ABSTRACT

Single-cell live imaging is a powerful method to investigate cell motion. Using this approach, Nanba and colleagues have elegantly demonstrated that motion can predict human epidermal keratinocyte (NHEKs) stem cell fate (Nanba et al., *J. Cell Biol.*, 2015). Human thymus, a primary lymphoid organ, contains numerous $TP63^{+}/EpCAM^{+}$ epithelial cells (hTECs) that are clonogenic when cultured. Interestingly, clonogenic hTECs give rise to tripotent precursor cells that can self-renew as refringent colonies, while generating scattered and stratified colonies. Furthermore, gene expression analysis has revealed that the stratified-forming cells expressed many markers of squamous differentiation, which demonstrate hTECs similarity with NHEKs. As hTECs possess a huge potential in the future regenerative application to regenerate not only thymus, but also the skin, this present work aimed to investigate whether motion can also predict stem cell fate in this model system. Individual hTEC and NHEK were seeded in 96 well-plate and imaged for 72 hours. Colonies were then stained with rhodamine B for types validation. The results demonstrate that each hTECs clonal type can be unambiguously distinguished by its motion pattern. Scattered colonies were highly mobile, migrating significant distances without any attachment, while refringent-forming cells were less mobile, but had a strong tendency to detach following rotation. Stratified-forming cells were not mobile but regularly rotated on themselves, resembling NHEKs. These differing motion patterns were reflected from balance between epithelial-mesenchymal transition (EMT) and stratification. In conclusion, this study confirm that single-cell live imaging can predict hTECs stem cell fate.

Keywords: human thymic epithelial cells, stem cells, cell motion, single-cell live imaging