Induction of Epithelial-Mesenchymal Transition Program in Cultured Epithelial Cells Derived from Oral Mucosa of Adult Albino Rat (Histological and Immunohistochemical Study)

Thesis

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بسم الله الرحمن الرحيم

﴿ قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

سورة البقرة الآيـــه رقم ٣٢

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Last but not least, I dedicate this work to my family, whom without their sincere emotional support, pushing me forward this work would not have ever been completed.

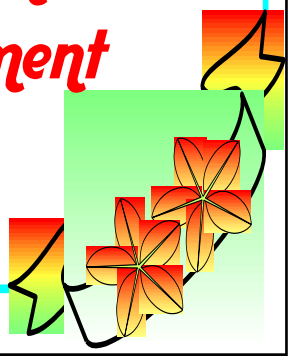
Reda Ahmed Hasan Hasan

Dedication

- *To my father*
- *To my wonderful mother*
- *To my husband*
- *To my lovely daughters*
- *To Dr. Asmaa Abdel El Moniem Mohamed*

I dedicate this work for their support and encouragement

Reda Ahmed Hasan Hasan



Contents

List of Abbreviations

α-SMA	:	α smooth muscle actin
APC gene	:	Adenomatous polyposis coli gene
BMP-7	:	Bone morphogenetic protein.
CAMs	:	Cell Adhesion Molecules
CD31	:	Cluster differentiation 31
Dkk-1	:	Dickkopf related protein 1
E cadherin	:	Epithelial cadherin
ECM	:	Extra cellular matrix
EGF	:	Epidermal growth factor.
EMT	:	Epithelial to mesenchymal transition
EndMT	:	Endothelial mesenchymal transition
FSP1	:	Fibroblast specific protein 1
ICS	:	Intercellular space
iPS	:	Induced pluripotent stem cells
MET	:	Mesenchymal to epithelial transition
MMP-9	:	Matrix metalloproteinase 9
N cadherin	:	Neural cadherin
PDGF	:	Platelet derived growth factor
sFRPs	:	Secreted Frizzled Related Proteins.
TCF	:	Transcription control factor
TEM	:	Transmission electron microscope
TGF-β	:	Transforming growth factor β
Wnt	:	Wingless

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ABSTRACT

Introduction : The origin of mesenchymal cells participating in tissue repair following pathological process (fibrosis) are poorly understood. EMT represents an important source of these cells. The identification of the signaling pathways involved in transition of epithelial to mesenchymal phenotype help to understand the plasticity of cellular phenotypes and the possible therapeutic interventions.

Aim of the work : This work aimed to induce EMT in different cell lines in order to study the morphological and immunohistochemical changes associated with this process.

Materials and Methods: Ten female albino rats were used in this study to provide a source for epithelial cells of oral mucosa. In addition, A549 human lung carcinoma cell line was used for EMT induction.

Cultured Cells used in the study were classified into two groups. **Group (I)**, oral mucosa group, which was subdivided into three subgroups: **Subgroup (Ia)** included cells of primary culture of oral mucosa. **Subgroup (Ib)** included subcultured cells of oral mucosa. **Subgroup (Ic)** included subcultured cells of oral mucosa after EMT induction. **Group (II)**, A549 human lung carcinoma cell line group, which further subdivided into two subgroups **Subgroup (IIa)** included cells of A549 human lung carcinoma cell line before EMT induction. **Subgroup (IIb)** included cells of A549 human lung carcinoma cell line after EMT induction. Morphologic and immunohistochemical changes were examined using phase contrast microscope.

Results :

Subcultured oral mucosa cells (group I) before EMT induction revealed polygonal cells with minimal intercellular spaces (ICS). They showed positive immunoreaction for E-cadherin and negative for vimentin. After EMT induction, the cells were spindle shaped with appearance of stress fibers in the cytoplasm. These cells showed negative immunoreaction for E cadherin and positive immunoreaction for vimentin.

Subcultured cells of carcinoma cell line (group II) before EMT induction, showed polygonal cells with minute surface projections. They showed positive immunoreaction for E cadherin and negative immunoreaction for vimentin. After EMT induction, all the cells were spindle shaped. They showed strong positive immunoreaction for vimentin and negative immunoreaction for E cadherin.

Conclusion: Epithelial Mesenchymal Transition (EMT) program can be induced in different types of cell lines, oral mucosa and A549 human lung carcinoma cell line. It was found that EMT induction was more prominent in A549 human lung carcinoma cell line. Activation of EMT programs can provide new insights to the plasticity of cellular phenotypes and possible therapeutic interventions.

Keywords: EMT, oral mucosa, A549 human lung carcinoma cell line.

Introduction

The tissues of human body are composed primarily of two types of cells which are epithelial and mesenchymal cells. Epithelial cells are surface cells that display a distinct polarity. They are strongly attached to one another by adhesion molecule namely E-cadherin. They are also attached to the underlying extracellular matrix (ECM) by another protein called intergrin molecule. In contrast, mesenchymal cells are loosely packed, have no polarity and are able to migrate freely ***(Kalluri and Neilson, 2003).***

Epithelial cells are able to undergo morphological and genetic changes collectively referred to as EMT (epithelial to mesenchymal transition). This occurs through the degradation of the ECM, dissolution of E-cadherin and other cell adhesion junctions and the loss of cell polarity. This results in the formation of migratory mesenchymal cells that have invasive properties ***(Lamouille at al., 2014).***

EMT has been assigned three distinct subtypes. The first type occurs during embryonic development. EMT program is recently recognized to be induced by a group of factors involved in the process of inflammation and tissue repair. This represents the second type of EMT. Moreover, it

is only recently recognized that a third type of EMT program leads to cancer progression ***(Kalluri and Neilson, 2003).***

Several signaling pathways as TGF- β and Wnt/ β-catenin can induce EMT. Many transcription factors such as Snail can induce EMT by repression of E cadherin ***(Lamouille et al., 2014).***

EMT represents also, one of the main sources of activated fibroblasts in many organ systems ***(Kalluri and Neilson, 2003).***

The cells within certain epithelial tissue appear to be plastic and thus able to move back and forth between epithelial and mesenchymal states via the process of EMT and MET ***(Lee et al., 2006).***

Recent therapeutic interventions in some organ fibrosis were directed toward inhibiting fibroblasts formation by shift local cytoplasm cytokines balance in favour of reversal MET ***(Ross and Pawlina, 2016).***

In the present study, EMT induction has been applied to both finite and continuous cell lines to investigate the technical development of such process, and to compare the results of two different cell lines.

Aim of the Work

Induction of EMT programs can provide new insights to the plasticity of cellular phenotypes and possible therapeutic interventions.

Thus we aim to induce EMT in cell line models and to study the structural remodeling and immunohistochemical changes associated with this process.

Historical perspective:

The idea that epithelial cells could downregulate their epithelial characteristics and acquire mesenchymal characteristics arose in the early 1980s from observation made by Elizabeth Hay. She described epithelial to mesenchymal phenotype changes in the primitive streak of chick embryos initially described as "epithelial to mesenchymal transformation". This differentiation process now commonly known as epithelial-mesenchymal transition (EMT) to emphasize its transient nature ***(Kalluri and Neilson, 2003).***

Mesenchymal-epithelial transition (MET) describes the reverse process. The ability of epithelial cells to transition into mesenchymal cells and back, either partially or fully, explain the phenomenon of plasticity of the epithelial phenotype ***(Lamouille et al., 2014).***

The epithelial cells are able to move back and forth between epithelial and mesenchymal states via the processes of EMT and MET ***(Lee et al., 2006).***

Characterization of epithelial cells:

The epithelial cells are closely adherent to each other by cell adhesion molecules that form specialized cell junctions. They show functional and morphological polarity. They have apical, lateral and basal domains. The properties of each domain are determined by specific lipids and integral membrane proteins. Their basal surface is attached to the underlying basement membrane.

The apical domain is directed toward the exterior surface or the lumen. The lateral domain communicates with adjacent cells through cell junctions.

The lateral domain is characterized by the presence of unique proteins for junctional specialization called cell adhesion molecules (CAMs). CAMs play an important role not only in cell - cell adhesion as E- cadherins but also, play a role in Cell - Extra cellular matrix (ECM) adhesions as integrins ***(Ross and Pawlina, 2016).***

- **E-cadherins:**

E- cadherins are calcium-dependent cell-cell adhesion molecules. They are transmembrane glycoproteins present in most epithelial cells of adult and embryonic tissues. They are required for normal embryonic development. They are

localized mainly within the zonula adherens. They maintain homotypic interaction with similar proteins from the neighbouring cell. They are associated with group of intracellular proteins called β-catenin. The cadherin-catenin complex is linked to actin filaments of cytoskeleton by vinculin and α actinin. Through this interaction, cadherins convey signals that regulate mechanism of growth and cell differentiation. They control cell to cell interactions and participate in cell recognition and embryonic cell migration ***(Ross and Pawlina, 2016).***

E- cadherins act as important suppressor of epithelial tumor cell. Their expression in carcinomas in vitro is sufficient to reduce the aggressiveness of tumor cells. Cadherins can be regulated at both the mRNA and protein levels, by means of changes in subcellular distribution, translational or transcriptional events, and degradation. ***(Vleminckx et al., 1991).***

- **Integrins:**

They are a main family of transmembrane proteins in the form of heterodimers formed of α and β subunits encoded by different genes. Almost every cell express one or several integrins. They are involved in focal adhesion. On