Polymorphisms in MDM2 and p53 Genes in Gliomas

Thesis

Submitted for the Partial Fulfillment of
MD in Clinical and Chemical Pathology

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2012

Acknowledgements

First and foremost, I thank ***Allah*** *for granting me the power, patience and health to finish this work.*

I am greatly honored to express my deepest thanks, gratitude and respect to ***Prof. Nadida Gohar****, for her kind guidance, advice and the example she has always set for me. It has been an honor to learn from her experience and wise counseling on the personal and professional levels.*

I am deeply thankful to ***Prof. Dina El Gayar****, for her great help and care. I am grateful for her valuable remarks and for the insights she has shared with me.*

I would also like to thank ***Assist. Prof. Mohamed Shehata*** *for his kind help and guidance.*

I owe my deepest gratitude to ***Assist. Prof. Khaled Anbar,*** *Assistant Professor of Neurosurgery, for his help and his steadfast encouragement to complete this study.*

No words can express my feelings towards ***Assist. Prof. Mervat Khorshied*** *for her help and ongoing encouragement up till the last moment of the study.*

A special thank you goes to my mentor ***Assist. Prof. Ahmed Mukhtar,*** *Assistant Professor of Anesthesia and ICU for his help in the statistical analysis of this study.*

Many thanks to ***Dr Mohamed El Borady****, Lecturer of Neurosurgery for his great effort and help with the patients and data collection.*

Moreover, I would like to extend my appreciation to my family and friends for tolerating with me through the times of hard work.

And finally, thank you to everyone who has helped me bring this study to light.

"No one is as blind as those who have sight without vision."

(Helen Keller)

To those who were to me what Anne Sullivan was to Helen Keller,

For showing me the right path,

For never giving up, and for teaching me never to give up,

And most of all,

For helping me become a better human being:

Thank you.

And,

To my family and my friends

For all that, and more

ABSTRACT

Gliomas are the most common primary tumors of the central nervous system. The molecular determinants of gliomas progression are still under investigation. p53 plays a major role in more than 50% of human cancers, among which are gliomas. In addition to this, the Murine-Double-Minute 2 (MDM2) gene was found to be amplified in about 10-15% of malignant gliomas. Genetic polymorphisms in MDM2 can modulate MDM2 expression, thereby impacting p53 tumor suppression and cause increased tumor progression. The aim of the current study is to verify the role played by each of MDM2 and p53 gene polymorphisms in glioma tumorigenesis, and the potential relation between both polymorphisms.

Genotyping of the candidate genes was performed by PCR-RFLP assay in 45 glioma patients and 50 subjects as a control group. Serum p53 level was assayed by ELISA.

The present study has shown that the genotype distributions of p53 Arg72Pro between glioma cases and control groups did not differ as well as their variant allele frequencies between cases and controls. As for MDM2 SNP309, our results support the hypothesis that there is no association between it and glioma tumorigenesis. Furthermore, there was no significant association detected between both p53 and MDM2 polymorphisms and glioma tumorigenesis.

Key words: Gliomas, p53 codon 72 polymorphism, MDM2 SNP309, PCR-RFLP

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Anaplastic Astrocytoma
Bid	BH3-interacting death agonist
bp	base pair
CBTRUS	Central Brain Tumor Registry of the United States
CDKIs	Cyclin-dependent Kinase Inhibitors
CDKs	Cyclin-Dependent Kinases
CIP/KIP	CDK interacting protein/Kinase Inhibitory Protein
CNS	Central Nervous System
DBD	DNA Binding Domain
EDTA	Ethylenediamine Tetra-Acetic Acid
EGFR	Epidermal Growth Factor Receptor
EGFRvIII	Epidermal Growth Factor Receptor variant III
ELISA	Enzyme-Linked Immunosorbent Assay
FGF	Fibroblast Growth Factor
Gadd45	Growth Arrest and DNA-Damage inducible protein 45
GBM	Glioblastoma Multiforme
HDAC1	Histone Deacetylase 1
HPV	Human Papilloma Virus
IAP	Inhibitor of Apoptosis Proteins
IARC	International Agency for Research on Cancer
IDH1	Isocitrate Dehydrogenase 1
INK4	Inhibitors of CDK4
LOH	Loss of Heterozygosity
MDM2	Murine Double Minute 2 (human)
mdm2	Murine Double Minute 2 (mouse)
MDR1	Multi-Drug Resistance 1
MEFs	Mouse Embryonic Fibroblasts
MRI	Magnetic Resonance Imaging
mRNA	messenger Ribonucleic acid
MSH6	mutS homolog 6
MW	Molecular Weight
NFKB2	Nuclear Factor Kappa-B2
PCR	Polymerase Chain Reaction
PDGF	Platelet-Derived Growth Factor
PI3K	Phosphatidylinositol 3-Kinase
pRb	protein of the Retinoblastoma gene

PTEN	Pathway/Phosphatase and Tension Homolog
RFLP	Restriction Fragment length Polymorphism
RING	Really Interesting New Gene
SDS	Sodium Dodecyl Sulphate
SNP	Single Nucleotide polymorphism
SV40[2]	Simian Virus 40
TAE	Tris-Acetate EDTA
TGF-β	Transforming Growth Factor-β
TP53	Total Protein 53
WHO	World Health Organization
wt p53	wild-type p53

Introduction and Aim of the Study

INTRODUCTION
AND AIM OF THE STUDY

Gliomas are the most frequent and deadly primary neoplasias of the central nervous system. Different studies suggest that distinct biological subtypes characterized by different prognostic factors may occur within histologically identical grade tumors. It is therefore of interest to identify genetic markers that might allow differentiation of subtypes of malignant gliomas ***(Schiebe et al., 2000).***

The molecular determinants of gliomas' progression are still under investigation, but it has become clear that the neoplastic evolution towards malignant gliomas is a multistep process. Several molecular mechanisms have been implicated in the progression of gliomas. These alterations include mutations of negative regulatory elements - such as the p53 tumor suppressor gene - and oncogene amplification. Murine Double Minute 2 (MDM2) gene was found to be amplified in about 10–15% of malignant gliomas ***(Schiebe et al., 2000)***. Genetic studies have shown that the MDM2 oncogene is a key negative regulator of the p53 tumor suppressor protein ***(Bond et al., 2006)***. MDM2 promotes degradation of p53, facilitates its nuclear export, and it interferes with its ability to bind DNA and thereby regulate transcription ***(Khatri et al., 2008)***.

A Single Nucleotide Polymorphism (SNP) in the promoter region of human MDM2 (SNP309) with a base change from T to G can modulate MDM2 expression, thereby impacting p53 tumor suppression and cause increased tumor progression ***(Binder, 2007)***. Concerning p53, a functional single nucleotide polymorphism at codon 72 of p53 gene results in the presence of either proline (Pro) or arginine (Arg) in the amino acid sequence

of p53. The pro72 variant results in stronger transcriptional activation, but weaker induction of apoptosis than the Arg72 variant. Thus, this polymorphism has been suggested to be associated with susceptibility to various cancers, among which are gliomas ***(El Hallani et al, 2009)***.

The aim of this study is to investigate the interrelation between p53 Arg72Pro SNP and MDM2 SNP309 and their role in the pathogenesis of gliomas in a sample taken from the Egyptian population.

Review of Literature

Glial Tumors: An Overview