ImmunoTools *special* Award 2024

Jay Mayatra, PhD-student

Supervisor: Prof. Dr. Daniel Johnston

Discipline of Anatomy, School of Medicine, Trinity College Dublin, the University of Dublin, Dublin 2, IRELAND

Creation of 3D organotypic *in vitro* models of the inflammatory skin disease hidradenitis suppurativa.

Scientific Abstract:

Hidradenitis suppurativa (HS) is a chronic skin disease which dramatically lessens patient quality of life and affects over 1% of Irish people. HS is characterised by the occlusion and rupture of hair follicles leading to flaring chronic inflammation which involves innate immune cell activation and Th1/Th17 driven disease pathology. The etiology of the disease is unclear, and a major limitation is the lack of an effective model to study the disease with research entirely reliant on invasive and scarce patient biopsies.

This project seeks to establish an 3D organotypic skin culture model as a platform to design a scalable *in vitro* model of HS. After establishing a fibroblast-keratinocyte co-culture model, we will use it to design larger funding proposals which will incorporate insights from new single cell and spatial transcriptomic datasets, available publicly and through collaborators, to accurately reproduce different cell states at different disease stages via pharmacological and/or genetic intervention. This novel approach will allow us to faithfully reproduce the critical aspects of biology HS in a controllable setting.

Project description:

Background:

Hidradenitis suppurativa (HS) is a poorly understood inflammatory skin disease with limited treatment options and a huge unmet clinical need. Affecting areas where the skin rubs together, such as the armpits, groin, buttocks and breasts, it is currently thought that excess growth of epithelial cells leads to hair follicle occlusion. This is

thought to lead to formation of painful subcutaneous nodules and abscesses which become chronically inflamed and lead to extensive scarring in severe cases [1,2]. Even in mild cases, the nodules and abscesses are painful and secrete embarrassing odorous pus, which is likely why HS is associated with significant social and psychological morbidity. Thus, HS patient quality of life is significantly impaired when compared to patients with other dermatological diseases such as psoriasis [3]. While the aetiology of HS is unknown, both genetic and environmental factors, including obesity and smoking, may increase disease risk. The incidence of HS in the Irish population has been estimated to be as high as 1.4%[4]. Treatment options for HS are limited and often the outcome of therapy is unsatisfactory, so there is an urgent need for new approaches.

In recent years, research into HS has gathered pace, but there is a dire lack of effective models to study the disease. This is very clear when compared to conditions like psoriasis where effective animal and *in vitro* cell culture models have allowed great strides to be made towards effective treatments. This leaves HS researchers limited to using patient samples which are difficult to acquire, invasive for the patient and of limited experimental utility[5,6].

Proposal concept:

As a platform for our novel HS model we will establish 3D organotypic skin equivalents with a dermis and fully differentiated epidermis using a previously validated method [7]. We will initially create the dermal layer through culture of commercially sourced human fibroblasts in a collagen matrix using Transwell inserts. We will then culture commercially sourced normal human keratinocytes on top of the established dermal matrix. Keratinocytes of the epidermis exist in differentiated layers, and we will induce this state with a media cocktail to generate a 3D skin equivalent in 10 days. We will treat these 3D skin equivalents with a cocktail of cytokines that have recently been described to be present in excess in HS skin (beta defensin[BD]-2, Interleukin [IL]-1α and β, IFN-gamma, IL-17, IL-36G, IP-10 and TNF-α). We will then assess changes to morphology and inflammation by H&E, immunohistochemistry and ELISA to detect key keratinocyte inflammation markers IL-6 and IL-8.

This proposal seeks to begin the develop models informed by that research, beginning with epithelial cells which are thought to be a key cell type in the disease [8].

References:

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