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Review

Cardiac to cancer: Connecting connexins to clinical opportunity

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ABSTRACT

Gap junctions and their connexin components are indispensable in mediating the cellular coordination required for tissue and organ homeostasis. The critical nature of their existence mandates a connection to disease while at the same time offering therapeutic potential. Therapeutic intervention may be offered through the pharmacological and molecular disruption of the pathways involved in connexin biosynthesis, gap junction assembly, stabilization, or degradation. Chemical inhibitors aimed at closing connexin channels, peptide mimetics corresponding to short connexin sequences, and gene therapy approaches have been incredibly useful molecular tools in deciphering the complexities associated with connexin biology. Recently, therapeutic potential in targeting connexins has evolved from basic research in cell-based models to clinical opportunity in the form of human trials. Clinical promise is particularly evident with regards to targeting connexin43 in the context of wound healing. The following review is aimed at highlighting novel advances where the pharmacological manipulation of connexin biology has proven beneficial in animals or humans.

1. Introduction

Evolution of the multi-celled organism demanded the coordinated integration of cell types and the intricate orchestration of the processes involved in cell coordination, synchronization, growth, differentiation, and programmed cell death. The formation of channels that directly link the cytoplasm of adjacent cells (gap junctions) or permit cell-extracellular communication (hemichannels), encoded by a set of highly evolutionarily conserved genes (connexins), appears ingeniously simple when considering the complex biology of the higher functioning organism. Scientific literature reveals the essential roles of these processes in cell and tissue homeostasis and angiogenesis, and by default, in a slew of pathological processes [1–3]. Research aimed at disclosing the dynamic, cell and disease specific roles involved in connexin transcription, translation, turnover, trafficking, and dysfunction barely scrape the surface in terms of deciphering the complexities associated with translating connexin biology to therapeutic opportunity. In reviewing the clinical promise associated with connexins, it seems best to keep in mind a quote by HL Mencken: "For every complex human problem, there's a solution that is simple, neat, and wrong. . .".

The canonical connexin is diagrammed as a tetraspan transmembrane protein with two extracellular loops, a cytoplasmic loop, and a cytoplasmic amino and carboxyl-terminus (N- and C-terminus). Six connexins oligomerize to form cored connexon transmembrane channels that couple with connexons on neighboring cells to form intercellular channels, which in turn aggregate to form gap junctions (GJs) [4,5]. Hemichannel recruitment to the preexisting GJ plaque is a critical determinant in the operational area, size, and stability of GJs [6]. The formation and dissolution of hemichannels and GJs is dynamic and regulates both junctional and non-junctional intercellular communication. It has been over half a century that the physiological relevance of these processes was first appreciated in studies that examined the electrical transmission of signals at the giant motor synapses in the crayfish [7]. We now know that intercellular communication is key in numerous processes critical for biological homeostasis including, but certainly not limited to, the rapid transmission of action potentials in heart and neuronal tissues [8–10], and the diffusion of metabolites, nucleotides, nutrients, and second messengers with roles in apoptosis, gene expression, inflammatory responses and cellular growth [11–28]. An additional level of complexity is bestowed in situations where heteromeric assembly of different connexin

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proteins in a single channel may result in unique and specific biophysical properties rendering preference with regard to passaging molecules [29,30].

Therapeutic intervention may be offered through the pharmacological and molecular disruption of the pathways involved in connexin biosynthesis, GJ assembly, stabilization, or degradation [31]. Chemical inhibitors aimed at closing connexin channels, peptide mimetics corresponding to short connexin sequences, and gene therapy approaches have been incredibly useful molecular tools in deciphering the complexities associated with connexin biology (Fig. 1). The therapeutic potential of these tools has only recently been evaluated in clinical trials. The following review will focus on discussing the translational relevance and clinical potential underlying the pharmacological manipulation of connexin biology.

2. Lessons learned from wound healing

The role of connexins in maintaining tissue homeostasis offers important lessons in both the scientific and commercial exploration of connexin–based therapeutics. A wide range of inherited human disorders stem from mutations in connexin genes (Table 1; reviewed in detail in [32,33]), highlighting the indispensable role of GJs in normal tissue function. Of the twenty-one identified connexin isoforms in the sequenced human genome, the epidermis expresses at least 10, including Cx26, Cx30, Cx30.3, Cx31.1, and Cx43, each correlated with a uniquely dynamic spatial and temporal expression pattern and functional role [34–37]. Given that the coordination and progression of wound healing and re-epithelialization is tied to a complex series of events that occur between various cell types, extracellular matrix components and signaling molecules, the critical role of connexins in regulating the metabolic coupling between cells and transfer of cell signaling molecules during the cutaneous injury response is palpable. These include roles in leukocyte diapedesis, re-epithelialization, wound contraction, fibroblast function, and collagen deposition and synthesis [38,39].

2.1. Connexin43 and therapeutic opportunity

The translational bridge connecting connexins to wound healing therapeutics was first made readily apparent with Cx43, the most highly expressed and widely studied connexin in human skin [39–42]. Experiments in murine wound models describe the transient expression pattern of Cx43 and GJ intercellular communication at the wound periphery following dermal injury. Following injury, Cx43 in the wound edge slowly downregulates over approximately 48 h, during which time keratinocytes adopt a migratory phenotype [37,39–42]. These same studies show that Cx43 downregulation is correlated with increased levels of TGF-β mRNA and collagen α-1, and decreased levels of chemokine ligand-2, tumor necrosis factor alpha (TNFα), infiltrating neutrophils and macrophages at the wound site, as well as the promotion of angiogenesis, fibroblast migration, and keratinocyte proliferation. Conversely, Cx43 expression is elevated in the blood vessels proximal to the wound site, with another rise several days post-wounding during granulation formation. The post-translational phosphorylation of serines (S364, S365, S325, S328, S330, S368, S279, S282, S262) in the C-terminal domain of Cx43 during these processes contributes to conformational changes and the formation or disruption of GJs and GJ intercellular communication depending on the site phosphorylated [43–46]. Additionally, Cx43 hemichannels release molecules such as glutamate, ATP, NAD+, prostaglandin E2, and glutathione, providing a paracrine route for intercellular

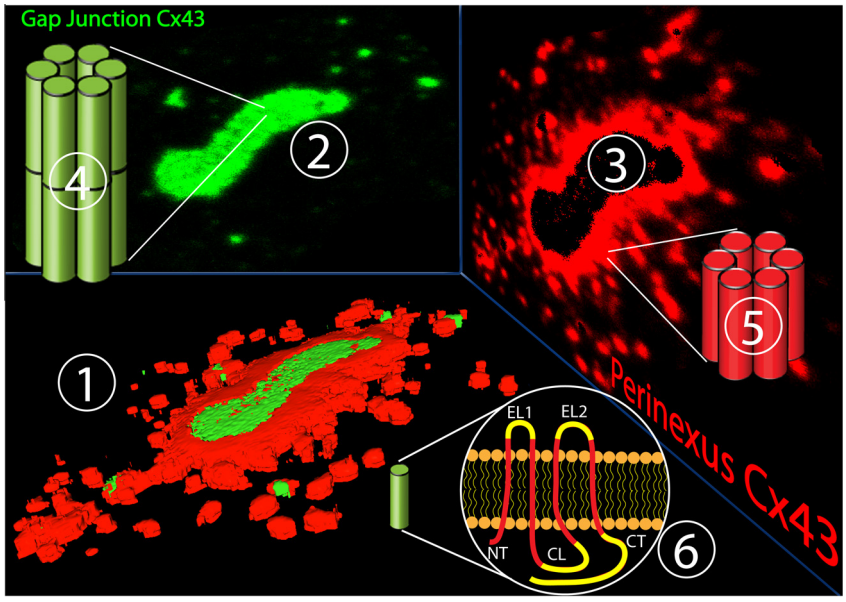

Fig. 1. Connexin-based therapeutics target both gap junctions and hemichannels through multiple domains on the Cx43 subunit. ① A three dimensional reconstruction of the Cx43 junctional complex generated from confocal optical sections of standard Cx43 immunofluorescence ②, and Cx43-Cx43 Duolink (Olink Bioscience, Uppsala, Sweden) ③. ② Standard Cx43 immunofluorescence highlights gap junctions, which are composed of aggregated intercellular channels ④. Drug targets may influence intercellular coupling by improving conduction in the heart or spatial buffering in the brain, for example. ③ Duolink imaging of Cx43 enables imaging of the perinexus, which is composed of diffuse hemichannels ⑤. Hemichannels are generally reduced in number or closed by connexin-based therapeutics as their opening in pathological systems participates in cell death signaling, and purinergic signaling of inflammatory cells. ⑥ Both intercellular channels ④ and hemichannels ⑤ are oligomers of connexin subunits. Each subunit has a cytoplasmic N-terminus (NT), 4 transmembrane domains, 2 extracellular loops (EL1 and EL2), a cytoplasmic loop (CL), and a cytoplasmic C-terminus (CT). Yellow areas indicate regions of the primary sequence that have been used to (or could be used to) generate connexin-based therapeutics. Specifically, Gap26 and Gap27 are mimetic peptides of the first and second extracellular loops respectively, and Gap19 is derived from the cytoplasmic loop [158]. ACT-1 mimetic peptide [25,64–66,96,99], the endogenous peptide discovered by Smyth and Shaw [177], and PEP-1 and PEP-2 mimetic peptides [173] are all found on the C-terminus. Furthermore, the C-terminus contains numerous phosphorylation sites that act as molecular switches for Cx43 dysregulation in injury.

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