 (Hsp70) in primary glioblastomas. In: *Journal of neuro-oncology* 135 (3), S. 443–452. DOI: 10.1007/s11060-017-2600-z.

Tissiéres, Alfred; Mitchell, Herschel K.; Tracy, Ursula M. (1974): Protein synthesis in salivary glands of Drosophila melanogaster: Relation to chromosome puffs. In: *Journal of Molecular Biology* 84 (3), S. 389–398. DOI: 10.1016/0022-2836(74)90447-1.

Todryk, Stephen M.; Gough, Michael J.; Pockley, A. Graham (2003): Facets of heat shock protein 70 show immunotherapeutic potential. In: *Immunology* 110 (1), S. 1–9. DOI: 10.1046/j.1365-2567.2003.01725.x.

Travis, Lois B. (2002): Therapy-associated solid tumors. In: *Acta oncologica (Stockholm, Sweden)* 41 (4), S. 323–333. DOI: 10.1080/028418602760169361.

van den Bent, Martin J.; Baumert, Brigitta; Erridge, Sara C.; Vogelbaum, Michael A.; Nowak, Anna K.; Sanson, Marc et al. (2017a): Interim results from the CATNON trial (EORTC study 26053-22054) of treatment with concurrent and adjuvant temozolomide for 1p/19q non-co-deleted anaplastic glioma: a phase 3, randomised, open-label intergroup study. In: *The Lancet* 390 (10103), S. 1645–1653. DOI: 10.1016/S0140-6736(17)31442-3.

van den Bent, Martin J.; Brandes, Alba A.; Taphoorn, Martin J. B.; Kros, Johan M.; Kouwenhoven, Mathilde C. M.; Delattre, Jean-Yves et al. (2013): Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. In: *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 31 (3), S. 344–350. DOI: 10.1200/JCO.2012.43.2229.

van den Bent, Martin J.; Smits, Marion; Kros, Johan M.; Chang, Susan M. (2017b): Diffuse Infiltrating Oligodendroglioma and Astrocytoma. In: *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 35 (21), S. 2394–2401. DOI: 10.1200/JCO.2017.72.6737.

Vega, Virginia L.; Rodríguez-Silva, Monica; Frey, Tiffany; Gehrmann, Mathias; Diaz, Juan Carlos; Steinem, Claudia et al. (2008): Hsp70 translocates into the plasma membrane after stress and is released into the extracellular environment in a membrane-associated form that activates macrophages. In: *Journal of immunology (Baltimore, Md. : 1950)* 180 (6), S. 4299–4307. DOI: 10.4049/jimmunol.180.6.4299.

Verhaak, Roel G. W.; Hoadley, Katherine A.; Purdom, Elizabeth; Wang, Victoria; Qi, Yuan; Wilkerson, Matthew D. et al. (2010): Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. In: *Cancer cell* 17 (1), S. 98–110. DOI: 10.1016/j.ccr.2009.12.020.

Vredenburgh, James J.; Desjardins, Annick; Kirkpatrick, John P.; Reardon, David A.; Peters, Katherine B.; Herndon, James E. et al. (2012): Addition of bevacizumab to standard radiation therapy and daily temozolomide is associated with minimal toxicity in newly diagnosed glioblastoma multiforme. In: *International journal of radiation oncology, biology, physics* 82 (1), S. 58–66. DOI: 10.1016/j.ijrobp.2010.08.058.

Watanabe, Takuya; Nobusawa, Sumihito; Kleihues, Paul; Ohgaki, Hiroko (2009): IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. In: *The American journal of pathology* 174 (4), S. 1149–1153. DOI: 10.2353/ajpath.2009.080958.

Wei, Y. Q.; Zhao, X.; Kariya, Y.; Teshigawara, K.; Uchida, A. (1995): Inhibition of proliferation and induction of apoptosis by abrogation of heat-shock protein (HSP) 70 expression in tumor cells. In: *Cancer immunology, immunotherapy : CII* 40 (2), S. 73–78. DOI: 10.1007/bf01520287.

Weller, Michael; Tabatabai, Ghazaleh; Kästner, Bärbel; Felsberg, Jörg; Steinbach, Joachim P.; Wick, Antje et al. (2015): MGMT Promoter Methylation Is a Strong Prognostic Biomarker for Benefit from Dose-Intensified Temozolomide Rechallenge in Progressive Glioblastoma: The DIRECTOR Trial. In: *Clinical cancer research : an official journal of the American Association for Cancer Research* 21 (9), S. 2057–2064. DOI: 10.1158/1078-0432.CCR-14-2737.

Weller, Michael; van den Bent, Martin; Tonn, Jörg C.; Stupp, Roger; Preusser, Matthias; Cohen-Jonathan-Moyal, Elizabeth et al. (2017): European Association for Neuro-Oncology (EANO) guideline on the diagnosis and treatment of adult astrocytic and oligodendroglial gliomas. In: *The Lancet Oncology* 18 (6), e315-e329. DOI: 10.1016/S1470-2045(17)30194-8.

Wick, Wolfgang; Gorlia, Thierry; Bendszus, Martin; Taphoorn, Martin; Sahm, Felix; Harting, Inga et al. (2017): Lomustine and Bevacizumab in Progressive Glioblastoma. In: *The New England journal of medicine* 377 (20), S. 1954–1963. DOI: 10.1056/NEJMoa1707358.

Wick, Wolfgang; Hartmann, Christian; Engel, Corinna; Stoffels, Mandy; Felsberg, Jörg; Stockhammer, Florian et al. (2009): NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. In: *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 27 (35), S. 5874–5880. DOI: 10.1200/JCO.2009.23.6497.

Wick, Wolfgang; Platten, Michael; Meisner, Christoph; Felsberg, Jörg; Tabatabai, Ghazaleh; Simon, Matthias et al. (2012): Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. In: *The Lancet Oncology* 13 (7), S. 707–715. DOI: 10.1016/S1470-2045(12)70164-X.

Yan, Hai; Parsons, D. Williams; Jin, Genglin; McLendon, Roger; Rasheed, B. Ahmed; Yuan, Weishi et al. (2009): IDH1 and IDH2 mutations in gliomas. In: *The New England journal of medicine* 360 (8), S. 765–773. DOI: 10.1056/NEJMoa0808710.

Zhang, Honghai; Liu, Rui; Huang, Weida (2007): A 14-mer peptide from HSP70 protein is the critical epitope which enhances NK activity against tumor cells in vivo. In: *Immunological investigations* 36 (3), S. 233–246. DOI: 10.1080/08820130600992073.

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10 Publications

“Potential Role of Hsp70 and Activated NK Cells for Prediction of Prognosis in Glioblastoma Patients”

Front. Mol. Biosci., 17 May 2021
DOI: 10.3389/fmolb.2021.669366

„Biomarkers in Adult-Type Diffuse Gliomas: Elevated Levels of Circulating Vesicular Heat Shock Protein 70 Serve as a Biomarker in Grade 4 Glioblastoma and Increase NK Cell Frequencies in Grade 3 Glioma“

Biomedicines, 7 December 2023
DOI: 10.3390/biomedicines11123235

11 Acknowledgement

I would like to thank everybody who contributed to the execution and success of this thesis.

Especially, I want to thank my thesis supervisor, Prof. Dr. rer. nat. Gabriele Multhoff, for conferring and realizing this interesting topic as well as continuous support until the completion of this work.

Sincere thanks also to the entire work group AG Multhoff for the kind cooperation and energetic support, without whom this would not have been possible to this extent.

Thank you!