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Epithelial to Mesenchymal Transition and Reepithelialisation in Wound Healing: A Review of Comparison

(Peralihan Epitelium kepada Mesenkima dan Pengepitelium Semula dalam Penyembuhan Luka: Ulasan Perbandingan)

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ABSTRACT

Skin wound healing is a complex physiological event, involving many cellular and molecular components. The event of wound healing is the coordinated overlap of a number of distinct phases, namely haemostasis, inflammatory, proliferative and remodelling. The molecular events surrounding wound healing, particularly the reepithelialisation, has been reported to be similar to the epithelial to mesenchymal transition (EMT). In this review, the mechanism between epithelialisation and EMT were compared. Both are characterised by the loss of epithelial integrity and increased motility. In terms of the signalling kinases, Smad and mitogen-activated protein kinase (MAPK) has been reported to be involved in both reepithelialisation and EMT. At the transcriptional level, SLUG transcription factor has been reported to be important for both reepithelialisation and EMT. Extracellular matrix proteins that have been associated with both events are collagen and laminin. Lastly, both events required the interplay between matrix metalloproteinases (MMPs) and its inhibitor. As a conclusion, both reepithelialisation and EMT shares similar signaling cascade and transcriptional regulation to exhibit decreased epithelial traits and increased motility in keratinocytes.

Keywords: Epithelial to mesenchymal transition; reepithelialisation; wound healing

ABSTRAK

Penyembuhan luka ialah proses fisiologi yang kompleks dan melibatkan pelbagai komponen sel dan molekul. Proses penyembuhan luka terdiri daripada tindihan beberapa fasa yang tersusun iaitu haemostasis, inflamasi, proliferasi dan pembentukan semula tisu. Proses molekul penyembuhan luka, khasnya pengepitelium semula, telah dilaporkan mempunyai persamaan dengan proses molekul yang terlibat dalam peralihan epitelium kepada mesenkima (EMT). Dalam kajian ini, persamaan proses molekul antara pengepitelium semula dan EMT dibincangkan. Penunjuk utama untuk kedua-dua proses ialah kehilangan keutuhan sel epitelium yang membawa kepada migrasi sel. Dua protein kinase telah dilaporkan sama-sama terlibat dalam pengepitelium semula dan EMT, Smad dan MAPK (mitogen-activated protein kinase). Pada tahap transkripsi genetik, faktor transkripsi SLUG dilaporkan diperlukan dalam kedua-dua proses. Matriks ekstrasel laminin dan kolagen juga dikaitkan dengan kedua-dua proses. Akhir sekali, keseimbangan antara enzim pencerna matriks ekstrasel dan perencatnya juga penting dalam proses pengepitelium semula dan EMT. Kesimpulannya, proses epitelium semula dan EMT berkongsi proses kawal atur yang sama di peringkat kinase dan faktor transkripsi yang membolehkan mobiliti sel dan pengurangan sifat epitelium untuk keratinosit.

Kata kunci: Pengepitelium semula; penyembuhan luka; peralihan epitelium kepada mesenkima

INTRODUCTION

The skin is the largest organ of the body that serve multiple functions; as the physical barrier that protect the body from external insults, helps in regulating the body temperature and permit the sensations of touch, heat and cold (Burr & Penzer 2005).

The skin is composed of three layers; the epidermis, dermis and hypodermis. The mammalian epidermis is made of integrated layers of stratified squamous epithelium, namely the epidermal keratinocytes (Maarof et al. 2016). The dermis is the layer of skin beneath the epidermis, that consists of connective tissue such as dermal fibroblasts (Idrus et al. 2014). The hypodermis is the layer beneath the dermis that contains loose connective tissue such as adipose tissue (Burr & Penzer 2005).

In the event of cutaneous wound, the skin cell has the capability to regenerate and restore the skin integrity via complex physiological process that involve soluble mediators, platelets, inflammatory cells, extracellular matrices (ECM) and parenchymal cells; in a series of coordinated events (Bielefeld et al. 2013). Wound healing can be divided into four overlapping phases; haemostasis, inflammatory, proliferative and the remodelling phase (Singer & Clark 1999).

The molecular events surrounding wound healing has been associated with epithelial to mesenchymal transition

(EMT) (Haensel & Dai 2018; Leopold et al. 2012). The idea of epithelial cell plasticity has emerged since the 1980s, when the first epithelial to mesenchymal phenotype change was observed in chick embryo (Hay 1995). Formerly thought to be a transformation, the paradigm has shifted to the notion of the reversible transition between epithelial and mesenchymal phenotype, EMT in one direction and mesenchymal to epithelial transition (MET) in another one (Kalluri & Neilson 2003).

Cell culture models of EMT has been used to identify and evaluate mechanism of action for novel therapeutics, particularly natural products such as honey (Chen et al. 2014, 2013a; Kao et al. 2013; Tseng et al. 2016). A systematic review of the literature concluded a positive effect of honey on the EMT process of wound healing (Nordin et al. 2017). In this review, the mechanism between epithelialisation and EMT were compared.

PHYSIOLOGY OF WOUND HEALING

Immediately upon wounding, the haemostasis phase happened. Damaged cells at the site of wound healing releases a plethora of signals that triggers the platelet activation and clotting cascade (Yau et al. 2015). The formation of the fibrin clot serves as a barrier that stops vascular leakage while protecting the wound site against microorganism invasion (Mazlyzam et al. 2007). Additionally, the fibrin clot acts as a temporary matrix for cell migration in the subsequent phase (Law et al. 2017). Activated platelet entrapped and aggregated in the blood clot, releasing a wide variety of factors that act as chemoattractant for cells involved in the inflammatory phase (Szpaderska et al. 2003). It is widely understood that the inflammatory response during normal healing is characterized by spatially and temporally changing patterns of various leukocyte subsets (Eming et al. 2007; Singer & Clark 1999).

In response to the signal releases by the platelet, neutrophils migrate to the wound site to debride the damaged tissue and clear up bacteria by releasing a large variety of highly active antimicrobial substances and proteases (Landén et al. 2016). Neutrophils cease from the wound site after few days, followed by the migration of macrophage into the wound site to phagocyte dead neutrophil (Koh & DiPietro 2011).

Macrophages also plays an integral role in ensuring a successful outcome of the healing response through the synthesis of numerous potent growth factors, such as transforming growth factor (TGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), which promote cell proliferation and the synthesis of extracellular matrix molecules by the parenchymal cells of the next phase of wound healing, within the wound site (Barrientos et al. 2014).

In response to the signals from the macrophages, fibroblast and epithelial cells migrate to the wound site (Minutti et al. 2017). Three distinct events characterized the proliferation phase; formation of granulation tissue, neovascularisation and reepithelialisation (Bielefeld et al. 2013).

The invading fibroblast repopulates the wound bed to restore the dermis layer. Fibroblasts degrades the temporary fibrin matrix via the secretion of matrix metalloproteinase and secrete ECM proteins such as collagen I-IV, XVIII, glycoproteins, proteoglycans, laminin, thrombospondin, glycosaminoglycans, hyaluronic acid and heparan sulphate to replace the degraded fibrin clot (Tracy et al. 2016).

Neovascularization, the formation of new blood vessel by the endothelial cells, occur concurrently throughout all the phases of wound healing (Tonnesen et al. 2000). In addition to attracting neutrophils and macrophages, numerous angiogenic factors secreted during the haemostatic phase promote angiogenesis (Landén et al. 2016).

The ECM matrix produced by fibroblasts in the proliferative phase, provided continuous support to the angiogenesis process (Tonnesen et al. 2000). The epithelium integrity is restored via the re-epithelialization process whereby, the epithelial cells proliferate, differentiate and migrate over the new matrix laid out by the fibroblasts (O'Toole 2001).

The final phase of wound repair is remodelling. The remodelling phase is the maintenance of balance between the degradation and the synthesis of the ECM (Lu et al. 2011). Matrix metalloproteinase (MMP), synthesized by neutrophils, macrophages and fibroblasts in the wound, is responsible for the ECM degradation (Xue & Jackson 2015). The degradation process is highly regulated with MMP inhibitor (Soo et al. 2000). Balance between activation and inhibition of MMPs continue until complete healing is achieved.

As the wound heals, density of fibroblasts and macrophages are reduced by apoptosis and eventually growth of capillaries stops, blood flow to the site decreases and metabolic activity decreases, resulting in a fully healed wound (Velnar et al. 2009).

BEHAVIOUR OF KERATINOCYTES IN REEPITHELIALISATION

Throughout the process of wound healing, the cells in the epidermis undergo a series of events to restore the epidermal integrity (Pastar et al. 2014). Together, known as reepithelialisation, the process involved migration, proliferation and differentiation of the epidermal keratinocytes.

Stages of reepithelialisation includes the formation of a provisional wound bed, the migration of keratinocytes from the wound edge, the proliferation of keratinocytes that feed the advancing migrating epithelial tongue, the stratification and differentiation of new epithelium and the reformation of the basement membrane zone (O'Toole 2001).

The epidermal basement membrane is made up of many collagen and laminin isoforms. Collagens are a family of glycoproteins containing triple helices that made up the bulk of the ECM. Twenty-eight different types of

collagen have been identified and at least 17 are known to be found in skin (Ricard-Blum 2011). Keratinocyte primarily synthesize type IV collagen and type VII collagen (anchoring fibril) (Lee & Cho 2005).

Laminins are large, cruciate, heterotrimeric molecules within the lamina lucida of the basement membrane zone. Laminin 1, 5, 6 and 7 are among the laminin isoforms that were found in the lamina lucida of the epithelium. Among them, laminin 5 has been associated with keratinocyte migration (Jones et al. 2000).

When the intact basement membrane zone is abrogated, migrating keratinocytes use the temporary matrix formed during the haemostasis phase of wound healing. This provisional matrix is rich in fibronectin, fibrin and vitronectin (Laurens et al. 2006).

Cultured keratinocyte demonstrated migrating behaviour on the surface coated with fibronectin (Clark et al. 1985). Upon completion of reepithelialisation, the keratinocyte can synthesize much of its own collagen and laminin, to restore the basement membrane (O'Toole 2001).

To initiate migration, the keratinocyte need to develop the necessary flexibility in order to move over the freshly deposited matrix (Pastar et al. 2014). Keratinocyte integrity is maintained through its attachment to each other via desmosomes and to the basement membrane via hemidesmosomes and focal adhesions (Fuchs & Cleveland 1998).

Analysis of different isoforms of desmosome on a regenerating epidermis model identifies E-cadherin as one of the downregulated desmosome during reepithelialisation (Moll et al. 1999). Keratinocyte at the wound edge removes its adhesion to each other and to the basal lamina, allowing them to start migrating from the wound edge over the denuded area (Werner & Grose 2003).

Upon the advancement of the first layer of migrating keratinocytes that covers the wound, termed as epithelial tongue, the keratinocytes behind the migrating tongue begin to proliferate to supply adequate number of cells to close the wound (Pastar et al. 2014) (Figure 1).

Growth factors that play a major role in the proliferative process during reepithelialisation include epidermal growth factor (EGF), TGF-β, insulin growth factor 1 (IGF-1) and keratinocyte growth factor (KGF) (Beer et al. 2000; Bhora et al. 1995; Gniadecki 1998). Application of topical TGF-β1, resulted in accelerated wound healing in aged rats (Puolakkainen et al. 1995). When TGF-β is absent, wound healing was impaired (Crowe et al. 2000).

Multiple signalling pathways are reported to be involved in the reepithelialisation process. TGF-β is known to activate Smad and MAPK signalling (Derynck & Zhang 2003). Activation of MAPK pathways was observed in EGF stimulated keratinocytes (Jost et al. 2001).

At the level of transcription, the transcription factor SNAI2 (SLUG) is reported to be important in reepithelialisation. EGF and TGF-β are known inducer of SLUG (Kusewitt et al. 2009). Absence of SLUG expression in both human and mouse compromised the cutaneous wound reepithelialisation (Hudson et al. 2009; Savagner et al. 2005).

For the wound to heal successfully, keratinocytes migration through the fibrin matrix requires the matrix metalloproteinases (MMPs), a group of enzymes that degrade ECM and pave the way for a smooth migration. Among MMPs involved are MMP-1, MMP-2, MMP-3, MMP-10, MMP-14, MMP-19 and MMP-28 (Caley et al. 2015).

More importantly, optimal keratinocyte migration during wound closure is achieved via the tight regulation of balance between MMPs and the tissue inhibitors of metalloproteinases (TIMPs) (Yalcinkaya et al. 2014).

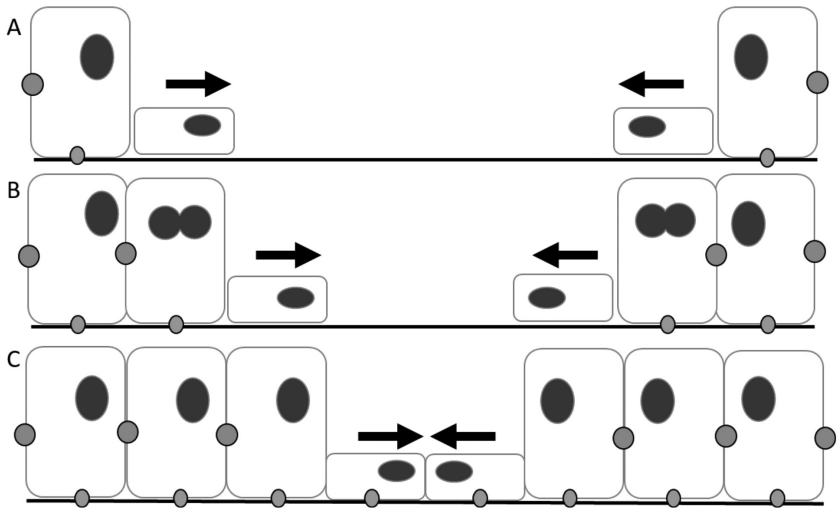

FIGURE 1. Illustration of the reepithelialisation process, (A) the keratinocyte at the wound edge removes its adhesion to each other and to the basal lamina, allowing them to start migrating from the wound edge over the denuded area, (B) the keratinocytes behind the migrating tongue begin to proliferate to supply adequate number of cells to close the wound, and (C) upon completion of the reepithelialisation process, migrating keratinocyte reform its adhesion to each other and to the basal lamina

Imbalance in the MMP and TIMP dynamics was evident in diabetic foot ulcers, an example of chronic wound pathology (Muller et al. 2008).

BEHAVIOUR OF KERATINOCYTE IN EPITHELIAL TO MESENCHYMAL TRANSITION

Prior to the observation of epithelial plasticity in the chick embryo, the histological similarity between tumour stroma generation and wound healing has been reported (Dvorak 1986). It was later known that the similarity of both wound healing and tumour metastasis was due to the epithelial plasticity, whereby the epithelial cell loses its rigid phenotype and transition towards the invasive mesenchymal-like phenotype (Byun & Gardner 2013).

Originally thought to be the irreversible transformation of the epithelial cell towards mesenchymal phenotype, epithelial to mesenchymal transition (EMT) has been widely accepted to be transient in nature (Kalluri & Neilson 2003).

The discovery of some early epithelial cells during embryogenesis and organ development, that is able to move back and forth between epithelial and mesenchymal states via the processes of EMT and MET, suggested the existence of a new category of EMT (Vićovac & Aplin 1996).

Consensus among EMT scientists in 2007 has classified EMT into three different sub-types according to the biological events where it was reported (Kalluri & Weinberg 2009). The EMT in embryogenesis and organ development is classified as type 1 EMT. The EMT related to wound healing and tissue regeneration, is classified as type 2 EMT. Lastly, the EMT related to cancer progression, is classified as type 3 EMT (Kalluri & Weinberg 2009).

Primary features of EMT include loss of cell to cell adhesion, loss of planar and apical-basal polarity, as well as an increase in cell mobility and migration capacity (Kalluri & Weinberg 2009). In type 2 EMT, polar epithelial cells turn into motile fibroblast under the induction of EMT ligand such as TGF-ß (Xu et al. 2009) and EGF (Okada et al. 1997). Removing EMT-inducing ligand restored epithelial phenotype of the cells suggesting reversibility of EMT, which is later known as MET (Janda et al. 2002).

Defining specific markers of EMT has been the subject of interest for the past decade to ensure the rigours of EMT investigation among the EMT researchers (Zeisberg & Neilson 2009). E-cadherin is an important feature of epithelial cells enabling it to form cell to cell adhesion (Li et al. 2012). Loss of the E-cadherin expression and increase expression of N-cadherin, known as cadherin switch has been widely accepted as a characteristic feature of the EMT (Gheldof & Berx 2013).

Following loss of cell to cell adhesion, cell motility is marked by the synthesis of various cytoskeletal proteins and rearrangement of actin fibres (Zeisberg & Neilson 2009). Vimentin is the intermediate filament protein that is expressed in various cells including fibroblasts and found to be essential in EMT-like process of wound healing (Cheng et al. 2016).

Other markers of EMT that has been summarised by Zeisberg and Neilson (2009) review includes the loss of epithelial markers such as CArG box-binding factor-A (CBF-A), lymphoid enhancer-binding factor (LEF), ß-catenin, cytokeratin and zona occludens 1 (ZO-1). The increase expression of mesenchymal/fibroblast markers such as fibroblast-specific protein-1 (FSP1), discoidin domain receptor tyrosine kinase 2 (DDR2), heat shock protein-47 (HSP47), collagen I, collagen II, or laminin, marks the other end of the EMT spectrum (Zeisberg & Neilson 2009).

Intracellularly, the increase expression of transcriptional factors such as SNAIL, SLUG, basic helix-loop-helix (bHLH) and zinc-finger E-box-binding (ZEB), indicated the initiation of EMT. Multiple signalling pathways cooperate in the initiation and progression of EMT including Smad, Rho-like GTPases, PI3K and MAPK (Lamouille et al. 2014).

Activation of EMT also triggers the release of various chemokines and matrix metalloproteinases (MMPs), notably MMP-2, MMP-3 and MMP-9 by the activated cells (Gialeli et al. 2011). Eventually, this will lead to basement membrane damage and focal degradation of collagen IV and laminin in the surrounding microenvironment which enables cell migration (Zeisberg et al. 2002). Inhibition of the regulator of the MMP, the tissue inhibitor of metalloproteinase (TIMP) is also a paramount feature of EMT (Yalcinkaya et al. 2014).

MORPHOLOGICAL CHANGES IN REEPITHELIALISATION AND EMT

Electron microscopic examination of the epithelial cells morphology at the initiation of migration after wounding showed that the migrating keratinocyte changes its shape dramatically as it goes from a stationary basal keratinocyte to a migrating cell. As the cell begins to migrate along the wound bed, it becomes flat and elongated (Odland & Ross 1968).

Long cytoplasmic extensions called lamellipodia are also observed. The migrating cell loses its hemidesmosomes and desmosomes and the gap junctions become more prominent. Keratin filaments retract from the cytoplasmic periphery and there is redistribution of the actin cytoskeleton into the lamellipodia (Odland & Ross 1968).

Enhanced migratory capacity of the epithelial cell is the one of the prominent features of EMT. In wound repair, migration capacity of keratinocyte is governed by the synthesis of various cytoskeletal proteins and rearrangement of actin fibres (Abreu-Blanco et al. 2012). Observation of human keratinocyte cell line induced with combination of cytokines, such as TGF-β and TNF-α, a known inducer of EMT, reiterated the many reports on the epithelial cells morphological changes in EMT (O'Kane et al. 2014). The rigid cobblestone morphology of the epidermal keratinocyte developed an elongated spindle-shaped morphology characteristic of a fibroblast-like cell when activated by the EMT inducer.

In the effort to establish the method to analyse motility of cells undergoing transient or stable EMT, Moreno-Bueno et al. (2009) suggested the utilisation of scratch assay. Scratch assay is a well-accepted *in vitro* model of cutaneous wound healing (Monsuur et al. 2016). Moreno-Bueno's team observed a distinct pattern between the migratory behaviour of the EMT-induced epithelial cells and the untreated epithelial cells.

Under EMT induction, the cells demonstrate an individual random migratory behaviour and repopulate the wound area as individual cells. In contrast, untreated epithelial cells migrate and repopulate the wound as a cohesive cell sheet without losing cell-cell contacts at the leading edge (Moreno-Bueno et al. 2009) as illustrated in Figure 2.

MOLECULAR CHANGES IN REEPITHELIALISATION AND EMT

Molecular changes in both wound healing and EMT, occurs at the level of cell junctions, cytoskeletons, kinases, transcriptional factors, extracellular matrices and matrix metalloproteinases (Figure 3).

A hallmark of EMT is the cadherin switch, indicated by the loss of E-cadherin and emergence of N-cadherin on the cell surface (Zeisberg & Neilson 2009). Similarly, in reepithelialisation, loss of E-cadherin is observed in regenerating epithelium and restored upon completion of reepithelialisation (Moll et al. 1999). Loss of E-cadherin in epidermal cell of both reepithelialisation and EMT resulted in the loss of cell to cell contact that leads to the motility of the epidermal cells.

Mobilisation of the epidermal cell is supplemented by the synthesis of various cytoskeletal proteins and rearrangement of actin fibres. Vimentin is the intermediate filament protein that is reported to be upregulated in EMT (Ivaska 2011). In reepithelialisation, vimentin also has been reported to be important in keratinocyte differentiation (Cheng et al. 2016). Upregulation of vimentin marks the transition between static to invasive phenotype of the epithelial cells in both EMT and reepithelialisation.

In terms of kinases, crosstalk between multiple signalling pathways has been reported in the activation of EMT including Smad, Rho-like GTPases, PI3K and MAPK pathways (Lamouille et al. 2014). In reepithelialisation, involvement of Smad, TGF-β and MAPK pathways is reported (Crowe et al. 2000; Derynck & Zhang 2003; Jost et al. 2001; Kusewitt et al. 2009). Both Smad and MAPK can be targeted to elucidate the molecular mechanism of novel therapeutics in the cell culture EMT models of wound healing (Figure 4).

Initiation of EMT is marked by the increase expression of transcriptional factors such as SNAIL, SLUG, bHLH, ZEB (Lamouille et al. 2014). In reepithelialisation, the

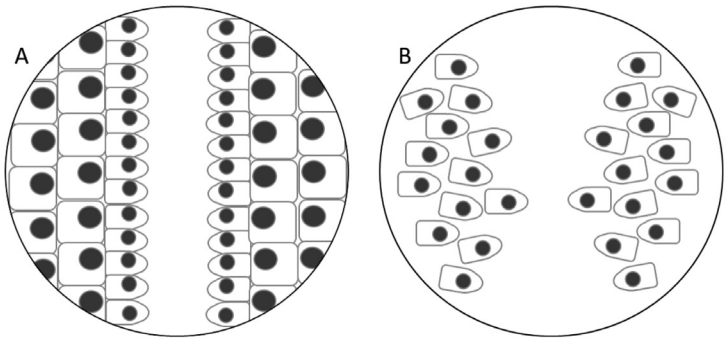

FIGURE 2. Migrating behaviour of keratinocyte in reepithelialisation and EMT, (A) without EMT induction, reepithelialising keratinocyte repopulate the wound as a cohesive cell sheet without losing cell to cell contact, and (B) under induction of EMT, the cells demonstrate an individual random migratory behaviour and repopulate the wound area as individual cells

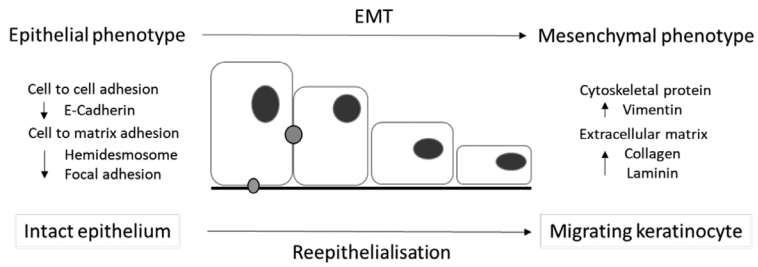

FIGURE 3. Comparison between reepithelialisation and EMT. Major molecular changes in both reepithelialisation and EMT, occurs at the level of cell to cell adhesion, cell to matrix adhesion, cytoskeletal protein and extracellular matrices synthesis

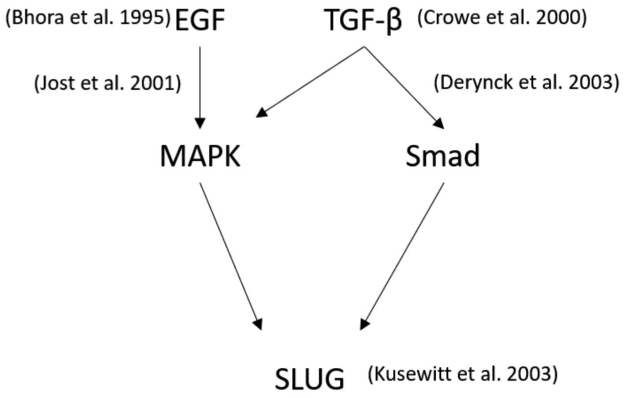

FIGURE 4. Signalling pathway shared by reepithelialisation and EMT. Schematic diagram of the common signalling pathway in both reepithelialisation and EMT

transcription factor SLUG is reported to be important (Hudson et al. 2009; Savagner et al. 2005). Only SLUG is reported to be involved in both reepithelialisation and EMT (Leopold et al. 2012). Thus, this suggested the importance of the transcription factor in the cell culture EMT models of wound healing.

Extracellular matrix was believed to regulates both reepithelialisation and EMT physiology (Chen et al. 2013b; O'Toole 2001). Among extracellular matrices that indicate EMT are collagen and laminin (Zeisberg & Neilson 2009). Similar to reepithelialisation, the keratinocyte is known to synthesize collagen and laminin, to restore the damaged basement membrane.

MMPs are a group of enzymes that is important in both wound healing and EMT process. Activation of EMT triggers the release of various MMPs, notably MMP-2, MMP-3 and MMP-9 by the activated cells (Gialeli et al. 2011). Among MMPs involved in the process of reepithelialisation to pave the way for migrating keratinocytes are MMP-1, MMP-2, MMP-3, MMP-10, MMP-14, MMP-19 and MMP-28 (Caley et al. 2015). Considering that, gelatine zymography, a technique to evaluate hydrolytic enzyme in culture, is of interest to investigate the effect of novel therapeutics on MMPs activity in cell culture EMT models of wound healing (Frankowski et al. 2012).

CONCLUSION

Cutaneous wound healing, more specifically, the reepithelialisation process, share many of the cellular and molecular changes with EMT, particularly decreased epithelial traits and increased motility.

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REFERENCES

Abreu-Blanco, M.T., Watts, J.J., Verboon, J.M. & Parkhurst, S.M. 2012. Cytoskeleton responses in wound repair. *Cellular and Molecular Life Sciences* 69(15): 2469-2483.

Barrientos, S., Brem, H., Stojadinovic, O. & Tomic-Canic, M. 2014. Clinical application of growth factors and cytokines in wound healing. *Wound Repair and Regeneration* 22(5): 569-578.

Beer, H.D., Gassmann, M.G., Munz, B., Steiling, H., Engelhardt, F., Bleuel, K. & Werner, S. 2000. Expression and function of keratinocyte growth factor and activin in skin morphogenesis and cutaneous wound repair. *Journal of Investigative Dermatology Symposium Proceedings* 5(1): 34-39.

Bhora, F.Y., Dunkin, B.J., Batzri, S., Aly, H.M., Bass, B.L., Sidawy, A.N. & Harmon, J.W. 1995. Effect of growth factors on cell proliferation and epithelialization in human skin. *Journal of Surgical Research* 59(2): 236-244.

Bielefeld, K.A., Amini-Nik, S. & Alman, B.A. 2013. Cutaneous wound healing: Recruiting developmental pathways for regeneration. *Cellular and Molecular Life Sciences* 70(12): 2059-2081.

Burr, S. & Penzer, R. 2005. Promoting skin health. *Nursing Standard* 19(36): 57-65.

Byun, J.S. & Gardner, K. 2013. Wounds that will not heal: Pervasive cellular reprogramming in cancer. *American Journal of Pathology* 182(4): 1055-1064.

Caley, M.P., Martins, V.L.C. & O'Toole, E.A. 2015. Metalloproteinases and wound healing. *Advances in Wound Care* 4(4): 225-234.

Chen, K.S., Shi, M.D., Chien, C.S. & Shih, Y.W. 2014. Pinocembrin suppresses TGF-beta1-induced epithelial-mesenchymal transition and metastasis of human Y-79 retinoblastoma cells through inactivating alphavbeta3 integrin/FAK/p38alpha signaling pathway. *Cell Bioscience* 4: 41.

Chen, M.J., Shih, S.C., Wang, H.Y., Lin, C.C., Liu, C.Y., Wang, T.E., Chu, C.H. & Chen, Y.J. 2013a. Caffeic acid phenethyl ester inhibits epithelial-mesenchymal transition of human pancreatic cancer cells. *Evidence-Based Complementary and Alternative Medicine* 2013: 270906.

Chen, Q.K., Lee, K., Radisky, D.C. & Nelson, C.M. 2013b. Extracellular matrix proteins regulate epithelial-mesenchymal transition in mammary epithelial cells. *Differentiation* 86(3): 126-132.

Cheng, F., Shen, Y., Mohanasundaram, P., Lindström, M., Ivaska, J., Ny, T. & Eriksson, J.E. 2016. Vimentin coordinates fibroblast proliferation and keratinocyte differentiation in wound healing via TGF-β–Slug signaling. *Proceedings of the National Academy of Sciences* 113(30): E4320-E4327.

Clark, R.A.F., Folkvord, J.M. & Wertz, R.L. 1985. Fibronectin, as well as other extracelllular matrix proteins, mediate human keratinocyte adherence. *Journal of Investigative Dermatology* 84(5): 378-383.

Crowe, M.J., Doetschman, T. & Greenhalgh, D.G. 2000. Delayed wound healing in immunodeficient TGF-β1 knockout mice. *Journal of Investigative Dermatology* 115(1): 3-11.

Derynck, R. & Zhang, Y.E. 2003. Smad-dependent and Smad-independent pathways in TGF-β family signalling. *Nature* 425(6958): 577-584.

Dvorak, H.F. 1986. Tumors: Wounds that do not heal. *The New England Journal of Medicine* 315(26): 1650-1659.

Eming, S.A., Krieg, T. & Davidson, J.M. 2007. Inflammation in wound repair: Molecular and cellular mechanisms. *Journal of Investigative Dermatology* 127(3): 514-525.

Frankowski, H., Gu, Y.H., Heo, J.H., Milner, R. & Del Zoppo, G.J. 2012. Use of gel zymography to examine matrix metalloproteinase (gelatinase) expression in brain tissue or in primary glial cultures. *Methods in Molecular Biology* 814: 221-233.

Fuchs, E. & Cleveland, D.W. 1998. A structural scaffolding of intermediate filaments in health and disease. *Science* 279: 514-519.

Gheldof, A. & Berx, G. 2013. Cadherins and epithelial-to-mesenchymal transition. *Progress in Molecular Biology and Translational Science* 116: 317-336.

Gialeli, C., Theocharis, A.D. & Karamanos, N.K. 2011. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *Federation of European Biochemical Societies Journal* 278(1): 16-27.

Gniadecki, R. 1998. Regulation of keratinocyte proliferation. *General Pharmacology: The Vascular System* 30(5): 619-622.

Haensel, D. & Dai, X. 2018. Epithelial-to-mesenchymal transition in cutaneous wound healing: Where we are and where we are heading. *Developmental Dynamics* 247(3): 473-480.

Hay, E.D. 1995. An overview of epithelio-mesenchymal transformation. *Cells Tissues Organs* 154(1): 8-20.

Hudson, L.G., Newkirk, K.M., Chandler, H.L., Choi, C., Fossey, S.L., Parent, A.E. & Kusewitt, D.F. 2009. Cutaneous wound reepithelialization is compromised in mice lacking functional Slug (Snai2). *Journal of Dermatological Science* 56(1): 19-26.

Idrus, R.H.B., Rameli, M.A.B., Low, K.C., Law, J.X., Chua, K.H., Latiff, M.B.A. & Saim, A.B. 2014. Full-thickness skin wound healing using autologous keratinocytes and dermal fibroblasts with fibrin. *Advances in Skin & Wound Care* 27(4): 171-180.

Ivaska, J. 2011. Vimentin. Central hub in EMT induction? *Small GTPases* 2(1): 51-53.

Janda, E., Lehmann, K., Killisch, I., Jechlinger, M., Herzig, M., Downward, J., Beug, H. & Grünert, S. 2002. Ras and TGFβ cooperatively regulate epithelial cell plasticity and metastasis: Dissection of Ras signaling pathways. *Journal of Cell Biology* 156(2): 299-313.

Jones, J.C.R., Dehart, G.W., Gonzales, M. & Goldfinger, L.E. 2000. Laminins: An overview. *Microscopy Research and Technique* 51(3): 211-213.

Jost, M., Huggett, T.M., Kari, C. & Rodeck, U. 2001. Matrix-independent survival of human keratinocytes through an EGF receptor/MAPK-kinase-dependent pathway. *Molecular Biology of the Cell* 12(5): 1519-1527.

Kalluri, R. & Neilson, E.G. 2003. Epithelial-mesenchymal transition and its implications for fibrosis. *Journal of Clinical Investigation* 112(12): 1776-1784.

Kalluri, R. & Weinberg, R. 2009. Review series the basics of epithelial-mesenchymal transition. *Journal of Clinical Investigation* 119(6): 1420-1428.

Kao, H.F., Chang-Chien, P.W., Chang, W.T., Yeh, T.M. & Wang, J.Y. 2013. Propolis inhibits TGF-β1-induced epithelial-mesenchymal transition in human alveolar epithelial cells via PPARγ activation. *International Immunopharmacology* 15(3): 565-574.

Koh, T.J. & DiPietro, L.A. 2011. Inflammation and wound healing: The role of the macrophage. *Expert Reviews in Molecular Medicine* 13: e23.

Kusewitt, D.F., Choi, C., Newkirk, K.M., Leroy, P., Li, Y., Chavez, M.G. & Hudson, L.G. 2009. Slug/Snai2 is a downstream mediator of epidermal growth factor receptor-stimulated reepithelialization. *Journal of Investigative Dermatology* 129(2): 491-495.

Lamouille, S., Xu, J. & Derynck, R. 2014. Molecular mechanisms of epithelial-mesenchymal transition. *National Review Molecular Cell Biology* 15(3): 178–196.

Landén, N.X., Li, D. & Ståhle, M. 2016. Transition from inflammation to proliferation: A critical step during wound healing. *Cellular and Molecular Life Sciences* 73(20): 3861-3885.

Laurens, N., Koolwijk, P. & De Maat, M.P.M. 2006. Fibrin structure and wound healing. *Journal of Thrombosis and Haemostasis* 4(5): 932-939.

Law, J.X., Chowdhury, S.R., Aminuddin, B.S. & Ruszymah, B.H.I. 2017. Role of plasma-derived fibrin on keratinocyte and fibroblast wound healing. *Cell and Tissue Banking* 18(4): 585-595.

Lee, D.Y. & Cho, K.H. 2005. The effects of epidermal keratinocytes and dermal fibroblasts on the formation of cutaneous basement membrane in three-dimensional culture systems. *Archives of Dermatological Research* 296(7): 296-302.

Leopold, P.L., Vincent, J. & Wang, H. 2012. A comparison of epithelial-to-mesenchymal transition and re-epithelialization. *Seminars in Cancer Biology* 22(5-6): 471-483.

Li, L., Hartley, R., Reiss, B., Sun, Y., Pu, J., Wu, D., Lin, F., Hoang, T., Yamada, S., Jiang, J. & Zhao, M. 2012. E-cadherin plays an essential role in collective directional migration of large epithelial sheets. *Cellular and Molecular Life Sciences* 69(16): 2779-2789.

Lu, P., Takai, K., Weaver, V.M. & Werb, Z. 2011. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harbor Perspectives in Biology* 3(12): 1-24.

Maarof, M., Law, J.X., Chowdhury, S.R., Khairoji, K.A., Saim, A.B. & Idrus, R.B.H. 2016. Secretion of wound healing mediators by single and bi-layer skin substitutes. *Cytotechnology* 68(5): 1873-1884.

Mazlyzam, A.L., Aminuddin, B.S., Fuzina, N.H., Norhayati, M.M., Fauziah, O., Isa, M.R., Saim, L. & Ruszymah, B.H.I. 2007. Reconstruction of living bilayer human skin equivalent

utilizing human fibrin as a scaffold. *Burns: Journal of the International Society for Burn Injuries* 33(3): 355-363.

Minutti, C.M., Knipper, J.A., Allen, J.E. & Zaiss, D.M.W. 2017. Tissue-specific contribution of macrophages to wound healing. *Seminars in Cell & Developmental Biology* 61: 3-11.

Moll, I., Houdek, P., Schäfer, S., Nuber, U. & Moll, R. 1999. Diversity of desmosomal proteins in regenerating epidermis: Immunohistochemical study using a human skin organ culture model. *Archives of Dermatological Research* 291(7-8): 437-446.

Monsuur, H.N., Boink, M.A., Weijers, E.M., Roffel, S., Breetveld, M., Gefen, A., van den Broek, L.J. & Gibbs, S. 2016. Methods to study differences in cell mobility during skin wound healing *in vitro*. *Journal of Biomechanics* 49(8): 1381-1387.

Moreno-Bueno, G., Peinado, H., Molina, P., Olmeda, D., Cubillo, E., Santos, V., Palacios, J., Portillo, F. & Cano, A. 2009. The morphological and molecular features of the epithelial-to-mesenchymal transition. *Nature Protocols* 4(11): 1591-1613.

Muller, M., Trocme, C., Lardy, B., Morel, F., Halimi, S. & Benhamou, P.Y. 2008. Matrix metalloproteinases and diabetic foot ulcers: The ratio of MMP-1 to TIMP-1 is a predictor of wound healing. *Diabetic Medicine* 25(4): 419-426.

Nordin, A., Sainik, N.Q.A.V., Zulfarina, M.S., Naina-Mohamed, I., Saim, A. & Bt Hj Idrus, R. 2017. Honey and epithelial to mesenchymal transition in wound healing: An evidence-based review. *Wound Medicine* 18: 8-20.

O'Kane, D., Jackson, M.V., Kissenpfennig, A., Spence, S., Damkat-Thomas, L., Tolland, J.P., Smyth, A.E., Denton, C.P., Elborn, J.S., McAuley, D.F. & O'Kane, C.M. 2014. SMAD inhibition attenuates epithelial to mesenchymal transition by primary keratinocytes *in vitro*. *Experimental Dermatology* 23(7): 497-503.

O'Toole, E.A. 2001. Extracellular matrix and keratinocyte migration. *Clinical and Experimental Dermatology* 26(6): 525-530.

Odland, G. & Ross, R. 1968. Human wound repair I . Epidermal regeneration. *The Journal of Cell Biology* 39(1): 135-151.

Okada, H., Danoff, T.M., Kalluri, R., Neilson, E.G., Danoff, T.M. & Neilson, E.G. 1997. Early role of Fsp1 in epithelial-mesenchymal transformation. *The American Physiological Society* 273(4): F563-F574.

Pastar, I., Stojadinovic, O., Yin, N.C., Ramirez, H., Nusbaum, A.G., Sawaya, A., Patel, S.B., Khalid, L., Isseroff, R.R. & Tomic-Canic, M. 2014. Epithelialization in wound healing: A comprehensive review. *Advances in Wound Care* 3(7): 445-464.

Puolakkainen, P.A., Reed, M.J., Gombotz, W.R., Twardzik, D.R., Abrass, I.B. & Helene Sage, E. 1995. Acceleration of wound healing in aged rats by topical application of transforming growth factor-beta1. *Wound Repair and Regeneration* 3(3): 330-339.

Ricard-Blum, S. 2011. The collagen family. *Cold Spring Harbor Perspectives in Biology* 3(1): a004978.

Savagner, P., Kusewitt, D.F., Carver, E.A., Magnino, F., Choi, C., Gridley, T. & Hudson, L.G. 2005. Developmental transcription factor slug is required for effective re-epithelialization by adult keratinocytes. *Journal of Cellular Physiology* 202(3): 858-866.

Singer, A.J. & Clark, R.A.F. 1999. Cutaneous wound healing. *The New England Journal of Medicine* 341(10): 738-746.

Soo, C., Shaw, W.W., Zhang, X., Longaker, M.T., Howard, E.W. & Ting, K. 2000. Differential expression of matrix metalloproteinases and their tissue-derived inhibitors in cutaneous wound repair. *Plastic and Reconstructive Surgery* 105(2): 638-647.

Szpaderska, A.M., Egozi, E.I., Gamelli, R.L. & DiPietro, L.A. 2003. The effect of thrombocytopenia on dermal wound healing. *The Journal of Investigative Dermatology* 120(6): 1130-1137.

Tonnesen, M.G., Feng, X. & Clark, R.A. 2000. Angiogenesis in wound healing. *The Journal of Investigative Dermatology. Symposium Proceedings* 5(1): 40-46.

Tracy, L.E., Minasian, R.A. & Caterson, E.J. 2016. Extracellular matrix and dermal fibroblast function in the healing wound. *Advances in Wound Care* 5(3): 119-136.

Tseng, J., Lin, C., Su, L., Fu, H., Yang, S. & Chuu, C. 2016. CAPE suppresses migration and invasion of prostate cancer cells via activation of non-canonical Wnt signaling. *Oncotarget* 7(25): 38010-38024.

Velnar, T., Bailey, T. & Smrkolj, V. 2009. The wound healing process: An overview of the cellular and molecular mechanisms. *Journal of International Medical Research* 37(5): 1528-1542.

Vićovac, L. & Aplin, J.D. 1996. Epithelial-mesenchymal transition during trophoblast differentiation. *Acta Anatomica* 156(3): 202-216.

Werner, S. & Grose, R. 2003. Regulation of wound healing by growth factors and cytokines. *Physiological Reviews* 83(3): 835-870.

Xu, J., Lamouille, S. & Derynck, R. 2009. TGF-beta-induced epithelial to mesenchymal transition. *Cell Research* 19(2): 156-172.

Xue, M. & Jackson, C.J. 2015. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. *Advances in Wound Care* 4(3): 119-136.

Yalcinkaya, E., Celik, M. & Bugan, B. 2014. Extracellular matrix turnover: A balance between MMPs and their inhibitors. *Arquivos Brasileiros de Cardiologia* 102(5): 519-520.

Yau, J.W., Teoh, H. & Verma, S. 2015. Endothelial cell control of thrombosis. *BMC Cardiovascular Disorders* 15: 130.

Zeisberg, M., Maeshima, Y., Mosterman, B. & Kalluri, R. 2002. Renal fibrosis. *The American Journal of Pathology* 160(6): 2001-2008.

Zeisberg, M. & Neilson, E.G. 2009. Review series personal perspective biomarkers for epithelial-mesenchymal transitions. *The Journal of Clinical Investigation* 119(6): 1429-1437.

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