IDENTIFICATION OF POTENTIAL AMINO ACIDS PROFILE WITH THE PROTEIN EXPRESSION OF IDH1, MGMT, ATRX AND OXIDATIVE STRESS MARKERS IN THE PRIMARY BRAIN TUMOUR

MICHELLE TIONG HUI LING

UNIVERSITI SAINS MALAYSIA

2025

IDENTIFICATION OF POTENTIAL AMINO ACIDS PROFILE WITH THE PROTEIN EXPRESSION OF IDH1, MGMT, ATRX AND OXIDATIVE STRESS MARKERS IN THE PRIMARY BRAIN TUMOUR

by

MICHELLE TIONG HUI LING

Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

August 2025

ACKNOWLEDGEMENT

Firstly, I would like to express my greatest praises to God for giving me blessing and strength endlessly in finishing this whole research. Special appreciation goes to my supervisor, Dr. Wan Norlina Wan Azman, for her supervision and constant support. Her invaluable assistance and constructive comments and guidance throughout the research and thesis work have contributed to the success of this research. Not forgotten my great appreciation to my co-supervisors, Assoc. Prof. Dr. Anani Aila Mat Zin, Prof. Dr. Zamzuri Idris, Dr. Wan Nur Syuhaila Mat Desa, and Dr. Maulidiani for their endless encouragement and knowledge throughout the research and thesis work. I would also like to extend my appreciation to Dr. Aidy Irman Yajid with his patience and knowledge particular in FISH method optimization. I would also like to thanks all medical laboratory technicians, science officers, and administrative staff of the School of Medical Sciences (USM), School of Health Sciences (USM), and INFORMM (USM), for their assistance and cooperation throughout the research endeavours. Special thanks for Miss Normayazi Mohemed Ali from Pathology Lab department for her guidance and patience in finishing this research. Sincere thanks also go to my friends, seniors, and lab mates, especially Ain, Jessy, Muslihah, Oliva, and others for their help, kindness, and support during my study. Last but not least, my deepest gratitude goes to my beloved parents for their endless love, encouragement and financial support. To those who indirectly contributed to this research, your kindness means a lot to make me who I am today. Thank you very much.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

[a]	Label for Chi-square test result in table
[b]	Label for Fisher's exact test result in table
%	Percentage
<	Less than
/	Per
+	Plus
℃	Degree Celcius
µl	Microliter
µm	Micrometre
ml	Millilitre
n	Number
nmol/ml	Nanomole per litres
R^2	R-squared

LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
Ala	L-Alanine
ALT	alternative lengthening of telomeres
Asp	L-Aspartic acid
ATRX	Alpha Thalassemia/Mental Retardation Syndrome X-Lin
α-KG	α-ketoglutarate
BBB	blood-brain barrier
BCAA	branched-chain amino acids
BCAT	branched-chain aminotransferase
BCKDH	branched-chain α-ketoacid dehydrogenase
CAT	Catalase
CNS	Primary central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
DNMT	DNA methyltransferase
ECM	extracellular matrix
EtOH	Ethanol
FAD	flavin adenine dinucleotide
FFPE	formalin-fixed paraffin-embedded
FISH	fluorescence in situ hybridization
GBM	Glioblastoma
G-CIMP	glioma CpG island methylator phenotype
GCMS	Gas Chromatography Mass Spectrometry
Gly	Glycine
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
GST	glutathione S-transferase
HGG	high-grade glioma
HIF	hypoxia-inducible factor
HIF1α	hypoxia inducible factor-1α

H_2O_2	hydrogen peroxide
HPUSM	Hospital Pakar Universiti Sains Malaysia
HRP	Horseradish peroxidase
IDH	isocitrate dehydrogenase
IDH1	isocitrate dehydrogenase 1
IHC	Immunohistochemistry
Ile	L-Isoleucine
Leu	L-Leucine
LGG	Low-grade glioma
LOD	limit of detection
LOQ	limits of quantification
Met	L-Methionine
MeOX	methoxyamine hydrochloride
MGMT	O6-Methylguanine-DNA Methyltransferase
MRI	magnetic resonance imaging
MSTFA	N-Methyl-N-(trimethylsilyl)trifluoroacetamide
mTOR	Target Of Rapamycin
mTORC 1	mTOR complex 1
mTORC 2	mTOR complex 2
m/z	mass-to-charge ratio
NAD+	nicotinamide adenine dinucleotide
NGS	next generation sequencing
NIST	National Institute of Standards and Technology
NO	Nitric oxide
NOS	not else defined
O_2	Oxygen
PCR	polymerase chain reaction
PCR-RFLP	polymerase chain reaction-restriction fragment length polymorphism
PCV	procarbazine, lomustine, and vincristine
Phe	L-Phenylalanine
PHGDH	phosphoglycerate dehydrogenase
PTM	histone posttranslational modification
Pro	L-Proline
PRODH	proline dehydrogenase

Pyr	L-Pyroglutamic acid
R^2	correlation coefficient
ROS	reactive oxygen species
SAM	S-adenosylmethionine
Ser	L-Serine
SHMT	serine hydroxymethyltransferase
SHMT1	serine hydroxylmethyltransfeerase 1
SHMT2	serine hydroxylmethyltransfeerase 2
SOD	Superoxide dismutase
SOD1	Cu-ZnSOD
SOD2	MnSOD
TCA	Tricarboxylic Acid Cycle
TET	ten-eleven translocation
THF	tetrahydrofolate
TIC	The total ion chromatograms
USM	Universiti Sains Malaysia
Val	L-Valine
2-HG	2-hydroxyglutarate

PENGENALPASTIAN PROFIL ASID AMINO BERPOTENSI DENGAN EXPRESI PROTIN IDH1, MGMT, ATRX DAN PENANDA TEKANAN OKSIDATIF DALAM TUMOR OTAK

ABSTRAK

Kanser sistem saraf pusat utama (CNS) mewakili kanser heterogenus yang berasal dari system saraf tunjang, di mana setiap kategori menunjukkan ciri histologi dan molekul yang berbeza yang mempengaruhi perkembangan klinikal dan prognostik pesakit. keabnormalan dalam protein memainkan peranan penting dalam perkembangan kanser sistem saraf tunjang, melibatkan IDH1, ATRX, MGMT, CAT, dan SOD1, bersama dengan perubahan profil asid amino sebagai penyumbang utama dalam proses perkembangan kanser. Kajian ini dijalankan untuk menentukan profil asid amino serta corak ekspresi ATRX, MGMT, IDH1, 1p/19q, CAT, dan SOD1 dalam kanser sistem saraf tunjang primer. Profil asid amino dianalisis menggunakan kromatografi gas-spektrometri jisim (GCMS), manakala ekspresi protein IDH1, ATRX, MGMT, CAT, dan SOD1 dinilai menggunakan kaedah imunohistokimia (IHC). Protokol pencahayaan hibridisasi in situ berpendarfluor (FISH) turut dioptimumkan untuk pengesanan delesi bersama 1p/19q. Analisis GCMS berjaya mengesan 11 jenis asid amino daripada 40 sampel tisu kanser saraf tunjang primer FFPE. Ujian IHC menunjukkan ekspresi ATRX Berjaya pada 65% (26 kes), MGMT pada 67.5% (27 kes), IDH1 pada 27.5% (11 kes), CAT pada 67.5% (27 kes), dan SOD1 dalam semua sampel (100%), dengan semua penanda menunjukkan hubungan signifikan antara ekspresi protein, gred, dan jenis tumor ($p < 0.001$). Protokol FISH yang dioptimumkan berjaya mengesan delesi bersama 1p/19q dalam 2 kes oligodendroglioma, manakala 2 kes anaplastik astrositoma menunjukkan keputusan

negatif dan 1 kes glioblastoma menunjukkan keputusan positif. Penemuan ini memberikan maklumat baharu berkaitan profil asid amino serta ekspresi protin ATRX, MGMT, IDH1, CAT, dan SOD1 dalam kanser. Kajian lanjut diperlukan untuk memperluaskan pemahaman terhadap biologi tumor dan potensi penemuan biomarker baharu untuk diagnosis serta rawatan kanser.

IDENTIFICATION OF POTENTIAL AMINO ACIDS PROFILE WITH THE PROTEIN EXPRESSION OF IDH1, MGMT, ATRX AND OXIDATIVE STRESS MARKERS IN THE PRIMARY BRAIN TUMOUR

ABSTRACT

Primary central nervous system (CNS) tumours are heterogeneous neoplasms originating within the CNS, each category exhibiting distinct histological and molecular features that influence clinical progression and prognosis. Protein alterations are central to CNS tumour development, involving IDH1, ATRX, MGMT, CAT, and SOD1, alongside amino acid disruptions as key contributors to tumour progression. This study aimed to determine the amino acid profile and expression patterns of ATRX, MGMT, IDH1, 1p/19q, CAT, and SOD1 in primary CNS tumours. Amino acid profiles were characterised using gas chromatography-mass spectrometry (GCMS), protein expression of IDH1, ATRX, MGMT, CAT, and SOD1 was evaluated using immunohistochemistry (IHC), and the fluorescence in situ hybridization (FISH) protocol for 1p/19q co-deletion was optimised. GCMS analysis identified 11 amino acids across 40 FFPE CNS tumour samples. IHC revealed ATRX expression in 65% (26 cases), MGMT in 67.5% (27 cases), IDH1 in 27.5% (11 cases), CAT in 67.5% (27 cases), and SOD1 in all cases (100%), with significant associations between marker expression, tumour grade, and type ($p < 0.001$). The optimised FISH protocol successfully detected 1p/19q co-deletion in 2 oligodendroglioma cases, with negative co-deletion in 2 anaplastic astrocytoma and positive co-deletion in 1 glioblastoma case. These findings provide new insights into amino acid profiling and the expression of ATRX, MGMT, IDH1, CAT, dan SOD1 proteins in primary CNS tumours. Further research is warranted to expand on these results, contributing to a deeper

understanding of tumour biology and the potential identification of novel diagnostic and therapeutic biomarkers.

CHAPTER 1

INTRODUCTION

1.1 Background of The Study

The Primary central nervous system (CNS) tumours represent heterogeneous neoplasms that originate within the brain or spinal cord. These tumours are typically classified as either glial or non-glial based on their cellular origin, with each category demonstrating unique histological and molecular characteristics that influence clinical progression and patient prognosis (Toader et al., 2023). Glial tumours, which include astrocytoma, oligodendroglioma, and ependymoma, arise from supportive glial cells and vary significantly in aggressiveness towards the patient and therapeutic complexity (Papadimitrakis et al., 2024). Glioblastoma is a highly aggressive subtype of astrocytoma and is incredibly challenging due to its invasive nature and resistance to conventional therapies(Obrador et al., 2024) . In contrast, non-glial tumours, such as meningioma, medulloblastoma, schwannoma, and pituitary adenoma, which develop in the pituitary gland, present distinct clinical implications related to their growth location and growth behaviour(Louis et al., 2021) . Although diagnostic and treatment methods have advanced, CNS tumours remain a significant source of morbidity and mortality, mainly due to their intricate biology and the limited efficacy of current treatment options (Alemany et al., 2020).

The clinical symptoms of CNS tumours are varied, often depending on the tumour's size and location. Common symptoms include chronic headaches, seizures, localized neurological deficits, cognitive changes, personality shifts, and motor or sensory impairments. These symptoms can significantly impact quality of life and are

often nonspecific, complicating early detection and underscoring the need for precise and reliable diagnostic tools (Zhou et al., 2024).

For early diagnosing of CNS tumours, advanced imaging techniques, such as magnetic resonance imaging (MRI) and computed tomography (CT), are essential as they provide detailed information on tumour size, location, and spread (Martucci et al., 2023). However, imaging alone does not yield a definitive diagnosis. Histopathological analysis of biopsy samples remains the gold standard for accurate diagnosis, determining tumour grade, and informing treatment strategies. This approach is enhanced by molecular and genetic studies, which is increasingly valuable for understanding tumour biology and improving patient care (Zhang et al., 2024).

Genetic alterations are central to the development and progression of CNS tumours and are crucial for determining prognosis and guiding treatment (Sarkar et al., 2023). For instance, isocitrate dehydrogenase 1 (IDH1) gene mutations are common in lower-grade gliomas and secondary glioblastomas. These mutations lead to the production of 2-hydroxyglutarate, an oncometabolite that affects cellular metabolism and epigenetic processes, generally correlating with a more favourable prognosis compared to IDH1 wild-type tumours (Hao et al., 2024). Similarly, mutations in the Alpha Thalassemia/Mental Retardation Syndrome X-Lin (ATRX) gene, which plays a role in chromatin remodelling and telomere maintenance, can result in alternative lengthening of telomeres, contributing to tumour progression in some gliomas(Pang et al., 2023). Additionally, methylation of the MGMT (O6-Methylguanine-DNA Methyltransferase) promoter, which silences this DNA repair enzyme, is associated

with increased responsiveness to alkylating chemotherapy, making it a valuable prognostic and predictive biomarker (Yu et al., 2020). A 1p/19q co-deletion is a simultaneous loss of genetic material from both chromosomes 1p and 19q, which is the diagnostic feature of oligodendrogliomas and generally signals a better prognosis and improved response to therapy (Kong et al., 2020).

Metabolomics, the comprehensive study of metabolites within a biological system, is an emerging field that offers insights into the biochemical shifts associated with tumour development and progression. Using Gas Chromatography-Mass Spectrometry (GCMS), metabolomic studies can analyse amino acid levels in cerebrospinal fluid (CSF), plasma, and tumour tissue, revealing fundamental changes in amino acid metabolism that reflect underlying tumour biology and may serve as diagnostic biomarkers or therapeutic targets (Nakamizo et al., 2013). Alterations in amino acid metabolism may indicate tumour-specific metabolic pathways that could be targeted in metabolic therapies (J. Chen et al., 2024).

Oxidative stress is characterized by an imbalance between reactive oxygen species (ROS) production and cellular antioxidant defence mechanisms. Oxidative stress is another critical factor in tumour biology. Elevated oxidative stress in tumour cells can influence growth, therapeutic resistance, and disease progression (Hayes et al., 2020). Catalase (CAT) is an antioxidant enzyme that decomposes hydrogen peroxide into water and oxygen, detoxifying hydrogen peroxide is particularly critical in cells that produce large amounts of ROS, such as rapidly dividing tumour cells. catalase activity is altered to match the increased ROS levels in the tumour

microenvironment (Glorieux and Buc Calderon, 2024). Superoxide dismutase (SOD), an enzyme that converts superoxide radicals into less reactive molecules, is crucial for managing oxidative stress, and fluctuations in SOD activity or expression may significantly affect tumour progression and treatment outcomes (Jomova et al., 2023).

Considering these complexities in defining brain tumours, this study aims to provide a comprehensive analysis of primary CNS tumours by characterizing the amino acid profiles using GCMS to uncover metabolic alterations, evaluating the expression of key genetic markers (IDH1, ATRX, and MGMT) and indicators of oxidative stress (CAT and SOD); and optimizing the fluorescence in situ hybridization (FISH) protocol for detecting 1p/19q co-deletion. This is because there is limited studies explored amino acid metabolic profiling in CNS tumours particularly using GC-MS on FFPE tumour samples, which could provide valuable diagnostic and metabolic insights. There is also insufficient local data on the expression patterns of key genetic markers such as IDH1, ATRX, MGMT and their association with tumour grade and type, especially within Malaysian brain tumour populations. Inadequately defined role of oxidative stress markers (CAT and SOD1) in CNS tumours with conflict result for their expression across tumour grades from previous studies. Optimisation and validation of fluorescence in situ hybridisation (FISH) protocols for 1p/19q co-deletion detection in FFPE brain tumour samples are not standardized yet this is critical for accurate oligodendroglioma diagnosis. By addressing these objectives, the research seeks to enhance our understanding of CNS tumour biology to improve diagnostic accuracy and develop a more targeted and effective therapeutic strategies.

1.2 Problem Statement and Originality of Research

Primary central nervous system (CNS) tumours are among the most complex cancers to diagnose and treat, largely due to their biological diversity and the limitations of current diagnostic tools. While imaging and standard pathology remain essential, they often fail to reveal the deeper molecular changes that drive tumour behaviour and treatment resistance. Increasingly, studies suggest that changes in amino acid metabolism and protein expression within tumours are closely linked to these underlying molecular alterations such as genetic mutations, chromosomal deletions, and epigenetic modifications. Protein expression often reflects these molecular changes and could provide valuable insight into tumour classification and prognosis. However, research exploring the connection between protein expression, amino acid profiles, and known molecular alterations in primary CNS tumours remains limited, especially using formalin-fixed paraffin-embedded (FFPE) tissue samples, which are the most common form of clinical specimen. In this study, we propose that the patterns of amino acid metabolism and protein expression differ depending on the specific molecular alterations present in brain tumours. By investigating these relationships through a combination of laboratory techniques, we aim to provide new insights that could improve early diagnosis and more precise tumour classification, ultimately supporting better clinical decision-making for patients with CNS tumours.

1.3 Research Questions

1. Is there an amino acid profile alteration in primary central nervous system tumours using FFPE brain tumour tissue?
2. Are IDH1, ATRX, MGMT, CAT, and SOD protein expression altered in the primary central nervous system tumours using FFPE brain tumour tissue?
3. Are there association of the protein expression of ATRX, MGMT, IDH1, CAT, and SOD using FFPE brain tissue samples in primary central nervous system tumours with the clinicopathology and histopathological variants?
4. Can the protocol for 1p19q fluorescence in situ hybridization (FISH) in using FFPE brain tumour tissue of primary CNS tumour be optimized?

1.4 Research Hypothesis

1. There is an alteration in the amino acid profile in the primary central nervous system tumour using FFPE brain tumour tissue using GCMS.

2. IDH1, ATRX, MGMT, CAT, and SOD protein expression are altered in the primary central nervous system using FFPE brain tumour tissue using IHC.

3. There is an association of the protein expression of ATRX, MGMT, IDH1, CAT, and SOD using FFPE brain tissue samples in primary central nervous system tumours with the clinicopathology and histopathological variants.

4. The 1p19q FISH protocol in primary central nervous system tumour diagnosis can be optimized using FFPE brain tumour tissue.

1.5 Research Aim and Objective

1.5.1 General Objectives

To determine the amino acid profile and protein expression of ATRX, MGMT, IDH1, 1p19q, CAT, and SOD in primary central nervous system tumours.

1.5.2 Specific Objective

1. To determine amino acid profile using FFPE brain tissue samples in primary central nervous system tumours using GCMS.
2. To determine the protein expression of ATRX, MGMT, IDH1, CAT, and SOD using FFPE brain tissue samples in primary central nervous system tumours using IHC
3. To determine the association of the protein expression of ATRX, MGMT, IDH1, CAT, and SOD using FFPE brain tissue samples in primary central nervous system tumours with the clinicopathology and histopathological variants.
4. To identify 1p19q FISH protocol using FFPE brain tissue sample in primary central nervous system tumours.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer Background

Cancer is one of the most significant health hazards not only in Malaysia but affecting millions worldwide and presenting a broad spectrum of complexities which make it difficult to recover but easily lead to death (Akhtari-Zavare et al., 2018). Despite remarkable progress in cancer research by researcher and scientist along the years, we are still not fully understanding the diseases moreover effectively managing the patients with cancers. Cancer is not a single disease, but a collection of cell mutation characterized by the uncontrolled growth and spread of abnormal cells. Key features of cancer according to (Hanahan and Weinberg, 2011) are mainly describe as suppress of programmed cell death, sustaining growth signals, activate invasion and metastasis, inducing angiogenesis, and enable unlimited replication. Cell has a programmed growth-and-division cycle to ensure a normal cell development and renewal but in the case of cancer cell they are avoiding the growths and suppressing signal. This process is more known as mitogenic signalling where cancer cell product autocrine proliferate stimulation (Ungefroren, 2021). The production of an extracellular mediator that will further promote the mediator itself and the process continue as a loop which promote abnormal signalling. Cancer cell also produce more receptor protein on the surface of the cell so it can be trigger by more signal compared to normal cells.

Cancer could also develop from epigenetic changes, like abnormal DNA methylation, chromatin remodels, and histone modification, can also drive tumourigenesis. These alteration in both cancer genetic and epigenetic are vice versa in promoting cancer development. Epigenetic alteration affect the pattern of histone

posttranslational modification (PTM) especially in wild types genes (Baylin and Jones, 2016). Drugs that target enzymes regulating epigenetic marks, such as DNA methyltransferase inhibitors, have shown success in blood cancers and are being studied for solid tumours (Ren et al., 2023). Recent studies focus deeply into the genetic and metabolite drivers behind these hallmarks. Mutations in genes like TP53, KRAS, and BRCA1 are most common in the development of tumourigenesis (Zhang et al., 2024). Advances in genomic sequencing have accelerated the discovery of other protein mutations such as ATRX, MGMT, and IDH1 which would be functioning in helping clinicians develop more tailored approaches to treatment in the future after more understanding. While the progress in cancer research is encouraging, significant challenges remain as tumour heterogeneity in which no two cancers are exactly alike, even within the same patient. This diversity complicates both diagnosis and treatment. Integrating data from multiple omics research such as genomic, proteomic, and metabolomic is helping researchers better understand these complexities, paving the way for more effective treatment (Mohr et al., 2024).

2.2 Primary CNS Tumours

2.2.1 Primary CNS Tumour Grading

Brain cancers are associated with notably poor survival rates (Miller et al., 2021). µ 2.1 shows the types of brain tumours according to the 4th edition of 2016 WHO Brain Tumour Grades (Komori, 2017). According to a review by Herholz et al. (2012), glioma tumours uniquely span all four grades, with each grade exhibiting distinct characteristics and target patient demographics. Grade I gliomas are predominantly observed in paediatric populations, while grade II gliomas, encompassing subtypes such as astrocytoma and oligodendroglioma, are more frequently diagnosed in young adults.

Grade III gliomas, classified as anaplastic gliomas, and grade IV gliomas, referred to as glioblastomas, are characterized by necrosis and pronounced cellular atypia.

Table 2.1 Types of Brain Tumours According to 2016 WHO Grades (Cosnarovici et al., 2021).

Grades	**Characteristics**	**Tumour type**	**Tumour subtype**
Grade I	• Benign • Can be cure by surgery • Slow growing • Non-infiltrative	Pilocytic astrocytoma	➢ Pilocytic astrocytoma ➢ High-grade astrocytoma with piloid features ➢ Pleomorphic xanthoastrocytoma ➢ Subependymal giant cell astrocytoma ➢ Chordoid glioma ➢ Astroblastoma, MN1-altered
		Craniopharyngioma	➢ Adamantinomatous craniopharyngioma ➢ Papillary craniopharyngioma
		Gangliocytoma	
		Ganglioglioma	
		Pediatric-type diffuse low-grade gliomas	➢ Diffuse astrocytoma, MYB- or MYBL1-altered ➢ Angiocentric glioma ➢ Polymorphous low-grade neuroepithelial tumour of the young diffuse low-grade glioma, MAPK pathway-altered
Grade II	• Less benign • Low infiltrative • Slow growing	Astrocytoma, IDH-mutant	
		Pineocytoma	
		Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted	
Grade III	• Malignant • Infiltrate • Fast growing	Anaplastic astrocytoma	
		Anaplastic ependymoma	➢ Supratentorial ependymomaa ➢ Posterior fossa ependymomaa
		Anaplastic oligodendroglioma	
Grade IV	• Most malignant • Rapid growth • Widely infiltrative • Rapid recurrence • Necrosis prone	Glioblastoma (GBM)	
		Pineoblastoma	
		Medulloblastoma	
		Ependymoma	

2.2.2 Classification and Pathology of Primary CNS Tumour

Primary CNS tumours represent a heterogeneous collection of neoplasms that arise from distinct cellular populations within the brain and spinal cord. These tumours can be broadly classified into two primary categories: glial and non-glial (Salari et al., 2023). This classification is refined into various tumour types based on genetic alterations, clinical manifestations, and histopathological characteristics. A comprehensive understanding of these classifications is crucial for accurate diagnosis and the formulation of effective treatment strategies (Zhou et al., 2024).

2.2.2(a) Glial Tumours

Glial tumours derived from glial cells are the predominant category of primary CNS tumours. Gliomas are categorized into confined gliomas, which exhibit a greater survival rate following complete surgical resection, and diffuse gliomas, which demonstrate a lower survival rate after surgical intervention and require additional therapeutic support (Yang et al., 2022). These tumours are primarily categorized by histological grading, from Grade I (benign) to Grade IV (malignant). Low-grade glioma (LGG) corresponds to CNS WHO grades I and II, whereas high-grade glioma (HGG) pertains to CNS WHO grades III and IV.

Astrocytomas, originating from astrocytes, represent the most prevalent category of glial tumours. The subtypes are classified based on grade and molecular characteristics. Pilocytic astrocytomas (Grade I) are generally well-defined and predominantly manifest in children or teenagers, frequently impacting the cerebellum, optic nerves, or hypothalamus (Collins et al., 2015). These tumours are slow-growing, facilitating favourable results post-surgical excision. Pilocytic astrocytoma often manifests as soft grey tissue characterized by round nuclei, Rosenthal fibres, and

eosinophilic granular masses commonly located in the cerebellum, spinal cord, basal ganglia, or cerebral hemisphere. Patients with pilocytic astrocytoma frequently experience loss of balance, dizziness, and gait instability. Pilocytic astrocytoma often contains mutations in the BRAF gene and is characterized by Rosenthal fibres, which serve as unique histologic indicator (Pizzimenti et al., 2024).

Diffuse astrocytoma (Grade II) consists of tiny tumour cells with irregular nuclei intermingling with adjacent brain tissue, infiltrating and complicating complete resection, generally manifesting in the cerebral hemispheres. The mutation in the IDH1 or IDH2 genes is prevalent and correlates with a more favourable prognosis (Gakinya et al., 2024). Anaplastic astrocytoma (Grade III) is defined by heightened mitotic activity, accelerated cell division, nuclear atypia, and cellular pleomorphism. Anaplastic astrocytoma comprises both IDH wild-type and IDH-mutant forms but lacks 1p/19q codeletion. These characteristics require intensive treatment modalities encompassing surgery, radiation, and chemotherapy (Caccese et al., 2020). Glioblastoma (GBM) is a Grade IV central nervous system cancer, recognized as the most aggressive variant of astrocytoma, characterized by fast proliferation and widespread infiltration. GBM can be categorized into three groups: GBM wild type, GBM IDH-mutant, and GBM not else defined (NOS) if IDH mutant testing was not done or inconclusive result. GBM exhibits histological heterogeneity characterized by pseudopalisading necrosis, which induces cell death near GBM cells and microvascular proliferation, forming aberrant blood vessels and accelerated growth (Delgado-Martín and Medina, 2020). Notwithstanding aggressive intervention, encompassing surgery, radiation, and chemotherapy, the prognosis is unfavourable, with a median survival of roughly 14 months (Mohammed et al., 2022).

Oligodendrogliomas, which arise from oligodendrocytes, are commonly found in the frontal and temporal lobes of the cerebral hemisphere, where these cells produce myelin for the central nervous system. These tumours are distinguished by their "fried-egg" appearance, characterized by round nuclei surrounded by clear cytoplasm and an exemplary network of branching capillaries (Wesseling et al., 2015). Their distinction lies in the 1p/19q chromosomal co-deletion and mutations in IDH1 and ATRX, which are significant for prognosis (Leeper et al., 2015). Grade II oligodendrogliomas are generally indolent and amenable to treatment, whereas Grade III anaplastic oligodendrogliomas display more aggressive characteristics (Wesseling et al., 2015).

Ependymomas, derived from ependymal cells that line the ventricles and spinal cord, are categorized as Grade I, Grade II, or Grade III (Korones, 2023). Grade I ependymomas are subependymomas, which are more prevalent in adults. Grade II ependymomas, encompassing myxopapillary ependymoma and conventional ependymoma, are well-circumscribed and suitable for surgical excision, yet they are prone to recurrence if not entirely resected. Grade III anaplastic ependymomas exhibit increased aggressiveness and may necessitate supplementary treatments. The prognosis depends on the tumour's location and the degree of surgical excision performed. Ependymomas are distinct masses with increased brightness relative to other glial tumour (Jünger et al., 2021).

2.2.2(b) Non-Glial Tumours

Meningiomas constitute the most prevalent non-glial brain tumours, accounting for 15–25% of all primary brain tumours, originating from the meninges that encase the

brain and spinal cord (Varlotto et al., 2015). These tumours are generally slow-growing and are categorized into three classes based on histological characteristics, primarily observable as a mass on the external lining of brain tissues known as the dura mater (Maggio et al., 2021). Grade I meningiomas are benign and frequently amenable to surgical resection, while atypical (Grade II) and anaplastic (Grade III) meningiomas exhibit greater aggressiveness and an elevated risk of recurrence. Grade II atypical meningiomas can be classified into choroid and clear-cell meningiomas. Grade III meningiomas necessitate a multifaceted approach involving surgery, radiation therapy, and, at times, chemotherapy. Both grade II and grade III meningiomas can readily disseminate cerebrospinal fluid (CSF) into adjacent bone tissue and other organs (Harter et al., 2017).

2.3 Brain Cancer Metabolic Pathway

The metabolic reprogramming of brain tumours is a hallmark of cancer. The Warburg effect as present in Figure 2.1, wherein tumour cells preferentially use glycolysis even in the presence of oxygen, is common in brain tumours (Devic, 2016). The Warburg effect function in many ways thru abnormal high rate of glucose uptake, production of lactate, and excess oxygen presence. This adaptation facilitates rapid ATP generation and biosynthesis, crucial for tumour proliferation. Glutamine metabolism, in particular, is essential for tumour growth in gliomas, where glutamine supports the synthesis of nucleotides, amino acids, and lipids (Trejo-Solis et al., 2023). There will be unlimited glucose consumption from the tumour cell and therefore increase cell proliferation as glucose was breakdown into carbon source for anabolic processes. One of the well-known processes would be de novo generation where again nucleotides, amino acids, and lipids were excessively produced (Devic, 2016). In the case of absent

of oxygen, the Warburg effect regenerate NAD+ from NADH which promote production of lactate from pyruvate and this process produce aerobic glycolysis but the side effect from this process was obviously increasing in acidity of the cell pH level (Li et al., 2022). Additionally, alterations in oxidative phosphorylation and elevated levels of reactive oxygen species (ROS) contribute to DNA damage and genomic instability, enhancing tumour progression (Singh and Manna, 2022).

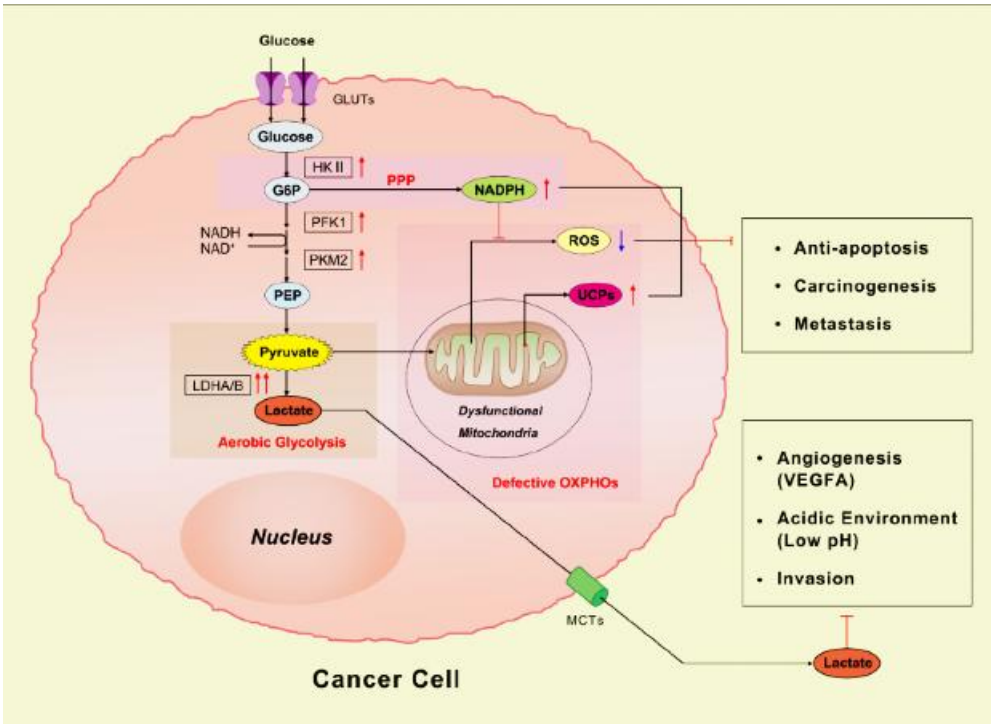

Figure 2.1 Overview of Warburg effect in cancer cells (Fu et al., 2017).

2.3.1 Amino Acid Metabolomic Profiling in Primary CNS Tumours

There has been mounting evidence in recent years that cancer cells also reorganise amino acid metabolism. Amino acids are essential for the synthesis of nucleotides, neurotransmitters, and alternative substrates for glycolysis in addition to their function as protein precursors. Tumour cells often have specific metabolic requirements for growth and survival. This may manifest as an elevated demand for certain amino acids. Targeted therapy can lessen collateral damage to healthy tissue by

focussing just on the cancer cells (Yang et al., 2022). The idea of focusing on the metabolism of amino acids has been researched for decades (Bishop et al., 2022). Metabolomic and lipidomic profiling offers valuable insights into the metabolic alterations driving tumourigenesis. Studies employing gas chromatography mass spectrometry (GCMS) have revealed significant changes in amino acid, fatty acid, and carbohydrate metabolism in brain tumours (Lubes and Goodarzi, 2018; Nakamizo et al., 2013; Ooi et al., 2011). These metabolic shifts enable rapid tumour growth and proliferation, with altered metabolites such as lactate and acetate associated with the Warburg effect (Alberghina, 2023). Amino acid metabolism have a crucial role in the altered metabolic activities of brain tumours. According to previous study, branched-chain amino acids (BCAA) such as leucine, isoleucine, and valine, are often dysregulated in brain tumours (J. Chen et al., 2024). These amino acids provide substrates for biosynthetic processes and act as signalling molecules that enhance tumour cell survival and proliferation.

2.3.1(a) Amino Acid in Tricarboxylic Acid (TCA) Cycle

The TCA cycle or more known as citric acid cycle is central to cellular metabolism, connecting energy production to biosynthetic processes essential for tumours growth. In brain tumours, alterations in amino acid metabolism profoundly influence this cycle to overproduce and overexpress (Pavlova and Thompson, 2016). Glucose is initially used for acetyl-CoA generation in normal tissue cells then promote oxidation process in TCA cycle (Pelley, 2012). Electron molecule extracted from TCA cycle oxidation process are promote into electron transport chain with nicotinamide adenine dinucleotide (NAD+/NADH) and flavin adenine dinucleotide (FAD/FADH2) (Deshpande and Mohiuddin, 2024). This process function to charge the electron

transport chain and promote ATP production in the cells. Electron transport chain are located in the inner mitochondrial membrane which exposed the chain to internal fluid with free flowing adenosine diphosphate (ADP) and NADH (Pelley, 2012). In order to supply unlimited energy for tumour cell development and growth, non-essential amino acid which included glutamine, glutamate, methionine, and phenylalanine will be used in the cycle as another supporting intermediates. Proliferation cell would be S-adenosylmethionine (SAM), cytosolic acetyl-CoA and one-carbon-carrying folate cycle units (Lieu et al., 2020). Figure 2.2 provide a clear picture of TCA cycle together with its relationship with urea cycle and DNA methylation.

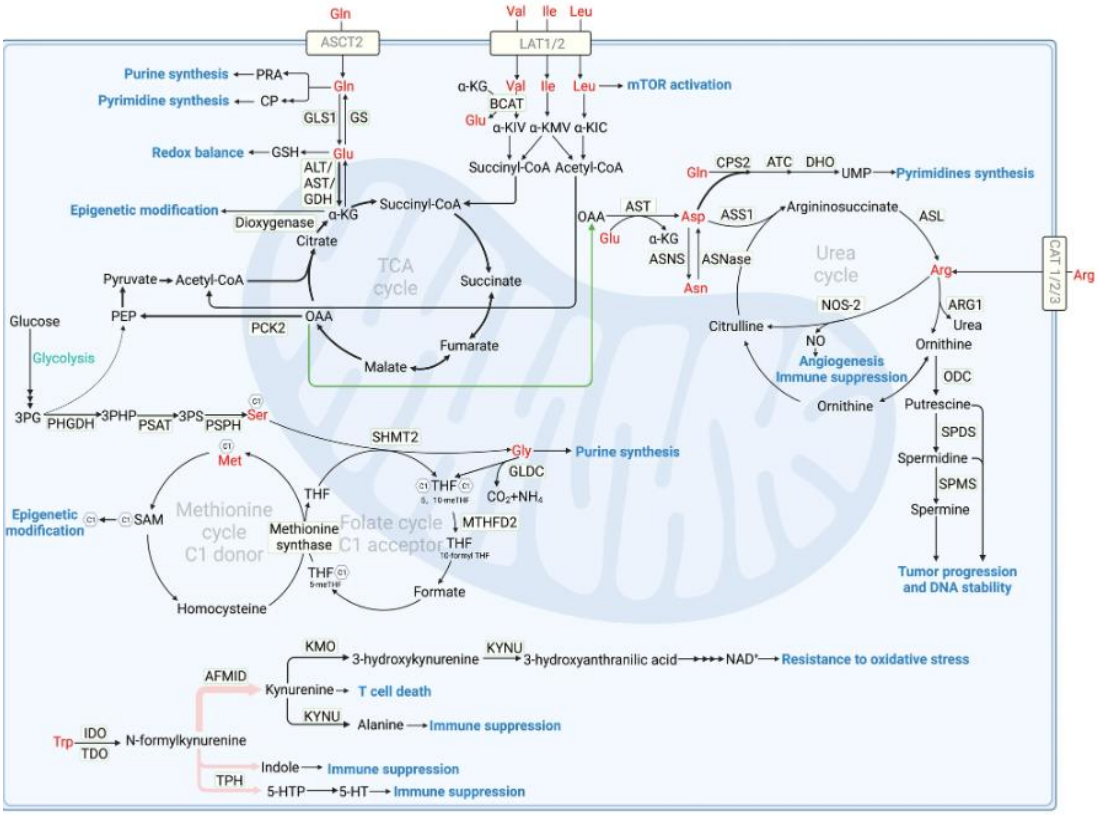

Figure 2.2 General amino acid metabolism in tumour cell (J. Chen et al., 2024) ASCT2 is primary glutamine transporter, take in glutamine to generate purines and pyrimidines. Glutaminolysis in GLS1 promote glutamate which synthesizes GSH to promote redox balance. Glutamate further transfers to α-KG into TCA cycle. With alteration of cell into tumour, amino acid glutamate uptake by α-KG acts as an alternative intermediate to run TCA cycle.

Glutamine serves as a critical nutrient for gliomas by supporting the synthesis of proteins, nucleotides, and lipids. Tumour cells exhibit an increased dependency on glutamine as a carbon and nitrogen source, enabling their rapid growth and survival under metabolic stress (Jin et al., 2023). Glutamine is one of the carbon and nitrogen donor in order to promote the production of purine (Lieu et al., 2020). convert glutamine into glutamate, replenishing TCA intermediates α-ketoglutarate (α-KG) (Lieu et al., 2020). In the situation of hypoxia, α-ketoglutarate is redirected to produce succinate and fumarate, which accumulate and act as oncometabolites, interfering with histone demethylases and hypoxia-inducible factor (HIF) regulation by serine hydroxylmethyltransfeerase 2 (SHMT2) and help to sustain tumour cell from hypoxia-associated oxidative stress (Porporato et al., 2018). Other than that, glutamine derived nitrogen directly promotes nucleotide biosynthesis and amino acid synthesis for cell proliferation (Yoo et al., 2020). Glutathione which synthesis by glutamine are a key molecule in defending tumour cells against reactive oxygen species (ROS) which function to reduce oxidative stress of the tumour cells (Kennedy et al., 2020). Brain tumours exhibit a phenomenon known as glutamine addiction, reflecting their reliance on glutamine for biosynthetic and antioxidant functions. Glutamine-derived glutamate is crucial for synthesizing glutathione, the primary intracellular antioxidant (Sappington et al., 2016). Glutathione neutralizes ROS, protecting tumours cells from oxidative damage caused by their heightened metabolic activity. Glutamate together with α-KG from glutamine promote reductive carboxylation to generate isocitrate, which is then converted into citrate back for cystine uptake, ensuring a constant supply of cysteine for glutathione synthesis (Lieu et al., 2020). This redox regulation mechanism supports tumours survival under hypoxic and nutrient-deprived conditions.

The role of branched-chain amino acids (BCAAs) like **leucine, isoleucine, and valine** is also significant in TCA cycle. BCAA will converted into different metabolite before entering TCA cycle, where all of BCAA convert into branched-chain α-ketoacids. Leucine promote into α-ketoisocaproate by branched-chain aminotransferase (BCAT) enzyme; valine promote into α-ketoisovalerate; and isoleucine into α-keto-β-methyvalerate (Zhang et al., 2017). Leucine and isoleucine contributes to acetyl-CoA production by combined with branched-chain α-ketoacid dehydrogenase (BCKDH) to generate acetyl-CoA which enter TCA cycle and further promote fatty acid synthesis (Mann et al., 2021). Succinyl-CoA derived from valine and isoleucine degradation integrates directly into the TCA cycle, sustaining energy and biosynthetic needs. This metabolic rewiring ensures tumours cells meet their proliferative demands (Schiliro and Firestein, 2021).

2.3.1(b) Amino Acid in One-carbon Metabolism and Serine-glycine Pathway

The serine-glycine pathway integrates with one-carbon metabolism and act as the backbone metabolite for the pathway, crucial for nucleotide synthesis and epigenetic modifications in brain tumours (Pan et al., 2020). **Serine** work as a major substrate in one carbon cycle. Serine synthesized from glucose and 3-phosphoglycerate via phosphoglycerate dehydrogenase (PHGDH), donates one-carbon units through folate-mediated reactions (Reina-Campos et al., 2019). The one carbon unit or γ-carbon would transfer to tetrahydrofolate (THF) which is promoted by the reaction of serine hydroxylmethyltransfeerase 2 (SHMT2) located in the mitrochondria and serine hydroxylmethyltransfeerase 1 (SHMT1) in cytosol to produce 5,10-methylene-THF and glycine (Petrova et al., 2023). **Glycine** which derived from serine, also promote into the development of nucleotide synthesis and contributes to glutathione production

(Hennequart et al., 2021), which is vital for maintaining redox balance under oxidative stress. Meanwhile the 5,10-methylene-THF undergoes a series of oxidative-reductive transformation and produce a final product of one-carbon-THF species (Lionaki et al., 2022). The one-carbon unit are essential for purine and pyrimidine synthesis. SAM is also produced from the one-carbon unit that further promote methylation process in TCA cycle. Increased activity of serine hydroxymethyltransferase (SHMT) in brain tumours highlights the dependency on this pathway for rapid DNA replication (Shunxi et al., 2023).

2.3.1(c) Branched-chain Amino Acids and Mammalian Target of Rapamycin (mTOR) Pathway

BCAA such as **leucine and isoleucine** are not only catabolized for energy but also act as key signalling pathway regulators of the mTOR pathway. BCAA are not usually produce by liver but can be produce from skeletal muscle metabolism (Zhang et al., 2017). mTOR pathway is a upstream signalling pathway that function in cell metabolism, growth and biosynthesis (Tian et al., 2019)**.** mTOR pathway function by two main protein complexes of mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC 2) in processing signal and promote actions (Zhou et al., 2018). **Leucine** is a key positive activator of mTORC1 where it promote mTORC1 to recruit to lysosomal surface and transfer signal to their downstream effector and further promote oncogenic protein translation (Rehman et al., 2023). **Isoleucine** promote mTOR phosphorylation by increasing ribosomal protein S6K1 phosphorylation linearly (Zhou et al., 2018). Hyperactivation of mTORC1 in brain tumours promotes anabolic processes, enabling rapid tumours growth. This pathway integrates nutrient availability with growth signals, emphasizing the importance of BCAA metabolism in sustaining tumours proliferation.

2.3.1(d) Amino Acid in DNA Methylation

DNA methylation involved in the epigenetic modification process of DNA of tumour cells which function in promote and silence gene expression (Sadida et al., 2024)**.** In DNA epigenetic modification the DNA sequence was not disturb but the chromosomal substructure of the relative chromosomes changes due to chemical modification (Sadida et al., 2024). This process promote alteration in the production of nucleotide and further function in disturbing result product produce from the alteration which in this case protein**. Methionine** is an essential amino acid for de novo methylation process (Zhang, 2018). Methionine is converted into SAM, the universal methyl donor for DNA and histone methylation and SAM act as one of the proliferation cells (Gao et al., 2018). Increased SAM availability in brain tumours drives hypermethylation of tumours suppressor gene promoters, silencing their expression and promoting malignancy (Wang et al., 2017). The production of homocysteine from DNA methyltransferase (DNMT) enzymes during the methionine cycle connects to the transsulfuration pathway, where cysteine is generated (Zhu et al., 2019).

Cystine which is a sulphur included proteinogenic amino acid is used as a precursor of glutathione synthesis, enhancing antioxidant defences and enabling tumours cells to thrive under oxidative stress (Bonifácio et al., 2021). DNA methyltransferase (DNMT) add an addition methyl group to cystine to promote cytosine methylation (Davletgildeeva and Kuznetsov, 2024). The cystine/glutamate antiporter is frequently upregulated, ensuring a continuous supply of cysteine for glutathione synthesis (Lewerenz et al., 2013). This metabolic plasticity highlights the interplay between amino acid metabolism and epigenetic regulation in tumours progression (J.

Chen et al., 2024). The critical role of cysteine in both redox balance and biosynthetic processes makes it a potential target for disrupting tumours metabolism.

2.3.1(e) Amino Acid in Urea Cycle and Polyamine Biosynthesis

The urea cycle classically associated with nitrogen disposal, is repurposed in brain tumours to sustain biosynthetic processes (Gropman et al., 2007). Arginine is form in urea cycle by argininosuccinase from argininosuccinate to form arginine and fumarate. **Arginine** function as a precursor of the polyamine synthesis by converting into urea and ornithine and subsequently into polyamines like putrescine, spermidine, and spermine which are essential for DNA stabilization, transcription, and cell proliferation (Xuan et al., 2023). The upregulation of arginase in tumours cells ensures a steady supply of these intermediates (Horák et al., 2020). Polyamines also contribute to extracellular matrix (ECM) remodelling and angiogenesis, processes critical for tumours invasion and metastasis (Holbert et al., 2022). Moreover, arginine metabolism generates nitric oxide (NO), enhancing vascularization and nutrient delivery to the tumour microenvironment, further supporting growth (Andrabi et al., 2023).

Proline which synthesis by glutamate via pyrroline-5-carboxylate synthase (P5CS) from ornithine function in providing for the development of ECM (Chalecka et al., 2021). Proline also function in collagen biosynthesis in order to maintain tumour microenvironment growth (Kay et al., 2023). Oxidation of proline by proline dehydrogenase (PRODH) which happened in mitochondria would also increate ROS level and promote oxidative stress (Kavi Kishor et al., 2022). The dual role of proline in structural support and redox signalling underscores its significance in tumours progression.

One nitrogen unit in the urea cycle is bind to oxaloacetate to form aspartate (Horák et al., 2020)**. Aspartate** is indispensable for the synthesis of nucleotides, which are critical for DNA replication and repair (Helenius et al., 2021). In brain tumours, the malate-aspartate shuttle facilitates the transfer of reducing equivalents across mitochondrial membranes, maintaining aspartate levels for biosynthetic needs (Broeks et al., 2023). Aspartate is also a precursor for arginosuccinate in the urea cycle, linking nitrogen metabolism to nucleotide production (Holeček, 2023). The reliance on aspartate highlights the interconnectedness of metabolic pathways in supporting tumours proliferation, particularly under hypoxic conditions where alternative pathways compensate for oxygen limitation.

2.3.2 Genetic Alterations in Brain Tumours

Molecular research on brain tumours has identified biomarkers of significant diagnostic, prognostic, and predictive value.

2.3.2(a) Isocitrate Dehydrogenase

Genetic modifications significantly contribute to the formation of brain tumours. In the Krebs cycle, isocitrate dehydrogenase (IDH) is a pivotal enzyme that plays a vital role in energy metabolism and biosynthesis. IDH catalyses the conversion of isocitrate into alpha-ketoglutarate and carbon dioxide. There are three isozymes of IDH, including IDH1, IDH2, and IDH3. The IDH1, IDH2, and IDH3 reside in different cellular compartments. Mutations in IDH1 and 2, combined with whole-arm deletions of 1p and 19q (Leeper et al., 2015) correlate with a favourable outcome in adult-type diffuse gliomas. IDH1 and IDH2 mutations are common in lower-grade gliomas and

subsequent glioblastomas and are frequently associated with improved prognosis outcomes. Among the most extensively studied genetic modifications in gliomas is the IDH1 mutation, characterized by an arginine-to-histidine substitution at position 132 (R132H), which alters the enzyme's activity (Sharma et al., 2023). IDH3 catalyse transformation of α-ketoglutarate (α-KG) to isocitrate in citric acid cycle which only indirectly impact cell mutation in gliomagenesis (Han et al., 2020).

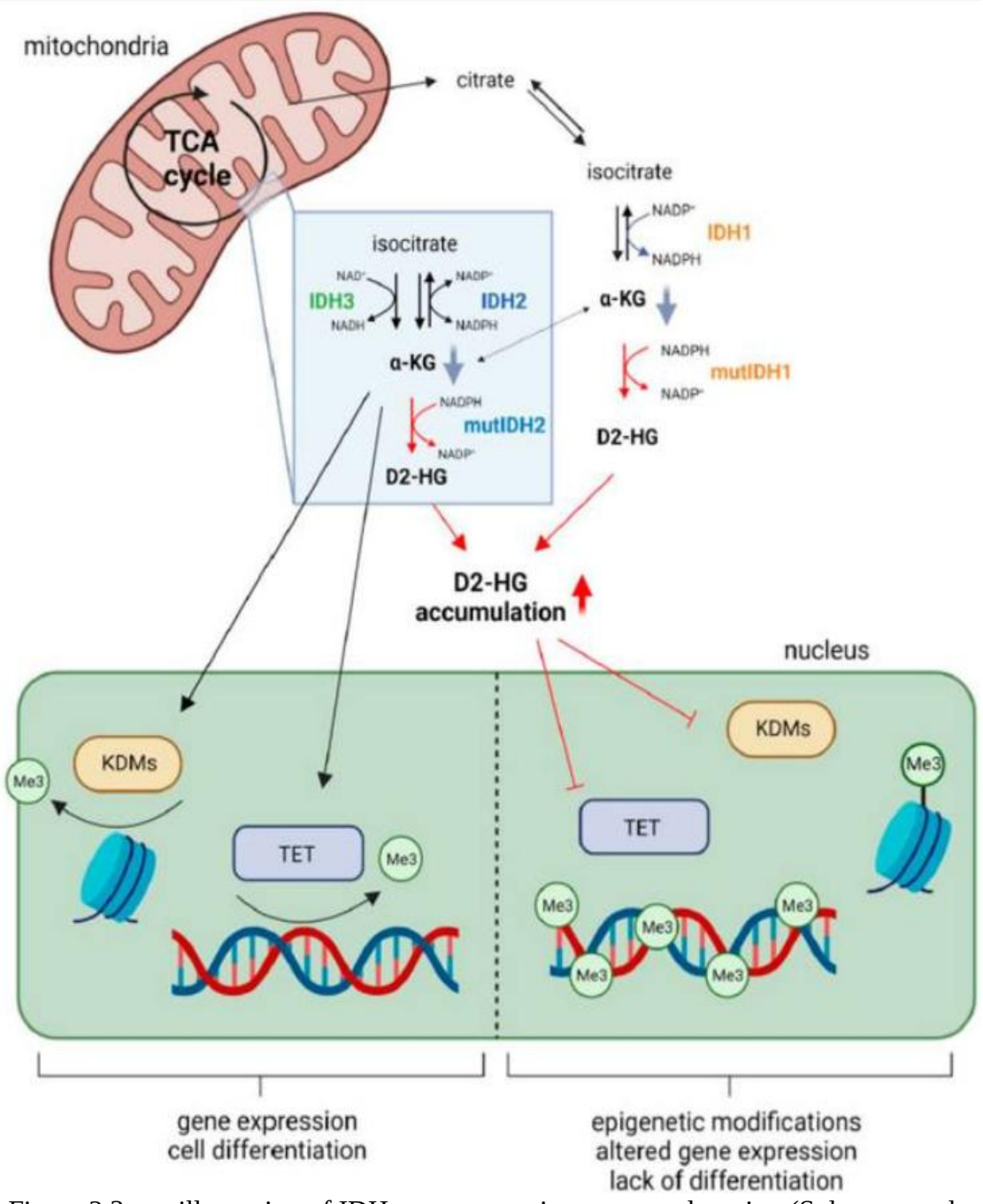

Figure 2.3 illustration of IDH mutant protein enzymes alteration (Solomou et al., 2023).