Frequency of BAP1 Gene Mutation in Egyptian Patients with Advanced Sporadic Malignant Pleural Mesothelioma and its Correlation with Clinical Outcome

The Thesis

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List of abbreviations

ABL	Abelson Murine Leukemia.
ALT	Alanine Amino-Transferase.
ANC	Absolute Neutrophil Count
ASS-1	Argino-Succinate Synthetase-1
AST	Aspartate Amino-Transferase.
AJCC	American Joint Committee on Cancer
BAP1	BRCA Associated Protein 1
BARD1	BRCA-Associated RING Domain 1
BCR	Breakpoint Cluster Region protein
BID	Bis In Die (twice daily)
CALGB	Cancer and Leukaemia Group B
CBC	Complete Blood Count
CD	Cluster of Differentiation.
CDKN2A/B	Cyclin-Dependent Kinase 2A/B
CEA	Carcino-Embryonic Antigen
CK	Cyto-Keratin
CM	Cutaneous Melanoma
CR	Complete Responce
CT	Computed Tomography
CTCAE	NCI common Terminology Criteria for Adverse Events
CTC	Circulating Tumer Cells
CTLA-4	Cytotoxic T Lymphocyte-Associated protein
CY 3	Cyanine Dye 3
DAPI	Di-Amidino-2-Phenyl-Indole plus
DDR	DNA Damage Response
DNA	Deoxy ribo Nucleic Acid
DSBs	Double Strand Breaks
DUBs	Debiquitinating enzymes
EBUS	Endotracheal Ultrasonography
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylene-Diamine-Tetra-Acetic acid
EGRF	Epidermal Growth Factor Receptor
EORTC	The European Organization for Research and Treatment of Cancer
EPD	Extended Pleurectomy and Decortication
EPP	Extra-Pleural Pneumonectomy

EZH2	Enhancer of Zeste Homolog 2
FAK	Focal Adhesion Kinase
FASL	FAS Ligand
FDG PET-CT	Fluoro- D-glucose integrated with Positron Emission Tomography and Computed Tomography
FGFR	Fibroblast Growth Factor Receptor
FHA	Fork-Head Associated Domain
FISH	Fluorescence In Situ Hybridization
HBM	HCF-Binding Motif
HCF-1	Host Cell Factor-1
HDAC	Histone Deacetylase
hTNF	Human Tumor Necrosis Factor
ICL	Interstrand cross-link
IF	Immuno-Fluorescence
IHC	Immuno- Histochemistry
IMIG	International Mesothelioma Interest Group
IMRT	Intensive Modulated Radiation Therapy
IV	Intra-Venous
LDH	Lactate Dehydrogenase enzyme
MM	Malignant Mesothelioma.
MPM	Malignant Pleural Mesothelioma
MTAP	Methyl-Thio-Adenosine Phosphorylase
mTOR	Mammalian Target of Rapamycin
MJDs	Machado-Joseph domain-containing proteins
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NF 2	Neuro-Fibromin 2
N/L ratio	Neutrophil/Lymphocyte ratio
NLS	Nuclear Localization Signal
OGT	O-linked N-acetyl -Glucosamine Transferase
OS	Overall Survival
OTUs	Otubain Domain-containing Proteases
PBS	Phosphate-Buffered Saline
PC	Pemetrexed plus Cisplatin
PCB	Pemetrexed plus Cisplatin plus Bevacizumab
PCNA	Proliferating Cell Nuclear Antigen

PD	Progressive Disease.
PDGFR	Platelet Derived Growth Factor Receptor
PD-L1	Programmed Cell Death Ligand-1
PDT	Photo Dynamic Therapy
PET-CT	Positron Emission Tomography and Computed Tomography
PFS	Progression Free Survival
PFTs	Pulmonary Function Tests
PO	Per oral
PARP	Poly ADP Ribose Polymerase
PR	Partial Responce
PRC	Polycomb Repressive Complex
PR-DUB	Polycomb Group Repressive Deubiquitinase complex
PS	Performance Status
PTM	Protein Post-Translational Modification
RCC	Renal Cell Carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RMH	Reactive Mesothelial Hyperplesia
RT	Radio Therapy
SAHA	Suberoyl-Anilide -Hydroxamic Acid
SD	Stable Disease
SMRP	Soluble Mesothelin Related Peptide
SV40	Simian Vacuolating Virus 40
TILs	Tumer Infiltrating Lymphocytes
TMT	Tri-Modality Treatment
TOPBP1	Topoisomerase Binding Partner 1
TTF-1	Thyroid Transcriptin Factor
UBDs	Ubiquitin-Binding Domains
UCHs	Ubiquitin C-terminal Hydrolases
UM	Uveal Melanoma
UNL	Upper Normal of Limit
UPS	Ubiquitin-Proteasome System
USP	Ubiquitin Specific Proteases
VATS	Video Assisted Thoracic Surgery
VEGF	Vascular Endothelial Growth Factor
WT-1	Wilms Tumor protein
YY1	Yin Yang 1

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Abstract

Background:

Malignant Pleural Mesothelioma (MPM) is a lethal cancer with limited therapeutic options. Patients with MPM have a poor prognosis, with estimated median survival 1 year and cure is rare. BRCA associated protein 1 (BAP1) has the highest prevalence of protein-altering mutations identified in MPM.

Aim of the study:

Assessment of the frequency and pattern of BAP1 gene mutations in Egyptian patients with Advanced Sporadic MPM in relation to response to treatment, disease progression and survival rates in order to identify a novel therapeutic target for MPM.

Methods:

A prospective, cohort study included 122 patients who were diagnosed and treated as advanced MPM at National cancer Institute. BAP1 gene mutations were assessed from circulating tumor cells (CTCs) and tissue blocks by Polymerase Chain Reaction (PCR) and Sequencing. BAP1 immunohistochemistry was performed using the Dako Envision visualization system. The relationship between BAP1 gene mutations, Progression Free Survival (PFS) and Overall Survival (OS) rates was assessed using the Log rank test. The relationship between BAP1 gene mutations, clinical response and patient's clinicopathological characteristics was assessed using Chi-Square test.

Results:

Forty seven (38.5%) MPM cases showed one or more mutations in BAP1 gene. The presence of BAP1 mutations associated significantly with BAP1 protein expression ($p < 0.001$), the incidence of organ metastasis ($p = 0.04$), PFS after second line treatment ($p = 0.04$) and clinical response after second line treatment ($p = 0.01$). There was no significant association between BAP1 mutation, PFS after first line treatment ($p = 0.3$), clinical response after first line treatment ($p =0.3$), OS ($p = 0.6$) and other clinicopathological features of the patient including age, gender, asbestos exposure, smoking and histology.

Conclusion:

BAP1 gene mutations are relatively common in Egyptian patients with Advanced Sporadic MPM. BAP1 mutations are associated with disease progression especially after second line therapy and the incidence of organ metastasis.

Key words:

Malignant Pleural Mesothelioma, BAP1 mutations, Circulating Tumor Cells, Immunohistochemistry, Overall Survival, Progression Free Survival, Overall Clinical Benefit.

Malignant Pleural Mesothelioma

Definition

Malignant Pleural Mesothelioma (MPM) is an insidious tumor arising from the mesothelial surfaces of the pleura. About 80% of Malignant Mesothelioma (MM) cases are pleural in origin **(NCCN guidelines, 2017).**

Epidemiology

Malignant Pleural Mesothelioma (MPM) affects approximately 2,500 persons in the United States every year. It was peaked around the year 2000 and is now declining, due to control the asbestos exposure **(Price et al., 2005).**

The incidence of MPM is increasing in many other places of the world, especially in Great Britain and Australia. The rate of MPM peaked around 2015 in England, but it declined gradually thereafter. These changes in the rate of MPM are associated with the understanding of the relationship between mesothelioma and asbestos exposure. In contrast, MPM incidence are predicted to increase dramatically in resource-limited countries due to poor regulation of asbestos mining , the utilization of industrial and household asbestos **(Park et al., 2011).**

MPM cases are also increasing in other countries such as Russia, Western Europe, China, and India **(Nishikawa et al., 2008).**

In Egypt it was estimated that there will be an increase in the incidence of MPM from 161 cases in 2013, to 456 cases in 2050 **(Ibrahim et al., 2014).**

MPM occurs mainly in elder men (median age of 72 years) who have been exposed to asbestos where it occurs 20–40 years later after asbestos exposure **(Taioli et al., 2015)**.

In 2011 a high incidence of mesothelioma was reported in Egypt especially in women (39.2%) and young adults ≤ 40 years (19.1%) **(Awad et al., 2011).**

The reported median age of MPM patients was 53 years. Residential exposure to asbestos was reported in 64.7% of cases from Shoubra El Kheima, 35.6% from El Maasara, 23.6% from El Zeytoon whereas 25% of

cases came from other Cairo areas and only 9.8% came from other governorates**.** A registry data from National cancer Institute (NCI) showed an increase in the relative frequency of MPM from 0.47% in the year 20 02 to 1.3% in the year 2003 **(Awad et al., 2011).**

The mortality rates from MPM are increasing with estimated median survival times ranging from 4 to 12 months. It reached to high levels in the United Kingdom, Netherlands, Australia, Japan, Argentina and Brazil **(Nishikawa et al., 2008).**

According to **Ibrahim et al., 2014** the Overall Survival (OS) of MPM patients in Egypt ranges from 1 to 70 months, and the median survival duration was 8±2.211months**.**

Risk and Prognostic factors

The risk of developing Malignant Pleural Mesothelioma (MPM) is age dependent where it is ten times higher in persons > 60 years than in persons < 40 years **(Neumann et al., 2013).**

Smoking isn't considered a risk factor for MPM; however smoking together with asbestos exposure is increased the risk for lung cancer development **(NCCN guidelines, 2017).**

Patients with MPM have a poor prognosis, with estimated median survival 1 year. The main prognostic factors in MPM are the age, sex, tumor subtype, and disease stage. Patients with Epithelioid tumors have a relatively favorable prognosis, as do women and patients who are less than 75 years **(Neumann et al., 2013).**

The World Health Organization (WHO) Classification of Tumors of the Pleura 2015 showed that the Pleomorphic and Sarcomatoid subtypes are usually associated with a poor prognosis **(Galateau-salle et al., 2015)**.

Malignant Pleural Mesothelioma (MPM) is an aggressive cancer that occurs more frequently in men, and is associated with longer survival in women **(De Rienzo et al., 2016).**

Within chemotherapy trials, two Prognostic scoring systems have been identified by the statistical analysis of large series of patients, The European Organization for Research and Treatment of Cancer (EORTC) as well as the Cancer and Leukaemia Group B (CALGB) which have been validated by Leicester series.

The EORTC series identified two risk groups, the high risk group were characterized with Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) more than one, high WBC count, low hemoglobin level, probable/ possible histologic diagnosis of mesothelioma, and having sarcomatous tissue as the histologic subtype. Age, Gender, Modified Butchart staging didn't affect survival significantly (**Curran et al., 1998**).

The regression tree of CALGB series characterized six prognostic groups of similar survival characteristics. Patients with pleural disease, LDH more than 500 lU/L, poor PS, platelet count >400,000/ fLL , nonepithelial histology , and increasing age more than75 years showed poor survival **(Herndon et al., 1998 and Edwards et al., 2000).**

For Leicester series median survival rates were comparable to the EORTC and CALGB series. A history of asbestos exposure was also reported in 81% of cases but wasn't considered as significant poor prognostic factor **(Fennell et al., 2005).**

In a revision of the seventh edition of TNM staging of MPM, The eighth edition has identified any metastasis as stage IV for MPM. The median survival with stage IV was 9.7 months compared to the median survival for stage III B which was 13.4 months **(Rusch et al., 2016).**

In addition, infection with Simian Vacuolating Virus 40 (SV40) together with asbestos exposure could contribute to poor Overall Survival (OS) in MPM patients **(Zekri et al., 2007).**

Some investigators showed that the Neutrophil /Lymphocyte (N/L) ratio represents another prognostic marker in advanced cases of MPM treated with second line chemotherapy as it is associated with lowering the 6 months Progression Free Survival (PFS) **(El Bastawisy et al., 2014).**