Current Research in Translational Medicine 64 (2016) 179–183

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Modelling atopic dermatitis during the morphogenetic process involved in reconstruction of a human epidermis

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ARTICLE INFO

Article history:
Received 10 October 2016
Accepted 12 October 2016
Available online 17 November 2016

Keywords:
Atopic dermatitis
Reconstructed human epidermis
Cholesterol
Interleukins
Epidermal barrier

ABSTRACT

Most crucial role of epidermis is to maintain efficient barrier between the organism and its environment. This barrier is however perturbed in inflammatory skin conditions like atopic dermatitis (AD), one common chronic disease. This review depicts characteristics of a model intending to reproduce epidermal features of AD in vitro. Firstly, methyl-β-cyclodextrin (MβCD) during reconstruction of epidermis was used to deplete cholesterol from plasma membrane because this condition reproduces characteristics of AD at transcriptomic level in monolayer cultures. Major changes are confirmed after same treatment inside reconstructed human epidermis (RHE). However, since early treatment do not reveal impairment to reconstruct a functional epidermal barrier and given the importance of the Th2 dysregulated immune response in AD, cholesterol-depleted RHE at day 11 of reconstruction were then incubated with three Th2-related cytokines (IL-4, IL-13 and IL-25) previously reported as playing important roles in the development of AD, as well as altering overall function of epidermal barrier. When combining both treatments, essential epidermal features of AD are observed. Indeed, RHE then exhibit spongiosis, disappearing granular layer, alteration of barrier function, as well as dysregulated expression levels for genes involved in AD pathogenesis. Moreover, while trying to identify individual roles for each component used to create AD-like alterations, incubation with IL-4 following cholesterol depletion from plasma membrane was found inducing most of the reported alterations. This model suggests potential for better investigations of epidermal AD features and may be considered for eventual in vitro screening of cosmetics or therapeutic compounds.

Plasma membranes of eukaryotic cells organize as phospholipid bilayers in order to define cell boundaries. Phospholipids constituting the plasma membrane are amphiphilic, allowing them to naturally create a bilayer as they orient hydrophobic tails in the centre of the bilayer where they can associate with each other and expose hydrophilic heads towards the exterior of the membrane, where they will be in contact either with the extracellular liquid in case of plasma membrane or with the cytosol. Phospholipids associate with sphingolipids and sterols to become the major lipid constituents of the plasma membrane.

It was reported that plasma membranes contain particularly high concentrations of cholesterol and sphingolipids within specialized specific regions of the membrane called membrane lipid domains. Such domains exhibit elevated affinities for multiple proteins involved in precise signalling pathways, such as the epidermal growth factor receptor (EGFR) as one example [1–3]. Cholesterol being one crucial component of the specialized membrane lipid domains in animals, a widely used experimental approach of the role of cholesterol in the function and structure of these lipid domains utilizes various cholesterol-depleting agents to extract cholesterol from the plasma membrane. One of these agents is the molecule called methyl-β-cyclodextrin (MβCD), commonly used as an externally hydrophilic molecule that is able to solubilize cholesterol in aqueous environments, like body fluids or culture media. MβCD is totally unable to cross cell membranes, but contains a central hydrophobic cavity which can, due to its steric characteristics, specifically extract cholesterol from plasma membranes. Thus, using MβCD to deplete cholesterol is supposed to effectively disrupt molecular organization of every cholesterol-containing membrane domain, as well as linked signalling pathways.

MβCD has become of interest in the context of atopic dermatitis (AD) when transcriptomic microarray analyses of cholesterol-depleted keratinocytes by MβCD have shown regulations of this cell type's phenotype that were quite similar to the abnormal

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http://dx.doi.org/10.1016/j.retram.2016.10.005
2452-3186/

phenotype of keratinocytes analysed in the skin of AD patients [4]. Such similarities were occurring in keratinocytes during the first hours of recovery following MβCD treatment. Those observations of similarities were further confirmed by using RT-qPCR to measure transcript levels produced by cholesterol-regulated genes in lesional and non-lesional AD skin biopsies. Thereby, elevated levels of involucrin (IVL), transglutaminase-1 (TGM-1), heparin-binding EGF-like growth factor (HB-EGF), interleukin (IL)-8 and plasminogen activator urokinase receptor (PLAUR) expression were observed, simultaneously with decreased levels of filaggrin (FLG) and loricrin (LOR) expression in keratinocytes which had been treated to create cholesterol depletion, as well as in keratinocytes analysed directly in lesional skin biopsies of AD patients.

In keratinocytes, cholesterol deprivation by incubation with MβCD is quickly followed by repletion of cholesterol in their plasma membranes since this cell-type exhibits a strong potential to resynthesize cholesterol, as can be expected from their normal behaviour in serum-free culture conditions. This property enables keratinocytes to recover normal cholesterol content within a few hours after the MβCD treatment [5–8]. Strong similarities, at transcriptomic levels, between keratinocytes bordered by membrane depleted in cholesterol on one hand, and keratinocytes in AD skin on the other hand, precisely occurs during the first hours of recovery that follow MβCD treatment [4]. In addition, studies of keratinocyte monolayer cultures revealed that, for several hours of recovery after MβCD, activation by dimerization and tyrosine transphosphorylation of the EGFR occurred in a ligand-independent way, which in turn induced activation through phosphorylation of extracellular signal-regulated kinase (ERK) [9]. Interestingly, mitogen-activated protein kinase p38 (p38 MAPK) was simultaneously activated after cholesterol depletion, together with enhanced expression of the differentiation markers IVL, as well as of the potential EGFR-ligand HB-EGF [5,6,8]. However, activation of p38 MAPK was demonstrated as independent of the activated tyrosine kinase of EGFR, suggesting thereby that unidentified signalling pathways unrelated to EGFR are activated after cholesterol-depletion in human keratinocytes. Whereas the involved mechanisms are still to be investigated in order to understand how multiple signalling processes are simultaneously activated in such circumstances, available data suggested that transient depletion of plasma membrane cholesterol is able to modulate the keratinocyte phenotype in a way that mimics certain characteristics of AD, at least at some transcriptomic levels.

Atopic dermatitis (AD) is probably the most common disease among chronic inflammatory skin conditions and is responsible for eczema in childhood. AD condition is characterized by dryness of the skin, intense pruritus and erythematous lesions, as a result created by scratching of itching dry skin. AD develops as a chronic, usually relapsing disease that most often starts early in childhood and may disappear during infancy in a significant proportion of children [10]. From an etiological point of view, AD is a complex multifactorial disease, still certainly not completely understood, which involves genetic predispositions and environmental triggering factors, but also multiple interplay between alterations of the epidermal barrier function and abnormal T helper 2 immune response [11–14]. So far, two different hypotheses have been proposed regarding the aetiology of the pathology. The first one is the "outside-inside" hypothesis which suggests that primary defects probably originate from a leaky epidermal barrier itself, allowing enhanced penetration of allergens and pathogens, thereby leading to inflammation and immune sensitisation. Conversely, the second hypothesis, known as "inside-outside" hypothesis, proposes that primary defects probably reside at the level of the immune system, causing inflammation of the skin and leading to subsequent barrier defects. As an evidence, precise aetiology of AD is still a puzzling question, even though it very likely involves together epidermal and immune actors, both of them being potentially responsible for induction of the other one, in a vicious circle that is prone for development of this disease, and in a rather complicated scheme where environmental as well as genetic factors are at some point involved [13,15,16] (Fig. 1).

Prevalence of AD has tripled over the last 30 years and it has been considered that between 15–30% of children and 2–10% of adults can be affected by the disease in most developed countries [17]. Recent reports highlight an increase in prevalence in developing countries also and even in low-income countries [18,19]. Moreover, AD can represent an initial step of the so-called "atopic march" which corresponds to the observation that between 30–50% of patients, particularly those suffering from severe AD, will develop asthma and/or allergic rhinitis during their life-course [20–22]. The "atopic march" can find some explanation in the potential role played by thymic stromal lymphopoïetin (TSLP), a cytokine induced either by trauma, infection by microbes, toll-like receptor (TLR) activation, or by a combination of inflammatory cytokines [23–27]. Induction of TSLP in skin has been shown to be accompanied by elevated circulating TSLP levels in blood. Thus, TSLP could be considered as a systemic driver of bronchial hyper-responsiveness because elimination of TSLP signalling [28], or inducible ablated TSLP in keratinocytes [29,30] of mice prevents occurrence of the atopic march, suggesting that TSLP produced by AD keratinocytes may be involved in the link between AD and allergic asthma.

AD is thus becoming a growing public and global health problem. Existing treatments are mainly aimed at restoring epidermal barrier defects and reducing skin inflammation, as well

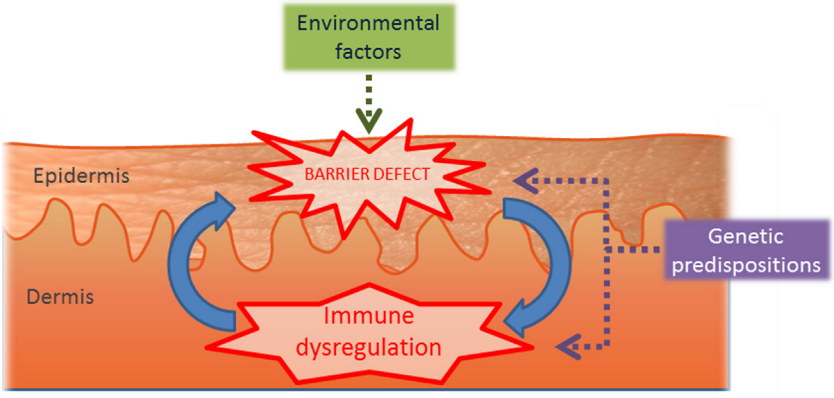

Fig. 1. Vicious circle observed in AD concerning barrier alteration and immune response dysregulation. Genetic predispositions are observed regarding both actors and this circle can further be influenced by environmental factors.