INTRODUCTION

Cell motility is an inherent event that defines the cell's locomotion, morphology and even cancer progression (Franz, Jones, & Ridley, 2002). The movement of a cell occurs mainly due to the actin cytoskeleton and microtubules along with the signaling cascades that prompt a polarity within the cell. This polarity is caused by Rho family GTPases (Franz, Jones, & Ridley, 2002), ROCK1 and ROCK2 in particular . The study of single cell migration is important since although it is beneficial for systemic differentiation for repairing damage areas (X. Trepat, 2012), single cell motility of leader cells in cancer can potentially cause the increased risks of metastasis, as it leaves the collective tumor cells.

The connections between cells signifies the overall structure of the cells and the tension of the junctions between each cell (Alberts, 2002). In the case of epithelial cells, the cell-cell adhesion is controlled by the adherens junction, where cell-cell contacts during migration are connected via E-cadherin (T. Lecuit, 2015). These cluster cell migrations are important for early embryogenesis and in adulthood as it regulates immune systems and heals areas that are damaged. However, in cancer cells, the cluster will follow the polarizing direction of the single leader cell. As the tumor increases, the single leader cell tends to dissociate and travel to other regions as a part of metastasis (S. Giampieri, 2009).

The article by (Huang & C. P. Brangwynne, 2005) discusses the motion governance of a cell-pair, which suggests that the cells move dependent of each other, where one cell pushes inward, allowing the other cell to fill in the opposing side as it rotates clockwise. The junction is also described as 's – shaped', suggesting a curving motion of the junctions. With epithelial cells as target and tumor progression as a factor, it is widely accepted that cancer progression alters the physiological architecture and morphology of cells. Affected epithelial cells can further allow metastasis due to tumor's invasive characteristic (D. Zink, 2004). Understandably, the cell-cell junctions and tensions are affected, allowing the morphological changes that occur during tumor invasion, but there is little proof suggesting this concept.

To conduct the research, a model for junctional unit was designed as per the manuscript written by (Ranjan, 2016), which provides a foundation to measure the morphological changes of the cell and its junction. The work suggests that keratinocyte doublets are placed in certain geometrical micropatterns, namely circular, square and triangular micropatterns, that will allow the doublet to take shape of these patterns and constrict them from the outside environment. It has been noted in the previous manuscript (Ranjan, 2016), that circular geometrical shapes create the weakest junctions, whereas triangular patterns had a greater tensile micro-environment of the junction than the square patterns (article under review). The cell-pairs are photographically recorded and their image is analyzed as datasets, using high – technology microscopes, to better identify the junction shape and tensions. Pixel measurements are used to further identify particular details from the image dataset.

Myosin II, found in the periphery of epithelial cells, is crucial in mammalian cell-cell contacts as it works on junction stability (Smutny, et al., 2010). Since E-caderin is essential for reorganizing thin bundles and therefore stabilizing the cell junction, myosin II in mammalian cells heavily influences the E-cadherin stability (Mege, Gavard, & Lambert, 2006). The Rho-associated coiled-coil kinase, or simply known as ROCK proteins have been identified to appropriately regulate elongated and polygonal epithelial sheets, due to its association as a serine / threonine structure, allowing the regulation of myosin II contraction (Jaffe & Hall, Rho GTPases: biochemistry and biology, 2005).With the intricacies that follow in understanding cell-cell contact during its motion and morphology, it is quintessential to prioritize the use of bioinformatics tools to better analyze the micropatterns and expressions that occur during such changes in the target cells. With the help of bioinformatics tools and the software analysis systems designed by the lab members of Imperial College London, we can further analyze junction activities of epithelial cells in controlled environments. Moreover, questions regarding the other types of proteins that regulate the cell and junction conformations can thus be answered.

Objective

Studies in the field of junction morphology and cell motility is **very important** for understanding the behavior of cells during cancer progression. With the help of computational tools developed to measure specific parameters of cell-cell contacts, the project aims to:

1. Validate the results for the computational tools used
2. Understand the significance of contractility with regards to cell adhesion and motility
3. Profile the motility patterns driven by different levels of cortical tension of cells in simulated condition

Hypothesis

With the help of bioinformatics tools to read cell sample images, parameters that define cell motility and junction morphology over time can be accurately analyzed. The accuracy will also further clarify the difference in cell behavior with regards to its contractility. The effects of ROCK inhibitor Y-27632 can also be measured through computational means and would provide a result suggesting the difference in cellular motility when compared to regular keratinocytes.

Scope of project

The scope of the project is to use cell tracking means to identify the cell migration patterns between regular cells and cells exposed to ROCK inhibitor Y-27632, to simulate the loss of cell contraction. The cells will be placed in confined regions of circular, square and triangular geometries and examine the effect on motility properties of the different cell samples. This project will provide and validate quantitative data that expresses the patterns of cell behavior based on the Keratinocyte Cell Tracking (KCT) program created by Andrew Loza, from the University of Washington, in collaboration with the Braga Lab, in Imperial College London, United Kingdom.

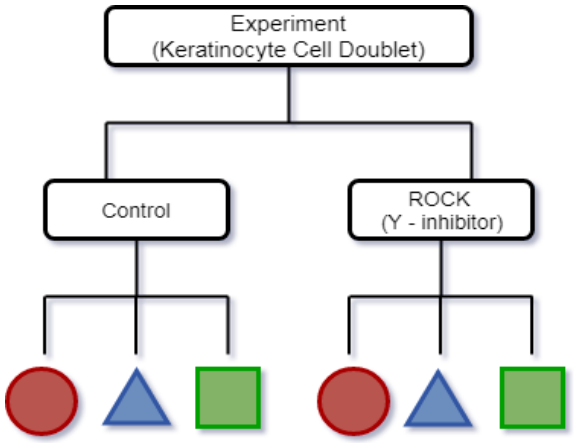

Figure.1. The experimental design of the project