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Invited Perspective

Connexin hemichannels influence genetically determined inflammatory and hyperproliferative skin diseases

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ABSTRACT

Connexin mutations underlie numerous human genetic diseases. Several connexin genes have been linked to skin diseases, and mechanistic studies have indicated that a gain of abnormal channel function may be responsible for pathology. The topical accessibility of the epidermal connexins, the existence of several mouse models of human skin disease, and the ongoing identification of pharmacological inhibitors targeting connexins provide an opportunity to test new therapeutic approaches.

1. Connexin channels and the epidermis

The recent decade or so has seen connexins claim heightened attention as key determinants of epidermal homeostasis and, unsurprisingly, also as effectors of a wide spectrum of genetic and acquired cutaneous pathophysiology [1,2]. The connexins are a multi-gene family of highly conserved integral membrane proteins that are dynamically expressed in virtually all vertebrate tissues [3]. Human skin is no exception, containing at least 9 of the 21 human connexin (Cx) isoforms identified to date, including members of both the alpha (Cx37, Cx40, Cx43, Cx45) and beta (Cx26, Cx30, Cx30.3, Cx31, Cx31.1) phylogenetic subgroups [4,5]. Connexin monomers hexamerize to create highly specialized aqueous pores in the plasma membrane, referred to as connexons or hemichannels, and through which messages may pass in the form of ions, small molecules, or cytoplasmic metabolites [6,7]. Hemichannels may associate with a cognate structure on the surface of a contiguous neighbor cell to make intercellular gap junctions (GJs) or may remain 'unpaired' to connect the cell interior with the extracellular microenvironment [8–12]. Channels in both configurations exhibit precise spatial and temporal expression patterns according to tissue-specific developmental and functional requirements for electrical and metabolic coupling of cell networks [13,14]. Furthermore, different channel compositions impart unique gating and permeability properties depending on the individual connexin protein constituents and their sensitivities and susceptibilities to factors such as membrane potential and post-translational phosphorylation events [15–18].

2. The case for epidermal connexins as drug targets

Pharmacological agents have been cleverly implemented in studies to probe for the physiologic relevance of gap junctions and hemichannels in both health and disease. Channel modulators have proven particularly useful as instruments to delineate the cellular consequences of deficient or altered junctional communication in genetic disorders. Mutations of 6 different connexin isoforms have been linked to autosomal dominant hereditary disorders of epidermal cornification, including Vohwinkel syndrome (VS, Cx26), Bart–Pumphrey syndrome (BPS, Cx26), hystrix-like ichthyosis with deafness (HID, Cx26) syndrome, keratitis-ichthyosis-deafness (KID, Cx26) syndrome, erythrokeratoderma variabilis (EKV, Cx30.3/Cx31/Cx31.1), hidrotic ectodermal dysplasia (HED, Cx30), and oculodentaldigital dysplasia (ODDD, Cx43) [2,19]. Interestingly, recent reports indicate that Cx43-related pathology additionally includes erythrokeratodermia variabilis et progressiva (EKVP) [20,21] and

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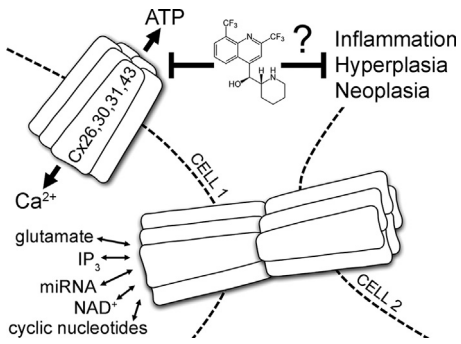

Fig. 1. Gap junctions allow for direct intercellular transfer of ions, small molecules, and second messengers including Ca^{2+}, ATP, cAMP, NAD^+, IP_3, glutamate, and prostaglandins. Connexin hemichannels display controlled exchange of some of these factors with the extracellular space. Mutations that precipitate inflammatory skin diseases show dysregulated Cx26, 30, 31, 43 hemichannels as evidenced by leakage of cytoplasmic ATP and excessive influx of calcium. Small molecule inhibitors capable of suppressing aberrant hemichannel activity, such as mefloquine (pictured), may placate hyperkeratoses in keratitis-ichthyosis-deafness syndrome (Cx26), hidrotic ectodermal dysplasia (Cx30), erythrokeratoderma variabilis (Cx31), and keratoderma-hypotrichosis-leukonychia totalis syndrome (Cx43).

keratoderma-hypotrichosis-leukonychia totalis syndrome (KHLS) [22], which share some clinical features with syndromic Cx26 diseases. ODDD manifests with neuropathies, craniofacial and digital anomalies and, occasionally, skin abnormalities such as palmoplantar keratoderma [23]. EKVP results in hyperkeratosis and transient figurate patches of erythema and KHLS is a subtype of palmoplantar keratoderma-congenital alopecia syndrome characterized by profound hyperkeratosis, congenital alopecia, and leukonychia. Generally stated, connexin mutations that cause human disease have been shown to mechanistically proceed through *either* loss of gap junction and/or hemichannel functionality (i.e. truncation/misfolding/defective oligomerization/defective trafficking) *or* by dysregulation of controlled channel activity and consequent acquisition of novel pathogenic behaviors [24–26]. Investigators of connexin-mediated cutaneous disease have worked industriously to functionally characterize greater than 30 distinct mutations with numerous reports now suggesting the latter, gain-of-function, scenario to be a common occurrence in the skin [19,27]. For these mutations, inhibitor strategies may hold therapeutic value in addition to serving as research tools to pin down errors in channel gating and permselectivity (Fig. 1). This concept is underscored by the topical accessibility of epidermal connexins and the fact that mouse models of Vohwinkel syndrome [28], EKV [29], ODDD [30], HED [31], and KID syndrome [32,33] already exist.

Specialized connexin-inhibitors have proven nontrivial to come by. This is in part due to the challenges of adapting high-throughput drug screens to directly incorporate a readout on membrane biophysics. In addition, the ubiquitous occurrence of connexin-family proteins necessitates inhibitory molecules possessing strict specificity and selectivity properties. Moreover, connexin hemichannels and gap-junctions are increasingly appreciated to operate in independent physiologic niches [6,7], implying a theoretical requirement for inhibitors to be capable of discriminating between the two channel conformations. Indeed, these frustrations have lead to the pursuit of antisense oligonucleotide [34] and siRNA [35,36] approaches as well as the development of the synthetic connexin-mimetic peptides [37–39]. Herein, we provide a perspective on the value of a continued search for pharmacological inhibitors of connexin hemichannels and GJs from among a pool of small molecules recognized to modulate traffic across the plasma membrane and, ideally, also boasting previously verified safety/tolerability profiles. Keratitis-ichthyosis-deafness syndrome will be discussed as an archetypal connexin-driven ectodermal dysplasia for which targeted therapeutic options are conspicuously absent. Within the framework of KID syndrome, our commentary will briefly explore the broader implications of connexin pathology relating to inflammation, wound healing, neoplasia, and innate immunity.

3. Molecular genetics of KID syndrome

KID syndrome is a rare multisystem genodermatosis caused by dominant mutations of *GJB2*, the gene that encodes Cx26 [40]. The disorder clinically manifests with coincident ichthyosiform dermatitis, vascularizing keratitis, and sensorineural hearing loss [41]. The incidence of patient cases is fewer than 1 in 100,000 live births, among which there seems to be substantial 'phenotypic' heterogeneity [42]. KID syndrome is also appropriately classified among the various forms of syndromic deafness, given the well-established importance of Cx26 in vestibulocochlear organ physiology. In fact, loss-of-function Cx26 mutation is the commonest cause of autosomal recessive prelingual nonsyndromic deafness in the world with greater than 225 sequence variations documented [43]. It is worth highlighting here that the scores of deaf individuals lacking viable Cx26 do not suffer notable skin abnormalities or defective wound healing. By contrast, Cx26 mutations presently appreciated to incite skin disease operate by engendering a gain or aberrance in protein activity and are vanishingly few in comparison [44].

10 distinct germline missense substitutions have been identified in the Cx26 sequence following a clinical diagnosis of KID syndrome [45]. KID mutations are concentrated in the amino-terminus, first transmembrane domain, and first extracellular loop of Cx26 with few exceptions. Electrophysiological functional analyses of several mutant channel forms via cell-based expression assays have suggested that high conductance hemichannels may represent a unifying pathomechanism [46]. Specifically, at least 7 of the Cx26 mutations linked to KID syndrome permit markedly larger hemichannel fluxes than the wild-type homomeric channel under the same experimental conditions [47–55]. Moreover, this phenomenon has been shown to exist without regard to the functional status of the corresponding homomeric–homotypic gap junctions. This is demonstrated clearly in the case of the most frequently detected KID mutation, Cx26-D50N, which forms highly active hemichannels but precludes gap junctional coupling altogether [50,54]. Recent work has elucidated transdominant effects of KID mutations exerted on co-localizing connexins to yield hyperactive Cx26/Cx43 heteromeric hemichannels. Notably, this was shown for the Cx26-S17F mutant that fails to form functioning homomeric Cx26 hemichannels [48]. Though it remains a theory, constitutively active hemichannels in KID syndrome are thought to harm cell viability and tissue integrity by allowing leakage of cytoplasmic contents (i.e. ATP) as well as excessive entry of electrolytes. In particular, corruption of the transepidermal extracellular calcium gradient unhinges signal transduction in differentiating keratinocytes required for successful cornification and turnover [56–60].

4. KID syndrome clinicopathology & current therapies

Patients harboring KID-inducing Cx26 mutations suffer significant morbidity associated with their cutaneous and extracutaneous disease and may encounter life threatening infectious and neoplastic sequelae of the former. Clinical presentation appears to vary in accordance with the specific causative mutation, though genotype-phenotype correlations are weak due to the paucity of patient cases. Two mutations, Cx26-G45E and Cx26-A88V, have been unequivocally linked with a lethal form of the disease [61–66].