Impact of EPLIN (Epithelial Protein Lost In Neoplasm) on HaCaT adhesion and migration rates: implications in wound healing

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Abstract

Introduction:
EPLIN (Epithelial Protein Lost In Neoplasm) is a cytoskeletal associated protein which has been implicated as a potential tumour suppressor due to its reduced expression in cancer cells and its impact on their invasive properties. Currently, no studies have examined the potential for EPLIN to influence the wound healing process. The aim of the study was to explore the impact of EPLIN on the HaCaT human keratinocyte cell line.

Methods:
EPLIN was over-expressed in the HaCaT keratinocyte cell line through the transfection of a mammalian expression construct containing the full EPLIN coding sequence. The attachment and migration rates of transfected HaCaT cells, displaying enhanced EPLIN expression, were examined in comparison to control cells using a electric cell-substrate impedance sensing (ECIS) system.

Results:
Transfection and selection of HaCaT cells with the EPLIN mammalian expression construct successfully resulted in the enhanced expression of EPLIN in these cells. Over-expression of EPLIN in HaCaT keratinocytes resulted in a reduction in the attachment of these HaCaT cells to a electrode compared to control HaCaT cells containing no EPLIN expression construct. Similarly, over-expression of EPLIN also substantially reduced HaCaT cell migration rates compared to control cells.

Conclusion:
EPLIN over-expression negatively impacted on HaCaT migration and attachment rates as assessed using an ECIS system. This data suggests that EPLIN may play a similar role in the regulation of cell-substrate adhesion and cell migration in keratinocytes to that suggested in cancer and endothelial cells. This effect on HaCaT cell migration and attachment may significantly impact on the wound healing process.

Background

EPLIN (epithelial protein lost in neoplasm) was initially discovered as a gene that displayed differential expression between normal oral epithelial cells and HPV-immortalised oral epithelial cell lines (1). The role of EPLIN in cancer cells has gathered recent interest and down-regulation of EPLIN is frequently observed in a number of cancer cells and tissues, being associated with poor NPI prognosis, lower patient survival rates and higher grade and TNM staging in breast cancer tumour samples (2,3). Recently, a role for EPLIN has also been indicated in angiogenesis where over-expression of EPLIN can negatively influence angiogenic traits *in vitro* (4).

EPLIN has been identified as a cytoskeletal protein, localised in a fibrillar pattern in the cytoplasm, similar to that of actin fibres (2). EPLIN has been identified as having a role in regulating actin turnover, stabilising actin filament structures (5) and in linking the cadherin-catenin complex to F-actin through its interaction with α-catenin (6). These studies highlight the potential for the loss of EPLIN expression in cancer cells to result in the disruption of cytoskeletal dynamics, potentially bringing about alterations in cell motility and invasiveness frequently demonstrated *in vitro*.

Currently, there are no studies in the literature focusing on the role of EPLIN in the wound healing process. The current study focused on identifying the impact of altering EPLIN levels in the HaCaT human keratinocyte cell line on cell processes such as cell-matrix adhesion and cell migration. These processes play important role in the complex cascade of wound healing. Thus, our study aims to evaluate the importance of this cytoskeletal protein in wound healing.

Materials & Methods

- ***Cell line:***
  The HaCaT human Keratinocyte cell line was used in this study

- ***Overexpression of EPLIN in HaCaT cells:***
  The full coding sequence of human EPLIN was cloned into a pEF6 plasmid as previously described (3). This EPLIN expression plasmid was used to transfect human keratinocyte HaCaT cells. Following transfection, cells were placed through a period of blasticidin selection to establish a stable cell line containing the plasmid and over-expressing EPLIN ($HaCaT^{EPLIN\ exp}$). Over-expression was verified in comparison to wild type ($HaCaT^{WT}$) and empty plasmid ($HaCaT^{pEF6}$) controls using RT-PCR. Following successful verification these cells were used to test the impact of EPLIN on HaCaT cell migration and adhesion..

- ***Impact of EPLIN over-expression on HaCaT cell migration and adhesion:***
  Cell adhesion and migrational rates following over-expression of EPLIN expression was assessed using the Electric cell-substrate impedance sensing (ECIS) model system.
  Cells were seeded into the ECIS array at a density of 400,000 cells per well and the array connected to the ECIS system. Changes in impedance were then recorded on the system as the cells attached to the electrodes present within the arrays. Cells were incubated for sufficient periods to allow complete adherence and formation of a confluent monolayer before undertaking migrational analysis.
  Migration rates were measured following the application of an electrical charge through the electrodes. This charge kills cells adhered to the electrode creating a number of "wounds" within the monolayer. Following the wounding the system was set up to record changes in resistance as cells migrated into this wound areas (plotted as normalised impedance) to recolonise the monolayer.

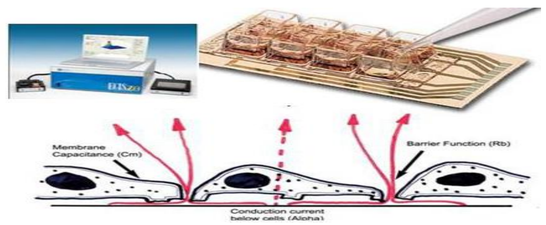

Figure 1: ECIS model system and arrays used in the study

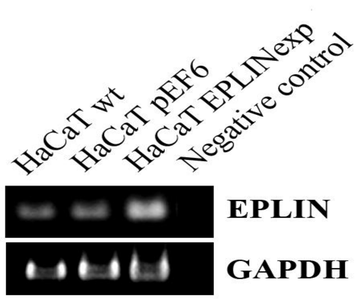

Figure 2: RT-PCR demonstrating the successful over-expression of EPLIN levels in cells transfected with EPLIN expression plasmid. Following transfection, a substantial increase in EPLIN levels was seen in $HaCaT^{EPLIN\ exp}$ compared to control cells.

Results

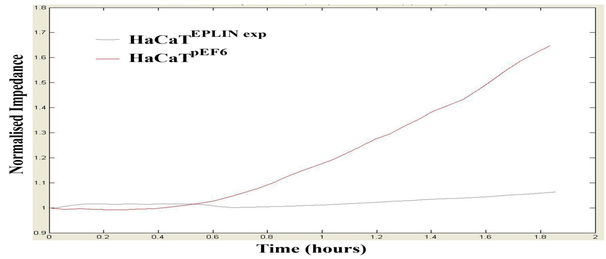

Figure 3: Impact of EPLIN over-expression on HaCaT cell adhesion to the ECIS array (*above*). Following over-expression of EPLIN, HaCaT cells were found to be less capable of adhering to the ECIS electrodes compared to the controls.

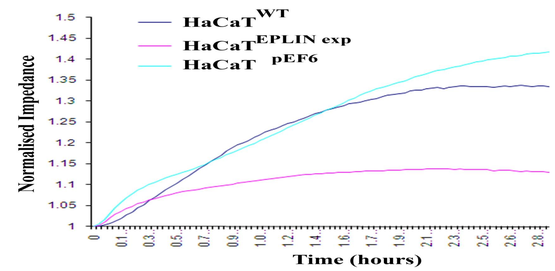

Figure 4: Similarly, over-expression of EPLIN also negatively impacted on HaCaT cell migration rates (*above*) and a substantial decrease was seen in comparison to the controls.

Discussion

EPLIN is a cytoskeletal molecule which is believed to be involved in regulating actin dynamics and hence influencing processes such as cell migration (5).
The role of EPLIN in cancer cells and endothelial cells is beginning to be identified, with this protein being down-regulated in cancer cells and tissue compared to normal cells and tissues, reducing the aggressive nature of cancer cells and having an anti-angiogenic affect (2-4).
Currently there is no information regarding EPLIN role in wound healing. Our study has implicated this protein in play a role in keratinocyte cell attachment and migration following electrical wounding. Cell migration plays a key role in re-epithelialisation of clinical wounds and thus, EPLIN may be an important factor in contributing to this process.

References

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