CHAPTER 1 INTRODUCTION

1.1 Research Background

Tissue architecture is maintained by cell adhesion molecules (CAMs) at both contacts: between two cells and towards the surrounding environment. The role of CAMs has been of importance in maintaining architecture of multicellular organisms as early as development and kept throughout life (Niessen, Leckband, & Yap, 2019). Skin epidermis serves as a protective layer for organisms, being the first line of defense, separating from external environment. Keratinocytes, the main cellular component of epidermis, upon differentiation and stratification establishes the beginning of formation of the stratified epidermal layers: basal layer, spinous layer, granular layer, and the cornified layer. When proliferating keratinocytes at basal layer commit to mature in differentiation, cell cycle progression is halted and soon differentiating cells that progressively undergo morphological changes and expression of differentiating proteins are established (Eckert & Rorke, 1989). This commitment of differentiation of keratinocytes involves not only changes in cell cycle and proliferation, but also dynamic changes in establishment of CAMs managing the cell interaction with the extracellular matrix (ECM) and cell-cell adhesiveness – collectively, these alterations promote the upward movement of differentiating keratinocyte (Braga, Hodivala, & Watt, 1995). Regulated changes in the level of expression, localisation or activation of CAMs such as E-cadherin which maintain cell-cell contact or integrin that maintain cell-ECM attachment are utilised not only in normal tissue homeostasis, but also during wound healing (Pastar et al., 2014; Safferling et al., 2013). During stratification, keratinocyte requires reduction of integrin-mediated attachment to the ECM along with an increase in cell-cell contact by E-cadherin (Braga et al., 1995). This allows the single proliferative monolayer of basal cells to detach from the basement membrane and start the progression of differentiation up the epidermal layers. Under normal condition, proliferating keratinocytes are maintained in their number and position through contact inhibition of proliferation (CIP) and contact inhibition of locomotion (CIL) which keep growth and tissue architecture in a controlled manner

(Mendonsa, Na, & Gumbiner, 2018). However, in early tumour progression, cells lose the ability to keep proliferation in check, resulting in uncontrollable growth. This outgrowth is likely a result of loss of CIP and CIL which are regulated by E-cadherin through a downstream cascades of signalling that control maintenance of cell-cell adhesion and polarity (Mendonsa et al., 2018). As such, the loss of stable junctions insinuates the loss of proper regulation of CAMs, undermining the structure, expression and function of proteins responsible for proper adhesion, resulting in loss of homeostasis in normal tissue architecture. It is hypothesised that the mechanism behind an early benign tumour formation is taking advantage of the dynamics in stratifying keratinocytes during differentiation, as proliferation had pushed past the threshold cell density that promotes the loss of CIP and thus, allowing cells to move upward. Regulation of functional CAMs are then topics of interest in perspective of cancer progression much to normal understanding of keratinocyte epithelialisation model.

Understanding the regulation of cadherin and integrin adhesiveness will shed insights into molecular pathways triggered in keratinocyte differentiation and potentially in tumour progression as well. Previously, RhoE, an atypical subtype of Rho small GTPase, has been found to regulate delamination of keratinocytes resulting in the stratification of the cells to form suprabasal layer. A transient upregulation of RhoE is observed during the induction of keratinocyte differentiation and stratification (Liebig et al., 2009). Although an interplay of RhoE to integrin has not been directly correlated in stratification, depletion of RhoE resulted in a higher attachment of keratinocytes to ECM despite an observed unchanged level of integrin expression, suggesting a regulation over integrin activation instead. Moreover, reduction of kindlin at basal level upon knockdown of RhoE has been found by the same group that suggests further that the reduction of integrin activation may be achieved by RhoE via regulation of kindlin (unpublished data). Kindlin is one of the key cytosolic proteins responsible for integrin activation alongside talin (Calderwood, 2013). Interestingly, kindlin-2 has been found to promote junction formation in vascular endothelial cell where kindlin-2 associates with catenins, indicating probable complex formation to adherens junction proteins (Pluskota et al.,

2017). To add, kindlin involvement in tumorigenesis has also been observed with both suppressing and promoting capabilities found across various cancer types (Djaafri et al., 2014; Sossey-Alaoui, Pluskota, Szpak, Schiemann, & Plow, 2018). Although the exact dynamics has not been elucidated, it is very likely that kindlin plays a role in cancer progression as integrin expression in tumour epidermal extend to the suprabasal layers in contrast to normal whose expression is confined to the basal cells (Watt, 2011). Thus, exploring the precise mechanism linking RhoE and kindlin promises further understanding into keratinocyte stratification that may benefit recovery model such as wound healing or disease model like early benign tumour formation.

1.2 **Research Objectives**

The objective of this project is to investigate the role of kindlin in regulating keratinocyte stratification and the functional interplay between small RhoE GTPase signalling and kindlin. More specifically, the study aims to determine kindlin function beyond focal adhesion and illustrate the relationship of RhoE and kindlin for upward movement of keratinocyte during stratification via knockdown and overexpression experiments.

1.3 **Research Scope**

The interplay between RhoE and kindlin functional expression in multilayering will be investigated through multiple assays assessing phenotype, expression, and functionality within the scope of stratification and the proteins involved via knockdown or overexpression experiments. The phenotype of the interaction between Kindlin and RhoE will be observed from imaging observation in stratification induced cells through quantification of stratifying or non-stratifying keratinocytes. Expression of proteins (RhoE, Kindlin-1, Kindlin-2, E-cadherin) will be assessed as a measure of Kindlin-RhoE dynamic upon the time course of calcium induced stratification model. Kindlin-RhoE dynamic towards integrin activity will be measured via adhesion assays to observe the ability of keratinocytes

adhering to ECM upon knockdown of kindlin expression and induced to stratify. The complete scope of methodology involved to determine relationship of Kindlin and RhoE in stratification is as below:

- Tissue culture: the study only focuses on *in vitro* model of normal human epithelial keratinocyte (NHEK) co-cultured with irradiated fibroblast and will not be exploring *in vivo* models. Only primary cell lines were used in the study and not immortalized cell lines.
- RNA interference: transfection of siRNA targeting kindlin-1 and/or kindlin-2 for knockdown
- Plasmid DNA transfections: transfection for overexpression of kindlin-1 or kindlin-2
- Western-Blot
- Immunofluorescence and microscopy
- Collagen adhesion assay