بسم الله الرحمن الرحيم

∞∞∞

تم رفع هذه الرسالة بواسطة / حسام الدين محمد مغربي

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات : لا يوجد

TGFb immunohistochemical expression in cancer associated fibroblasts in urinary bladder carcinoma

Thesis Submitted for fulfillment of M.D
in Pathology

By

Noha Helmy Ghanem

M.B.B.Ch, MSc of Pathology

Supervisors

Prof.Dr. Nafissa Mohammed Amin El-Badawy

Professor of Pathology

Faculty of Medicine, Ain Shams University

Prof. Dr. Sahar Saad El Din

Professor of Pathology

Faculty of Medicine, Ain Shams University.

Prof. Dr. Iman Hamed Mostafa Hewedi

Professor of Pathology

Faculty of Medicine, Ain Shams University

Prof.Dr. Lobna Sadek Shash

Professor of pathology

Faculty of Medicine, Ain Shams University

Faculty of medicine

Ain Shams University

2022

Acknowledgement

. I wish to express my profound gratitude and sincere thanks to Almighty **Allah** His magnificent help is the first factor in everything we can do in our life.

I am overwhelmed in all humbleness and gratefulness to acknowledge my depth to all those who have helped me to put these ideas into something concrete.

It is a genuine pleasure to express my deep sense of thanks and gratitude to my supervisor **Prof. Dr. Nafissa El-Badawy professor** of pathology, Faculty of Medicine, Ain Shams University. Her dedication and keen interest above all her overwhelming attitude to help her students had been mainly responsible for completing my work. Her timely advice, meticulous scrutiny, scholarly advice and scientific approach have helped me to a very great extend to accomplish this task.

I would like to express my appreciation and sincere gratitude to **Prof Dr. Sahar Saad El Din** professor of pathology, Faculty of Medicine, Ain Shams University, for her continuous efforts, advice, patience and continuous constructive criticism throughout this thesis and during publication.

I would like to express my deep and sincere gratitude to my research supervisor **Prof Dr. Iman Hamed Mostafa Hewedi** professor of pathology, Faculty of Medicine, Ain Shams University. Her invaluable guidance throughout this research, dynamism, vision, sincerity and motivation had deeply inspired me.

I'd like to thank **Ass. Prof.Dr. Lobna Sadek Shash,** Assistant professor of pathology, Faculty of Medicine, Ain Shams University, for her timely suggestions with kindness, enthusiasm, dynamism, patience, technical skilful supervision and encouragement had

List of Abbreviations

AJCC	*American Joint Committee on Cancer*
AMH	*anti-mullerian hormone*
APC	*Antigen representing cell*
APOBEC	*Apolipoprotein B mRNA editing enzyme*
ASK1	*apoptosis signal-inducing kinase 1*
BMPs	*bone morphogenic proteins*
CAFs	*Cancer associated fibroblasts*
CDKN2A	*cyclin-dependent kinase inhibitor 2A*
CIS	*Carcinoma in situ*
coSMAD	*the common SMAD*
CSCs	*cancer stem cells*
CTGF	*connective-tissue growth factor*
DAB	*Diaminobenzidine*
EAU	*European Association of Urology*
ECM	*extracellular matrix*
EGFR	*epidermal growth factor receptor gene*
ELF3	*E74-like ETS transcription factor 3*
EMT	*Epithelial-to-mesenchymal transition*
EndMT	*endothelial-mesenchymal transition*
ERCC2	*excision repair cross-complementing rodent repair deficiency, complementation group 2*
FAP	*Fibroblast activation protein*
F.E	*Fisher's exact test*
FGFR3	*fibroblast growth factor receptor 3*
FSP-1	*Fibroblast specific protein-1*
GCO	*Global Cancer Observatory*
GDFs	*growth and differentiation factors*

GIT	*Gastrointestinal tract*
H&E	*Haematoxylin and eosin*
HER2	*human epidermal growth factor receptor 2*
HG	*High grade*
HIER	*Heat-induced epitope retrieval*
HIF1	*hypoxia-inducible factor 1*
IHC	*Immunohistochemistry*
IFNγ	*Interferon gamma*
ISUP	*International Society of Urological Pathology*
iTregs	*induced Tregs*
IUC	*Invasive urothelial carcinoma*
JNK	*the Jun amino-terminal kinase*
KD	*Kilo Dalton*
KDM6A	*lysine-specific demethylase 6A*
LAB	*Labeled avidin–biotin*
LAP	*latency associated peptide*
LDL	*Low-density lipoprotein*
LP	*Lamina propria*
LTBP	*Latent TGF-B-Binding Protein*
LLC	*larger complex called Large Latent Complex*
MIBC	*muscle invasive urinary bladder carcinoma*
MM	*Muscularis mucosa*
MMP	*matrix metalloproteases*
MP	*Muscularis propria*
NCCN	*National Comprehensive Cancer Network*
NIP LG	*Noninvasive papillary Low Grade urothelial carcinoma*
NIP HG	*Noninvasive papillary high grade*

NMIBC	*Non-muscle invasive urinary bladder carcinoma*
NPV	*Negative predictive value*
NSCs	*normal stem cells*
OS	*overall survival*
PBS	*Phosphate buffer saline*
PCR	*Polymerase chain reaction.*
PI 3-kinase	*Phosphoinositide 3-kinase*
PMN	*pre-metastasis niches*
PPV	*Positive predictive value*
PUNLM	*Papillary Urothelial Neoplasm of Low Malignant Potential*
PTC	*papillary thyroid carcinoma*
PyMT	*polyoma middle T*
RAF	*Rapidly Accelerated Fibrosarcoma.*
RAS	*Rat sarcoma virus*
R-SMAD	*receptor regulated SMAD*
ROC	*Receiver operating characteristic*
r	*Spearman's rho*
Rb	*retinoblastoma protein*
RT-PCR	*Reverse transcription polymerase chain reaction*
S	*Significant*
SLC	*Small Latent Complex*
SCC	*Squamous cell carcinoma*
SD	*Standard deviation*
SMA	*Smooth muscle actin*

Smads (or SMADs)	*abbreviation refers to the homologies to the Caenorhabditis elegans SMA ("small" worm phenotype) and MAD family ("Mothers Against Decapentaplegic") of genes in Drosophila.*
SPSS	*Statistical Package for the Social Sciences*
SNPs	*single-nucleotide polymorphisms*
TGFB- ***TGFb*** ***TGF-B***	*Transforming growth factor beta*
TGFBRI	*TGF-B receptor I*
TME	*Tumor microenvironment*
TSP-1	*thrombospondin-1*
t- test	*Student t test*
TURBT	*Transurethral resection*
UBC	*cancer of the urinary bladder*
UC	*Urothelial carcinoma*
VEGFC	*vascular endothelial growth factor*
WHO	*World health organization*
X2	*Chi square*

List of Figures

List of Tables

List of diagrams

ABSTRACT

BACKGROUND: Urinary bladder carcinoma (UBC) is one of the most common malignancies in Egypt and all over the world. Transforming growth factor beta (TGFB) levels in plasma and urine were proved to connote predictive and prognostic attributes in UBC patients. Furthermore, Cancer-associated fibroblasts (CAFs) are, now, recognized as a key player in carcinogenesis. Yet, TGFB1 expression in CAFs of UBC had not been elucidated. Moreover, TGFB1-targeted therapy is now emerging with potential benefits for TGFB1 expressing cancers.

AIM OF THE STUDY: We dedicated this study to explore potential implications of TGFB1 immunohistochemical expression in CAFs of UBC by correlating it to relevant clinical and pathological data.

MATERIALS AND METHODS: This retrospective study included 48 UBC specimens. Different tumor grades were presented in balanced groups. TGFB1 immunohistochemical expression was evaluated, categorized as low or high and compared in CAFs among different UBC grades, statistical analysis of the results was then followed.

RESULTS: TGFB1 expression in CAFs was significantly different among tumor histologic types ($p = 0.01$), high tumor grade ($p \leq 0.01$), presence of muscle invasion ($p \leq 0.001$), higher tumor stage ($p = 0.01$), presence of preceding bilharziasis ($p = 0.003$), and necrosis ($p = 0.03$). There was a highly significant difference between TGFB1 expression in both tumor cells and CAFs ($p = 0.002$). Intense CAFs TGFB1 staining was also strikingly observed along the muscle invading frontside of UBC cells further emphasizing the pivotal role of CAFs expressing TGFB1 in invasion.

CONCLUSION: This study demonstrates significant predictive implications of TGFB1 in UBC, thus emphasizing its potential benefits in management and therapy.

Keywords: Transforming growth factor beta1; Urinary bladder cancer; Cancer-associated fibroblast; Cancer-associated fibroblasts; Immunohistochemistry.

Introduction

Urinary bladder cancer (UC) is the 10th most common form of cancer worldwide with an estimated 573,000 new cases and 213,000 deaths of all sites in both sexes in 2020 according to Global Cancer Observatory (GCO). In Egypt, according to GCO, UC is ranking the 3rd most common cancer after liver and prostatic cancers in both sexes of all ages in 2020. The number of new cases in Egypt in 2020 in both sexes and all ages is 10655/134632 representing 7.9% of all cancers **(Sung et al., 2021).**

More than 95% of bladder tumors are of epithelial origin. The urothelial neoplasms are the most common type followed by squamous and glandular neoplasms **(Humphrey et al., 2016).**

Cancer cells can't survive nor grow without hospitable microenvironment. This tumor microenvironment (TME) is an active participant in tumorigenesis rather than passive observer. It consists mainly of resident cells, secreted growth factors, and extracellular matrix proteins **(Bhowmick et al., 2004).** Recognition of TME concept had revolutionized cancer treatment modalities **(Hainaut et al., 2013).**

Fibroblasts association with cancer cells attain special phenotype and are called carncer associated fibroblasts (CAFs). CAFs are usually derived from the resident fibroblasts in the