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Determining cellular and biochemical function of a novel adhesion molecule in kidneys.

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Boston University
DETERMINING CELLULAR AND BIOCHEMICAL FUNCTION OF A NOVEL ADHESION MOLECULE IN THE KIDNEY.

by

EMAD ARAFA.

Submitted in partial fulfillment of the requirements for the degree of Master of Arts 2015
Approved by

First Reader

Nader Rahimi, Ph.D.
Associate Professor of Pathology and Laboratory Medicine

Second Reader

Vipul Chitalia, M.D., Ph.D.
Assistant Professor in Medicine
DEDICATION

This thesis is dedicated to my parents, Hala Hidmi and Isam Arafa and to the rest of my family Heba, Samer, Rawan and Esam. I am very grateful to my family for their encouragement, support and the sacrifices they made during my graduate program.
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DETERMINING CELLULAR AND BIOCHEMICAL FUNCTION OF A NOVEL ADHESION MOLECULE IN THE KIDNEY.

EMAD ARAFA.

ABSTRACT

Acute kidney injury is an abrupt loss of kidney function that develops in short time with limited effective treatments other than kidney transplantation. We have identified TMIGD1 (Transmembrane immunoglobulin domain 1) as a novel receptor expressed in various organs and tissues, mainly in cell with epithelial origin. TMIGD1 regulates cell morphology and adhesion and its extracellular domain mediates its activity. Knocking down of TMIGD1 using short hairpin RNA (shRNA) increased cell death in human kidney epithelial cells (HK2). On the other hand, HEK293 cells over expressing TMIGD1 protected cells from oxidative stress and nutrient deprivation induced injuries. Furthermore, TMIGD1 expression is reduced in vivo and in vitro kidney injury models. TMIGD1 expression was regulated by ubiquitination and degradation by proteosome 26s. Thus, we present TMIGD1 as a novel receptor that plays important roles in regulation of cell morphology, cell- cell interaction and cell survival.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TITLE</th>
<th>COPYRIGHTPAGE</th>
<th>READER APPROVALPAGE</th>
<th>DEDICATION</th>
<th>ACKNOWLEDGMENTS</th>
<th>ABSTRACT</th>
<th>TABLE OF CONTENTS</th>
<th>LIST OF FIGURES</th>
<th>LIST OF ABBREVIATIONS</th>
<th>INTRODUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>1.3</td>
</tr>
<tr>
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<td>1.4</td>
</tr>
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<td>1.5</td>
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<td></td>
<td>7</td>
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<td></td>
<td></td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.2</td>
</tr>
</tbody>
</table>

viii
2.3. Immunoprecipitation and western blotting……………………………………8
2.4. In Vitro GST Pull-down Assay…………………………………………………8
2.5. Cell survival Assay………………………………………………………………9
2.6. Immunohistochemistry analysis…………………………………………………9
2.7. Cell Traction Force Microscopy…………………………………………………9
2.8. Animal models: DOCA salt uninephrectomy model…………………………10

RESULTS.................................................................................................................. 12

3.1. Development and characterization of polyclonal anti-TMIGD1
antibody……………………………………………………………………………………12

3.2. TMIGD1 regulates cellular morphology………………………………………………12

3.3. The extracellular domain mediates dimerization of
TMIGD1………………………………………………………………………………………13

3.4. TMIGD1 expression is down-regulated in kidney hypertensive
model…………………………………………………………………………………………13

3.5. TMIGD1 reduces cell death in response to H₂O₂ induced cell injury
and nutrient deprivation in human kidney cells……………………………………14

3.6. Hydrogen peroxide induces down regulation and ubiquitination of
TMIGD1………………………………………………………………………………………15

Figures………………………………………………………………………………………18

4.1. Figure 1: Development and characterization of polyclonal anti-TMIGD1
antibody……………………………………………………………………………………18
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Development and characterization of polyclonal anti-TMIGD1 antibody.</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>TMIGD1 regulation of cellular morphology.</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>GST fusion of TMIGD1 binds TMIGD1.</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>TMIGD1's down regulation in kidney hypertension model.</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>TMIGD1 over-expression promoting cell survival.</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>TMIGD1 knockdown increases cell death.</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>Hydrogen peroxide induction of down regulation and ubiquitination of TMIGD1.</td>
<td>24</td>
</tr>
<tr>
<td>8</td>
<td>Predicted model of oxidative stress induced downregulation, ubiquitination and degradation of TMIGD1.</td>
<td>25</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

BSA ........................................................................................ Bovine Serum Albumin
CKR ............................................................................. CSF-1 FLK-1 Receptor- Chimeric Flk-1
DMEM .............................................................. Dulbecco’s Modified Eagle Medium
DNA........................................................................... Deoxyribonucleic acid
ER................................................................................. Endoplasmic reticulum
FBS........................................................................... Fetal bovine serum
GST..................................................................... Glutathione S-Transferase
HEK................................................................. Human Embryonic Kidney
HK2...................................................................... Human Kidney 2
Hsp...................................................................... Heat Shock Protein
PBS...................................................................... Phosphate Buffered Saline
PLC-γ1.............................................................. Phospho Lipase C γ1
RNA....................................................................... Ribonucleic acid
shRNA....................................................................... Small hairpin RNA
siRNA....................................................................... Small interfering RNA
SDS-PAGE........ Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TMIGD1......................................................... Transmembrane immuno globulin domain 1
INTRODUCTION

1.1. The human genome is composed of about 20,000 protein coding genes and approximately the same number of genes for non-coding RNAs (rRNA, tRNA)\(^1\). Every tissue and cell has a complete set of genes, however at any given time cells use only a fraction of the genes in their genome\(^1\). The characteristic pattern of transcribed genes is what defines the phenotype of the tissue or cell\(^1\). This characteristic pattern changes once exposed to a signal such as a dietary or a stress molecule\(^1\). Moreover, tissue and signal specific gene expression provides a central mechanism to control the general properties of a cell and its response to environmental perturbations\(^1\). A detailed understanding of gene expression and regulation can therefore bear the potential for understanding dysregulation in diseased state and can provide possible therapeutic treatments\(^1\).

The importance of gene expression and regulation becomes apparent when cells become specialized for certain tasks and it allow cells to adjust and adapt to the ever changing conditions\(^1\). Regulated genes opposite to constitutive genes that are always active represent the blueprints for proteins that are needed by the cells to function under diverse conditions\(^1\). Gene Expression can be regulated on different levels; most commonly on the transcriptional level where either tightly condensed chromatin prevents or limits DNA binding transcription factors from gaining access to promoter, enhancer or silencer regions in DNA, or
preventing RNA polymerase from binding and transcribing DNA\textsuperscript{2}. In addition, gene expression can be regulated on the translational level by controlling the ability of pre-mRNA to be converted into a mature mRNA molecule and by controlling the stability of mRNA and how long it remains viable to be translated\textsuperscript{2}. Finally, gene expression can be regulated on the protein level, where a protein can be subjected to post translational modifications, which can activate, deactivate, modify function, modify location or degrade the protein\textsuperscript{2}.

1.2. Oxidative stress and cell injury: a condition caused by the accumulation of reactive oxygen species and the inability of the different biological systems to readily detoxify the reactive intermediates or to restore the ensuing damage\textsuperscript{3}. This imbalance in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA\textsuperscript{3}. Furthermore, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling\textsuperscript{3}.

Oxidative stress is associated with increased production of oxidizing species or a significant decline in the effectiveness of antioxidant defenses, such as Glutathione\textsuperscript{4}. The severity of oxidative stress depends upon the size of these changes, with a cell being able to tolerate small perturbations and to recover to its original state\textsuperscript{5}. However, more severe oxidative stress can lead to cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis\textsuperscript{5}.
In humans, oxidative stress is thought to be associated in the development of cancer\(^6\), several kidney diseases\(^7\), cardiovascular diseases\(^8,9\), and several systemic diseases such as hypertension\(^{10}\), diabetes and mellitus\(^7\). In cancer, oxidative stress is likely to be involved in age-related development of cancer\(^6\). The reactive species produced in oxidative stress can cause direct damage to the DNA and are therefore mutagenic, and it may also suppress apoptosis and promote proliferation, invasiveness and metastasis\(^3\).

In addition, in cardiovascular diseases, oxidation of LDL in the vascular endothelium is a precursor to plaque formation\(^{11}\) where it plays a significant role in the ischemia injury due to oxygen reperfusion following hypoxia\(^{11}\).

On the contrary, reactive oxygen species can be beneficial when the immune system uses the lethal effects of oxidants by exploiting the generated oxidizing species to kill pathogens through a mechanism involving activated phagocytes producing both ROS and reactive nitrogen species\(^{12}\). Although the use of these highly reactive compounds in the cytotoxic response of phagocytes causes damage to host tissues, the non-specificity of these oxidants is an advantage since they will damage almost every part of their target cell\(^{12}\). This prevents a pathogen from escaping this part of immune response by mutation of a single molecular target\(^{12}\). Furthermore, reactive oxygen species play important roles in cell signaling, a process termed redox signaling \(^{13}\). Thus, to maintain proper cellular homeostasis, a balance must be struck between reactive oxygen production and consumption\(^{13}\).
1.3. The Kidneys; Two bean shaped kidneys are retroperitoneal organs on either side the vertebral column consisting of 1 million nephrons, which represents the fundamental functional units of kidney.\textsuperscript{14} The kidneys have a tough fibrous capsule (irregular dense connective tissue) for protection. Otherwise, they have very little connective tissue between the nephrons\textsuperscript{14}. The kidney contains about 1 million functional units called nephrons, which are continuous with a system of collecting tubules\textsuperscript{15-17}. The nephrons with several segments and compartments are responsible for distinct functions including filtration, excretion, resorption, and regulation of ion balance, and water content. Hence, their function extends to stabilize blood pressure\textsuperscript{15-17}. This regulation of ion balance and water content (osmoregulation) of the blood plasma means that all the other body fluids will also be regulated\textsuperscript{15}.

On a histological section, the kidney can be identified by the presence of the renal corpuscule and kidney tubules\textsuperscript{14}. The tubules are lined by cuboidal epithelium\textsuperscript{14}. The renal corpuscule is surrounded by Bowman’s capsule which is made of squamous epithelium\textsuperscript{14}. The kidney can also be identified by a tuft of capillaries appearing as a large cellular mass known as glomerulus. The kidneys can be subjected to both acute and chronic injuries, which if not managed properly can lead to chronic kidney disease (CKD) failure, a condition compromising several renal functions and is associated with high morbidity and mortality\textsuperscript{15-17}.
1.4. **Cell Adhesion Molecules**: Cell adhesion molecules are cell surface proteins involved in cell-cell interaction, cell-extracellular matrix binding and organization of tissue structure. Adhesion molecules are commonly transmembrane receptors composed of an intracellular domain that stabilizes the protein in the cytoskeleton, a trans-membrane domain, and an extracellular domain that is responsible for these proteins interactions with other cell adhesion molecules or the surrounding extracellular matrix\(^{18}\). Adhesion molecules can be divided into distinct families with adhesive interactions varying in strength from strong binding involved in the maintenance of tissue architecture to a more transient, less avid, dynamic interactions observed in leukocyte biology\(^{19}\). Adhesion molecules have a wide range of physical and biochemical functions providing signals that can regulate cell proliferation, gene expression, differentiation, apoptosis and migration\(^{19}\). Reduced expression and abnormalities of adhesion molecules have been implicated in many disease including cancer\(^{21}\), cardiovascular diseases\(^{22}\) and renal diseases\(^{20,23}\).

1.5. **Immunoglobulin and proline rich receptor (IGPR-1)**: Recently, IGPR-1 was identified as a novel cell adhesion molecule encoded by TMIGD2 (transmembrane and Immunoglobulin domain containing 2)\(^{24}\). IGPR-1/TMIGD2 was found to be expressed in human endothelial and epithelial cells but was found to be absent in mouse genome\(^{24}\). IGPR-1 has been implicated in angiogenesis, regulation of endothelial capillary tube formation and cell migration\(^{24}\). Further investigation resulted in the identification of a second family member of IGPR-1.
TMIGD1 (trans-membrane and immunoglobulin domain containing 1) unlike IGPR-1 is conserved among most species including human and mouse. The overall goal of this thesis project was to determine expression and biological function of TMIGD1 in human kidney epithelial cells. The work presented in this thesis work for the first time demonstrates that TMIGD1 is expressed in kidney tubular epithelial cells and acts to protect kidney epithelial cells from oxidative cell injury as exhibited by the data shown in this manuscript.
METHODS AND MATERIALS

2.1. Plasmids, siRNA and antibodies:

Mouse TMIGD1 clone (cDNA clone MGC: 74197, IMAGE: 30311543) was purchased from Open Biosystems and subsequently was cloned as a c-myc tag in its C-terminus into retroviral pQCXIP vector via NotI and BamH1 sites (vetrogen Inc.). C-myc tagged TMIGD1 was further sequenced to confirm its sequence identity. Rabbit polyclonal anti-TMIGD1 antibody was made against a peptide corresponding to 20 amino acids in the extracellular domain of TMIGD1.

2.2. Cell lines and culture:

All cell lines HK2 (human kidney tubular epithelial cells), HEK293 (human embryonic kidney epithelial cells)) were grown in DMEM supplemented with 10% FBS and the antibiotic Streptomycin. HEK 293 cell lines were engineered to express either an empty vector (PMSCV), TMIGD1 with a Myc tag. HK2 cell lines were engineered to express an empty vector or had TMIGD1 knocked down from it. pMSCV puro retroviral vector was used to clone Myc-tag TMIGD1. Viruses were produced in 293-GPG cells. The viral supernatant was collected for 7 days, concentrated by centrifugation, added to the HEK293 cell line, 24 hours later DMEM media supplemented with 10% FBS, Streptomycin and 1 ug/ml Puromycin was used to select the newly engineered HEK293 cells. Cells expressing the chimeric VEGFR-2 (herein referred to as CKR) were established by a
retroviral system. Briefly, the cDNA for CKR was cloned into a retroviral vectors (pLNCX2) and transfected into 293GPG cells. The viral supernatant was collected for 7 days, concentrated by centrifugation, and used as described previously (25).

2.3. **Immunoprecipitation and western blotting:**

Cells were prepared and lysed as described 25. Briefly, cells were washed twice with H/S buffer (25 mM HEPES (pH 7.4), 150 mM NaCl, and 2 mM Na3VO4) and lysed in EB lysis buffer (10 mM Tris-HCl, 10% glycerol (pH 7.4), 5 mM EDTA, 50 mM NaCl, 50 mM NaF, 1% Triton X-100, 1 mM PMSF, 2 mM Na3VO4, and 20 μg/ml aprotinin). The normalized cell lysates were immunoprecipitated by using the appropriate antibodies and were subsequently subjected to Western blotting using the appropriate antibody as indicated in the figure legends. In some instances, the membranes were stripped by incubating them in a buffer containing 6.25 mM Tris-HCl (pH 6.8), 2% SDS, and 100 mM β-mercaptoethanol in 50 °C for 30 min and reprobed with the desired antibody.

2.4. **In Vitro GST Pull-down Assay:**

The extracellular domain of TMIGD1 encompassing the immunoglobulin domains was cloned into pGX2T vector and recombinant protein was prepared as described 2. The purified GST-fusion TMIGD1 protein subsequently was used for GST pull down assay. The assay was performed as described 26. Briefly, HEK-293 cells expressing TMIGD1
were grown in 10-cm plates. The plates were washed with H/S buffer and lysed in EB lysis buffer. The normalized cell lysates were incubated with equal amounts of immobilized GST fusionTMIGD1 or GST control for 3 h at 4 °C. The beads were washed with phosphate buffered saline solution with protease inhibitors. The eluted proteins were boiled in sample buffer and analyzed by Western blotting using the appropriate antibody.

2.5. **Cell survival Assay:**

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole (MTT) assay was used to measure cell survival as described 27. In brief, cells (5X10^4) were seeded in 24-well plates after 24 hours of transfection (quadruple wells/group). Cells were washed with PBS and replaced with serum-free medium or other conditions as indicated in the figure legends. MTT assay was added in a 5ul/100ul concentration for 2 hours, then a stop solution was added for 1 hour and finally MTT assay was read using an ELISA plate reader at 570 wavelength absorbance.

2.6. **Immunohistochemistry analysis:**

Immunohistochemistry staining was performed as per the manufacturer’s instruction using the EXPOSE Rabbit specific HRP/DAB detection IHC kit (Abcam, Cambridge, USA).

2.7. **Cell Traction Force Microscopy:**

A Polyacrylamide gel substrate was prepared in a circular dish by mixing fluorescent microbeads with acrylamide/ bis-acrylamide in a pre-
determined bis-acrylamide to acrylamide ratio\textsuperscript{28}. The gel disk is further coated with collagen type I before cells are plated on it. Cells were afterwards plated in two concentrations 1:10000 and 1:20000. Later on several separate cells in each dish were chosen for imaging at different time intervals on an inverted microscope (Nicon deconvolution wide field epifluorescence microscope). This yields “force loaded” images. The cells in the dish are detached afterwards by trypsinization, and an image of the same location is taken. This is denoted as the “null force” image. The force loaded and null force images are then aligned using Adobe photo-shop and the fluorescent micro-beads serve as markers for tracking the movement of the substrate under CTFs. By locating the micro-beads in the images taken before and after straining poly-acrylamide gel substrate, micro-bead movements are determined.

2.8. **Animal models: DOCA salt uninephrectomy model:**

Ten to twelve week old 129/SVE mice were obtained from Taconic Farms. After a week of acclimatizing, surgeries were performed on mice. Mice were anesthetized by isoflurane inhalation after which right uninephrectomy was performed followed by implantation of a 21-day release DOCA salt pellet (Innovative research of America). After recovery from anesthesia, animals were housed singly in cages and fed on standard chow and given 1\% saline as source of drinking water. Control animals were maintained on standard chow and tap water. For
immunohistochemistry studies, mice were killed at 0, 24 hours and 2 weeks after DOCA-salt uninephrectomy treatment.
RESULTS

3.1. Development and characterization of polyclonal anti-TMIGD1 antibody:

To study the TMIGD1 gene, we developed a polyclonal rabbit anti-TMIGD1 antibody. We tested the ability of anti-TMIGD1 antibody to detect TMIGD1 using cell lysates derived from HEK-293 cells over-expressing c-Myc tagged TMIGD1. The initial western blot analysis showed that the anti-c-Myc antibody detects TMIGD1 with an approximate molecular weight of 45kDa (Figure 1A). The same protein band in cells expressing TMIGD1 and a weaker band in the cells expressing control vector were also detected when immunoblotted with anti-TMIGD1 antibody (Figure 1A). The weaker band detected in the HEK-293 cells expressing empty vector most likely corresponds to endogenously expressed TMIGD1 (Figure 1A). To determine whether TMIGD1 antibody was specific, the anti-TMIGD1 antibody was pre-incubated with the peptide that was used to generate anti-TMIGD1 antibody. The pre-incubation of the anti-TMIGD1 antibody with peptide blocked the detection of both endogenous and over-expressed TMIGD1 (Figure 1B), indicating that the anti-TMIGD1 antibody specifically recognizes TMIGD1. The predicted molecular weight of TMIGD1 is 29kDa, however both anti-c-myc and anti-TMIGD1 antibodies detected TMIGD1 with approximate molecular weight of 45kDa. The higher molecular weight of TMIGD1 suggests that TMIGD1 likely undergoes post-
translational modifications (PTM) such as glycosylation, which is a common PTM for cell surface proteins. The data demonstrate that the anti-TMIGD1 antibody specifically recognizes TMIGD1.

3.2. TMIGD1 regulates cellular morphology:

Interesting, we noticed that over-expression of TMIGD1 in HEK-293 cells alters cell morphology. HEK-293 cells expressing TMIGD1 appear to be more spreading, more flattened, larger and with less podia compared to HEK-293 cells expressing empty vector (Figure 2A). The data suggests that TMIGD1 is involved in the regulation of cell morphology. Immunohistochemistry analysis using anti-TMIGD1 antibody showed that TMIGD1 tends to be expressed in epithelial cells, most specifically in epithelial cells of the kidney (Figure 2B). Furthermore, examination of the expression of TMIGD1 in different epithelial tumor cells line, revealed that TMIGD1 is expressed in colorectal and clear cell renal cell carcinoma cell lines (Figure 2C).

3.3. The extracellular domain mediates dimerization of TMIGD1:

Immunoglobulin domains are known to be involved in protein-protein interaction\textsuperscript{29}, consistent with its known function, a GST-fusion recombinant protein of extracellular domain of TMIGD1 encompassing its two immunoglobulin domains showed that it binds to TMIGD1 (Figure 3A). The data suggests that immunoglobulin domains of TMIGD1 act as self-dimerizing domains to mediate its function.
Given that cell adhesion molecules are known to regulate cell motility, we investigated the role of TMIGD1 in cellular traction. HEK-293 cells over-expressing TMIGD1 showed significantly lower cell traction values of (94.71±27.89 Pa) compared to HEK-293 cells expressing the empty vector of (64.87±38.23 Pa). Confluent cell layers expressing TMIGD1 plated on polyacrylamide gel substrate exhibited increased cell spreading similar to that observed on collagen coated plastic plates. Thus, indicating that there is less motility and migration of HEK293 over-expressing TMIGD1. No obvious differences were observed in cellular spreading in single cells present on the collagen coated polyacrylamide gel substrate (Figure 3B). On the whole, these findings suggest that the functional effects observed in the presence of TMIGD1 requires cell to cell interaction a typical feature of proximal tubular/kidney function. Thus, TMIGD1 undergoes trans-dimerization and regulates cellular traction (Figure 3C).

3.4. TMIGD1 expression is down-regulated in kidney hypertensive model:

We studied TMIGD1 expression in a hypertensive mouse model of kidney disease. A uninephrectomy was done on 8-10 weeks old mice after which DOCA-salt pellet was planted subcutaneously and the mice given 1% saline. Kidney tissues were harvested 24 hours and 2 weeks after treatment to study differences in earlier protein expression changes as compared to late changes. After 24 hours and 2 weeks of inducing hypertension, there was a marked decline in TMIGD1 staining in morphologically normal proximal and/or distal
tubules compared to tubules in non-hypertensive mice (Figure 4). I thought you had data about its localization in the tubular cell--- is it apical or basolateral? The data suggests that TMIGD1 expression is affected in the mouse experimental model of hypertension.

3.5. TMIGD1 reduces cell death in response to H\textsubscript{2}O\textsubscript{2} induced cell injury and nutrient deprivation in human kidney cells:

Considering that expression of TMIGD1 was down regulated in the mouse experimental model of hypertension, we decided to examine possible function of TMIGD1 in the kidney epithelial cells. For this purpose, we knocked down TMIGD1 in HK2 cells (Figure 5C) and examined the effect of nutrient deprivation (hypoglycemia) and H\textsubscript{2}O\textsubscript{2}-induced cell injuries. Oxidative stress is a uniform underlying pathogenic mechanism of renal progression irrespective of inciting factor. Several previous studies and animal models show H\textsubscript{2}O\textsubscript{2} induce nascent oxygen and compromises survival of renal tubular epithelial cells. Since TMIGD1 expression is down regulated in hypertensive CKD model and that TMIGD1 enhances tubular survival, we hypothesized that TMIGD1 abrogates H\textsubscript{2}O\textsubscript{2}'s effect on renal epithelial cell\textsuperscript{7}. The results showed that engineered HK2 with TMIGD1 knocked down had a considerable less survival (12% survival \textit{versus} 28% survival with control SiRNA) when treated with hydrogen peroxide (Figure 5A). Furthermore, engineered HK2 with TMIGD1 knocked down had a reduced
survival when grown in a nutrient deficient media (serum free, 5mM glucose) (Figure 5B). Our data suggests the importance of TMIGD1 for the survival of kidney epithelial cells in response to cell injuries induced by hydrogen peroxide and nutrient deprivation. To support our

Since, knockdown of TMIGD1 increased susceptibility of HK2 cells to $\text{H}_2\text{O}_2$-induced cell injury, we over expressed TMIGD1 in HEK293 and examined the effect of over-expression of TMIGD1 (Figure 6D). Over-expression of TMIGD1 in HEK293 cells improved cell survival in response to hydrogen peroxide induced injury as depicted in (Figure 6A). Addition of N-acetyl cysteine antioxidant (free radical scavenger) markedly reversed the effect of hydrogen peroxide (Figure 6B). In addition, TMIGD1 over expressed in HEK-293 cells increased cell survival in response to nutrient deprivation (hypoglycemia) as well (Figure 6C). The data suggest that TMIGD1 significantly contributes to survival of kidney epithelial cells in response to cell injury.

3.6. Hydrogen peroxide induces down regulation and ubiquitination of TMIGD1:

To study the molecular mechanism of downregulation of TMIGD1 in kidney cells, we tested whether hydrogen peroxide treatment induces down regulation and ubiquitination of TMIGD1 as a mean to stimulate cell death. Our results show that TMIGD1 is down regulated in response to hydrogen peroxide treatment in both HK2 and HEK293 cell lines (Figure 7A). Treatment of cells with
proteosome inhibitors, like Bortezomib and MG132, restore the hydrogen peroxide–mediated down-regulation of TMIGD1 (Figure 7B). Furthermore, ubiquitination of TMIGD1 in HK2 cells was robustly increased in response to hydrogen peroxide treatment (Figure 7C). To support our work, we examined the effect of Bortezomib treatment on the survival of HK2 cells. Our results show that when HK2 cells are treated with both hydrogen peroxide and Bortezomib, the Bortezomib treatment helps HK2 cells recover from the cell death induced by hydrogen peroxide (Figure 7D).

Overall, our work demonstrates that TMIGD1 protects kidney epithelial cells from cell induced injury by hydrogen peroxide and nutrient deprivation. Thus, our work shows that hydrogen peroxide down regulates TMIGD1 expression and increases ubiquitination and degradation of TMIGD1 (Figure 8A).
Development and characterization of polyclonal anti-TMIGD1 antibody: A) Whole cell lysates from HEK-293 cells expressing empty vector (pMSCV) or c-Myc-tag-TMIGD1 were blotted with anti-c-Myc antibody and anti-TMIGD-1 antibody. B) To demonstrate the specificity of the anti-TMIGD1 antibody was also pre-incubated with the TMIGD1 peptide, which was used to develop anti-TMIGD1 antibody.
Figure 2

**TMIGD1 regulation of cellular morphology**: A) Shown is the typical morphology of HEK-293 cells expressing TMIGD1 and control cells. Pictures were taken under light microscope (40X). B) Immunohistochemistry of Kidney epithelial cells showing expression level of TMIGD1 at 20X and 100X under light microscope (Images courtesy of Philip Bondzie). C) TMIGD1 expression in human kidney cells and different tumor cell lines.
GST fusion of TMIGD1 binds TMIGD1: A) GST-fusion recombinant protein of extracellular domain of TMIGD1 encompassing its two immunoglobulin domains showing TMIGD1 dimerization. B) The role of TMIGD1 in cellular traction. HEK-293 cells over-expressing TMIGD1 showed significantly lower cell traction values of (94.71±27.89 Pa) compared to HEK-293 cells expressing the empty vector of (64.87±38.23 Pa). C) Predicted model for TMIGD1 dimerization and activation.
**Figure 4**

**TMIGD1 ‘s down regulation in kidney hypertensive model:** Mouse kidney tissues from DOCA salt uninephrectomy models were subjected to immunohistochemistry using anti-TMIGD1 antibody and representative pictures are shown. Also shown are the H&E staining of control mouse kidney. (Images courtesy of Philip Bondzie).
**Figure 5**

**A)** HEK-293 cells expressing TMIGD1 or control vector were plated in 24-well plates in quadruple in medium plus increasing concentration of hydrogen peroxide as indicated. The result is the representative of three independent experiments. *p< 0.05 compared to control cells expressing empty vector.

**B)** HEK-293 cells expressing TMIGD1 or HEK-293 cells expressing TMIGD1 +100mM NAC were plated in 24-well plates in quadruple in medium plus increasing concentration of hydrogen peroxide as indicated. The result is the representative of three independent experiments. *p< 0.05 compared to control cells expressing empty vector.

**C)** HEK-293 cells expressing TMIGD1 or control vector were plated in 24-well plates in quadruple in low glucose (5mM) and serum-free medium for indicated days and cell survival was measured with MTT assay. The result is the representative of three independent experiments. *p< 0.05 compared to control cells expressing empty vector at day 3.

**D)** TMIGD1 over-expression in HEK293 cell.

**TMIGD1 over-expression promoting cell survival:** A) HEK-293 cells expressing TMIGD1 or control vector were plated in 24-well plates in quadruple in medium plus increasing concentration of hydrogen peroxide as indicated. The result is the representative of three independent experiments. *p< 0.05 compared to control cells expressing empty vector. B) HEK-293 cells expressing TMIGD1 or HEK-293 cells expressing TMIGD1 +100mM NAC were plated in 24-well plates in quadruple in medium plus increasing concentration of hydrogen peroxide as indicated. The result is the representative of three independent experiments. *p< 0.05 compared to control cells expressing empty vector. C) HEK-293 cells expressing TMIGD1 or control vector were plated in 24-well plates in quadruple in low glucose (5mM) and serum-free medium for indicated days and cell survival was measured with MTT assay. The result is the representative of three independent experiments. *p< 0.05 compared to control cells expressing empty vector at day 3. D) TMIGD1 over-expression in HEK293 cell.
**TMIGD1 knockdown increases cell death:** A) HK2 cells transfected with control siRNA or TMIGD1 siRNA. Cells were seeded in 24-well plates (4 wells/group) in medium containing 10% FBS. After 12 hours serum containing medium was replaced with serum-free medium plus hydrogen peroxide (0.1mM). Cells were incubated for additional 8 hours and cell survival was measured by MTT assay. The result is representative of three independent experiments. *p< 0.05 compared to control siRNA transfected cells. Shown is the knockdown effect of TMIGD1 siRNA on TMIGD1 expression. B) HK2 cells transfected with control siRNA or TMIGD1 siRNA were incubated in low glucose (5mM) and serum-free medium for overnight and cell survival was measured by MTT assay as panel A. The result is the representative of three independent experiments. C) TMIGD1 expression knockdown using siRNA.
Hydrogen peroxide induction of down regulation and ubiquitination of TMIGD1: A) HK2 cells and HEK293 over-expressing TMIGD1 treated with different concentrations of hydrogen peroxide and whole cell lysates were subjected to western blot analysis using anti-TMIGD1 antibody and loading control Hsp70. (B) HK2 cells and HEK293 over-expressing TMIGD1 were treated with hydrogen peroxide in the absence or presence of proteosome inhibitors, Bortezomib or MG132 Cells respectively, were lysed and blotted for TMIGD1 and loading controls, Hsp70 and PLCγ1 respectively. (C) HK2 cells were incubated in serum-free medium or hydrogen peroxide and cells were lysed and subjected to immunoprecipitation using anti-TMIGD1 antibody followed by western blot using anti-ubiquitin (anti-Ub) antibody. (D) HK2 cells seeded in 24-well plates for overnight in 10%FBS followed by incubation of cells in serum-free medium (vehicle), serum-free medium plus hydrogen peroxide plus or minus proteosome inhibitor, Bortezomib. After 8 hours survival of cells were evaluated with MTT assay. The result is the representative of three independent experiments. *p< 0.05 compared to control cells treated hydrogen peroxide only.
Predicted model of oxidative stress induced downregulation, ubiquitination and degradation of TMIGD1.
DISCUSSION

Kidneys consist of 1 million of functional units called nephrons which are responsible for filtration, excretion, resorption, and regulation of ion balance and water content, which all together function to stabilizes blood pressure.\textsuperscript{15,16,17} Nephron's functions are highly dependent on epithelial cell-cell and cell-extracellular matrix adhesion\textsuperscript{20,29}, adhesion molecules such as cadherins and integrins are known to play key roles as mediators of the adhesion processes\textsuperscript{30,31}. Consistent with the conventional function of cell adhesion molecules; previous data obtained on TMIGD1 (unpublished data) and data presented in this work suggests that TMIGD1 regulates kidney epithelial cell permeability, reduces cell migration, inhibits cell proliferation, changes actin fibril assembly and promotes survival of kidney epithelial cells. We propose TMIGD1 as a novel gene, which is expressed in epithelial cells and functions as a cell adhesion molecule.

Consistent with the linkage between the loss of cell adhesion in renal ischemia and cell death\textsuperscript{32,33}. The newly obtained data (Figures 5 and 6) in this research work on TMIGD1 suggests that TMIGD1 protects and promotes cell survival of kidney tubular epithelial cells against oxidative stress and nutrient deprivation. These findings show (Figure 5) that the reduced expression of TMIGD1 in human kidney epithelial cell (HK-2 cells) leads to a decrease in survival of epithelial cells in response to hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and nutrient
deprivation. Moreover, the data (Figure 6) demonstrates that over-expressing TMIGD1 in human embryonic kidney cells (HEK293) promotes survival of HEK293 cells in both hydrogen peroxide and nutrient deprivation induced injuries. Thus, our observation (Figure 5 and 6) suggests that TMIGD1 plays a significant role in renal cell injury.

Moreover, the data also shows (Figure 7) that TMIGD1 is down regulated in oxidative induced injury through ubiquitination and degradation. The data also demonstrates that using proteasomal inhibitors such as Bortezomib and MG132 reduces TMIGD1 degradation in HEK-293 over-expressing TMIGD1 and therefore, promotes HEK-293 survival in oxidative stress induced cell injury. Here, it is important to note that recent literature on Bortezomib shows that it has been explored as a possible treatment in acute renal failure\textsuperscript{34}. Although Bortezomib is a general proteosome inhibitor, it is reasonable to speculate that agents that could up-regulate expression of TMIGD1 could potentially lead to the development of a new class of drugs for treatment of renal failure. Furthermore, the data gathered from cell culture allows (Figure 6) us to speculate that the loss of TMIGD1 in part may contribute to loss of cell adhesion and death in kidney epithelial cells.

Despite recent progress in renal therapy and critical care medicine, acute kidney injury still carries a high morbidity and mortality rate\textsuperscript{35}, underscoring the need for a better understating of the biological process involved in renal cell injury and the type of bio-molecules associated in the process. Identification of
TMIGD1 as a putative molecule involved in renal cell injury and further characterization of its role and mechanisms involved, should provide new insight on the molecular mechanism of renal cell injury. In this respect, this work describes a newly identified gene TMIGD1 which tends to reside in epithelial cells in general and specifically in tubular epithelial cells of the kidneys.

In general, our work predicts that TMIGD1 functions as an adhesion molecule that is expressed in the epithelial cells where it plays a major role in cell-cell and cell-extracellular matrix interactions.
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<th>Abbreviation</th>
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<tr>
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<td>American Journal of Physiology – Cell</td>
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REFERENCES


CURRICULUM VITAE

EMAD ARAFA (01/02/1987)

8122 Avalon Drive, Wilmington, MA 01887 / C: 617-642-5532 / emad2187@bu.edu

Professional Overview


In 2012, I joined a Pathology Research Laboratory focused on studying molecular mechanisms of angiogenesis and its application to human disease at Boston University.

In September 2013, I enrolled in the Master of Arts program in Pathology at Boston University. I continued to work as a researcher in the same Laboratory.

Core Qualifications

- Basic Life Support
- Preventive Dentistry.
- Conservative Dentistry.
- Dental Surgery.
- Endodontics.
- Geriatric Dentistry.
- Periodontics.
- Molecular and Cell Biology.

Accomplishments

Successfully passed the National Board Dental Exam Part I (2011) and National Board Dental Exam Part II (2012).
**Education**

**DDS: Dentistry.**
Jordan University of Science and Technology
Irbed, Jordan.

**Masters of Arts: Pathology/ Medical Sciences.**
Boston University
Boston, MA, United States.

**Experience**

During my six-month internship at different Jordan Health Ministry hospitals and clinics, I examined and treated hundreds of patients of varying ages with diverse dental conditions. This allowed me to exercise and to improve my dental skills in Preventive Dentistry, Conservative Dentistry, Endodontics, Periodontics and Dental Surgery. During my work as a Molecular and Cell Biology researcher, I worked on functional and biochemical characterization of a variety of proteins including TMIGD-1 and IGPR-1. This allowed me to increase my understanding of how the cell functions, how to ask the critical questions and design suitable experiments to answer those questions. This experience also allowed me to improve my analytical skills and to learn various biochemical skills.

**Publications**

*Emad Arafa1*, *Philip Bondzie1*, *Kobra Rezazdeh1*, *Rosana D Meyer1*, *Edward Hartsough1*, *Joel Henderson1*, *John H. Schwartz2*, *Vipul Chitalia2*, *Nader Rahimi1,3* “TMIGD1 is a novel adhesive molecule that protects kidney epithelial cells from oxidative cell injury”.
Skills

- Cell Culture.
- Western Blotting.
- Immunoprecipitation.
- Cell Lysis.
- MTT cell proliferation assay.
- Immunohistochemistry.
- Transfection.
- Scratch migration assay.
- Microsoft Excel.
- Microsoft Word.

Standardized Test Scores

- NBDE I: 88.
- GRE: 320 (Quantitative: 163(86 Percentile), Verbal: 157(74 Percentile)).
- NBDE II: Pass.
- Toefl: 110.

Memberships/ Scholarly Societies

Member of the Jordanian Dental Association.