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Review

The biochemistry and function of pannexin channels ☆

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ABSTRACT

Three family members compose the pannexin family of channel-forming glycoproteins (Panx1, Panx2 and Panx3). Their primary function is defined by their capacity to form single-membrane channels that are regulated by post-translational modifications, channel intermixing, and sub-cellular expression profiles. Panx1 is ubiquitously expressed in many mammalian tissues, while Panx2 and Panx3 appear to be more restricted in their expression. Paracrine functions of Panx1 as an ATP release channel have been extensively studied and this channel plays a key role, among others, in the release of "find-me" signals for apoptotic cell clearance. In addition Panx1 has been linked to propagation of calcium waves, regulation of vascular tone, mucociliary lung clearance, taste-bud function and has been shown to act like a tumor suppressor in gliomas. Panx1 channel opening can also be detrimental, contributing to cell death and seizures under ischemic or epileptic conditions and even facilitating HIV-1 viral infection. Panx2 is involved in differentiation of neurons while Panx3 plays a role in the differentiation of chondrocytes, osteoblasts and the maturation and transport of sperm. Using the available Panx1 knockout mouse models it has now become possible to explore some of its physiological functions. However, given the potential for one pannexin to compensate for another it seems imperative to generate single and double knockout mouse models involving all three pannexins and evaluate their interplay in normal differentiation and development as well as in malignant transformation and disease. This article is part of a Special Issue entitled: The communicating junctions, roles and dysfunctions.

Contents

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1. Introduction

The pannexin family of channel proteins consists of three members, namely Panx1, Panx2 and Panx3. This family of integral membrane proteins was first identified in the mammalian genome in the year 2000 by Panchin and colleagues [1]. Pannexins were discovered due to their limited sequence homology (25–33% identity) to the invertebrate gap junction proteins, innexins (Inxs) [1,2], and were initially proposed to share functional features with the vertebrate gap junction proteins, connexins (Cxs). While no sequence homology exist between Panxs and Cxs all three families of proteins are predicted to exhibit similar topology with four α-helical transmembrane (TM) domains, two extracellular loops (EL), and one intracellular loop (IL), with their amino (NT) and carboxyl (CT) termini exposed to the cytoplasm [1–3] (Fig. 1).

Among the pannexin family members, the NT region is the most highly conserved domain while the highest sequence variability is found in the CT domain [2]. Panx1 and Panx3 are more homologous to each other than to Panx2 [4]. Panx2 exhibits a much larger CT domain that is speculated to convey unique functions to Panx2 regulation, targeting or macromolecular interactions [2]. Additionally, Inxs and Panxs have two cysteine residues in each of their EL domains (Fig. 1) with the exception of *Drosophila* Inx4 that has 3 cysteines in each loop [2,5]. On the other hand, all members of the Cx family (except Cx23 [6]) possess 3 cysteine residues within each EL which form intramolecular disulfide bonds [7].

Analogous to the connexin family of gap junction proteins, initial characterization of Panx1 oligomerization revealed that 6 subunits were required to form a channel [8]. However, similar analysis of Panx2 using a C-terminal truncation mutant revealed that this pannexin most likely assembles into heptamers or octomers [9]. The field is still awaiting an oligomer analysis of Panx3 but given its close polypeptide sequence relationship to Panx1 it is predicted to form hexamers. Recently the substituted cysteine accessibility method was used to identify the pore lining residues of Panx1 channels. This approach revealed that the outer pore structure of Panx1 is lined by portions of the first TM and first EL regions whereas the inner pore lining is contributed by the CT domain and not the NT as it is for connexins [10].

Pannexin oligomers are often called pannexons [8] following the nomenclature established for connexins where oligomers are termed connexons [11,12]. While 'connexons' are also referred to as 'hemichannels' (i.e. a structure constituting half of a cell–cell channel); the term 'pannexons' refers to single-membrane channels. In fact, many authors strongly discourage calling pannexons, hemichannels, as this infers that they are destined to proceed to assemble into an intercellular channel which is not well supported by many recent publications as reviewed by Sosinsky and colleagues [13].

We and others have reported that Panx1 is projected to have a very long half-life [4,14]. The cell surface population of Panx1 remained relatively unchanged when protein secretion was blocked with brefeldin A (BFA) suggesting that this pannexin was not subject to rapid displacement and renewal [4]. In fact, it took up to 32 h of BFA treatment before some clearing of Panx1 from the cell surface was evident with a concomitant increase in newly synthesized Panx1 localized to an ER-like pattern, while clearing of Cx43 from the cell surface was evident within 3 h of BFA treatment (Fig. 2A). This eventual clearing of Panx1 from the cell surface was further correlated with a reduction in the highly glycosylated Gly2 species and an increase in the high-mannose Gly1 species of Panx1 after 20 h of BFA treatment (Fig. 2B). Further evidence that Panx1 is a long-lived molecule was provided as there was a negligible change in the Panx1 species banding pattern upon BFA washout (Fig. 2B).

2. Pannexin expression and genomics

In addition to the human and mouse genomes, the expression of all three pannexin members has now been identified in at least 5 more species including: *Rattus norvegicus* (rat), *Canis familaris* (dog), *Bos taurus* (cow), *Danio rerio* (zebrafish) and *Tetraodon nogrovirdis* (puffer fish) [2]. Despite the vast inter-species distribution, pannexins are most well characterized in human and murine tissues. Overall, the human PANX1 (accession number NP_056183, 426 amino acids, 47.6 kDa), PANX2 (accession number NP_443071, 677 amino acids, 74.4 kDa) and PANX3 (accession number NP_443191, 392 amino acids, 44.7 kDa) have conserved sequence homologies of up to 94% with murine pannexins [15].

2.1. Pannexin1 (Panx1)

Various levels of Panx1 are ubiquitously expressed in human tissues such as the brain, heart, skeletal muscle, skin, testis, ovary, placenta, thymus, prostate, lung, liver, small intestine, pancreas, spleen, colon, blood endothelium and erythrocytes, as it was initially reported by Northern blot analysis [3]. Specifically in the central nervous system Panx1 transcripts were detected in the cerebellum, cortex, lens (fiber cells), retina (retinal ganglion, amacrine and horizontal cells), pyramidal cells, interneurons of the neocortex and hippocampus, amygdala, substantia nigra, olfactory bulb, neurons and glial cells [3,16–26]. At the protein level, a tissue survey using custom-designed affinity purified anti-Panx1 antibodies revealed a robust expression of Panx1 in the brain, with variable levels of Panx1 in the lung, kidney, spleen, heart ventricle, skin and sources of cartilage from the ear and tail of 3-week old mice [4]. More recently, Panx1 protein expression was also detected in the rodent cochlea, specifically in supporting cells of the organ of Corti, spiral limbus, cochlear lateral wall, strial blood vessels [27] and in vascular smooth muscle cells of thoracodorsal arteries [28]. In addition, Panx1 has also been reported in the basal compartments of the seminiferous epithelium and epididymis, and in the apical region of efferent ducts of adult rats [29].

2.2. Pannexin2 (Panx2)

In comparison to Panx1, Panx2 mRNA appears more restricted to several areas of the human adult brain including: cerebellum, cerebral cortex, medulla, occipital pole frontal lobe, temporal lobe and putamen [3]. While Northern blot analysis revealed high levels of Panx2 transcript in the rodent brain, spinal cord and eyes, other tissues such as thyroid, kidney and liver revealed low levels of Panx2 transcripts [16–18]. Interestingly, in situ hybridization revealed co-expression of

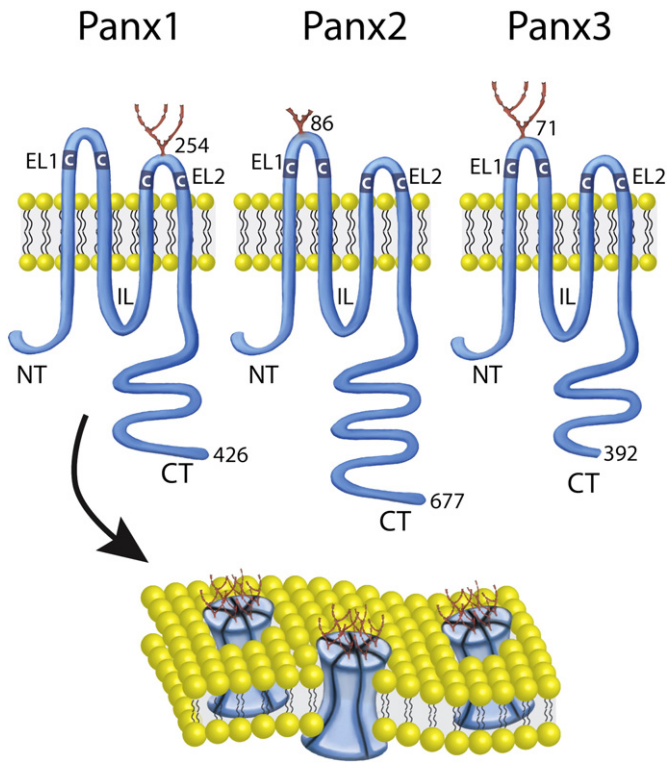

Fig. 1. Schematic representation of the three pannexin family members. Panx1 (426), Panx2 (677) and Panx3 (392) of varying amino acid lengths are all tetra-spanning integral membrane proteins with N-glycosylation sites at amino-acid 254 (Panx1), 86 (Panx2, predicted), and 71 (Panx3). Panx1 and Panx3 are glycosylated to both a high-mannose form and a complex glycoprotein, while Panx2 has only been reported as a high-mannose glycoprotein or a palmitoylated isoform. All pannexins have two cysteine residues in each extracellular loop (C). Panx1 has been shown to oligomerize into a hexamer to form single-membrane channels at the cell surface of many mammalian cells. EL1, EL2 (extracellular loops 1 and 2), IL (intracellular loop), CT (carboxy-terminus) and NT (amino-terminus).

Panx2 with Panx1 in various regions of the adult rat brain, such as hippocampus, olfactory bulb, pyramidal cells, dentate gyrus, Purkinje cells of the cerebellum, pyramidal cells and interneurons of the hippocampus and neocortex [23]. Unlike Panx1, low levels of Panx2 transcripts were detected in the prenatal brain while transcript levels substantially increased in postnatal brains [23]. Recently, Panx2 protein expression was identified in the basal cells of the stria vascularis and spiral ganglion neurons of the rat cochlear system [27], as well as in neural stem and progenitor cells of the mouse hippocampus [30].

2.3. Pannexin3 (Panx3)

Based on expressed sequence tags, Panx3 was found in osteoblasts, synovial fibroblasts, whole joints of mouse paws and cartilage from the inner ear [3]. In addition, low levels of Panx3 transcripts were detected in human hippocampus extracts [3]. Panx3 protein was reported in murine cochlear bone [27] while in situ hybridization of embryonic day 16.5 mice strongly revealed Panx3 expression in prehypertrophic chondrocytes, perichondrium and osteoblasts [31]. Furthermore, we demonstrated that while Panx3 protein exhibits two forms at ~43 kDa and ~70 kDa in skin, cartilage, and ventricle only the ~70 kDa species was detected in lung, liver, kidney, thymus and spleen of 3 week old mice [4]. It is however yet to be determined if the occurrence of the ~70 kDa immunoreactive species is due to Panx3 dimerization. Panx3 has also been shown to be endogenously expressed in osteoblasts, both in primary culture from murine calvaria and in reference cell lines [32–34]. Recently, Panx3 was also reported to be present in Leydig cells, the epididymis and efferent ducts of adult rats [29]. Overall, endogenous Panx1, Panx2 and Panx3 expression has been identified in a number of cultured reference cell lines as summarized in Table 1.

3. Biochemistry of pannexins

3.1. Post-translational modifications of pannexins

Panx1 and Panx3 protein sequence analysis followed by site-directed mutagenesis revealed that N-linked glycosylation occurs at asparagine 254 in the second extracellular loop of Panx1, and at asparagine 71 in the first extracellular loop of Panx3 [4] (Fig. 1). N-glycosidase F and endoglycosidase H (Endo H) enzymatic digestion studies confirmed the glycosylation status of Panx1 and Panx3 [4,8,15]. Three distinct species of Panx1 and Panx3 were revealed: the non-glycosylated core species — Gly0, the predominant endoplasmic reticulum (ER) resident high mannose species — Gly1 and the complex glycosylated species — Gly2. In addition, Panx2 also encodes a potential N-linked glycosylation consensus site in its first EL domain at asparagine 86 but glycosylation of this residue has yet to be confirmed by a site-directed mutagenesis study [15] (Fig. 1). However, Endo H digestion supports the premise that Panx2 is glycosylated to a high mannose species [15]. Glycosylation of Panx1 and Panx3 was found to be important for proper trafficking of these pannexins to the cell surface. This conclusion is based on reduced [4] or absent [8] cell surface localization of the glycosylation-deficient mutants $Panx1^{N254Q}$ or $Panx3^{N71Q}$. Nevertheless, the population of N-glycosylation-deficient Panx1 and Panx3 mutants that do reach the cell surface are functional in their ability to uptake dye [15] thus indicating that these pannexins can form functional channels even in the absence of glycosylation.

Interestingly, in another study Endo H treatment did not alter the mobility of Panx2, rather the ~80 kD species observed in neural progenitor cells was found to be sensitive to hydroxylamine suggesting Panx2 was palmitoylated [30]. Although Panx2 does not contain a clear palmitoylation consensus sequence, the in silico analysis revealed a potential site (SYLCTYY) at residue 246 [30]. Further site-directed mutagenesis studies will be necessary to confirm the palmitoylated status of Panx2.

3.2. Pannexin intermixing

Panx family members share significant sequence homology and have been reported by us and others to interact and form potential intermixed channels [15,35]. This intermixing is more evident between Panx1 and Panx2 and is governed by their level of N-glycosylation, as only the core and high-mannose species of Panx1 co-immunoprecipitate with Panx2. Panx1 and Panx3 interact to a lesser extent with no observed change to the channel function while channels are attenuated when Panx1 and Panx2 intermix [15,35]. However, since Panx1 forms hexamers while Panx2 is proposed to form octomers it is difficult to speculate what type of oligomer occurs when Panx1 and Panx2 intermix. Ambrosi et al. [9] recently attempted to address this question by purifying Panx1/Panx2 heteromeric channels. However, the isolated channels were found to be unstable. These authors also observed a reduction in current when Panx1 and Panx2 were co-expressed in *Xenopus* oocytes indicating a mutual inhibition between these pannexins. It was further postulated that due to the formation of unstable heteromeric channels it is possible that they do not co-oligomerize in vivo. Given the known physical interaction between Panx1 and Panx2 it is possible that this interaction causes them to mis-traffic or mis-fold. [9]. There is no evidence for Panx2 and Panx3 intermixing at the biochemical level [15] and they have not been reported to be co-expressed in the same cell types.

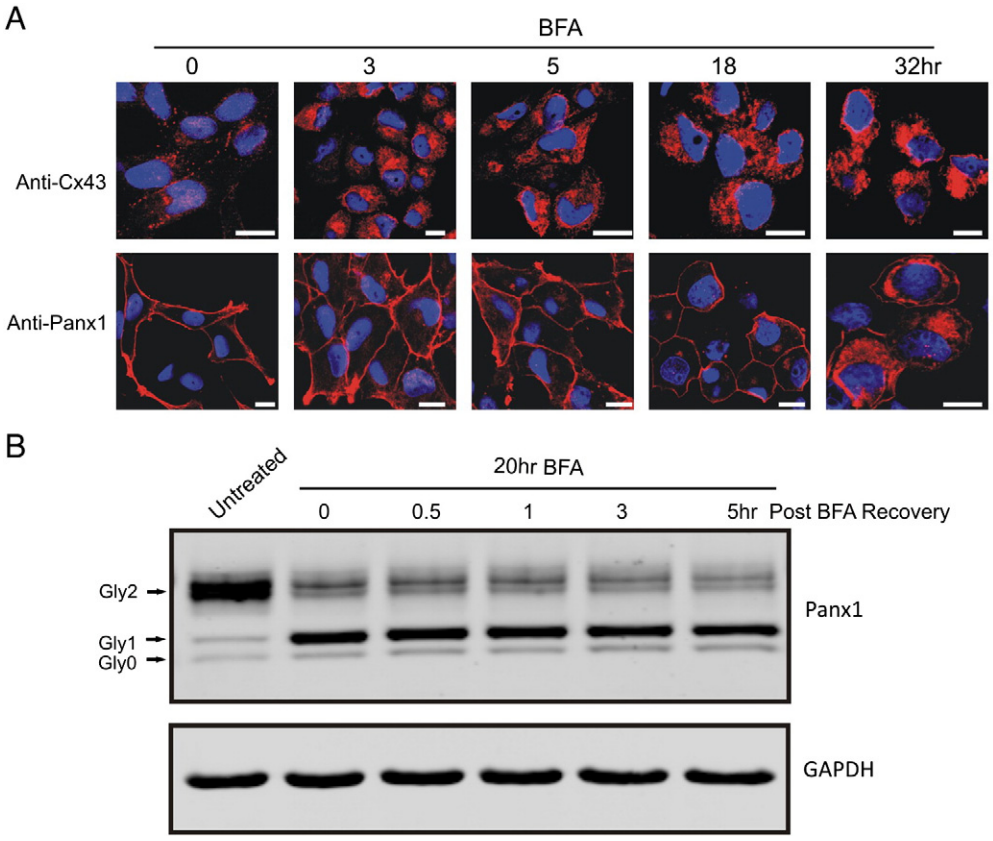

Fig. 2. Panx1 is a long-lived protein compared to Cx43. Long-term brefeldin-A (BFA) treatment of BICR-M1R$_k$ rat mammary tumor cells revealed a clearing of the cell surface population of exogenously expressed Panx1 and the accumulation of an intracellular pool of Panx1 after 32 h of BFA treatment. On the other hand there was a clearing of cell surface Cx43 as early as 3 h post-BFA treatment. Anti-Panx1 (CT-395) and anti-Cx43 antibody labeling are shown in red. Nuclei (blue) are stained with Hoechst 33342. Bars, 10 μm (A). Western blotting for Panx1 revealed an accumulation of the high mannose species of Panx1 (Gly1) with a noticeable reduction in the higher molecular weight glycosylation species (Gly2) after 20 h of BFA treatment. GAPDH was used as a loading control (B). Results shown are representative of three independent experiments.

3.3. Pannexin binding partners

In addition to the protein–protein interactions described among pannexin family members new members are being added to the pannexin interactome. Panx1 was shown to interact with actin and this interaction was demonstrated to be direct and involves the Panx1 C-terminal tail. It has been proposed that Panx1 depends on actin microfilaments for proper trafficking and stability at the plasma membrane [36]. In another study, a bacterial two-hybrid system was used to identify the potassium channel subunit Kvβ3 as a potential interacting partner of Panx1 [37]. This interaction was later confirmed by co-immunoprecipitation experiments in neuroblastoma cells where Kvβ3 was further proposed to be involved in the regulation of Panx1 channel sensitivity to redox potentials [38]. In another study, α1D-adrenergic receptors were reported to interact with Panx1 in mouse thoracodorsal resistance arteries [28]. Panx1 was further shown to play a role in phenylephrine-induced vasoconstriction of resistance arteries through this association with α1D-adrenergic receptors [28]. Finally, Panx1 was found to also interact with several components of the multiprotein inflammasome complex including the P2X7 receptor, caspase-1, caspase-11, NLRP1, ASC, and the inhibitor of apoptosis XIAP [39]. Further studies should define what role Panx1 plays in the function of the inflammasome.

4. Channel functions of pannexins

In 2003, it was reported that Panx1 has the ability to form non-junctional membrane channels in single *Xenopus* oocytes [16]. Later, the unitary conductance of Panx1 channels was determined to be 550 pS [40], which was approximately 200 pS larger than that of any of the connexins [41]. Since these early studies, we and others have reported that all pannexins can form channels capable of dye uptake [15]. Although Panx2 was initially reported to be incapable of forming functional single membrane channels [16], a recent study using a cytochrome c based proteoliposome assay as well as electrophysiological recordings in *Xenopus* oocytes revealed that Panx2 can

Table 1
Reference cell lines reported to endogenously express pannexins. Endogenous pannexin expression reported in cultured cell lines by RT-PCR (mRNA level, "r") or Western blot (protein, "p") as specified in the corresponding references. Note that many of these cells lines have only been shown to express pannexin mRNA and may not express detectable levels of pannexin protein.

Cultured cell line	Panx1	Panx2	Panx3
Mouse neuroblastoma — N2A		[30]$^{r,\,p}$	
Mouse pituitary cells — AtT-20	[76]$^{r,\,p}$	[76]$^{r,\,p}$	
Mouse osteoblast — MC3T3-E1	[4]^{p}		[32,34]$^{r,\,p}$
Mouse chondrogenic — ATDC5 and N1511			[31]$^{r,\,p}$
Mouse osteoprogenitor — C2C12			[33]$^{r,\,p}$
Mouse macrophage — J774	[44]^{r}		
Rat epidermal keratinocyte — REK	[54]^{p}		
Canine kidney — MDCK	[8,34]^{p}		[34]^{p}
Human bronchial epithelial — WD-HBE	[73]^{r}		
Human cervical-HeLa S3 cells (ATCC number CCL-2.2); HeLa	[18,77]$^{r,\,p}$		
Human prostate cell — LNCaP	[57]^{r}		
Human kidney-HEK293	[57,77]^{r}		
Human monocyte-THP-1	[77]^{r}		
Human Jurkat T cells	[42]$^{r,\,p}$		

form functional homomeric channels that open at high voltages [9]. Functional single membrane channels composed of homomeric Panx3 were also proposed to play a role in ATP release into the extracellular space and act as Ca^{2+} channels in the ER of C2C12 cells and primary calvarial osteoblasts [33]. However, electrophysiological evidence for Panx3 homomeric channel function has yet to be reported. While Panx1 channels can be activated by mechanical stimulation, caspase cleavage, cytoplasmic Ca^{2+}, membrane depolarization, extracellular ATP and K^+ [39,40,42–46]; its ability to function is abolished by CO_2-mediated cytoplasmic acidification [43], negative feedback from ATP release [47], mimetic peptides [48] and channel blockers such as carbenoxolone, probenecid and fluefenamic acid [49,50]. It is now quite well established that the activation of pannexin channels allows for cellular communication with the extracellular environment to fulfill a diverse range of functions [43,51–54].

Currently, there are conflicting reports in the literature as to whether pannexins are ever involved in direct intercellular communication. It was initially reported that the expression of Panx1, but not Panx2 or Panx3 in paired *Xenopus* oocytes led to intercellular channels, albeit, 24–48 h after cell pairing [16,35,55]. In a parallel study, Boassa et al. [8] reported that Panx1 intercellular channels did not form under similar conditions while robust Cx46 intercellular channels readily assembled [8]. To date two other laboratories have provided some evidence that Panx1 forms intercellular channels when overexpressed in C6 glioma cells [56] and LNCaP prostate cancer epithelial cells [57]. Also, Panx3 was recently reported to form gap junctions in osteoblasts [33]. However, these latter studies revealing the passage of sulfurodamine 101 dye in C6 gliomas [56] and Ca^{2+} permeability in LNCaP neighboring cells [57] or osteoblast cells [33] were all performed under very specific conditions raising questions as to whether background levels of connexins may have contributed to the evidence that intercellular channels had indeed formed. Evidence against pannexins forming intercellular channels continues to grow as a dye transfer assay in Panx1-enriched and connexin negative Madin–Darby Canine Kidney cells failed to reveal the presence of intercellular channels [8]. This study was in line with our findings that revealed no electrical coupling in gap junction-deficient neuro-2A cells expressing either Panx1 or Panx3 [4]. Additional reports involving erythrocytes [58] as well as neuronal and glial cells [52] have also failed to provide evidence that pannexins can in fact form intercellular channels thus providing further indications that the pannexin family of proteins does not likely serve a redundant role to connexins by forming intercellular channels [13]. Finally, it is notable that there remains no in vivo evidence for the existence of pannexin-based intercellular channels.

4.1. Paracrine signaling mediated by Panx1

Indirect communication between cells is typically mediated through activation of single-membrane channels and the releasing of molecules such as ATP [59] into the extracellular space.

4.1.1. Calcium waves

Ca^{2+} is a versatile, ubiquitously found second messenger that can regulate several cellular responses [60]. Initiation of Ca^{2+} waves is mediated by the activation of ATP-sensitive purinergic receptors, P2Y and P2X [61]. Binding of ATP to the P2Y receptors increases inositol 1,4,5-triphosphate, which in turn releases Ca^{2+} from the ER stores. Once released, Ca^{2+} activates single-membrane channels leading to further release of ATP and propagation of signals to neighboring cells [49]. Essential to this notion is the fact that Panx1 has been shown to be a part of the $P2X_7$ receptor complex necessary for ATP release [44,58,62]. Furthermore, ATP-induced ATP release was also reported when Panx1 channels were activated through P2Y receptors and cytoplasmic Ca^{2+} [43], thus supporting the role of Panx1 in the initiation and propagation of regenerative Ca^{2+} signaling. The interaction of Panx1 channels with NMDA receptors has also been shown to participate in the regulation of Ca^{2+} in hippocampal pyramidal neurons and is proposed to play a role in epilepsy [53]. The role of Panx1 in epilepsy was recently confirmed in vivo using Panx1 null mice [63].

4.1.2. Vasodilation and vasoconstriction

Release of ATP from erythrocytes occurs during stress and hypoxic conditions [64]. It was reported that erythrocytes endogenously express Panx1 and mechano-sensitive activation of Panx1 channels regulates ATP release [58]. It is hypothesized that under conditions of stress activation, Panx1 channels control blood flow by releasing ATP from red blood cells and initiating Ca^{2+} wave propagation through the stimulation of purinergic receptors on the endothelial cells [58]. Elevation of Ca^{2+} subsequently releases NO onto the smooth muscle leading to vasodilation [58]. Recently, Billaud et al. [28] showed that Panx1 is expressed in both endothelial and vascular smooth muscle cells (VSMC) and plays a key role in phenylephrine-induced vasoconstriction of resistance arteries through an association between Panx1 and α1D-adrenergic receptor in VSMCs. This indicates that Panx1 may be directly linked to the coordination of vascular smooth muscle cell constriction and potentially the regulation of blood pressure.

4.1.3. Taste sensation

Taste buds are comprised of two distinct populations of cells, some that express taste receptors (receptor cells) and others that contain synapses (presynaptic cells) [65]. It has been reported that Panx1 is expressed in the receptor cells and upon taste stimulation ATP is released through Panx1 channels [50,65]. Once released in the extracellular medium, ATP stimulates P2 receptors of the presynaptic cells to release serotonin (5-HT) [50,65] thereby providing a mechanism for cell–cell signaling and information processing within the taste bud.

4.1.4. Airway defense

Airway epithelium provides a defense mechanism by controlling mucociliary clearance which depends on maintaining adequate airway surface liquid volume and ciliary activity [66]. In the differentiated human airway epithelium, expression of Panx1 channels has been linked to ATP release under hypotonic stress [66]. Panx1-evoked ATP release is proposed to be crucial for regulating the ciliary beat frequency and surface liquid volume for mucous clearance [66].

4.1.5. Viral infection

ATP release by Panx1 channels has also been reported to play a role in HIV-1 viral infection of $CD4^+$ cells [67]. HIV-1 envelope proteins are proposed to interact with specific $CD4^+$ cell receptors and trigger the release of ATP via Panx1 channels into the extracellular milieu where ATP then acts on P2Y2 purinergic receptors and activates Pyk2 kinase. This interaction causes membrane depolarization and facilitates membrane-to-membrane fusion to allow viral entry into the cell as well as cell-to-cell transmission. Interestingly, Panx1, P2Y2 and Pyk2 are all physically recruited to the contact site between Env-containing and CD4/CDXCR4-containing membranes indicating that they form part of the infection synapse that is orchestrated by the virus to facilitate infection [67]. Inhibiting any of the components of this interaction could reduce HIV-1 infection and impair viral replication indicating their potential use as a novel antiretroviral therapy.

4.2. Cellular responses mediated by Panx1

4.2.1. Immune response

Interaction of Panx1 with $P2X_7$ receptors elicits an immune response by releasing the pro-inflammatory cytokine interleukin (IL)-1 β in response to receptor stimulation by ATP followed by a subsequent

activation of caspase-1 [44]. Panx1 is also reported to trigger the Toll-like receptor-independent inflammasome (comprising cryopyrin) based on recognition of bacterial molecules passing from endosomes to cytosol [68]. Furthermore, $P2X_7$-mediated activation of Panx1 channels was found to be potentiated by high extracellular K^+ levels in neuronal/astrocytic inflammasomes [39]. However, in a recent study using Panx1 null mice [69] the authors found that Panx1 is not required for the assembly of caspase-1-activating inflammasome complexes.

4.2.2. Tumorigenesis

Exogenous expression of Panx1 has been linked to tumor-suppressive properties in C6 gliomas [56]. In the presence of Panx1, C6 gliomas exhibit a flattened morphology and a reduction in proliferation, motility and anchorage-independent growth [56]. In addition, Panx1 overexpressing cells exhibited reduced in vivo tumor growth in nude mice as compared to controls [56]. Taken together, it is suggested that Panx1 plays a role as a tumor suppressor. Later, these same authors reported a similar tumor suppressive role for Panx2 [70].

4.2.3. Ischemic cell death and epileptic seizure

The rapid decrease of O_2 and glucose in mouse hippocampal pyramidal neurons has been associated with the opening of Panx1 channels that lead to conductance of large currents [71]. This, in turn, causes a profound ionic dysregulation leading to neuronal death [71]. In addition, the N-methyl-D-aspartate receptor-based opening of Panx1 channels augments epileptiform seizure activities in pyramidal neurons [53] further supporting the importance of precise regulation of Panx1 channel opening. As will be discussed later, studies using Panx1 knockout mice have now confirmed that Panx1 contributes to the severity of epileptic seizures in vivo [63].

4.2.4. Apoptosis

Panx1 has been proposed to form the pore unit of the $P2X_7$ death complex [62]. The co-expression of Panx1 with $P2X_7$ receptor revealed ATP-induced zeiosis in *Xenopus* oocytes which was not observed upon the injection of Panx1 transcript alone or together with P2Y receptor [43,62]. These results suggest that although activation of the purinergic P2Y receptor can mediate Panx1 currents, the specific cell death signaling is through an interaction with ionotropic $P2X_7$ receptors. Quite recently, Panx1 channels have been documented to mediate the release of nucleotide signals from apoptotic cells for the recruitment of activated monocytes [42]. Mechanistically, the authors showed that Panx1 channels get activated by caspases which cleave Panx1 and open the channel for ATP and UTP release as a "find me" signal to attract phagocytes [42].

4.2.5. Keratinocyte differentiation

We have previously shown that ectopically expressed Panx1 reduces the proliferation rate of rat epidermal keratinocytes (REKs), however, it does not significantly alter their migratory properties [54]. Furthermore, over-expression of Panx1 in organotypic cultures (generated from monolayer REKs) disrupts the overall architecture of the epidermis, reduces the thickness of the living cell layer and re-localizes the basal cell marker, cytokeratin 14, throughout the living cell layer [54]. Thus, this study argues for a role of Panx1 in the regulation of keratinocyte differentiation.

4.3. Cellular responses mediated by Panx2 and Panx3

4.3.1. Neuronal differentiation

Swayne et al. [30] demonstrated that Panx2 is expressed by progenitor neural cells as well as mature neurons. However, this expression is post-translationally regulated by the generation of S-palmitoylated Panx2 species that appear to be localized within intracellular compartments of stem-like neural progenitor cells, but not in mature neurons. Following maturation, neurons were shown to re-express Panx2 at the plasma membrane. Additionally, the authors found that Panx2 knockdown in Neuro2a cells significantly accelerated the rate of neuronal differentiation [30].

4.3.2. Differentiation and development

In contrast to Panx1, much less is known about the channel capabilities of Panx3. We have shown that, similar to Panx1, Panx3 also reduces the proliferation of REKs without altering their migration rates [54]. However, unlike Panx1, Panx3 maintains the integrity of the organotypic epidermis and keratinocyte differentiation upon its over-expression [54]. In addition, Panx3 expression in cartilage has recently been associated with chondrocyte differentiation [31]. Specifically, Panx3 was found to promote ATP release into the extracellular medium and reduce the parathyroid hormone-mediated proliferation of chondrocytes by decreasing cAMP levels and inhibiting phosphorylation of CREB [31]. Thus, these results support a role for Panx3 in switching the properties of chondrocytes from proliferation to differentiation. Two recent reports further highlight the role of Panx3 in chondrocyte/osteoblast differentiation where the Panx3 promoter was found to be responsive to Runx2 activation [32] and may function as an ER calcium channel, plasma membrane channel or even as a gap junction [33]. It is notable that Panx3 expression caused a concomitant increase in Cx43 leaving one to wonder if the gap junction function was due to this connexin and not due to Panx3. Recently, Panx3 was also reported to be present in rat Leydig cells, the apical region of the epididymis and in efferent ducts raising the possibility that Panx3 plays a role in sperm transport and maturation [29].

5. Pannexin knockout mice as models for determining the physiological roles of pannexins

As a result of the increased interest in uncovering the physiological roles of pannexins, several groups have now generated Panx1 knockout mouse models (Panx1-KO). Surprisingly, given the ubiquitous expression of Panx1 in many human and mouse tissues most authors report no major abnormalities in the anatomy and overall health of the Panx1 knockout mice. One of the first published reports of a Panx1 knockout mouse was provided by Anselmi et al. [72] using a mouse generated by Hannah Monyer's laboratory in Germany. In that study, Panx1-KO-derived cochlear organotypic cultures were used to show that the ATP-induced intercellular Ca^{2+} signal propagation was due to Cx26 and Cx30 hemichannels and did not involve Panx1 channels [72]. The same Panx1-KO mouse was acquired by Santiago et al. [63] along with a heterozygote Panx1-knockout '(KO) first' mouse generated by the knockout mouse project (KOMP) at the University of California-Davis. These two mouse models were used to demonstrate that Panx1 channels contribute to status epilepticus in vivo by increasing the severity and duration of seizures [63]. This data is consistent with previous reports of Panx1 channel opening in hippocampal neurons exposed to NMDA potentiating seizure-like activity [53]. A third Panx1-KO mouse also derived from embryonic stem cells obtained from KOMP was used by Seminario-Vidal [73] to test the hypothesis that Panx1 mediates ATP release from hypotonically swollen airway epithelia as was reported in vitro by Ransford et al. [66]. Using primary cultures of bronchial epithelial cells as well as freshly excised tracheas from Panx1-KO mice they saw a significant reduction in the hypotonicity-induced ATP release as well as dye uptake indicating a key role for Panx1 channels in the airway epithelia [73]. Finally, a fourth Panx1-KO mouse was generated by Genentech Inc. and used to discover that, contrary to previous reports, Panx1 is not required for inflammasome activation but it is needed for the release of chemoattractants as "find-me" signals for phagocytic cells for appropriate apoptotic cell clearance as was earlier described by Chekeni et al. [42]. Interestingly, these authors also demonstrated that ATP engagement of the $P2X_7$ receptor increases

membrane permeability independent of Panx1 indicating that they belong to distinct signaling pathways [69].

Given the apparent normal morphology and physiology of Panx1 KO mice that are not under stress or harboring an additional pathology, it is possible that compensation by other members of the pannexin family could be at play. A recent study on cerebral ischemia using either single ($Panx1^{-/-}$or $Panx2^{-/-}$) or double knockout ($Panx1^{-/-}$ $Panx2^{-/-}$) mice revealed that only the double knockout mice had a significant effect on the function of cortical neurons [74]. The ablation of just one of the pannexins had no effect but the double knockout mouse had smaller infarcts than the control mice during ischemic stroke [74]. This raises the possibility that double or triple pannexin knockout mice may be needed in future studies to resolve the physiological roles of pannexins in different cellular systems. Additionally, since connexin hemichannels have been postulated to carry out similar ATP release and paracrine signaling functions [75] as pannexin channels, it will also be important to assess any potential cross-talk between the pannexin and connexin families of channel-forming proteins using combinations of pannexin and connexin knockout mouse models.

6. Conclusion

The initial interest in the pannexin family of proteins was derived from their limited homology to the innexin family of gap junction proteins. Consequently, they were first thought to be a third family of gap junction proteins with similar tetra-spanning topologies but very diverse sequences. With the exception of a few reports in which Panx1 and Panx3 were suggested to form intercellular gap junction channels, the vast majority of the literature supports what is thought to be their primary role in forming single membrane channels. These channel forming glycoproteins are all capable of dye uptake and Panx1 has been extensively reported as an ATP/UTP release channel even under conditions of physiological levels of calcium. Post-translational modifications appear to regulate their expression, intracellular localization, intermixing and perhaps their ultimate function in different tissues. We are only beginning to uncover the interesting physiological roles that pannexins may play as independent channels necessary for normal paracrine signaling, regulation of differentiation and cell death, and immune function. Evidence suggests that they are involved in pathologies possibly as potential tumor suppressors or ischemic cell-death and seizure enhancers. With the arrival of more single and double Panx-KO mouse models that will allow for compensatory mechanisms to be assessed, the field will continue to further elucidate the true nature of pannexin channels and their key roles in health and disease.

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