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Review

Connexins: Sensors of epidermal integrity that are therapeutic targets

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ABSTRACT

Gap junction proteins (connexins) are differentially expressed throughout the multiple layers of the epidermis. A variety of skin conditions arise with aberrant connexin expression or function and suggest that maintaining the epidermal gap junction network has many important roles in preserving epidermal integrity and homeostasis. Mutations in a number of connexins lead to epidermal dysplasias giving rise to a range of dermatological disorders of differing severity. 'Gain of function' mutations reveal connexin-mediated roles in calcium signalling within the epidermis. Connexins are involved in epidermal innate immunity, inflammation control and in wound repair. The therapeutic potential of targeting connexins to improve wound healing responses is now clear. This review discusses the role of connexins in epidermal integrity, and examines the emerging evidence that connexins act as epidermal sensors to a variety of mechanical, temperature, pathogen-induced and chemical stimuli. Connexins thus act as an integral component of the skin's protective barrier.

1. Overview

The mammalian epidermis forms a resilient, water-impermeable barrier that protects internal organs from the external environment. This highly differentiated, stratified structure consists of basal, spinous, granular and cornified keratinocyte layers that are characterised by the differential expression of keratins and a range of cell-to-cell adhesion and junctional proteins, including the connexins (Fig. 1) [1]. This barrier provides an interface with the external environment, the surface of which is colonised by a diverse array of microorganisms that exist in specialised topographical niches, with a fine balance between commensal and potentially opportunistic pathogenic organisms [2]. Disruption in the balance of this host–microbe interaction can result in skin disorders and infection [3]. In recent years, observed changes in connexin expression and signalling in a number of epidermal conditions have indicated a central role for connexin-mediated events in maintaining the integrity of this tissue. This review focuses on recent evidence supporting the concept that the epidermal connexin network plays a central role in sensing and maintaining epidermal integrity, and that connexins are emerging as prime therapeutic targets for a diverse range of epidermal conditions.

2. Connexins and the epidermis

Connexins are a family of highly conserved transmembrane proteins that assemble to form connexon hemichannels in the cell plasma membrane. Under 'normal' conditions these channels are closed, however they can be triggered to open under environmental stress conditions, providing a conduit between the intra- and extra-cellular environments. Ultimately, connexons align and dock with connexons from neighbouring cells to form dodecameric gap junction channels, which facilitate the direct exchange of inorganic ions, small metabolites and cellular messenger molecules (<1 kDa in size) between cells [4,5]. Twenty-one connexin subtypes have

Abbreviations: Cx, connexin; HPV, human papilloma virus; NSHI, non–syndromic hearing impairment; ODDD, oculodentodigital dysplasia; ZO-1, zona occludens 1; E1 and E2, extracellular domains 1 and 2; PPK, palmoplantar hyperkeratinisation; KID, keratitis ichthyosis deafness; EKV-P, erythrokeratodermia variabilis et progressiva; CaR, Calcium-sensor receptor; UPR, unfolded protein response; ROS, reactive oxygen species; Panx, pannexin; IP_3, inositol triphosphate; PGN, Peptidoglycan; IL-6, interleukin 6; TLR2, toll-like receptor 2; ECM, extracellular matrix; PKC, protein kinase C; asODNs, antisense oligonucleotides; CMP, connexin mimetic peptide

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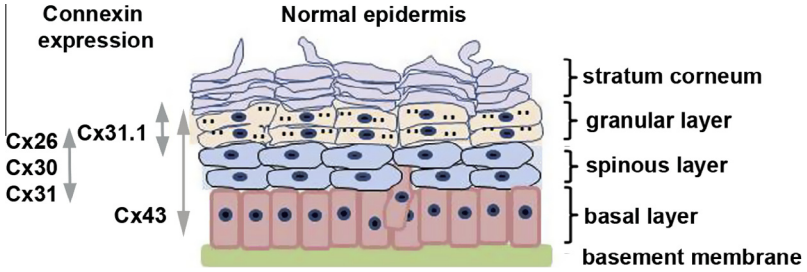

Fig. 1. Representative image of expression profile of key connexins in normal human epidermis. Of note Cx26 is generally expressed at very low levels in normal epidermis but is enhanced under a range of hyperproliferative skin disorder. Mutations in Cx26, Cx30 and Cx31 are associated with hyperproliferative skin disorders falling into two main groupings: 'loss of function' or 'gain of function'. These mutations give insight into the functional role of connexins in epidermal integrity. See text for details.

been identified in the human genome. Connexins are classified according to their phylogenetic origins into the 'alpha' subgroup connexins (including connexin43 (Cx43), Cx40 and Cx45) and the 'beta' subgroup connexins (including Cx26, Cx30 and Cx31), each having unique tissue expression patterns and permeability properties [4,6]. In the stratified, avascular epidermis these proteins provide a mechanism for cells to co-ordinate their activities.

The integrity of the epidermal barrier is maintained by continuous renewal of the cell layers from a pool of epidermal stem cells, with a steady state existing between the rate of production of new cells in the basal layer and the rate of loss of terminally differentiated cells from the skin surface [7]. Up to 10 different connexin proteins are differentially expressed throughout the terminal differentiation programme [8], with Cx43 the main connexin found in basal proliferating cells. In the human epidermis connexin expression is at its highest in the spinous layer with the expression of Cx26, Cx30, Cx30.3, Cx31, Cx31.1, Cx40, Cx43 and Cx45, and in the granular layer where there are high levels of Cx30.3, Cx31 and Cx43 and lower levels of Cx26, Cx31.1, Cx40 and Cx45, although species-specific variations occur [9–11] (Fig. 1).

The role of these proteins in establishing and maintaining the epidermal barrier is significant as exemplified by the many skin states and diseases associated with altered connexin expression and mutations in Cx43, Cx31, Cx31.1 Cx30.3, Cx30 and Cx26 (reviewed by [12,13]). During wound healing, distribution patterns of both Cx43 and Cx26 are remodeled, and Cx43 emerges as a therapeutic target to improve wound closure events (reviewed by [14]). Of particular interest is Cx26, the shortest connexin, which, although abundant in other stratified epithlia, e.g. vagina and oral cavity, is generally found at low levels throughout the stratified epidermis. Expression of Cx26 is enhanced in a range of hyperproliferating skin disorders including chronic non-healing wounds [15], psoriasis [16], and in human papilloma virus (HPV)-induced cutaneous warts [17]. It is also upregulated in the early stages of HPV16-associated cervical cancer progression [18], although downregulated in advanced stages [19]. Certain dominant-acting mutations in Cx26 manifest in distinctive skin disorders that range from mild to lethal forms, leading to suggestions that maintaining the Cx26 balance is crucial for epidermal integrity.

3. Mutations in connexins and epidermal dysplasia

Many mutations in Cx26 are present in the human population, with recessive mutations throughout the Cx26 coding region now recognised as one of the most common genetic causes of non-syndromic hearing impairment (NSHI) [20], and genetic screening for Cx26 mutations is often offered (e.g. http://www.arupconsult.com/Topics/NSHL.html#tabs=2). Cx26 mutations are believed to have remained in the gene pool due to heterozygous advantage, possibly by improving epithelial barrier function (see below) [21]. Patients harbouring recessive mutations, or extensive Cx26 gene deletions, suffer from deafness but no skin pathology, suggesting that Cx26 is not an absolute requirement in human skin, although epidermal thickening of carriers has been reported [22,23]. This is exemplified by the 35delG mutation, which is the highest penetrating Cx26 mutation. It is highly likely that in the absence of Cx26 expression, substitution by the closely related Cx30 carries some of the responses, as reported in studies using mouse models of the cochlea [24,25].

In addition to deafness, a spectrum of skin disorders is associated with autosomal dominant Cx26 mutations. Autosomal dominant mutations in other beta connexins including Cx31, Cx30 and Cx30.3 are also linked with unique epidermal dysplasias, while up to 73 mutations in Cx43 are associated with oculodentodigital dysplasia (ODDD) (recently reviewed by [26]), that along with other tissue defects sometimes manifests in palmar plantar hyperkeratosis. The impact of these mutations on connexin assembly and function are beginning to provide clues as to the functional importance of the connexin family in the epidermis.

To understand the role of connexins in the epidermis it is important to first consider the key steps in the connexin life cycle. Translation of connexin mRNA occurs on endoplasmic reticulum (ER)-bound ribosomes, ensuring co-translational insertion into the membrane enabling the protein to adopt its correct topology. Four transmembrane domains are linked by two extracellular loops (E1 and E2), which project into the lumen of the ER, with intracellularly-located amino and carboxyl termini and a linking intracellular loop. Six connexins then oligomerise in the ER/Golgi environs to form a connexon hemichannel [27,28], which is trafficked in a closed state to the plasma membrane via a microtubule-dependent pathway with the aid of plus end motor proteins [27,29,30] and chaperones such as consortin [28] and CIP75 [31]. It is important to note that alpha and beta connexin subtypes are incompatible and are unable to form heteromeric channels [6]. The beta connexins Cx26 and Cx30 have also been reported to be recruited to the plasma membrane via a microtubule-independent route, bypassing the classical secretory pathway [32,33]. Once inserted into the plasma membrane, hemichannels laterally accrete and dock with hemichannels on the neighbouring cell at the edge of an established gap junction intercellular plaque [34]. Close association of the carboxyl terminal domain of the mature connexin protein with cytoskeletal adaptor proteins including zona occludens 1 (ZO-1) at the plasma membrane [35,36] and post-translational modification (particularly for Cx43) suggest that connexins are also involved in cell migration and cell-to-cell adhesion responses [37–40]. The beta connexins, particularly Cx26 and Cx30, with short carboxyl termini, are less likely to be post-translationally modified on their carboxyl termini and Cx30 has been reported to lack any interactions with ZO-1 [33]. The connexin life cycle is typically short (with a half-life of 2–4 h), such that new connexons are continuously added to the edges of the plaque and old connexons are removed from the central region by invagination into one cell and formation of a connexosome [5] that