Connexin 26

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Connexin 26, one of the smallest connexins, is expressed in diverse epithelial tissue and mutations in this protein are associated with hearing loss, skin and eye conditions of differing severity.

epithelial tissue connexin gap junction

1. Introduction

Epithelial tissues line the outer surface of organs and the inner surface of cavities such as the digestive tract and secretory glands, where they project into the lumen connecting with the external environment. Thus, the epithelium separates tissue compartments forming barriers, regulates molecule exchange between those compartments and protects from biological, physical and chemical aggressions [1]. The integrity of the epithelium is maintained by intercellular junctional complexes composed of tight junctions (TJs), adherens junctions (AJs), and desmosomes [2][3]. These junctions aid in the formation of tight seals or barriers between the external environment. Since the epithelium is avascular, it is believed that the delivery and co-ordination of intercellular signals directly between cell layers is conducted via gap junction intercellular communication channels (GJIC) and paracrine signalling pathways [4][5].

Connexins (CXs) are the structural building blocks of gap junctions, four-transmembrane domain spanning proteins with intracellular N- and C-tails (Figure 1). In humans, connexins are encoded by a multigene family containing twenty-one members classified by their molecular weight ranging from 23 to 62 kDa in size (CX23-CX62) [6]. Six connexins oligomerise forming hemichannels or connexons linking the cytoplasm with the extracellular space. Two connexons from adjacent cells connect head-to-head to form an axial channel or gap junction, thereby allowing the interchange of ions and water-soluble molecules with a relative molecular mass up to 1.2 kDa. They play a central role in the control of tissue development, homeostasis and a diverse range of cellular functions [7][8][9][10].

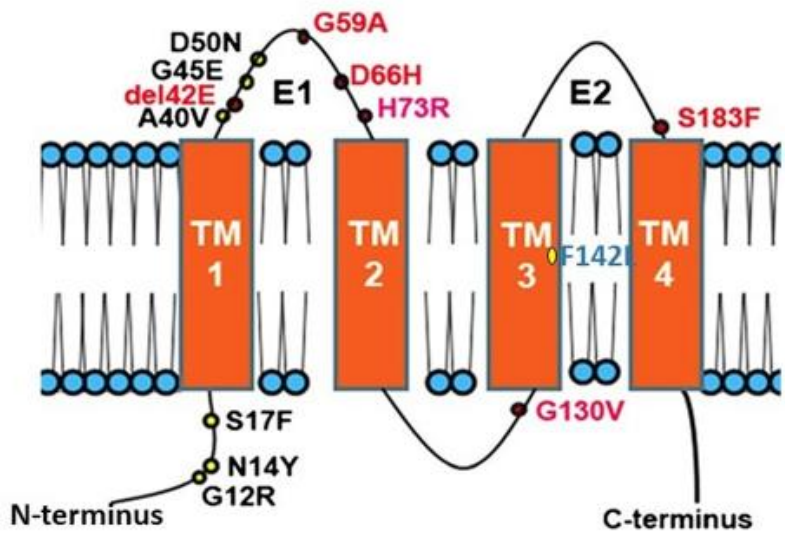

Figure 1. Topology of Connexin 26 and examples of mutations in CX26 associated with epidermal dysplasia and hearing loss. N amino terminus; E1 extracellular loop 1; E2 extracellular loop 2; TM transmembrane domain. Mutations in red indicate 'Class 2′ black, 'Class 3′ and blue 'Class 4′ mutations (see Section 2.2).

The Epithelium and the Tissue Barrier

The structure of epithelial tissue depends on its function. In general, epithelial tissues are classified by their stratification, being simple, transitional or stratified. Simple epithelium composes surface-forming epithelia in contact with the basement membrane (e.g., epithelium of nephric tubules, trachea and secretory glands). The transitional epithelium has some cells in contact with the basement membrane and are surface-forming (e.g., epithelium of urinary bladder). Stratified epithelium is composed of a basal layer, in contact with the basement membrane and the only cells which can divide, and a superficial stratum, where the cells undergo two processes of differentiation: keratinisation non-cornification (e.g., epithelium of oral cavity, oesophagus, vagina) or keratinisation and cornification (e.g., epidermis, nail plate) [1]. The epithelium also plays a central role in body fluid secretions including heat and emotional responses via eccrine, apocrine and sebaceous glandular secretions that are tightly regulated and express a range of connexin proteins [11][12][13]. Skin appendages including the hair follicles and nails also extend from the stratified epidermis where connexins play a significant role in the hair follicle cycle and reviewed elsewhere [13][14]. This review will focus primarily on examples of the role of CX26 and CX43 in epithelial tissue including stratified epithelium such as the epidermis and cornea and simple epithelium including the lining of the respiratory and intestinal tracts. Table 1 summarises the expression profile of CX26 in human tissue.

Table 1. Expression of CX26 in human tissue.

System or organ	Tissue or structure	Cell type	References

Skin	Epidermis		
	Spinous layer	Keratinocyte	[13][15]
	Granular layer		
Appendages	Sebaceous gland		
	Eccrine sweat gland and ducts		
	Hair follicle.		
	Outer root sheet		[13][15]
	Inner root sheet: Henley and Huxlye		
	Hair shaft: Cortex and Medulla		
	Matrix		
Brain	Occipital cortex	Astrocyte (glia)	
	Diencephalon	Leptomeningeal cells	[16]
	Medulla oblongata		
	Caudate nucleus		

Digestive system	Stomach	Epithelial cells	[17]
	Small intestine	Muscularis externa cells	
	Colon		
Endocrine and exocrine glands	Salivary glands	Acinar cells	[18][19][20]
	Pancreas (serous acini)	Beta cells	
	Pituitary (adenohypophysis)		
	Parathyroid	Principal cells	
	Thyroid (follicles)		
	Preputial ducts		
	Lacrimal (serous acini)		
	Parotid (serous acini)		
	Liver	Periportal hepatocytes	
Kidney	Proximal tubule		[21]
Reproductive system	Endometrium (luminal epith.)	Basal glandular cells	[22][23]
	Preimplantation embryo	Blastocysts	

	Placenta	Syncytiotrophoblasts	
	Myometrium	Uterine myocytes	
Endocrine system	Adrenal cortex		[24]
Ear	Spiral limbus	Fibrocytes	[25]
	Spiral ligament		
	Striavascularis		
	Cochlea	Claudius cells	
		Hensen’s cells	
		Inner sulcus cells	
Lung	Alveolar epithelium		[26]

2. Connexins and the Skin

The skin is the human body’s largest organ and is composed of three layers: the hypodermis, dermis and epidermis. The hypodermis and the dermis are connective tissues, while the epidermis is epidermal tissue composed mainly of epithelial cells known as keratinocytes. Keratinocytes are subclassified into four layers: basal (proliferative cells), spinous, granular and corneous (anucleated squamous cells) [27]. The skin provides four different types of barrier: physical, redox, bio-chemical (innate immunity) and the adaptive immune barrier. The physical barrier consists of protein-enriched cells and is mainly located in the stratum corneum and the granular layer, with strong adhesive interactions via tight, adheren and gap junctions. The bio-chemical or antimicrobial barrier consists of lipids, acids, lysozymes and antimicrobial peptides. These two barriers protect from external

aggressions (outside-inside barrier), while also avoiding loss of water and solutes (inside-outside barrier) [28][29] (Figure 2).

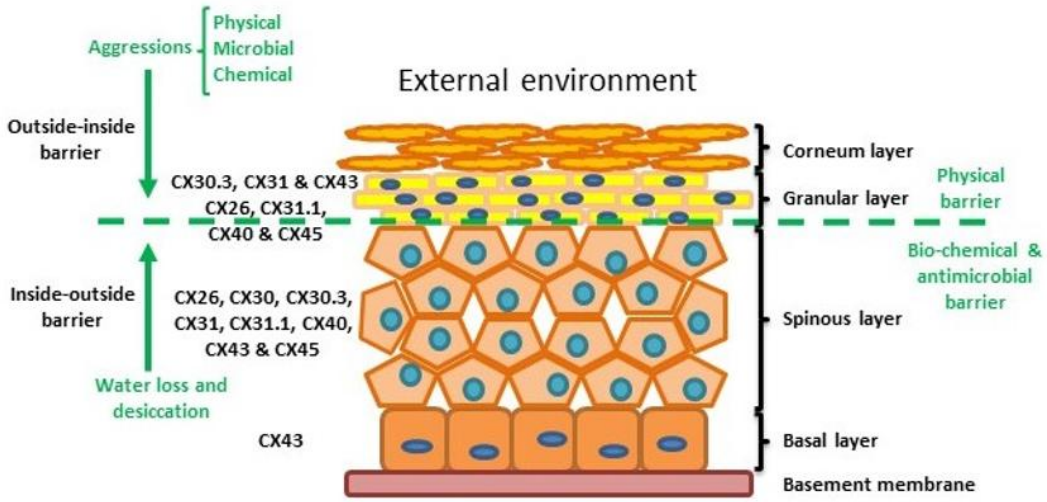

Figure 2. Structure of the epidermis indicating connexin expression profile and barriers.

The epidermis, like all other stratified epithelial tissues, is avascular so gap junctions play an important role in cell-to-cell communication and coordination. Up to 10 different connexins are differentially expressed throughout the epidermis, each presenting a characteristic expression profile dependent on the species, conditions and keratinocyte differentiation status. These Connexin profiles allow the establishment of specific gap junction communication compartments in the different strata. Alteration of connexin activity and compatibility may provoke problems in keratinocyte differentiation [5][30][31]. Several studies have demonstrated that connexins are involved in skin conditions such as chronic non-healing wounds, psoriasis and a variety of genetically related skin syndromes (e.g., [32][33][34]).

Within normal healthy human skin CX30.3, CX31 and CX43, and at lower levels CX26, C31.1, CX40 and CX45 are expressed in the granular layer. In the spinous layer, CX26, CX30, CX30.3, CX31, CX31.1, CX40, CX43 and CX45 are expressed with CX43 the predominant connexin in the basal layer. [5][30][35] (Figure 2). CX26 is expressed at low levels in proliferating keratinocytes in tissue culture and readily expressed in stratified 3D epidermal cultures by immunocytochemistry [36][37][38].

2.1. Connexin 26, Trafficking and Assembly

CX26, a 26 kDa protein consisting of 226 amino acids, is encoded by the *GJβ2* gene, located on Chromosome 13q12.11 in Homo sapiens [39]. The gene is formed by the non-coding exon 1 (160 bp), an intron (3 kb) and exon 2 containing the complete connexin coding region and the subsequent 3′-UTR [40]. The promoter P1 (−128 bp:+2 bp) upstream of exon 1 has a number of transcription regulatory domains including SP1/SP3 and AP1 [39]. CX26 is also responsive to the NFkB transcription factor, which plays a role in inflammation and immunity, as well as in cell proliferation, differentiation and survival [41][42]. Human CX26 has a short C-terminal tail, with only 18 amino acids,

this characteristic is relevant because it affects its interactome and post-translational modification, where CX26 interacts with a variety of proteins including those associated with the tight junction network [20]. Gap Junction assembly depends on the oligomerisation of connexins to form a closed hexameric connexon or hemichannel in the ER-Golgi environs, which are then escorted and inserted into the plasma membrane via the microtubule network, in association with motor proteins such as consortin [7][43][44]. Evidence suggests that CX26, and closely related CX30, can also follow an alternative Golgi-independent trafficking pathway, possibly providing a means for translation on free ribosomes and an ability to be rapidly translated at site-specific plasma membrane locations when required [45][46][47][48][49][50]. Entire gap junction plaques are removed from the plasma membrane by the formation of annular gap junctions [4][51] (Figure 3). In addition, CX26 is unique to the connexin family as it does not contain phosphorylation sites in its C-terminal tail that play a significant role in protein turnover, particularly well characterised for Cx43 [51]. Nevertheless, CX26 presents a variety of putative post-translational modifications, including carbamylation [52], hydroxylation, phosphorylation and methylation, some of which happen at sites of deafness-causing mutations and may be associated with CX26 biogenesis and channel function [53][54]. Furthermore, hemichannels are normally closed during normal conditions; however, human CX26 hemichannels are an exception as they tend to be open under basal conditions. The 3D molecular structure of CX26 suggests this could be because, in contrast to other species, human CX26 presents an asparagine (uncharged) at amino acid position 159 in place of aspartic acid found in other species [55][56].

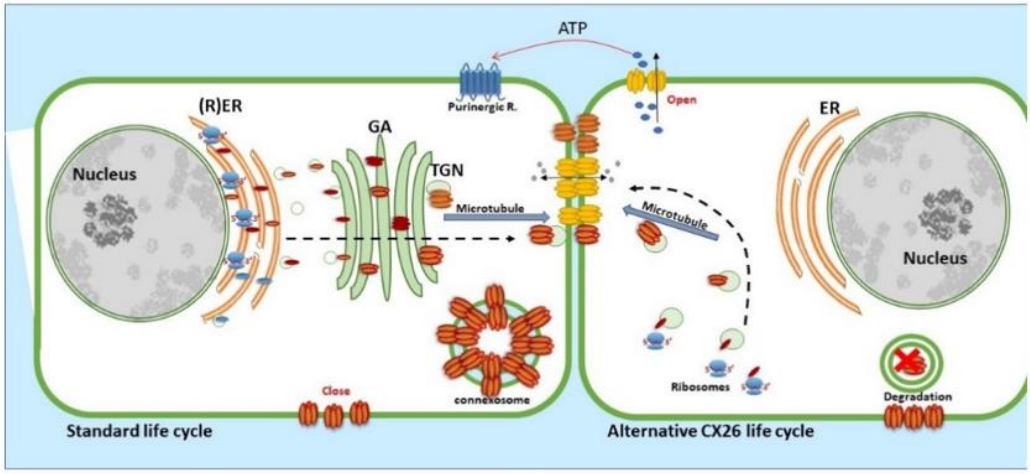

Figure 3. Life cycle of CX26: CX26 can be co-translated and trafficked through the secretory route (standard life cycle). It can also be post-translationally incorporated into ER microsomes and trafficked by Golgi-independent pathway (alternative CX26 life cycle). GJIC mediated communication is indicated between cells and paracrine signalling permitting ATP release indicated via the open hemichannels.

2.2. The Effects of CX26 Mutations

Many diseases have been linked with mutations in connexins, commonly termed connexin channelopathies [57][58][59][60]. CX43 is the most abundant connexin in the human skin; however, mutations and dysregulation of CX26, which is expressed at very low levels in healthy human epidermis, are related to skin disease characterised by abnormal keratinisation and hyperproliferation of the stratum corneum. Mutations in CX26 are among the most

prevalent mutations associated with inherited non syndromic deafness (see Section 4.1) [61][62][63]. In addition to deafness, dominant mutations are also linked with a range of skin conditions of differing severity, suggesting a complex interrelationship between functional changes in connexin genotype and the phenotypic outcome [59][64][65][66] (Figure 1). Other mutations in the beta connexin subgroup including CX31 and CX30 cause similar epidermal dysplasia [59].

Mutations fall into four main classes. Class 1: trafficked to the plasma membrane with non-functional channels; Class 2: non-functional channels and protein trafficking deficiencies; Class 3: mutations associated with 'leaky' hemichannels and inflammatory skin disease; Class 4: trafficking deficiency and cell death, associated with mucositis, inflammatory disease and deafness. Class 1 mutations tend to be linked with non-syndromic deafness and have limited skin pathology and may be related to heterozygous advantage (see Section 4.1). Class 2 mutations align with non-inflammatory skin disorders such as Bart-Pumphrey and Vohwinkel syndrome and deafness. Vohwinkel syndrome (OMIM#124500) is a non-inflammatory disorder caused by CX26 mutations located predominantly on the first portion of EL1 (e.g., D66H). The disease is characterised by keratodermas with constriction bands around the phalanges, which induces autoamputation of the digits [67][68][69]. Class 3, gain of CX26 function mutations, are associated with inflammatory disorders such as Keratitis ichthyosis deafness (KID), Hystrix-like ichthyosis-deafness (HID). KID syndrome (OMIM#148210) is caused by CX26 mutations on the N-terminal tail, the EL1 (e.g., G12R, N14Y, G45E and D50N) [70][71][72]. In addition to hearing loss, the disease is characterised by: hyperkeratosis of the palms and soles, erythrokeratoderma on the extremities and face, follicular hyperkeratosis, photophobia and corneal vascularisation that ultimately leads to blindness. Patients experience severe and chronic bacterial and fungal skin infections and are susceptible to the development of squamous cell carcinoma [73][74]. The molecular mechanisms underlying the condition likely relate to "leaky" hemichannels, with differing sensitivities to pro-inflammatory mediators, and ionic sensitivity including calcium and zinc levels thereby altering channel function [66][75][76][77][78][79][80][81][82]. Several mutations induce lethal phenotypes and are associated with loss of cell viability [83][84][85]. Recently, we proposed a further Class 4 mutation group associated with hyperkeratosis, mucositis and deafness, where cell model studies revealed cell death and a collapse of the microtubule network, but limited connexin channel function (e.g.,F142L, CX31G45E) [86][87].

Accumulating evidence suggests that, in addition to changes in CX26 trafficking and channel behavior, a key pathological trigger in the diverse CX26 mutations is alteration in connexin oligomerization compatibility. Normally, oligomerization is connexin subtype-specific with 'alpha' and 'beta' subgroups being incompatible. As such, CX26 and CX43, critical connexins in epithelial tissue are unable to form heterotypic structures [88][89]. Recent studies report changes in CX26 mutation oligomerization compatibility, allowing aberrant interactions with CX43 with exacerbated hemichannel activity but non-functional gap junction channels [78][86][90], with each mutation uniquely altering the 3D structure in terms of charge, pore size, hydrophobicity, etc. It is also conceivable that altered CX43:CX26 heteromeric channels influence unique metabolic exchange and influence asymmetric cell division required for the stratification of the epidermis, thereby contributing to the hyperproliferative status of the skin [91]. Further characterisation and understanding of the impact of such aberrant connexin signalling is required to enhance understanding of these complex conditions. Interestingly, a recent report by Laird and colleagues suggest that hearing loss caused by CX26 mutations does not depend on any interaction with CX43 [92].

2.3. The Effects of CX26 Dysregulation

Up-regulation of CX26 is a characteristic of hyperproliferative epidermis in physiological conditions, such as vaginal and buccal epithelium, which shows a high proliferation rate, and pathological conditions, such as psoriatic epidermis, chronic non-healing wounds epidermis and viral warts [93][94][95][96]. CX26 expression is also induced during wound healing and skin hyperplasia stimulated by tumor promoters [40]. Transgenic mice over-expressing CX26 in the suprabasal layer developed a hyperproliferative phenotype, providing models for several epidermal human CX26 diseases, such as psoriasis [97].

2.3.1. Connexins and Wound Repair

Connexins are closely involved in normal wound repair and show dynamic changes in expression after wounding. After 6 h of injury, CX43 is down-regulated in keratinocytes and fibroblast at the wound edge to allow cell migration followed by recovery of CX43 levels [95]. In contrast, CX26 and CX30 are upregulated in keratinocytes until the wound is closed [96][98][99] in the granular cell layer near the wound margins and in the basal cell layer at some distance from the wound [95]. Thus, connexins play a pivotal role in a variety of aspects of the acute wound healing process and each step is associated with a different connexin environment [51].

At the edge of chronic wounds, epidermal CX43, CX26 and CX30, and dermal CX43 are strikingly up-regulated around wound margins and at some distance from the chronic wound [95]. The up-regulation of CX43 may disrupt fibroblast migration to the wound bed and as a result failure of granulation tissue formation occurs [100][101][102][103][104]. The up-regulation of CX26 may contribute to the inflammatory and hyperproliferative status of the wound [95][105]. The overexpression of Cx26 in mouse skin (under the control of the involucrin promoter) kept wounded epidermis in a hyperproliferative state, blocked the transition to remodelling, and led to an infiltration of immune cells. This overexpression also induced ATP release from keratinocytes, which delayed epidermal barrier recovery and promoted an inflammatory response in resident immune cells [97].

2.3.2. Connexins and Psoriasis

Psoriasis is a chronic hyperproliferating skin disorder that manifests sporadic skin lesions characterised by loss of the granular layer and incomplete keratinocyte differentiation associated with a thickened cornified layer. Overexpression of CX26 in psoriatic lesions was originally reported by Labarthe and Lucke in the late 1990s [93][94]. Subsequent studies in mouse models overexpressing Cx26 in the skin showed mildly acanthotic and hyperkeratotic skin, with a thicker and more compact cornified layer. Areas with frictional trauma, such as axillary areas, presented with scaling and desquamation and hyperkeratotic plaques developed [97]. Other studies revealed that tape stripping of normal human epidermis induced CX26 expression and hyperproliferation [106]. More recently, publication of the psoriatic transcriptome permitted in-depth RNAseq analysis [107] and many of the upregulated genes are related with cell-to-cell adhesion complexes. *GJB2*, encoding CX26 was the 98th most up-regulated gene detected and its overexpression is used as a marker of genetic predisposition in psoriasis [108][109]. A variety

of other transcriptomic studies confirm this overall increase in CX26 expression in psoriatic tissue yet no changes in CX43 gene expression are reported [110][111].

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