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IDENTIFICATION OF NOVEL GENES WITH IMPORTANT FUNCTIONS IN GLIOBLASTOMA MULTIFORME AND ACUTE MYELOID LEUKEMIA

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Karolinska Institutet

Stockholm 2018

Published by Karolinska Institutet.
Printed by E-print AB 2018

ISBN 978-91-7831-207-8

Front cover: Acrylics on canvas by Josefine Enneby, 2018

IDENTIFICATION OF NOVEL GENES WITH IMPORTANT FUNCTIONS IN GLIOBLASTOMA MULTIFORME AND ACUTE MYELOID LEUKEMIA

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Glioblastoma multiforme (GBM) is one of the most common and aggressive brain tumors. The cancer stem cells of GBM (GSCs) are notorious for being invasive as well as resistant to radiation and chemotherapy. The current treatment options for GBM lack specificity and result in severe side effects. Therefore, the need for finding novel and efficient treatments with high specificity in GBM has become paramount.

In study I, to assess the role of various cytoskeletal regulators as potential biomarkers for prognosis and treatment design in GBM, we analyzed gene expression through a bioinformatics approach. Using this method, we identified six genes having a potential value as biomarkers. In combination, we performed a small molecule screen using various Food and Drug Administration (FDA) approved oncology drugs and compared the effects of these compounds, on GBM cytoskeleton, to the conventional chemotherapeutic temozolomide (TMZ). Our results show the importance of cytoskeletal regulators in GBM and the need for combinatorial therapies.

In study II, to identify novel genes required for the growth of acute myeloid leukemia (AML), we performed a large scale short hairpin RNA (shRNA) screen in AML cells and non-transformed bone marrow (BM) cells. We identified the chromatin remodeler, CHD4, as essential for leukemic growth and validated its role in-vitro and in-vivo using RNA interference and CRISPR-Cas9 approaches. More importantly, we found that CHD4 was not required for the growth of normal hematopoietic cells. The study suggests CHD4 as a novel therapeutic target in childhood AML.

To identify genes that are required for the growth of GCSs, in studies III and IV, we performed an unbiased functional shRNA screen. Using this approach, in study III, we identified ubiquitin C-terminal hydrolase-L1 (UCHL1) as being selectively essential for the growth of GSCs. Inhibition of UCHL1 was detrimental to GBM cells, caused cell cycle arrest at G0 phase and induced apoptosis. Small molecule inhibitors of UCHL1 effectively and specifically reduced viability of GBM cells and did not affect viability or function of mature neurons.

In study IV, we also developed a new shRNA/CRISPR-Cas9 modular vector system for efficient and multiplex validation of genes. From the screening data, we identified Ariadne RBR E3 Ubiquitin Protein Ligase 1 (ARIH1) and Ariadne RBR E3 Ubiquitin Protein Ligase 2 (ARIH2) to be specifically required for the growth of GBM cells. Inhibition of ARIH1 and ARIH2 effectively reduced cell growth of various GBM cell lines, and loss of ARIH2 specifically induced cell cycle arrest and sensitivity to DNA damage. Our data from studies III and IV suggests UCHL1, ARIH1 and ARIH2 as novel targets for future GBM therapies.

LIST OF SCIENTIFIC PAPERS

I. Masoumi S*, **Harisankar A***, Gracias A, Bachinger F, Fufa T, Chandrasekar G, Gaunitz F, Walfridsson J, Kitambi SS. *Understanding cytoskeleton regulators in glioblastoma multiforme for therapy design*. Drug Design Development and Therapy, 2016, 10: p. 2881-2897.

II. Heshmati Y, Türköz G, **Harisankar A**, Kharazi S, Boström J, Dolatabadi EK, Krstic A, Chang D, Månsson R, Altun M, Qian H, Walfridsson J. *The chromatin-remodeling factor CHD4 is required for maintenance of childhood acute myeloid leukemia*. Haematologica, 2018, 103(7): p. 1169-1181.

III. **Harisankar A**, Desroses M, Marks C, Papadia D, Fisahn A, Almlöf I, Boström J, Nelander S, Westermark B, Forsberg-Nilsson K, Uhrbom L, Altun M, Walfridsson J. *Identification of UCHL1 as being selectively essential for growth of glioblastoma cancer stem cells*. Manuscript

IV. **Harisankar A**, Boström J, Sanjiv K, Nelander S, Westermark B, Forsberg-Nilsson K, Uhrbom L, Altun M, Walfridsson J. *E3 ubiquitin ligases ARIH1 and ARIH2 are required for growth of glioblastoma multiforme*. Manuscript

* Authors contributed equally to this study

CONTENTS

LIST OF ABBREVIATIONS

AKT1	AK Strain Transforming Serine/Threonine Kinase 1
AML	Acute Myeloid Leukemia
ARIH1	Ariadne RBR E3 Ubiquitin Protein Ligase 1
ARIH2	Ariadne RBR E3 Ubiquitin Protein Ligase 2
ATP	Adenosine Triphosphate
BBB	Blood Brain Barrier
BM	Bone Marrow
BTK	Bruton's Tyrosine Kinase
c-abl	Abelson murine leukemia viral oncogene homolog 1
CCND2	Cyclin D2
CD133	Prominin-1
CD95	Cluster of Differentiation 95
CDKN2A	Cyclin Dependent Kinase Inhibitor 2A
CDKN2B	Cyclin Dependent Kinase Inhibitor 2B
CDKN2C	Cyclin Dependent Kinase Inhibitor 2C
CEBPA	CCAAT Enhancer Binding Protein Alpha
CEND1	Cell Cycle Exit And Neuronal Differentiation 1
CFU	Colony Forming Units
CHD4	Chromodomain Helicase DNA Binding Protein 4
cKIT	KIT Proto-Oncogene Receptor Tyrosine Kinase
c-PARP	cleaved Poly(ADP-Ribose) Polymerase 1
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
D-2HG	D-2-hydroxyglutarate
DNA	Deoxyribonucleic Acid
DSF	Differential Scanning Fluorimetry
DUB	Deubiquitinase
EGFR	Epidermal Growth Factor Receptor
EGFRvIII	Epidermal Growth Factor Receptor Variant III
FDA	Food and Drug Administration
FGFR1	Fibroblast Growth Factor Receptor 1

FGFR3	Fibroblast Growth Factor Receptor 3
FGFR4	Fibroblast Growth Factor Receptor 4
FGMS	Fluorescence-Guided Multiple Sampling
FISH	Fluorescence In Situ Hybridization
FLT3	Fms Related Tyrosine Kinase 3
FLT3-ITD	Fms Related Tyrosine Kinase 3-Internal Tandem Duplication
FUCCI	Fluorescence Ubiquitination Cell Cycle Indicator
GBM	Glioblastoma Multiforme
GEO	Gene Expression Omnibus
GFAP	Glial Fibrillary Acidic Protein
GP	Glial Progenitor
GSC	Glioma Stem Cell
GSK3β	Glycogen Synthase Kinase 3 Beta
GTR	Gross Total Resection
H2AXɣ	ɣH2A Histone Family, Member X
H3F3A	Histone H3.3
HSC	Hematopoietic Stem Cell
IC_{50}	Inhibitory Concentration 50
IDH	Isocitrate Dehydrogenase
KDM2B	Lysine-Specific Demethylase 2B
KDM4D	Lysine-Specific Demethylase 4D
KDM5A	Lysine-Specific Demethylase 5A
KDM6B	Lysine-Specific Demethylase 6B
LIC	Leukemia Initiating Cells
LSC	Leukemic Stem Cells
MAPK	Mitogen-Activated Protein Kinase
MDM2	Mouse Double Minute 2 Homolog
MET	MET Proto-Oncogene
MGMT	O-6-methylguanine-DNA Methyltransferase
mTORC1	Mammalian Target of Rapamycin Complex 1
NEUROD1	Neurogenic Differentiation 1

NES	Neuroepithelial like Stem Cell
NEUROG2	Neurogenin 2
NF1	Neurofibromin 1
NPM1	Nucleophosmin
NSC	Neural Stem Cell
PDGFRA	Platelet-Derived Growth Factor Receptor α
PDGFRB	Platelet-Derived Growth Factor Receptor β
PFS	Progression Free Survival
PI3K	Phosphoinositide 3-Kinase
PI3KR2	Phosphoinositide-3-Kinase Regulatory Subunit 2
PIK3C2A	Phosphatidylinositol-4-Phosphate 3-Kinase Catalytic Subunit Type 2 Alpha
PTEN	Phosphatase and Tensin Homolog
qPCR	Quantitative Real time Polymerase Chain Reaction
RBBP4	RB Binding Protein 4
RNA	Ribonucleic Acid
RT	Radiation Therapy
RTK	Receptor Tyrosine Kinase
SCGE	Single Cell Gel Electrophoresis
SGZ	Sub Granular Zone
shRNA	Short Hairpin Ribonucleic Acid
SOX2	Sex Determining Region Y-box 2
STR	Sub Total Resection
SVZ	Sub Ventricular Zone
TCGA	The Cancer Genome Atlas
TMZ	Temozolomide
TP53	Tumor Protein P53
UCHL1	Ubiquitin C-Terminal Hydrolase L1
ulRFE	Ultra-low Radio Frequency Energy
UPS	Ubiquitin Proteasome System
VEGF-A	Vascular Endothelial Growth Factor α

WHO	World Health Organization
α-KG	α-Ketoglutarate

1 INTRODUCTION

1.1 GLIOBLASTOMA MULTIFORME

1.1.1 An Overview

Glioblastoma multiforme (GBM) is one of the most common and highly malignant types of primary brain tumors. This disease is associated with poor prognosis and approximately 95% of the patients do not survive more than 5 years after diagnosis [1-4]. Standard care of treatment for GBM patients consists of surgical resection of the tumor, followed by several weeks of radio- and chemotherapy. However, the extent of surgery and intensity of therapy is considered carefully as the organ has mostly terminally differentiated cells and may be damaged irreversibly.

Several pharmacological drugs have been developed against GBM and are currently in or have completed clinical trials. These inhibitors mainly target receptors of the receptor tyrosine kinase (RTK) family involved in processes like angiogenesis, cellular proliferation, DNA repair among others [5]. Although some of these drugs have promising efficacy, clinical outcome still remains dismal [6]. The main reasons for treatment failure are the rapid, diffuse and heterogeneous nature of the tumors and the presence of the blood brain barrier (BBB) hindering transport of chemotherapeutic agents [7, 8]. As a result of these complicated and distinct characteristics of GBM, with no known risk factors and poor understanding of the cellular origins of these tumors [9, 10], dissecting the biology of GBM becomes critical especially in designing novel targeted therapies.

1.1.2 Histopathological and Molecular Stratification of GBM

Classifying gliomas into different grades is clinically important for purpose of diagnosis, prognosis and makes it easier to compare treatments between different clinical trials [11]. A working group of the World Health Organization (WHO) has created a histo-morphological classification of gliomas to distinguish different tumor types and this has helped in improving prognosis and treatment strategies across the world [12, 13]. Gliomas are broadly classified into astrocytomas, oligodendrogliomas and mixed gliomas. They are also graded based on the

presence of important features like nuclear atypia, mitotic index, endothelial proliferation (microvascular) and necrosis (Table 1) [12, 14]. The number of features displayed by a tumor are directly correlated to the malignancy or its grade. Grade I tumors are associated with low proliferative potential while grade II tumors, in addition are infiltrative and recur. Grade III tumors are malignant and show increased mitotic activity and nuclear atypia. Grade IV, being the highest grade, is associated with all the above features including extensive necrosis, infiltration of tissue surrounding the tumor and rapid clonal evolution (pre- and post-operative) leading to fatality [14].

Astrocytic tumors	Grade
Pilocytic Astrocytoma	I
Diffuse Astrocytoma	II
Anaplastic Astrocytoma	III
Glioblastoma	IV
Oligodendroglial tumors	Grade
Oligodendroglioma	II
Anaplastic oligodendroglioma	III
Mixed gliomas	Grade
Oligoastrocytoma	II
Anaplastic oligoastrocytoma	III

Table 1: Grading of different gliomas according to the WHO classification of central nervous system tumors. Table content adopted from [12].

GBM is a grade IV tumor characterized by presence of necrotic tissue and increased vascularization around the tumors. GBM cells could vary from being small in size to giant cells, poorly differentiated and stain positively for glial-fibrillary acidic protein (GFAP) and vimentin due to their astrocytic nature [14]. The tumors are polymorphic and hence the term ‘multiforme’. This is because even within the same tumor, the cells are heterogenous and display mixed histological features [15]. Cells could be multinucleated and stain strongly for Ki-67 proliferation marker. The cells are often shiny in appearance and hyperchromatic. One of the unique features of this tumor is the presence of pseudopalisading necrosis. Proliferating tumor cells line up around a central area of necrotic cells and form a palisade [16]. Clinically, a majority of these tumors are *de novo* and rapidly advance to a malignant state in elderly

patients. These are termed primary GBM, whereas secondary GBM usually progresses from lower grade astrocytomas and are often associated with younger patients [17].

Chromosomal aberrations such as gain of whole chromosome 7 and loss of chromosome 10 are very common events in GBM. But the need for stratifying GBM into different prognostic groups pushed research towards identifying genetic events like somatic mutations and copy number variations to classify GBM into different molecular subtypes. With emergence of the next generation sequencing technology, GBM genomes were sequenced for identifying amplification and deletions of protein coding genes [18]. This landmark study led to the discovery of several genes that were not implicated in human glioblastoma. One of the major findings from this study was the identification of recurrent mutations in the *IDH1* gene. Following studies showed that the mutations on IDH1-R132 and IDH2-R172 hindered the generation of α-KG [19]. More precisely these were gain of function mutations in which the mutations conferred the IDH1/2 the ability to convert α-KG to D-2-hydroxyglutarate (D-2HG) [20, 21]. Not only does reduction in α-KG or up-regulation of 2HG in IDH1/2 mutations increases oxidative stress [22], but also alters the genome wide epigenetic pattern [23].

In an alternate approach, gene expression analysis was used to identify specific gene expression patterns correlating to patient outcome. GBM was roughly classified into three subtypes: proneural, proliferative and mesenchymal [24]. It was observed that the mesenchymal subtype showed worse prognosis than the other two subtypes and had a gene expression signature characteristic of mesenchymal differentiation. This type of sub-classification relating the molecular profiles to clinical outcomes set up the base for developing targeted therapies.

The Cancer Genome Atlas (TCGA) was initiated as a pilot project by the National Cancer Institute and the National Human Genome Research Institute in an effort to identify and catalogue cancer-causing somatic alterations in various tumor types. TCGA Research Network released an interim report in 2008 and defined the major genes and pathways involved in human GBM based on gene expression and DNA methylation patterns [25]. Three major pathways were identified to be aberrant in most human GBMs- receptor tyrosine kinase, p53 and retinoblastoma protein signaling (Figure 1).

By genomic profiling, the different subtypes were defined and classified as classical, mesenchymal, proneural and neural [26]. Each subtype was defined by an aberrant expression of a specific gene or genes. Following this, hot spot mutations were identified on the H3F3A

and IDH1 genes which further expanded and revamped the classification of GBM subtypes to six [27]. The new subtypes were based on the mutations in the gene H3F3A at K27 and G34 on histone 3.3 and accounts for one-third of pediatric patients.

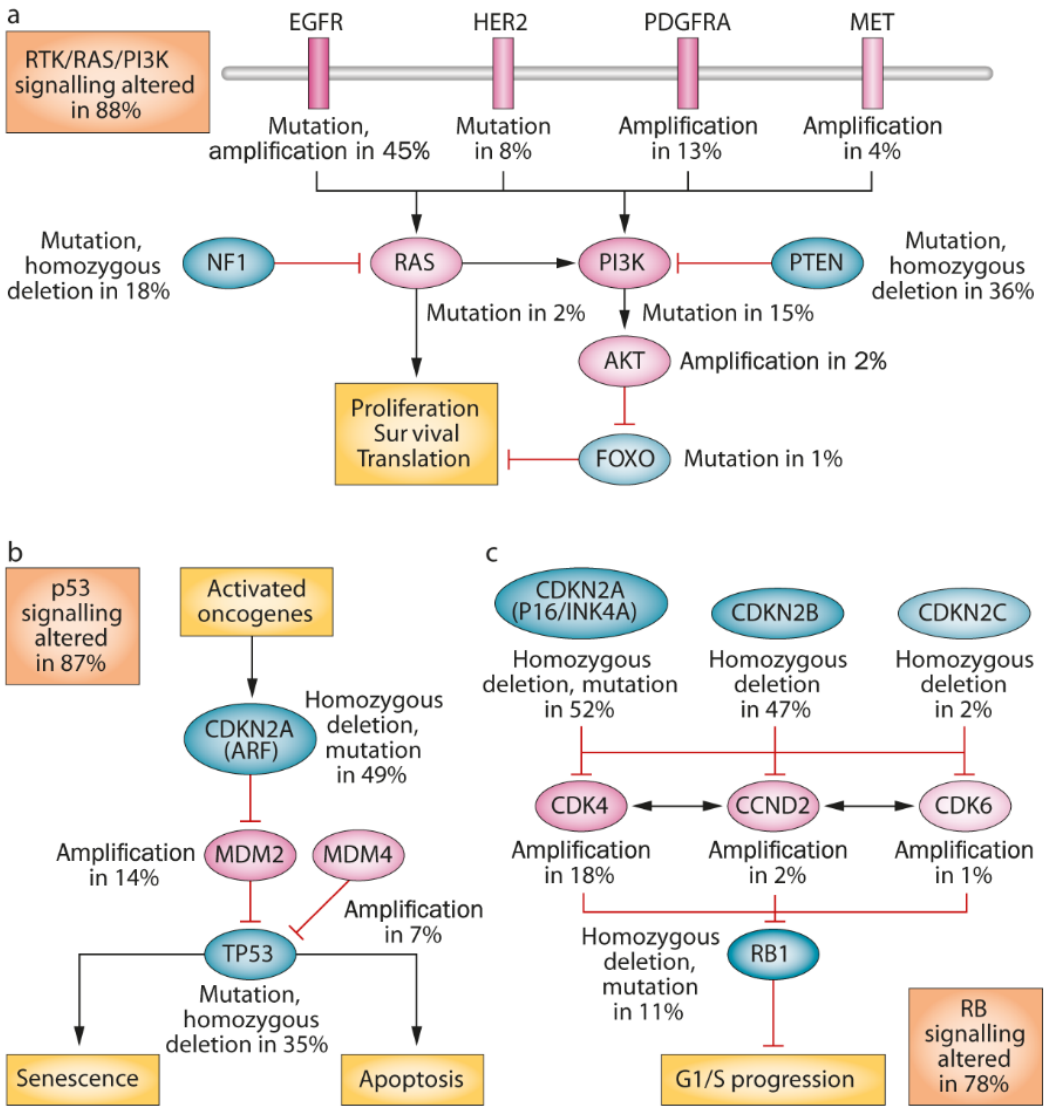

Figure 1: Signaling pathways commonly altered in malignant gliomas. a. RTK/RAS/PI3K, b. p53 and c. Rb signaling pathways. Red and blue colors indicate activating and inactivating genetic alterations respectively. Figure reprinted with permission from publisher [28].

The recent multidimensional study by the TCGA network describes the somatic genomic landscape of GBM [29]. Analyzing and comparing over 500 GBM tumor samples, the study provides comprehensive genomic data linked to clinical outcome which can be utilized for discovering novel biomarkers and identifying disease related pathways for targeted therapies.

In light of these advancements, the latest edition of the WHO classifications for the first time incorporated molecular features as well to define diagnostic categories [13, 30-32]. GBM is currently classified into two categories namely IDH wild-type and IDH mutant which are associated with primary and secondary GBMs respectively (Table 2). Since the disease progresses rapidly, an efficient diagnosis to distinguish the different subtypes becomes critical.

	GBM-IDH-Wildtype	GBM-IDH-Mutant
Synonyms	Primary GBM	Secondary GBM
Tumor development	De novo	From diffuse or anaplastic astrocytomas
Relative Frequency	~ 90%	~ 10%
Median age at diagnosis	~ 62 years	~ 44 years
Clinical history (mean length)	4 months	15 months

Table 2: Table summarizing the latest WHO classification of GBM into two categories based on IDH status. Table data adopted from [13].

1.1.3 Tumor Heterogeneity

Cancer cells are in general a very heterogeneous population of cells. GBM is not an exception and it in fact displays extensive intra-tumoral heterogeneity. Understanding how this is brought about as a result of tumor evolution will explain why treatments usually fail. By using fluorescence guided multiple sampling (FGMS) technique, multiple samples from the same tumor separated in space were collected in real time to conserve spatial information [33]. Analysis of these tumor samples showed that the different parts of the same tumor had different genotypes and molecular profiles. This study, by integrating multiple sampling with genomic data, showed the clonal evolution of GBM tumor at the level of an individual patient.

Following this, a landmark study in 2014 showed that individual cells within the same tumor can show diverse expression patterns relating to different cellular processes, such as proliferation, immune response, hypoxia and oncogenic signaling [34]. Using single cell RNA-sequencing it was shown that different subtypes can be represented by individual cells within the same tumor sample. A more interesting finding was that the individual cells can also have ‘hybrid states’ meaning that they can have expression pattern of two or more

different subtypes at the same time, usually being classical and proneural or mesenchymal and neural. This subtype heterogeneity was also useful in being a prognostic marker and showed that pure proneural subtype tumors have an overall survival better than tumors with a more heterogeneous mix of proneural subtype with others. Identifying these various subclasses using these multi-dimensional approaches has made an impact on development of therapies and designing more efficient treatments.

1.1.4 Cancer Stem Cells and Cell of Origin

More than two decades ago, the clonal expansion model of cancers was the prevalent dogma where one cell had to pick up all the required mutations for malignant transformation and gave rise to bulk tumors [35-37]. It was also postulated that each cell in this bulk tumor population retained tumorigenic potential. This belief was succeeded by the cancer stem cell hypothesis where a subset of the tumor cells gained unlimited replicative potential, immortality and self-renewal capacity and could give rise to and maintain tumor population [38-42]. This paradigm shift meant a leap in targeted therapy of cancers.

Stem cell populations in the adult brain are found within the sub-ventricular zone (SVZ) or in the sub-granular zone (SGZ) of the dendate gyrus (DG) at high density and are regions of active neurogenesis throughout life [43-47]. However, the glial progenitor cells have been found in different regions of the brain at lower numbers indicating that gliomas could potentially also arise from outside the SVG or SGZ [48, 49].

Glioma cancer stem cells (GSCs) represent a small population of cells within the tumor, which are capable of self-renewal, can form neurospheres, differentiate into various cell types and form tumors upon xenotransplantation [50-55]. CD133 (Prominin-1) together with SOX2 and Nestin has been widely used as glioma stem cell markers [56-59]. However, opposing studies show that not the expression of CD133 but rather the ability to form neurospheres defines the aggressiveness of the tumor [60], that CD133+ cells are not required for tumor initiation [61] and that they do not represent the whole self-sufficient tumor initiating cells [62].

GSCs are similar to neural stem cells (NSC) or progenitor cells, in terms of their capacity to migrate long distances within the central nervous system [63]. A pathologist named Hans-Joachim Scherer in the 1930s observed that the glioma cell migration pattern was not random and were selective to certain paths made of myelinated fibers or blood vessels. These paths were eventually termed secondary structures of Scherer and they closely mirror the same migration pattern and behavior of glial progenitor (GP) cells [64]. Also, the morphology of the migrating glioma cells resemble that of the migrating GPs [65]. Adult neural stem cells and transit amplifying cells have been shown to populate and regenerate at vascular niches in the sub-ventricular zone (SVZ) and sub-granular zone (SGZ) [66-69]. Strikingly, GSCs (CD133+/Nestin+) were also found to utilize these vascular niches for self-renewal and proliferation [70, 71]. These suggest possible related mechanisms for the utilization of nutrient rich angiogenic sites in NSC/GSC maintenance.

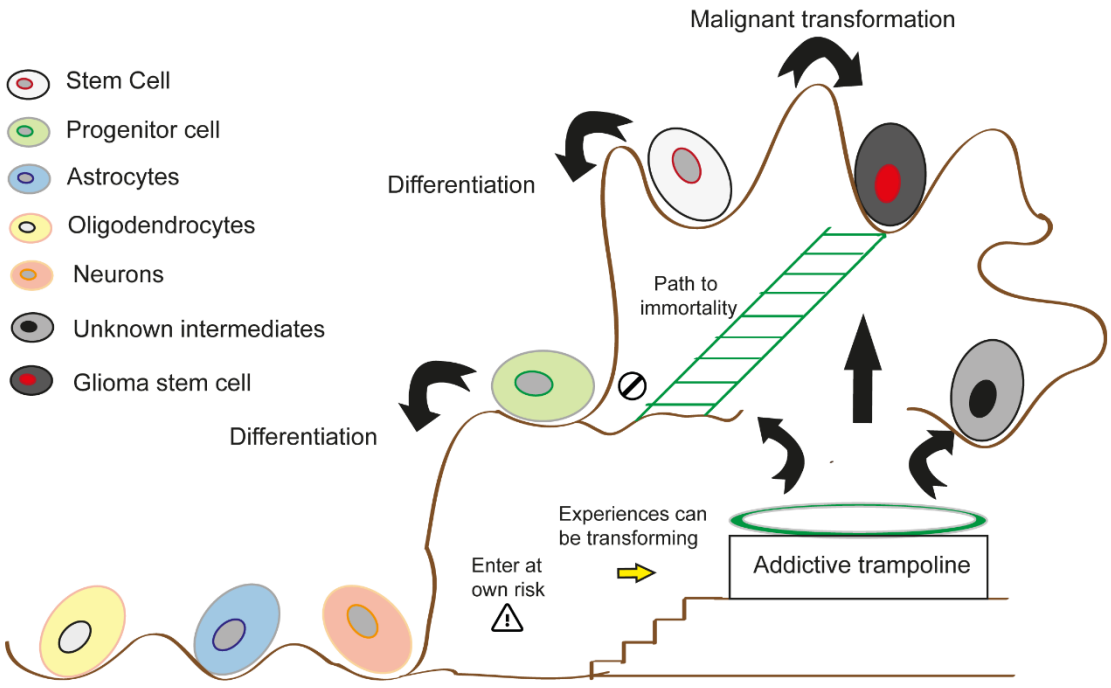

Figure 2: Schematic illustration of probable cells of origin in GBM. Landscape represents a steep hill with NSCs at the top. Its natural movement is down, indicating differentiation. However, mutations and microenvironment influences cells and could transform them to a malignant state (by climbing uphill). Figure illustrated by Aditya Harisankar.

Defining the cell type of origin and elucidating the tumor hierarchy helps understand tumor biology and the specific targeting of cancer stem cells (Figure 2). Although, in GBM the cell of origin has remained elusive, the most probable candidates have been established namely the neuroglial stem cells (type B cells) and the transit amplifying cells (type C cells) [72]. However, they could also arise from the de-differentiation of mature cells as previously shown [73].

With similar cellular and molecular features to that of NSCs and the ability to generate recurrent tumors causing disease relapse, GSCs have stolen the spotlight in GBM research. Identifying the cell of origin which gives rise to GSCs and understanding the underlying pathways required for such a transformation, have become a priority in order to develop novel targeted therapies against this deadly disease.

1.1.5 Treatment strategies for GBM

1.1.5.1 Conventional treatment

The standard treatment options for newly diagnosed GBM patients include maximal safe resection of the tumor followed by radiation and chemotherapy. The extent of surgery usually depends on the location of the tumor and patients receiving a gross total resection (GTR) have better survival over those receiving subtotal resection (STR) [74-76]. However, this could simply be due to differences in tumor subtype or performance status of the patient.

Temozolomide (TMZ), an alkylating agent with potential anticancer activity was developed three decades ago by Malcolm Stevens and his research team [77, 78]. Being small in size, it is readily absorbed in the digestive tracks and crosses the blood brain barrier (BBB) at high efficiency [79]. This has made TMZ a valuable drug against high grade gliomas like GBM [80]. The *MGMT* gene encoding the O^6-alklyguanine DNA alklytransferase protein, is critical for repair of methylated guanine residues and therefore protects cells from alkylating agents [81, 82]. However, this mechanism also exists in the cancer cells particularly CSCs making them resistant to alkylating chemotherapeutic agents [83, 84]. Since MGMT is responsible for repair of TMZ-induced DNA damage, epigenetic silencing of MGMT gene promoter has been linked to increased survival of patients upon TMZ treatment [85-87]. In conclusion, although TMZ has been shown to improve overall survival in post-operative patients in

combination with radiation therapy (RT), the median survival is still very low at 14.6 months [88].

1.1.5.2 Novel and targeted treatments

With the establishment of the important chromosomal aberrations and recurrent mutations in GBM, it is evident that the major pathways being deregulated are involved in signal transduction, cell cycle control, angiogenesis and metabolism [18, 26, 29]. At least one genetic alteration has been observed in genes belonging to the PI3K pathway, RTK genes and PTEN gene in approximately 89% of GBM cases. Also, in 67.3% of GBM cases, at least one RTK gene has been found to be altered with EGFR being the most frequent (57.4%) [29]. Several different RTK gene amplifications have been observed to be mutually expressed in different cells within the same tumor indicating intratumoral heterogeneity [89-92]. Therefore, these pathways have been highly targeted for novel treatments in GBM.

The involvement of vascular niches and endothelial cells in maintenance and proliferation of GSCs [70, 71], has led to targeting factors involved in angiogenesis. Bevacizumab, a monoclonal antibody targeting VEGF-A, has been considered successful in targeted therapy of GBM with increased progression free survival (PFS) [93-96], yet not significantly increasing overall survival [97, 98].

Targeting other RTKs, like PDGFR alpha/beta, c-kit, c-abl and FLT3, using small molecule inhibitors like sunitinib and imatinib mesylate have also shown limited success in GBM treatment [99-102].

EGFR is a frequently mutated gene in GBM [29, 103] and in around 40% of the cases, *EGFRvIII* is expressed, depleted of ligand binding domain by gene rearrangement, leading to ligand independent constitutive activation [104-106]. Since EGFR signals through different signaling pathways [107], the constant activation leads to increased proliferation and aggressiveness of the tumors including radio-resistance [108]. Due to these reasons several inhibitors targeting EGFR like erlotinib and gefitinib and cetuximab (monoclonal antibody) have been tested clinically but have shown no improvements in overall survival [109-115].

Several new drugs are currently in phase I/II clinical trials for safety and efficacy studies. These include drugs targeting RTKs such as Acalabrutinib (BTK inhibitor from Acerta Pharma), AZD2014 (mTORC1/mTORC2 inhibitors from AstraZeneca) and immunotherapies like ICT-107 (dendritic cell vaccine, ImmunoCellular Therapeutics, Ltd.) and CAN008 (fusion protein with extracellular domain of CD95 receptor, CANbridge Life Sciences Ltd.).

There are also novel and non-invasive treatments like use of Nativis Voyager® which delivers ultra-low radio frequency energy (ulRFE) in disrupting signal transduction and metabolic pathways and Optune® (Novocure) which delivers low energy electric field in waves to slow down tumor cell division. However, as previously mentioned in paragraph 1, several oncogenic mutations and activation of several different signaling pathways within the same tumor create intratumoral heterogeneity. These indicate the oncogenic dependencies of tumor cells through multiple redundant pathways to establish cell survival and resistance to therapy. Since each patient is unique with respect to their tumor profile, adapting a combinatorial approach in targeting several of these receptors/pathways, tailored to the patient profile, could prove effective in fighting this deadly disease [116-122].

In line with this, a second generation proteasome inhibitor, marizomib is currently being tested in phase III clinical trials on patients with newly diagnosed GBM in combination with TMZ-RT and previously in phase II with bevacizumab (Avastin®). Unlike Bortezomib (Velcade®), proteasome inhibitor approved by FDA for treatment of multiple myelomas, marizomib binds and affects all three proteolytic subunits of the proteasome, thereby shows more potency [123-126]. Compared to bortezomib and carfilzomib, one of the major advantages of marizomib is its ability to cross the blood-brain barrier and also affect proliferation and invasion of glioma cells in rodent and non-human primate models [127]. In conclusion, exploiting the proteasome system and utilizing combinatorial therapies could improve selectivity and treatment efficacy in GBM.

1.1.6 Ubiquitin Proteasome System

The ubiquitin proteasome system (UPS) is a highly regulated and fundamental part of cellular machinery necessary for protein turnover and localization by controlling ubiquitination and de-ubiquitination of target proteins. Ubiquitination is a process which occurs as a post-translational modification regulated stepwise by ubiquitin activating enzymes (E1), ubiquitin conjugating enzymes (E2) and ubiquitin ligases (E3) [128]. These post-translational ubiquitin (Ub) tags are crucial for the recognition by the 26S proteasome for target protein degradation (Figure 3). However this process is reversible, similar to protein phosphorylation, and is carried out by deubiquitinating enzymes (DUB). Several cellular processes like cell cycle, apoptosis, DNA damage repair, protein metabolism etc., depend on the UPS for the timely disposal or recycling of proteins [129-131].

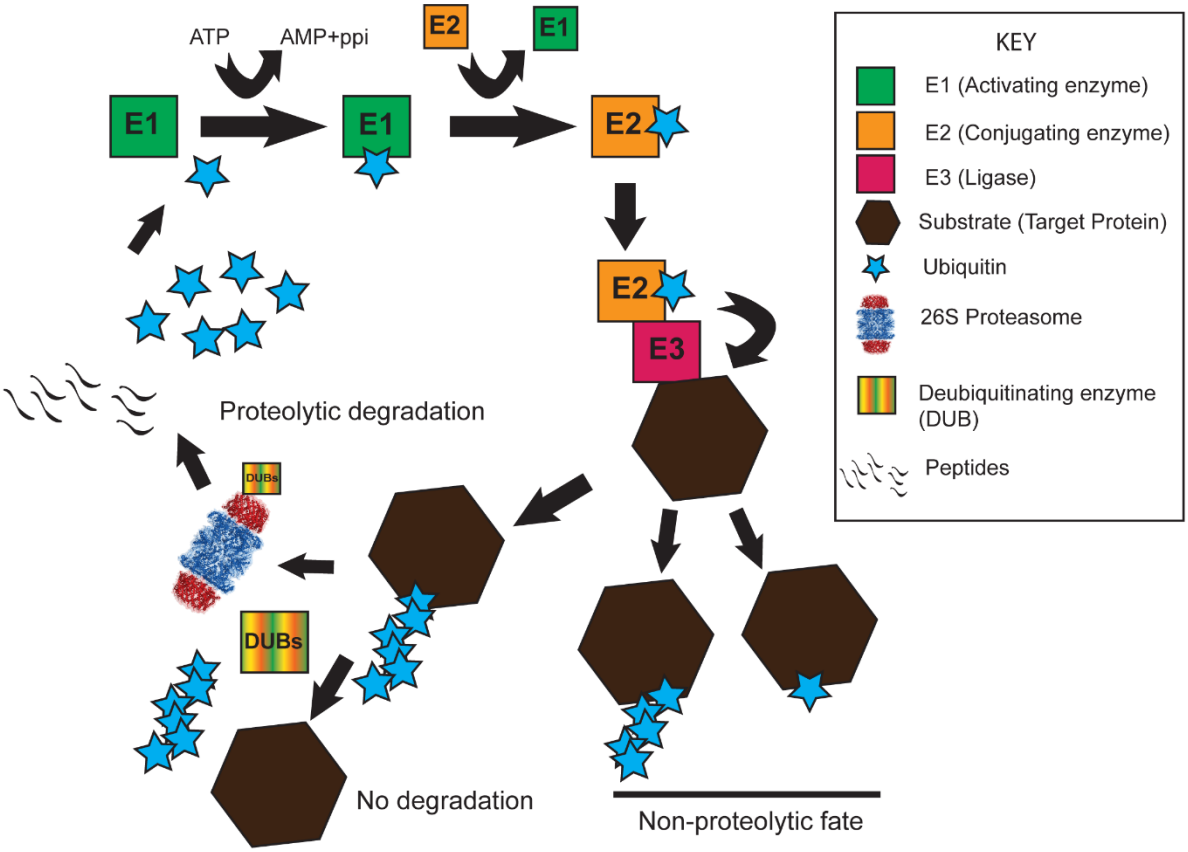

Figure 3: Schematic illustration of the ubiquitin proteasome system (by Aditya Harisankar).

To date, there are only two E1-activating enzymes, thirty eight E2-conjugating enzymes, around 700 E3-ligases and approximately 100 putative DUBs identified in the human genome [132]. The large number of E3-ligases and DUBs provide high substrate specificity for the entire ubiquitin proteasome system. And not surprisingly, the UPS is deregulated in several malignancies and neurodegenerative disorders [133-149].

Although the similarities between ubiquitination and protein phosphorylation are striking, the pharmaceutical industry has mainly focused its resources on developing drugs against protein kinases while very few drugs targeting the ubiquitin system have made it to the clinics [150]. Also, the field of protein phosphorylation has already been heavily exploited through medicinal chemistry leaving little room for developing novel drug targets. Therefore, in the recent years, the UPS has become a major target for drug development against several malignancies [132, 151-155] including leukemia [156-158] and GBM [127, 159-163].

1.2 ACUTE MYELOID LEUKEMIA: AN OVERVIEW

Acute myeloid leukemia (AML) is a hematological malignancy arising from infiltration of the bone marrow and peripheral blood by proliferating and poorly differentiated hematopoietic cells (myeloid blast cells) [164]. Previously considered incurable, now up to 40% of patients below the age of 60 years and up to 15% of patients over the age of 60 years are cured [165]. Symptoms of AML manifest as anemia, weight loss, fever etc., and are mainly due to lack of healthy blood cells which are outcompeted by leukemic blast cells. The diagnosis for AML is primarily by morphological examination of blood smears and characterized by the presence of 20% or more of blast cells in the bone marrow or peripheral blood [166, 167]. Cytogenetic examinations to detect important chromosomal aberrations by karyotyping or fluorescent in situ hybridization (FISH) are still used and in combination with targeted sequencing of important biomarkers for AML diagnosis [168].

Large scale sequencing efforts by TCGA has helped identify genetic and epigenetic changes in adult de novo AML [169]. From this data it is evident that AML, in general, has very few mutations per genome compared to other forms of cancers. However, several of these are in recurrently mutated genes and nearly all AML samples analyzed had at least one potential mutation for AML pathogenesis. These findings have helped improve AML evaluation and mutations in *KIT*, *NPM1*, *CEBPA* and *FLT3*-ITD are now used for AML diagnosis [164, 170].

The treatment strategy has remained the same in over three decades, beginning with induction therapy and followed by consolidation therapy. The intensity and type of these treatments are decided based on factors like age, performance status of patient, biomarkers etc. [165].

Similar to GBM, AML is organized in a hierarchical manner with leukemic stem cells (LSC) or leukemic initiating cells (LIC) at the apex [171]. These cells are characterized by their ability to initiate AML in serial transplantation experiments, ability to self-renew and display surface markers similar to HSCs [38, 171, 172]. Similarly, LSCs were thought to originate from hematopoietic stem cells (HSC) but they have also been shown to arise from transformation of differentiated hematopoietic cells by certain mutations [173, 174]. The importance of these findings are that LSCs, similar to GSCs, are a rare population of quiescent cells displaying intratumoral heterogeneity which develop resistance to treatment and cause tumor relapse [175-177]. Therefore, to ensure complete remission (CR) and long-term cure for AML, elimination of these elusive LSCs is critical.

GBM and AML are surprisingly similar in the nature of their aggressiveness, predominantly occurring de novo [178, 179] and having poor 5-year survival rates [1, 3, 4, 180, 181]. Both diseases display cancer stem cell hierarchy, intratumoral heterogeneity and recurrent deregulation of genes like TP53, IDH1, MET and NF1 [29, 50, 51, 169, 171, 175-177]. Cancer stem cells are the predominant reason for disease relapse but targeting them have been difficult. By drawing parallels between GBM and AML, basic research from either field can be transposed for identifying dual targets and shorten time required for developing novel therapies.

2 AIMS OF THE THESIS

The overall aim of the thesis was to identify novel genes with important functions in glioblastoma multiforme and acute myeloid leukemia.

Study I

The aim of this study was to understand the role of various cytoskeletal regulators in glioblastoma multiforme (GBM), how they are affected by various cancer drugs including temozolomide (TMZ) and assess these regulators for use as biomarkers in GBM.

Study II

The aim of this study was to identify novel cancer specific genes using childhood acute myeloid leukemia as a model system.

Study III

The aim of this study was to identify and characterize novel target genes required for the growth of glioblastoma multiforme (GBM) cells by utilizing a functional genomics approach.

Study IV

The aim of this study was to identify novel targets in glioblastoma multiforme using a large-scale functional shRNA screen. We also aimed to develop a new dual CRISPR-Cas9/shRNA vector system for multiplexing and efficient validation of target genes.

3 SUMMARY OF PRESENT INVESTIGATIONS

3.1 STUDY I

Methodology

For the bioinformatics analyses, we selected 85 genes encoding different cytoskeletal regulators and classified them into 10 groups based on their molecular function and compartments. To understand their role in GBM, we then analyzed their expression levels in several gene expression data sets comparing patient derived tumors versus non-tumor tissue, CD133 positive and negative GBM and neural stem cells (NSC) and stem versus differentiated GBM cells. We also compared the association of these genes to clinical parameters such as percent genetic alterations and overall patient survival using various databases. To understand the effects of various FDA approved drugs including TMZ on GBM cells, we performed a small molecule screen and monitored effects on the cytoskeleton by immunofluorescence and performed gene expression analysis by qPCR.

Results

To assess if cytoskeletal regulators can be used as prognostic markers in GBM, a list of known cytoskeleton genes were analyzed in different gene expression GBM data sets available from GEO and the TCGA data. We observed that most of these genes were differentially expressed compared to normal tissue and harbored at least some type of genetic aberration. Using the TCGA survival data we were able to shortlist 6 genes that were significantly correlated with overall patient survival. Having established these as potential prognostic markers, we wanted to test if they have predictive value. To do so, we performed a small molecule screen and evaluated the potency of several FDA approved drugs in comparison to TMZ, a standard chemotherapeutic agent used for treatment of GBM. Compounds were classified based on their molecular function they inhibit and shortlisted based on their ability to affect viability of GBM cells. Compounds belonging to classification ‘kinase inhibitor’, ‘antimetabolite’ and ‘alkylation agents’ drastically reduced GBM cell viability within 4 days of treatment. We next investigated if TMZ or any of these shortlisted compounds exert effects on GBM cells by affecting the cytoskeleton. We measured viability and performed immunostaining on GBM cells after 2 days of drug treatment. We also measured gene expression changes of the selected 6 genes and from our study, it is evident that TMZ does not have the best potency as many of the other FDA approved drugs on the cytoskeletal regulators but further evaluation shows that it is consistent in its effect.

Conclusions

Our study highlights the importance of cytoskeleton regulators in GBM and suggests that combinatorial therapies, specifically targeting the cytoskeleton, might be more efficient in GBM.

3.2 STUDY II

Methodology

In order to identify AML specific vulnerabilities, we performed a negative selection RNAi screen in AML cells and non-transformed bone marrow cells with a library of pooled barcoded shRNAs. To validate the findings from our screen, we used several leukemia cell lines and performed loss of function studies in mixed growth competition assays. By using qPCR and western blots to quantify relative gene expression and protein levels, we ensured a good knockdown efficiency. To circumvent the possible off-target effects of using shRNAs, we also performed CRISPR-Cas9 based knockout studies. In addition, to rule out effects on normal cells, we functionally tested the effects of gene inhibition in primary normal human and mouse cells by using colony formation assays (CFU). To show the importance of the target gene in disease progression, we transplanted AML knockout cells in immune competent mice. Using flow cytometry, we were able to assess the effects on disease maintenance in primary leukemia-initiating cells and bulk cancer cells. To elucidate the cellular and molecular mechanisms by which the target gene contributes to childhood AML cells, we used cell cycle analysis, apoptosis assays and RNA-sequencing.

Results

CHD4, a chromatin remodelling factor, severely affected cell growth of AML cells upon deregulation but did not affect growth of non-transformed bone marrow cells. We used knockdown and knockout experiments to functionally validate the importance of CHD4 in the growth of leukemic cells and demonstrated that CHD4 inhibition arrested leukemic cells in G0 phase of the cell cycle. Inhibition of CHD4 downregulated genes important for cell cycle progression, particularly, through Myc and its downstream effectors. Loss of CHD4 function prevented disease progression in xenotransplantation models and was shown to have anti-leukemic effects on primary childhood AML cells when targeted by shRNAs. Interestingly, CHD4 was essential in driving growth of leukemia initiating cells (LIC) but not in normal hematopoietic cells.

Conclusions

Overall, our findings indicate the importance of CHD4 for maintenance of LICs and thereby emergence and development of childhood AML. This AML-specific dependency suggests that CHD4 may represent a novel therapeutic target in childhood AML.

3.3 STUDY III

Methodology

To identify genes specifically required for the growth of GBM cells, we performed a negative selection shRNA screen in proneural glioma stem cell (GSC) lines. Fibroblast cells were used to identify genes that were GBM specific. We utilized inducible constructs to validate our findings from the screen and used qPCR and western blot analysis to confirm knockdown efficiency at the mRNA and protein levels. Using commercially available cell lines and a flow cytometry based cell growth assay, we functionally validated the role of our target gene. To prove that the target gene was specifically required for the growth of GBM cells, we performed rescue experiments. In addition, to identify the cellular mechanisms by which the target gene operates, we performed cell cycle analysis and apoptosis assays. We also performed small molecule inhibition of the target protein utilizing several active and inactive tool compounds. Compounds were tested for their ability to bind and stabilize target protein by differential scanning fluorimetry (DSF) assays and IC_{50} values were established by using Ub-Rho[110] assays. To assess the potency of the tool compounds, we measured viability of different GBM cell lines after treatment. To ensure there is no effects on healthy neural cells, we utilized the neuroepithelial-like stem cells (NES) and NES-derived neurons and functionally tested effects of target gene inhibition. To understand how UCHL1 functions at the molecular level in GBM and dissect downstream pathways, we performed RNA-sequencing and validated results by functional enrichment analysis and qPCR.

Results

UCHL1 is an enzyme in the ubiquitin proteasome pathway with ubiquitin hydrolase and ligase functions. shRNAs targeting UCHL1 showed significant effects on GSC growth but did not result in pronounced effects on fibroblast cell growth. By using inducible shRNAs and over-expression plasmids in rescue experiments, we were able to demonstrate the specific effects of UCHL1 in GBM cell growth. Loss of UCHL1 arrested GSCs in the G0 phase of the cell cycle which also caused apoptosis. Transcriptomic profiling by RNA-sequencing showed that genes positively correlating with UCHL1 inhibition were important for cell proliferation and involved in cell invasion while the genes negatively correlating with UCHL1 inhibition were important in stress response and regulation of cell death. Quantitative real time PCR (qPCR) analysis of these findings showed a significant correlation with the RNA-seq data. Further, inhibition of UCHL1 using various active and inactive control tool compounds showed that pharmacological inhibition of UCHL1 prevents growth of GBM cells. In contrast to GBM cells and GSCs, UCHL1 downregulation did not increase cell death in NES cells or NES derived neurons. Also, chemical inhibition of UCHL1 in NES-derived neurons demonstrated that two of the three compounds tested did not affect neuronal functions like membrane potential and action potential.

Conclusions

From our study it is evident that UCHL1 is crucial for the growth and survival of GBM cells while being expendable in NES cells or NES derived neurons for their function or survival. Our results highlight the important and cancer-specific functions of UCHL1 in GBM and indicate that UCHL1 may represent a novel therapeutic target in GBM.

3.4 STUDY IV

Methodology

A large scale shRNA screen was performed in two patient derived glioma stem cell (GSC) lines and fibroblast cells with the aim to identify GBM-specific dependencies. We also developed a new dual shRNA/CRISPR-Cas9 multiplex vector system capable of switching between 9 different fluorescent reporters, 3 different antibiotic selection markers and a luciferase/FUCCI system. Using this new vector system developed in-house, we validated target genes by knockdown and assessed gene inhibition using qPCR and western blots. We used several commercially available glioma cell lines and U2OS cells for growth and colony formation assays. In addition, we were able to validate our vector system by multiplexing several target genes at the same time and distinguished their effects on glioma cells by immunofluorescence. To understand the cellular mechanisms by which the target genes contributed to the growth of GBM cells, we utilized the FUCCI reporter to assess cell cycle status after gene inhibition in glioma cells. To delineate molecular function of the target genes, we performed transcriptomic analysis using RNA-sequencing.

Results

ARIH1 and ARIH2 are two E3 ubiquitin ligases identified from the shRNA screens for their specific contribution to GBM cell growth. Downregulation of ARIH1 or ARIH2 in GSCs or different glioma cell lines severely affected growth compared to scramble control and inhibition of ARIH2 but to a lesser extent ARIH1 decreased colony formation in U2OS cells. Downregulation of ARIH2 significantly affected viability of MO59K cells compared to ARIH1 targeted cells or negative non-targeting controls and arrested U2OS cells in S and G2-M phases of the cell cycle. Previous reports of involvement of ARIH1 and ARIH2 in DNA damage response led us to investigate if ARIH1/2 inhibition will sensitize cells to DNA damage. Only inhibition of ARIH2, not ARIH1, led to increase in fragmented DNA in single cell gel electrophoresis (SCGE or COMET) assays compared to control cells. In addition, increased protein levels of DNA damage markers c-PARP and γ-H2AX was observed, which are known indicators of single and double stranded DNA breaks respectively. RNA sequencing of ARIH1 and ARIH2 knockdown samples identified genes which were

positively correlated to gene sets enriched in post translational protein modifications and cell proliferation. Loss of ARIH1 lead to upregulation of genes associated with regulation of cell death and apoptosis and downregulated genes involved in regulation of cell proliferation and neuron parts. Genes negatively correlated to ARIH2 inhibition were enriched for gene sets such as positive regulation of cell differentiation, neurogenesis and neuronal differentiation. Further, qPCR analysis confirmed our findings from the RNA-seq data. Overall, the RNA-sequencing data suggests the involvement of ARIH1 and ARIH2 in GBM cellular proliferation through different cellular processes.

Conclusions

Here we show, in part, that ARIH1 and ARIH2 contribute to GBM cell growth through different pathways. Although further validation is required to establish this, our data demonstrates the importance and possible cancer-specific dependencies of ARIH1 and ARIH2 and suggest them as novel therapeutic targets in GBM.

3.5 UTILIZATION OF RELEVANT MODEL SYSTEMS AND COMPARATIVE ANALYSIS TO BUILD SPECULATIVE MODELS

The purpose of this section is to highlight and discuss the use of appropriate model systems and the choice of experimental techniques to address relevant scientific questions.

In addition, findings from the functional genomics screen have been compared with the transcriptomic data to build a speculative model outlining the possible role of identified genes in GBM. Some of these findings are purely speculative and have not been discussed in studies III and IV for this reason.

To refresh, studies III and IV demonstrate the use of a functional genomic screening approach in identifying novel targets required for the growth of GBM CSCs. By doing so, we have identified three genes belonging to the ubiquitin proteasome system to be important for the growth of GBM cells.

3.5.1 Model system and functional screening approach

It is evident from the TCGA data that patients with a classical or mesenchymal subtype respond better to high doses of temozolomide (TMZ) and radiation and have increased survival, compared to the proneural subtype of GBM [26]. Interestingly, the two patient derived proneural GSC lines used for this study showed worse survival in xenotransplantation experiments compared to other subtypes [55]. Studies III and IV utilize the aforementioned model system, which faithfully represents the parent tumors, in screening for novel GBM targets.

Functional screens utilizing siRNAs, shRNA or CRISPR-Cas9 based knockdown/knockout systems, have been successfully used to identify vulnerabilities in cancer cells including GBM and AML [182-190]. However the caveats of using such systems are the possibilities of off-target effects leading to false positive discoveries. We addressed this issue by using human fibroblast cells to filter for effects in control cells and identify GBM specific effects. Although they are not representing the most relevant cancer-specific control cells, fibroblasts are still useful in identifying possible side effects. From our screening data, we found shRNAs targeting numerous genes important for GBM cell growth and survival, like AKT1 [191], CCND2 [192], EGFR [90, 193-197], FGFR1 and FGFR3 [198, 199], IDH1 [200], MDM2 [201, 202], KDM2B and KDM5A [203-206] etc., to be reduced in abundance, specifically in the GSCs and not in the fibroblast cells. Thus, these findings from study III and IV validate the use of a shRNA-based screening methodology and presents a resource of possible GBM-specific vulnerabilities.

3.5.2 Comparing transcriptomic and functional genomics data

Transcriptomic analysis of UCHL1 inhibition in GSCs showed that genes involved in cell cycle, PI3K/Akt and Myc signaling were affected. PI3K/Akt signaling pathways is one of the three major pathways deregulated in GBM accounting for at least one altered genetic event in 86% of patient samples [25]. We observed that upon UCHL1 inhibition, *EGFR*, *FGFR1*, *FGFR3*, *FGFR4*, *PDGFRA*, *PIK3C2A* and *PI3KR2* transcripts are downregulated, which are genes involved in the activation and downstream signaling of PI3K/Akt/Ras-MAPK pathways. Strikingly, the same genes were found to be reduced more than two-fold in shRNA abundance in GSCs and did not affect fibroblast cell growth. Such comparative analysis strengthens the validity of our data and the pathways identified.

3.5.3 Speculative modeling to generate new hypothesis

More in depth analysis of this data revealed pathways which require further validation, especially at the proteomic level. However they were used to build a speculative model showing interaction between the genes identified from the RNA-seq data.

Study III

There has been previous reports of UCHL1 physically interacting with β-catenin, a Wnt signaling molecule, through which a positive feed-back loop is established as UCHL1 deubiquitinates β-catenin and stabilizes it while β-catenin/TCF4 binds and upregulates UCHL1 transcript levels [207, 208]. β-catenin is usually kept inactive by GSK3β mediated phosphorylation under normal conditions but in GSCs unphosphorylated forms of β-catenin are found to be in high levels compared to adult human neural stem cells [209]. Previous studies have shown that PI3K/Akt signaling pathways interact and inhibit GSK3β by phosphorylation [210-213]. One of the downstream targets of β-catenin, cyclin D1 [214], a major cell cycle regulator of G1-S transition, was also downregulated upon UCHL1 inhibition. Therefore, we speculate that UCHL1 inhibition leads to downregulation of PI3K/Akt signaling axis leading to loss of proliferative potential.

Study IV

ARIH1 downregulation showed a strong downregulation of RBBP4, a protein regulating DNA repair components and modulating chemo-sensitivity to TMZ treatment in GBM cells [215] and significantly upregulated CEND1, a protein involved in cell cycle exit and differentiation of neuronal precursors [216, 217]. We also noticed that genes required for transcriptional activation and neuronal determination and differentiation, NEUROG2 and NEUROD1 were found to be negatively correlated to ARIH2 expression. These two proneural genes have been shown to be important for the progression of neurogenesis in the inner ear [218] while NEUROD1 is important in the terminal differentiation of proneural precursors in the olfactory bulb [219]. In contrast, activation of NEUROG2 is linked to cell cycle exit by repression of D and E type cyclins [220]. In vivo experiments have also shown that NEUROD1 expression can reprogram reactive glial cells, which are formed after neuronal injury/death, into functional neurons [221].

Combining data from studies III and IV, including the data validated by qPCR, a speculative schematic model of interaction between the different genes has been illustrated (see below). Such models can help describe novel molecular mechanisms of action for genes identified from omics studies and conceive hypothesis for further testing.

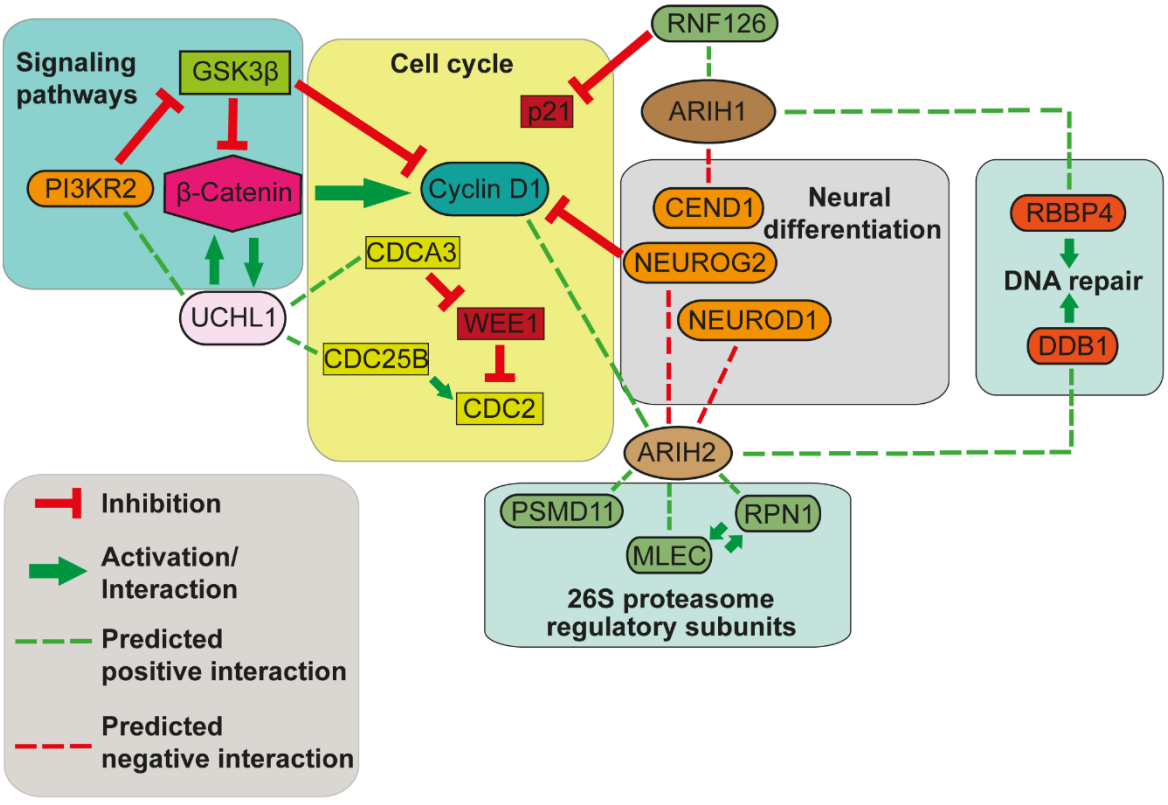

Figure 4: Schematic illustration of the speculative model showing interactions between genes identified from RNA-sequencing in studies III and IV. Green and red dashed lines indicates unknown but speculated interactions based on RNA-seq data. Green arrow and red inhibition symbols are drawn based on evidence from literature.

4 FUTURE PERSPECTIVES

Even though the incidence of GBM is considered rare in comparison to other cancers, it is a truly fatal disease. In the last two decades several drugs have entered clinical trials and have failed to improve the overall survival of GBM patients, due to lack of specificity and inability to eradicate the GSCs. Therefore the need for a targeted therapy has grown exponentially.

Evidence points to GSCs, which cycle slowly and acquire radio- and chemotherapeutic resistance, in tumor relapse. GSCs have been observed to migrate along and proliferate at vascular branch points. Additionally, pushing GSCs to enter cell cycle can sensitize them to chemotherapeutic agents aimed at proliferating cells. Identifying and targeting critical molecules required for migration and quiescence of GSCs will significantly reduce tumor invasiveness and increase treatment efficiency.

Use of next generation genomics and proteomics have helped paint a detailed GBM landscape. However, it is hard to obtain brain tissue from healthy individuals, let alone isolate NSCs or GPCs. With the help of single cell genomic and proteomic analysis, one can catalog the cellular and molecular profiles of NSCs or GPCs from thousands of healthy donors. With time and large scale efforts, this data can be pooled to create a database. By data mining, we can then identify unique cellular and molecular profiles of GSCs and NSCs which can then be thoroughly and functionally interrogated. This would help in designing targeted therapies with minimal side effects.

One of the biggest problems with treating GBM is the delivery of the chemotherapeutic agents across the blood brain barrier (BBB). With advances in high resolution imaging, a map of the patient’s brain can be created. This generates spatial data and much like a GPS, a brain positioning system can be developed. With advancements in the field of nanotechnology, nanobots could be engineered to mobilize to any given position in the brain simply by using spatial coordinates and arrive at the tumor site for targeted drug delivery.

As the tumor cells proliferate rapidly in a background of post-mitotic neurons, use of microchips to monitor, in real time, parameters like pH, temperature and cellular ATP levels can help identify minute differences between normal and malignant cells. With quantum computing capabilities in the near future, instruments can be trained to identify abnormalities including GBM cells at very early stages.

Several oncogenic events and activation of different signaling pathways within the same tumor create intratumoral heterogeneity. Tumor cells utilize such redundant pathways to establish cell survival and resistance to therapy. Since each patient is unique with respect to their tumor profile, adapting a combinatorial approach in targeting several of these receptors or pathways, tailored to the patient profile, could prove effective in fighting this deadly disease.

5 POPULAR SCIENCE SUMMARY

Cells are the most basic building blocks of the human body. They are like tiny machines with thousands of moving parts and are constantly being repaired and rebuilt while performing routine work. They produce the energy required for us to perform daily activities, produce proteins to build muscles and so on. Cells can also multiply and give rise to more cells. But this is tightly controlled by the body to make sure not too many are produced too quickly. Cells also get damaged from daily wear and tear, by what we eat and by the environment we are exposed to. If the cells are damaged too badly, they are automatically destined to die while those with repairable damages are rescued. On rare occasions, such repair mechanisms fail and the cells continue to function with damages. Such events change the behavior of the cells and makes them go rogue. They begin to disobey the laws of the human body and begin to produce more cells, avoid the body's police system (immune cells) and eventually form cancers. This thesis describes studies performed in two different types of cancers, namely brain cancer and blood cancer.

Glioblastoma is an aggressive form of cancer arising in the brain. Patients feel severe headaches and can experience problems with other body functions due to damage to the brain cells. Even with intensive treatments and good care, the life expectancy of these patients are short and the disease is incurable.

Acute myeloid leukemia is a form of blood cancer, where cancer cells steal the nutrients and the space in the bone marrow so normal cells become a minority. Patients feel weak and anemic due to this reason and must be treated quickly. However only 40% of young patients and 15% of old patients are cured from this disease.

Treatment for both diseases are often associated with severe side effects. It means that the treatment affects normal healthy cells and cause more damage to the body. There are also special cancer cells (cancer stem cells) which are like guerrilla warriors and go into hiding. It is hard to identify and destroy these cells. Even a single cancer stem cell can give rise to an army of dangerous cells with time. Therefore, complete elimination of these cells is a priority in curing these diseases.

By identifying vulnerabilities for such sneaky cancer cells and targeting those weak points, we can specifically and effectively eliminate them. By turning off genes one at a time, we have in this thesis, identified three genes that are vulnerable for the glioblastoma cells and one gene that is the weak point for leukemia cells. We also saw that turning off these genes in normal cells did not affect their cell growth or other activities. Therefore, the thesis presents three new genes that could be targeted in glioblastoma and one gene in acute myeloid leukemia for future treatments.

6 ACKNOWLEDGEMENTS

Looking back at the last six years of my life only reminds me of how lucky I am to be surrounded by kind people and good friends. Throughout this journey, I have experienced ups and downs but I could not have gone through this without the help of my close friends and my family. In one way or another, a lot of people have contributed to this thesis.

First and foremost, I would like to thank my main supervisor **Julian Walfridsson**. I will always be thankful to you for believing in me and taking me as your student and investing your time and energy in nurturing me through these years. From the first time we met, I enjoyed your sense of humor which made me feel at ease and made our interactions much more relaxed and not too formal. You have always been critical in a positive way and encouraged the same thinking process from me and others. I admire your 'hej-ho-lets-go' mentality and I have to say it is very catchy. My opinions were always heard and you have always appreciated my work in the lab. You have shared your immense experience and knowledge with me and helped me develop as a person and an independent scientist. I have also learnt that when I enter an argument with someone, I will remember to bet my left arm. Personally I feel very confident in myself and about my future, and I am thankful to you for that.

I would like to thank my co-supervisors, **Indranil Sinha** and **Satish Srinivas Kitambi**, for helping me with all my projects and fruitful collaborations that has helped me complete my thesis work. Thank you **Robert Månsson**, for your encouragement and scientific discussions during the early stages of my PhD when I was a newbie at the research center. Thank you **Andreas Lennartsson**, my mentor, for your support and checking on me before my half-time.

I would like to thank **Mikael Altun**, **Johan Boström** and **Carolyn Marks** for the wonderful and successful collaborations. Thank you **Mikael** for sharing your expertise with the ubiquitin proteasome system and timely help in completion of major experiments. Thank you **Johan** for performing all the cloning required for our collaboration and the good scientific discussions we had while exchanging data. Thank you **Carolyn** for letting me work under your wing at the Scilife lab and taking care of me during those weeks of cell culture.

Being mainly behind the scenes, I would like to thank the people at MedH administration. Thank you **Jan Bolinder**, **Klas Karlsson**, **Ulrika Markne**, **Anastasia Urban**, **Therese Lind**, **Elenor Nyman** and **Gulaid Ismail** for taking care of all the paper work and resolving issues quickly.

I would also like to thank the core facility at NEO, Bioinformatics and Expression Analysis (BEA) core facility members **Fredrik Fagerström-Billai**, **Marika Ronnerhölm**, **Anastasius Damdimopoulos**, **David Brodin** and **Thais de Castro Barbosa** for heling me out with affymetrix analysis, RNA-sequencing and the extensive bioinformatics support.

Unlike many other places I have seen, HERM is very special where different people and cultures blend beautifully and exist symbiotically. You are always greeted by happy faces and experience good hospitality. Thank you **Eva Hellström-Lindberg**, **Petter Höglund** and all senior members of HERM for your leadership and making all this happen. Thank you **Monika Jansson**, **Ann-Sofie Johansson** and **Sri Sahlin** for taking care of the routine non-academic work and making it easy for all of us. Thank you **Iyadh Douagi** for maintaining a wonderful flow cytometry core facility, sharing your expertise and training everyone interested. Thank you **Hong Qian, Robert Månsson** and **Evren Alici** for being critical at SAP seminars and promoting a good scientific environment.

Tack **Gunilla Waldin**, för att du motivierar mig till att alltid prata svenska. Tack för ditt stöd!

Thank you **Yaser Heshmati**, **Gözde Turköz** and **Shabnam Kharazi** for being such good team players and providing scientific and moral support when needed. Thank you **Shabnam** for helping me set up flow cytometry panels for cell cycle and apoptosis assays. Thank you also for the wonderful conversations we had in the cell culture room and in the corridors.

To the first generation PhDs, **Michael Chrobok**, **Edda Maria Elvarsdottir**, **Erle Refsum**, **Caroline Gavin**, **Deepika Nair**, **Hani Adbulkadir**, **Pingnan Xiao**, **Simona Conte** and **Ayla De Paepe,** thank you for all the wonderful moments we have shared during these years at work and outside at socials. Thank you **Teresa Mortera-Blanco** for all the interesting lunch conversations. Thank you **Thibault Bouderlique**, **Heinrich Schlums**, **Isabel Hofman**, **Hongya Han**, **Caroline Leijonhufvud** and **Monika Dolinska** for a positive and exciting office environment. Thank you **Stephan Meinke**, for generally sharing your positive energy, wisdom and scientific expertise.

To my most favorite people, **Huthayfa Mujahed**, **Monika Dolinska** and **Jennine Grootens (The Gang)**, thank you for being a big part of my life. I cannot imagine the last few years without the three of you. **Huthayfa**, you are like a brother to me and we have made people envy us for being so chilled out at work. The everyday coffee on the couch, early lunch and the afternoon fika are all part of an everyday ritual that I am so happy to have. The days you are not at work, I feel like a part of me is missing. You will forever be my Bro! **Monika** and **Jennine,** I cannot count all the exciting afterworks, parties and dinners we have had together. I am thankful for all your moral support and general life advice. I wish you all good luck in finishing your PhDs successfully.

To my dearest **Josefine Enneby**, you have been a storm that swept me off my feet. I cannot imagine going through the last year without your undeterred commitment to taking care of me and providing me with energy to go on with my PhD. You are a wonderful and talented artist and I cannot thank you enough for the beautiful art on the cover of my thesis. Thank you for being patient, loving and always standing by my side.

To my best friend, **Suhasini Udayakumar**, life would be way different without you. I met you eight years ago and our friendship has only grown stronger. Not a day has passed when I

have not thought about you and all that we have experienced in Sweden together. You were there for me through thick and thin and made me who I am today. And I am forever thankful for that.

To **Harisankar Krishnamurthy** and **Padmini Harisankar**, I feel grateful for having you two as my parents. You have given me unconditional love and affection and always encouraged me with all my life decisions. It must have been so hard for you two to stay away from your children and yet you were only cheering and enthusiastic when I had to move to Sweden for my higher education. To my sister **Sandhya**, thank you for being the best little sister ever. I cannot imagine growing up without a sister like you. It makes me happy that we share many quirks as it reminds me of our teenage years and generally life at home. I am blessed with such a loving family and I would not have achieved all this without your love and support. Thank you for that!

This thesis was supported by the Wallenberg Foundation, Karolinska Institutet, Åke Wiberg Foundation, Åke Olsson Foundation for Hematological Research, Magnus Bergvall Foundation, Swedish Cancer Society, Vinnova and The Swedish Childhood Cancer Foundation.

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