**THE ROLE OF DESMOPLAKIN DURING EPIDERMAL DEVELOPMENT**

**I. Introduction:**

 Cellular adhesion, particularly in the skin, is vital to human life. The skin is the body’s largest organ and it serves as protection against potential threats from the surrounding environment (Stalder, 2014 et al). It is comprised of three layers-the epidermis, dermis and hypodermis (*Human Skin*, 2016). Of particular interest is the epidermis, which is the outermost layer of the skin. The epidermis is responsible for the creation of new skin cells and acts as the skin’s primary layer of defense (Forni & Trombetta-Lima, 2012). Without proper cell adhesion in the epidermis, the skin would fail to maintain its protective state, ultimately leading to disease (Stalder, 2014 et al).

One important type of cellular adhesive is known as the desmosome (Garrod & Chigdey, 2008). The desmosome is primarily found in the epidermis and it consists of proteins that help to form its structural component, the desmosome-intermediate filament complex (DFIC). Without proper function of the desmosome and its constituents, the skin can become blistered, abnormally calloused and vulnerable to infection/disease (**Figure 1**). In order to help prevent or treat such skin disorders it is important to understand the components of the DIFC and the role that they play in cell growth and differentiation, especially during embryological development (Garrod & Chigdey, 2008).

**Fig. 1: Skin-blistering diseases caused by desmosomal defects. Taken from (Stanley & Amagai, 2006) and (Whittock et al. 2012)**

The desmosome-intermediate filament complex is made up of three main protein groups-desmocollins, desmogleins, and desmoplakin. Of these three proteins, this investigation focuses on desmoplakin and its role in cell differentiation during development. Desmoplakin contains three critical protein domains-the rod domain, plakin domain, and the tail domain (**Figure 2**). The tail region of desmoplakin is particularly important because it directly associates itself with keratin (Garrod & Chigdey, 2008). Keratin is a vital structural component of the skin that helps to maintain tissue integrity (*Keratin*, 2016). The goal of this experiment is to determine how disrupting desmoplakin structure may affect its linkage to keratin, and in turn affect the differentiation of the epidermal cells during prenatal development.

Whittock et al (2002) previously conducted a study in which an individual displaying a skin abnormality was observed from birth into adulthood. The patient was found to have a mutation in desmoplakin, ultimately leading to blistering and an early onset of skin fragility. Immunohistochemistry and electron microscopy revealed that while desmoplakin proteins were still present in the cells of the affected individual, the association of desmoplakin with the desmosome-intermediate filament complex and keratin was not consistent with that of a normal wild-type individual (Whittock et all, 2002). While this experiment helped to characterize the role of desmoplakin in cells after birth, there is little to no knowledge of its role during prenatal development. This project is therefore filling a major knowledge gap in the understanding of desmosomes during the early stages of life.

**II. Background:**

*Xenopus* laevis, also known as the African clawed frog, is an innovative and tractable system, with free-living embryos and an epidermis that is very similar to the epidermis of mammalian skin. It is a widely used species in the area of developmental research due to its cost efficiency and easy embryonic manipulation (James-Zorn, 2015 et al). Many desmosomal genes of *Xenopus*, including desmoplakin, have been annotated and are described on Xenbase (Bowes et al, 2010). Also, it has many structural similarities to humans, which makes it an ideal model organism for this investigation (James-Zorn, 2015 et al).

**III. Experiment:**

The aim of this experiment is to determine how a deletion in the desmoplakin tail domain affects its affinity to bind with keratin in a developing *Xenopus* embryo. The purpose of this is to understand the role of desmoplakin in cell differentiation. It is thought that if the tail region of desmoplakin is missing, it will no longer be able to link to keratin. Without this cellular linkage, proper cell structure should not exist, ultimately affecting the structural support of the epithelial cells that make up the epidermis.

**Figure 3: A schematic of a plasmid vector. The mutant desmoplakin gene is inserted into the plasmid, and replicated upon insertion into the host cell. Taken from (Morgan & Juchheim, 2014)**.

 This experiment will follow similar steps as describe by Kowalczyk et al (1997). The full *Xenopus* desmoplakin gene will be isolated in order carry out a deletion of the tail domain through the use of PCR. The mutant desmoplakin gene will then be subcloned and inserted into a plasmid vector (**Figure 3**). The purpose of the vector is to serve as a way to transfer the mutant desmoplakin gene, via microinjection, into *Xenopus* embryos at the one cell stage. Once the mutant gene is introduced, the embryos will then incubate to allow cell growth and replication. The cells of the developing embryos will then be stained using tubulin antibodies to observe cell differentiation through the use electron microscopy **(Figure 4)**.

**Figure 4: A photographic representation of desmoplakin and the desmosome using electron microscopy. Taken from (Dickinson lab, 2016).**

**IV. Discussion:**

Deletion of the tail region of desmoplakin should have a devastating affect on cell adhesion and differentiation. It is thought that if desmoplakin lacks the tail region, it will no longer be able to attach to keratin. It has been observed, as in the study of Whittock et al (2002), that loss of desmoplakin function with its surrounding protein counterparts, ultimately leads to blistering diseases and skin fragility. However, there is much uncertainty of how the mutation will affect the developing embryo.

 If the experiment goes as planned, the embryo should present epithelial cells that are not differentiating correctly. If this is the case, failure of cell differentiation should be easily visualized using electron microscopy. Without proper cell differentiation, I predict that the embryo will not be able to sustain any kind of mechanical stress. I also think that there is a high chance that there will be problems during the developmental shaping of the fetus. If this is the case, the knowledge gained from this experiment will be valuable in predicting similar problems with cell differentiation in humans. By understanding the potential results of this study, there could be further investigation upon how various desmoplakin related diseases could be treated/prevented to further enhance the quality of life for affected individuals.

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