Review

Connexins in Cancer: Jekyll or Hyde?

Erin E. Mulkearns-Hubert [1,*] , Ofer Reizes [1,2,3] and Justin D. Lathia [1,2,3,4]

[1] Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA; reizeso@ccf.org (O.R.); lathiaj@ccf.org (J.D.L.)
[2] Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH 44106, USA
[3] Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Case Western Reserve University, Cleveland, OH 44195, USA
[4] Rose Ella Burkhardt Brain Tumor and Neuro-Oncology Center, Cleveland Clinic, Cleveland, OH, 44195, USA
* Correspondence: mulkeae@ccf.org

check for updates

Abstract: The expression, localization, and function of connexins, the protein subunits that comprise gap junctions, are often altered in cancer. In addition to cell–cell coupling through gap junction channels, connexins also form hemichannels that allow communication between the cell and the extracellular space and perform non-junctional intracellular activities. Historically, connexins have been considered tumor suppressors; however, they can also serve tumor-promoting functions in some contexts. Here, we review the literature surrounding connexins in cancer cells in terms of specific connexin functions and propose that connexins function upstream of most, if not all, of the hallmarks of cancer. The development of advanced connexin targeting approaches remains an opportunity for the field to further interrogate the role of connexins in cancer phenotypes, particularly through the use of in vivo models. More specific modulators of connexin function will both help elucidate the functions of connexins in cancer and advance connexin-specific therapies in the clinic.

Keywords: connexins; cancer; gap junctions; cancer stem cells; hemichannels

1. Introduction

Communication between cells is essential for normal tissues to maintain the ability to grow and respond to their environment. However, this process is frequently altered in cancer cells. Over 50 years ago, Loewenstein and Kanno observed that liver cancer cells displayed a lack of cell–cell communication [1], and further studies supported this observation in other tumor types. This led to the long-standing historical dogma that connexins, the proteins that make up gap junctions (GJs), are functionally tumor suppressive. Over time, additional evidence has suggested a more complex system where connexins serve multiple cellular functions and individual connexins can act as both tumor promoters and tumor suppressors depending on context. In this review, we discuss the mechanisms—insofar as they are known—by which the connexin family of GJ proteins mediates the key phenotypes of cancer as laid out by Hanahan and Weinberg [2], including roles in more recently appreciated cancer phenotypes such as immune evasion and metabolic reprogramming.

1.1. Canonical and Non-Canonical Functions of Connexins

1.1.1. Gap Junctions

Connexins are tetraspanin transmembrane proteins that assemble into a circular hexameric structure, termed a connexon, arranged around a central pore. Each connexin subunit contains two extracellular loops, which mediate docking between connexons on adjacent cells, and three intracellular regions: an intracellular loop and N- and C-terminal tails. When docked, the pore of the

GJ allows molecules such as adenosine triphosphate (ATP) and other nucleotides, amino acids, small metabolites (including glucose), miRNAs (including miR-142, miR-223, miR-34a, and miR-124-3p [3–5]), second messengers (including cyclic adenosine monophosphate (cAMP) and inositol trisphosphate (IP_3)), reactive oxygen species (ROS), glutathione, ions (Ca^{2+} and K^+), and small proteins less than approximately 1.5 kDa to pass from the cytoplasm of one cell to another. Importantly, this transfer of materials is driven by simple diffusion gradients and is not an active transport. The opening and closing of GJ channels are mediated by multiple factors, including cross-channel pH and voltage, connexin phosphorylation, and intracellular Ca^{2+} concentration. There is evidence that channels composed of different connexin proteins display some varying selectivity to molecules, although the challenges associated with understanding exactly which molecules pass through GJs in a specific situation have limited a full understanding of channel selectivity. Furthermore, there is an emerging recognition that, in addition to their function in communication, GJ structures can function as adhesive anchors between cells (see [6]), particularly during cell motility, as well as protein scaffolds, as detailed in the section on non-junctional roles for connexins.

1.1.2. Connexin Hemichannels

While it was originally postulated that connexons were only able to open for communication while docked as GJs, more recent work has suggested that undocked connexons, or hemichannels, do open and close, at least in some situations, to exchange material between a cell and the extracellular space (reviewed in [7,8]). It remains controversial whether hemichannels are active only during pathological states or whether they also open during normal physiological states. Investigating hemichannel function in cultured cells is complicated by the question of whether the effects of hemichannel inhibition are due to beneficial small molecules not able to get into the cell, toxic small molecules not able to get out of the cell, or a combination of the two. Additionally, the study of connexin hemichannel biology is complex due to the presence of pannexin hemichannels. Pannexins form channels that are similar to those composed of connexins, although hexameric pannexin channels in the plasma membrane do not form GJs and instead function as single-membrane channels [9]. It has recently become appreciated that many inhibitors of GJs and connexin hemichannels also inhibit pannexin hemichannels, confounding the interpretation of inhibitor studies in cells that express both connexins and pannexins [10].

1.1.3. Non-Junctional Connexin Functions

In addition to their channel function, connexins are known to mediate extensive protein–protein interactions, which occur primarily through the connexin C-terminal tail. Early work showed a lack of correlation between GJIC and growth suppression [11], suggesting that connexins might have additional functions. Numerous proteins that bind to the intracellular domains of connexins have since been identified, and the importance of some of these interactions in cancer phenotypes is detailed below. Many connexins are also substantially phosphorylated, which alters their ability to interact with other proteins and also affects the gating of the channels they comprise. In fact, channel regulation has been suggested to be the main function of connexin protein–protein interactions, although it is now recognized that there are additional intracellular non-junctional functions as well. However, it can be difficult to separate channel regulation from other connexin activities; ideally, downstream effects of disrupting connexin–protein interactions would be compared to inhibition of GJIC to determine whether the binding affects channel function. Non-junctional functions have also been reported for truncated forms of connexin 43 (Cx43), particularly in the nucleus [12]. Connexins in cancer cells also often become relocalized away from the plasma membrane to intracellular compartments, where they may acquire novel, non-canonical functions. There are many potential intracellular functions of connexins, and this is an emerging area that deserves more in-depth study.

2. Mechanistic Roles of Connexins in the Major Cancer Phenotypes

There is an abundance of comprehensive reviews of connexins in cancer [13–25]. Although the majority of reports on connexins in cancer do not delve into the mechanistic role of connexins, there is a growing number of studies, particularly with the advancement of more specific peptide mimetics and antibodies, that do attribute either GJ activity, hemichannel activity, or a non-channel function of the connexin to the cancer phenotype. Many studies report an association between decreasing connexin expression and increasing tumor grade or show a mislocalization of connexins within the cell and conclude that a loss of GJIC is pro-tumorigenic. However, this could easily be attributable to either connexin hemichannel or non-channel activity, and these alternatives should be tested. Here, we focus on those studies that attempt to isolate which of the connexin functions is responsible for an observed effect on cancer cells, with the aim of adding to our understanding of how connexin expression and function impact cancer phenotypes.

2.1. Maintenance of Proliferation and Evasion of Anti-Proliferative Signals

2.1.1. Gap Junction Intercellular Communication (GJIC)

We envision that gap junction intercellular communication (GJIC) could affect cancer cell proliferation through direct effects on proliferative signaling pathways by molecules entering or exiting the cell through the channel—from other tumor cells, from host microenvironmental or immune cells, or through exosomes or tunneling nanotubes, the functions of which have been reviewed elsewhere [26–32]. Several recent reviews provide a good discussion of what is known about the role of connexins in cancer cell proliferation [13–15,17]. Due to the decreased expression of connexin proteins in many cancer cell types, numerous studies hypothesize that GJIC is tumor suppressive, but fewer directly test this hypothesis using specific connexin function modulators rather than adding or removing the proteins. Willebrords et al. provided a comprehensive review of the status of current connexin inhibitors, antibodies, and peptide mimetics [10], some of which have been used in cancer cells. The inhibitor 18-α/β-glycyrrhetinic acid (18-GA), or its derivative carbenoxolone (CBX), while relatively non-specific and non-selective, has been shown to inhibit cell growth in a variety of cancer cell types, including glioma [33], leukemia [34,35], hepatomas [36], and thyroid [37], gastric [38,39], lung [40], bladder [41], prostate [42] and breast cancers [43,44]. Some of these tumors were shown to express connexins (glioma and thyroid), suggesting that the effect might be due to an inhibition of GJIC. However, in other studies, connexin expression was not assessed (leukemia, gastric, breast, lung, and bladder), and the effect of 18-GA may be due to GJIC inhibition or other non-specific effects. Similar to 18-GA, the GJ inhibitor heptanol decreased the proliferation of multiple myeloma cells, which express high levels of Cx43 and demonstrate GJIC, suggesting a pro-proliferative, pro-tumorigenic role for Cx43 in these cells [45]. However, heptanol can also inhibit connexin hemichannels, so this effect cannot be definitively linked to Cx43 or GJIC.

These previous studies suggested that that the degree of GJIC does not correlate with tumorigenicity, which has also been directly observed [46,47], suggesting that the relationship between cell–cell communication and tumor cell proliferation is more complex than simply causative. Subsequently, GJIC has been shown to be pro-proliferative in certain contexts. Treatment of breast cancer cells with the Cx43 targeted peptide mimetic aCT1 increased GJIC and subsequently decreased proliferation [48]. Again supporting a link between alterations in GJIC and proliferation, overexpression of Cx43 decreased proliferation of melanoma cells, and, consistently, treatment with a mimetic peptide that specifically blocks Cx43 GJIC increased proliferation [49]. The use of both inhibitors and peptide mimetics in addition to connexin modulation via genetic approaches increases confidence that the observed effect is truly due to GJIC.

Interestingly, one way that GJs may affect proliferation involves asynchronous cell divisions. Decades ago, it was observed that expression of Cx26 was able to inhibit the proliferation of HeLa cells, while expression of other connexins had little effect [11]. In an elegant mechanistic study,

Chandrasekhar et al. showed that this was due to the maintenance of Cx26 GJs at the plasma membrane during cell division, while other connexins are typically internalized during this process [50]. These Cx26 junctions allow the passage of cAMP from cells in M phase into neighboring cells. cAMP then activates protein kinase A (PKA) signaling in G1/S-phase surrounding cells, slowing their cell cycle and suggesting an anti-proliferative role for Cx26. Strikingly, this type of effect may explain how modulation of GJs can affect the growth of homogeneous cultures of cells that should have similar cytoplasmic contents.

Transfer of miRNAs through GJs has also been described to affect cell proliferation. While it is likely that this effect could be pro- or anti-proliferative depending on the miRNA and the cell type, the effects observed thus far have been primarily anti-proliferative. In one example, macrophages coupled with hepatocellular carcinoma cells transferred miR-142 and miR-223 into the cancer cells, inhibiting proliferation [3]. More recently, miR-34a and miR-124-3p were also shown to decrease glioma cell proliferation [4,5], and several miRNAs targeting CXCL12 that were transferred from bone marrow stromal cells slowed proliferation of breast cancer cells [51]. There may be some selectivity to this process, as Cx43 was shown to efficiently transfer miRNAs between cells, with lower levels of transfer for Cx26 and Cx31 and little transfer for Cx32 and Cx37 [5,52]. Thus, the effects of GJIC appear to be both pro- and anti-proliferative depending on the setting.

2.1.2. Connexin Hemichannels

Studies have also described functions for connexin hemichannels in cancer cell growth control, although, as for GJs, specific inhibition of hemichannels remains challenging. However, some studies (e.g., [53]) have used peptide mimetics; pharmacological agents that are relatively specific for hemichannels, such as bisphosphonates; and antibodies specific for the extracellular loops of Cx43, which specifically inhibit hemichannel activity when bound. One critical cargo of connexin hemichannels is ATP. ATP released from hemichannels composed of Cx43 by osteocytes inhibited breast cancer cell proliferation and migration [53], indicating the importance of microenvironmental hemichannel function for tumor cells. However, in other systems, ATP released from pigment epithelial cells through hemichannels increased proliferation of neural retinal progenitor cells [54]. These contradictory effects may in fact be due to the amounts of ATP released into the extracellular space: high concentrations of ATP activate purinergic receptors to induce cell death, while low concentrations may promote cell proliferation [21]. In a non-cancer situation, hemichannel inhibition has also been shown to increase proliferation of rat cardiomyocytes [55] and decrease proliferation of smooth muscle cells [56].

2.1.3. Non-Junctional Roles

As mentioned above, caution should be exercised when assuming that the effects of proteins binding to the intracellular regions of connexins are independent of connexin channel functions. One model of connexin gating proposes that the C-terminal tails of the proteins physically gate the channel, and there is experimental evidence to support this for some connexins [57]. For example, both the channel activity and C-terminal tail of Cx37 are required for rat insulinoma cell proliferation [58,59], but these are not independent; instead, phosphorylation of the C-terminus appears to regulate opening and closing of the GJ, thus regulating proliferation [60,61]. Similarly, phosphorylation of the Cx43 C-terminal tail at S368 by PKA and at Y247 and Y265 by v-src decreases GJIC [62].

However, and perhaps surprisingly, mechanistic studies investigating the non-canonical roles of connexins in cancer cell growth have actually been numerous, likely owing to the relative ease of disrupting protein–protein interactions via genetic means compared to the difficulty involved in studying connexin channels. Many of these functions have been discovered upon observation that the addition of connexin proteins to cancer cells affects cell proliferation without conferring the ability for GJIC, for example, for Cx43 [63] and Cx26 [64] in breast cancer cells. Cx43 inhibits the proliferation of multiple cancer types through channel-independent mechanisms. Expression of only the tail in

HeLa cells decreased proliferation [65]; as HeLa cells do not express endogenous Cx43, this suggests a tail-intrinsic effect rather than a dominant-negative effect on GJs or hemichannels. Reports have suggested that Cx43 inhibits basaloid carcinoma cell proliferation through a cytoplasmic role [66], colorectal cancer cell proliferation through an effect on the WNT/β-catenin pathway independent of GJIC [67], glioma cell proliferation by decreasing the activity of SRC through interaction with the Cx43 C-terminal tail [68,69], epidermal growth factor (EGF)-induced ovarian cancer cell proliferation [70], and neuroblastoma cell proliferation [71], among others. Recently, Cx43 was shown to coimmunoprecipitate with both β-catenin and casein kinase, suggesting that Cx43 may exist as part of the "destruction complex" responsible for β-catenin degradation in the absence of WNT ligands [72]. Some of these situations are accompanied by an altered localization of connexin protein, in cases of both overexpressed and endogenous proteins. This has been shown in many cancers, including for Cx43 in glioma [73], rat liver epithelial cells [74], and transformed keratinocytes [75]; Cx26 in breast cancer [64,76]; and Cx32 in liver cancer [77].

Truncated forms of Cx43 have also been shown to affect cell cycle progression. Translation initiating at internal AUG codons within the GJA1 mRNA can produce at least four, and possibly six, different N-terminal truncations of the protein [78,79]. When overexpressed, the 11 kDa form, GJA1-11k, localizes to the nucleus and inhibits transition from G0/G1 phase into S phase of the cell cycle [80]. Other connexins also affect cell cycle progression; for example, Cx37 inhibits the growth of human renal cell carcinoma cells by blocking G1-S phase transition through a channel-independent mechanism involving HER-2 activation [81].

Thus, while the study of connexin functions in the proliferation of cancer cells is difficult due to the frequent decreased expression of connexins, there is evidence that connexins can be both pro- and anti-proliferative. For cells that have lost connexin expression, the ideal type of experiments to study connexin function would be to overexpress a connexin and then use an inhibitor in an attempt to "rescue" the cancer cells, thus attributing the proliferation of the cells to a specific connexin function. The further development of targeting molecules that are connexin and connexin function specific will aid in this process.

2.2. Resistance to Cell Death

2.2.1. GJIC

Similar to its role in proliferation, the function of GJs seems to both promote and inhibit cell death. Many studies have linked decreases in GJIC to an induction of cell death, particularly by apoptosis. Treatment of mouse hepatoma cells with chlordane, a benzene metabolite that decreases GJIC [82], enhanced the apoptosis of cells treated with benzo[a]pyrene [83]. In non-transformed cells, treatment of cardiomyocytes with CBX also increased apoptosis [84], and inhibition of GJs via 18-GA and octanol led to apoptosis of endometrial stromal cells [85]. 18-GA and CBX also induced cell death in mouse embryonic stem cells [86]. Furthermore, treatment of thyroid cancer cells with CBX sensitized cells to anoikis, a form of detachment-induced cell death [37]. Knockdown of Cx25 in leukemia cells sensitized cells to cytosine arabinoside chemotherapy [87]. These increases in cell death upon GJ inhibition are perhaps a bit surprising when considering the frequency at which GJ communication is lost in cancer—one might expect that cells that lose expression of connexin proteins would die rather than become transformed. This is consistent with the notion that the ability to evade these apoptotic signals is an essential cancer phenotype.

The presence of functional GJIC is also able to induce cell death, particularly in situations where cancer cells are treated with radiation or chemotherapies. Studies using engineered cells expressing thymidine kinase provided evidence that cells are able to spread pro-apoptotic molecules, in this case likely phosphorylated ganciclovir, through GJs [88–90], supporting the idea of a "bystander effect" where cells treated with anti-cancer agents, particularly radiation therapy, are able to induce death in directly adjacent neighboring cells. Subsequent studies have shown that endogenous molecules are also

able to spread apoptosis in a GJIC-dependent manner (for example, through Cx43 [91], Cx37/40/43 [92], and Cx32 [93]). The mechanistic basis of how this occurs in vivo is not certain, but some studies have suggested that this effect is potentiated by Ca^{2+}/IP_3 signaling; IP_3 is required but not sufficient for the activation of apoptosis through GJs [94], and prolonged exposure to high Ca^{2+} levels as induced by IP_3 in the ER can contribute to cell death [95]. In addition to the bystander effect mediated by radiation, GJIC has been shown to increase chemosensitivity in many models. Use of a dominant-negative Cx43 that blocks GJIC showed that GJIC sensitizes prostate cancer cells to tumor necrosis factor-alpha (TNFα)-induced apoptosis [96]. Cx26 also enhanced hepatocellular carcinoma cell sensitivity to oxaliplatin [97]. Together, these results suggest that GJIC might function to suppress apoptotic stimuli in some cells as it also drives apoptosis in other situations.

2.2.2. Connexin Hemichannels

Similar to GJs, hemichannels can also mediate the bystander effect and spread apoptotic signals between cells. Elegant work in rat C6 glioma cells showed that, while, as one might expect, GJs are able to spread apoptotic signals directly from one cell to another, hemichannel release of pro-apoptotic molecules was able to induce apoptosis in cells up to 100 μm away, suggesting that hemichannels may have a broader scope of an effect on cell death compared to GJs [98]. This effect was negated by Ca^{2+} buffering, suggesting an involvement of calcium ions in this process. It has also been suggested that, due to their effect on intracellular ATP levels, hemichannels may regulate a balance between necrosis and apoptosis [99]; depleted cellular ATP has been shown to drive cells away from apoptosis and toward necrotic cell death [100].

2.2.3. Non-Junctional Roles

In addition to GJ- and hemichannel-dependent roles in apoptosis, channel-independent roles have been described for several connexins. Recently, Cx32 was shown to localize intracellularly in both hepatocellular carcinoma and cervical cancer cells and suppress apoptosis in a channel-independent manner [101,102]. In both cancers, Cx32 bound to SRC, which activates the EGFR signaling pathway, leading to an inhibition of apoptosis induced by the chemotherapy streptonigrin and/or cisplatin. Cx32 also suppressed the induction of apoptosis by TNFα and TNF-related apoptosis-inducing ligand (TRAIL) through activation of the nuclear factor kappa B (NF-κB) pathway in cervical cancer cells [103]. Cx43 has also been shown to induce apoptosis in a channel-independent manner by binding to the apoptotic regulator BAX in both pancreatic cancer and mesothelioma cells [104,105]. In contrast, Cx43 also reduced hydrogen peroxide-induced apoptosis in C6 rat glioma cells by inhibiting caspase activation, perhaps through an interaction with apoptosis signal-regulating kinase 1 (ASK1) [106]. A final example again shows how interconnected connexin functions may be: bisphosphonates inhibit apoptosis of osteoblasts and osteocytes through the activation of extracellular-signal-regulated kinase (ERK) signaling. Plotkin et al. determined that Cx43 hemichannels mediate this signal [107]. The bisphosphonates stimulated Cx43 hemichannels to open, which altered the conformation of the connexins so that SRC could bind, leading to downstream activation of ERK. Based on their frequent loss of expression in cancer cells, one would expect connexins to suppress cancer cell apoptosis, but evidence clearly indicates that they may also be pro-apoptotic in some situations, perhaps dependent on the ability of cancer cells to evade those apoptotic signals.

2.3. Replicative Immortality

Only a few studies have reported a mechanistic role for connexins or connexin channels in canonical properties of replicative immortality of cancer cells, such as senescence and immortalization via telomere extension or telomerase expression. More has been reported regarding functions for connexins in the more recently appreciated field of cancer stem cell biology (see below). Several studies have shown changes in connexin gene expression in response to immortalization [108–110] but have not investigated the underlying mechanism of connexin function in this situation. A decrease in GJIC with

age and with senescence has also been reported [111–114], as well as changes in connexin expression during senescence [115]. In the context of untransformed cells, knockdown of Cx43 in glomerular mesangial cells increased senescence-associated β-galactosidase staining [116], and Cx43 gene knockout in mesenchymal stem cells also increased cellular senescence. Inhibition of GJIC via 18-GA and octanol decreased telomere length in human endometrial stromal cells [85], suggesting a promoting role for GJIC in immortalization. Nicotinamide adenine dinucleotide (NAD^+), which can be transferred through GJs and taken up/released by hemichannels, also stabilizes telomeres through its role as a cofactor for sirtuins [117]. Furthermore, specific inhibition of Cx43 hemichannels via the mimetic peptide TAT-Gap19, which inhibits hemichannel activity by binding to the intracellular tail, preventing interaction with the intracellular loop [118,119], decreased radiation-induced senescence [120]. However, an additional study observed no changes in senescence of glioma cells upon overexpression of Cx43 [121]. Together, these studies suggest that connexins and connexin channels may affect the immortality of some cell types, but the direction of this effect may be cell-type specific. This is an area that is primed for future study.

Cancer Stem Cells

Cancer stem cells (CSCs, also called cancer- or tumor-initiating cells) are defined as tumor cells that exhibit sustained proliferation; the ability to self-renew or generate an additional stem cell during cell division; and the ability to generate a tumor with similar heterogeneity to the original tumor [122]. Thus, CSCs are important not only for the immortality of a CSC population but also for persistence of a tumor after therapy. The role of GJIC in these cells is complicated not only by the many types and anatomical sites of human cancer but likely also by the variety of CSC populations found within a given cancer. In some instances, it has been observed that CSCs have low expression of connexin genes and exhibit low GJIC (e.g., [123–125]; see also reviews on the topic [125,126]). This is supported by some observations in CSCs or CSC-like cells from glioma [127,128], liver cancer [129,130], lung cancer [131,132], triple-negative breast cancer [76], and adenoid cystic carcinoma [133]. However, other studies have shown the presence of GJIC in glioblastoma [33,134], gastric cancer [135], lung cancer [136], and breast cancer [137,138].

Only a few studies have gleaned insight into the mechanisms by which connexin functions affect CSC phenotypes, and, as for other cancer hallmarks, some are contradictory. While studies tend to agree that glioma CSCs express low levels of Cx43, they differ for example on whether and how this is mechanistically important. Cultured glioma CSC-like cells have been shown to express low levels of all connexins tested and to exhibit little GJIC, and re-expression of Cx43 inhibited CSC characteristics including proliferation, self-renewal, and tumor initiation by increasing and binding to E-cadherin, which correspondingly lowered WNT/β-catenin signaling [127]. Other studies have shown similar effects of Cx43 expression in glioma CSCs but attributed its effects to a Cx43-mediated inhibition of SRC kinase activity [139]. This work suggested that Cx43 may suppress the CSC state through channel-independent mechanisms. However, another study observed GJIC in glioblastoma CSCs and found that inhibition of GJIC via CBX or octanol inhibited their growth and self-renewal, as well as tumor growth in vivo [33]. These CSCs expressed lower levels of Cx43 compared to non-stem cells, as previously reported [127], but relatively higher levels of Cx46 [33]. Blockage of GJIC by the seemingly Cx46-specific phenazine dye clofazimine or expression of a Cx46 GJIC-incompetent mutant phenocopied the use of more general GJ inhibitors and suggested that glioblastoma CSCs may rely on Cx46-mediated GJIC [134].

While, to our knowledge, no role for connexin hemichannels in CSC biology has yet been reported, several other interesting non-membrane functions have been described. Cx26 is expressed at relatively higher levels in triple-negative breast cancer CSCs compared to non-stem cells and maintains the properties of these cells via an intracellular complex with focal adhesion kinase (FAK) and the pluripotency transcription factor NANOG [76]. Intracellular Cx32 expression in hepatoma cells also increased the self-renewal of cells, suggesting that Cx32 may play a cytoplasmic role in

maintaining a CSC-like state [140]. Thus, it is likely that connexin proteins exert both CSC-promoting and CSC-inhibitory effects depending on the connexin and the biological system, and, as the field of CSC biology continues to mature, further study may shed additional light on this phenomenon and the mechanisms underlying it.

2.4. Angiogenesis

The growth of new blood vessels within a tumor is critical for the delivery of oxygen and other nutrients. Knockdown of any of the major connexins expressed in endothelial cells, Cx43, Cx37, and Cx40, compromises endothelial branching in vitro [141], and Cx43, Cx37, and Cx40 knockout mice also exhibit vasculogenic/angiogenic defects [142–144]. While these studies show the importance of connexins for vessel growth and remodeling, they do not provide mechanistic insights into the functions of connexins that are necessary for these processes. Although coupling of tumor cells with endothelial cells is also important for cancer cell migration and extravasation (discussed next), here, we focus specifically on angiogenic processes.

2.4.1. GJIC

Communication between tumor cells and endothelial cells in the microenvironment is necessary for stimulation of angiogenesis. In glioma, Cx43 has been suggested to be both pro-angiogenic and anti-angiogenic. On the pro-angiogenic side, co-culture of glioma cells expressing Cx43 with human umbilical vein endothelial cells (HUVECs) stimulated HUVEC tube formation, an angiogenic process [145]. Functional GJs formed between the two cell types, and, because the effect on tube formation required direct cell–cell contact, it is likely that this effect was due to GJIC. Similarly, in a non-cancer setting, bone marrow mononuclear cells activate angiogenesis by GJIC with endothelial cells [146]. However, anti-angiogenic roles have also been reported. Knockdown of Cx43 in human glioma cells increased their ability to induce angiogenesis [147], although the mechanism of this effect was not clear. Similarly, breast cancer cells release the proliferative inhibition of endothelial cells by mural cells by secreting a signal that inhibits Cx43-mediated GJIC between these two cell types [148]. miRNAs transported through GJs have also been shown to affect angiogenesis. GJ-mediated transfer of miR-145-5p from human microvascular endothelial cells to colorectal cancer or glioblastoma cells inhibits the ability of the cancer cells to stimulate angiogenesis [149,150]. This is accompanied by a reciprocal transfer of miR-5096 from tumor cells to endothelial cells, which stimulates tubulogenesis of HMECs and therefore appears to be pro-angiogenic [150]. Thus, interestingly, GJIC may be both pro- and anti-angiogenic even in the same cancer. Finally, Cx40 has been shown to have a pro-angiogenic function in tumor cells. Inhibition of Cx40-mediated GJIC in a mouse lung tumor model via treatment with [40]Gap27, a GJIC-specific peptide mimetic inhibitor of Cx40, reduced tumor growth and angiogenesis [143]. These results suggest then that GJIC, particularly that mediated by Cx43, has a range of effects on endothelial cell proliferation and tumor angiogenesis.

2.4.2. Connexin Hemichannels

While, to our knowledge, no documented effects of connexin hemichannel function on tumor angiogenesis have been reported, it is tempting to speculate that many molecules found in the tumor microenvironment that would be permeable to hemichannels may affect vessel formation. Nucleotides including ATP and UTP released from breast cancer cells stimulated P2Y purinoceptor 2 (P2Y2R) [151], which can induce vascular sprouting [152]. Ca^{2+} [153,154] and the cAMP-dependent kinase PKA [155] have also been shown to affect angiogenesis. Overexpression of Cx43 in mouse melanoma and breast cancer cells inhibited angiogenesis, and this was mediated by a soluble factor in conditioned medium, suggesting that this could be due to hemichannel release of some molecule [156–158]. Thus, while no clear roles for hemichannel activity of connexins have been documented at this point, it is possible and even likely that hemichannels present on tumor cells or other microenvironmental cells may play a role in tumor angiogenesis.

2.4.3. Non-Junctional Roles

Similar to hemichannel involvement in angiogenesis, no clear roles for non-junctional functions of connexins have been described in angiogenesis, but these effects likely occur. Comparison of a GJIC-incompetent mutant of Cx26 with wild-type Cx26 suggested that Cx26 controls the expression of angiogenesis-associated genes through both GJ-dependent and GJ-independent mechanisms [159], as expression of both forms of the protein upregulated expression of thrombospondin, an anti-angiogenic protein. This GFP-Cx26 appears to be non-functional for GJIC or as a hemichannel, suggesting that this is a non-junctional role [159]. Together, it is clear that GJIC is the best understood role of connexins in tumor angiogenesis, but there is potential for the discovery of additional roles.

2.5. Invasion and Metastasis

Other than functions in cancer cell proliferation, the role of connexins in migration, invasion, and metastasis is the most frequently studied and has been comprehensively reviewed (e.g., [13,17,160–163]). Here, again, we focus on developing a mechanistic understanding of how connexin functions promote or inhibit invasion and metastasis.

2.5.1. GJIC

The role of GJs during invasion and metastasis likely relates back to the role of connexins during different stages of tumorigenesis (diagrammed nicely in [14,20]). It has been proposed that connexins more frequently function to suppress the early stages of transformation (although, again, this is cancer and connexin dependent) but enable later stages such as metastasis. Particularly in the context of invasion and metastasis, GJs may serve an adhesive function in addition to their communicative role (reviewed in [6,164,165]). One could envision a situation where, during metastasis, a decrease in any homocellular GJs present between cancer cells would first need to occur, followed by an increase in heterocellular GJs between cancer cells and microenvironmental cells, both to enable migration and to establish a metastatic lesion in a satellite location.

One of the most striking examples of a role for heterocellular GJIC during tumor metastasis was provided by the Massagué group for breast cancer metastasis to the brain. This model suggests that metastatic breast cancer cells form functional heterocellular GJs composed of Cx43 with astrocytes, and transfer of cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) from tumor cells to astrocytes through these GJs stimulates the production of inflammatory cytokines by the astrocytes [166]. These cytokines then reciprocally activate the signal transducer and activator of transcription 1 (STAT1) and NF-κB pathways in the tumor cells, ensuring the proliferation of the metastatic cells. Similarly, work in glioma suggested that homocellular GJs between glioma cells inhibit metastasis, while heterocellular GJs formed between glioma cells and astrocytes promote metastasis in a GJIC-dependent manner [167]. However, this contrasted a study by the Naus laboratory indicating that, while functional GJIC between astrocytes is required for glioma invasion, GJIC between astrocytes and glioma cells is dispensable [168]. Furthermore, both homocellular GJIC between breast cancer cells and heterocellular GJIC between breast cancer cells and endothelial cells promoted cancer cell diapedesis and thus invasion and metastasis [169]. Other studies also support the idea that GJIC promotes tumor metastasis (in breast cancer [170] and lymphoma [171], Cx43 and Cx26 in breast cancer and melanoma [172], Cx26 in melanoma [173,174], Cx43 in breast cancer [175], Cx43 in glioma [168,176,177], Cx43 in non-small cell lung cancer [178], and Cx43 in prostate cancer [179], among other studies). However, suppression of metastasis by GJIC has also been reported, often even for the same connexins in the same types of cancer (Cx26 and Cx43 in breast cancer [157], Cx43 in glioblastoma [180], Cx43 in breast cancer [181], and Cx32 in cervical cancer [182]). A recent study used a GJIC-incompetent Cx43 mutant to show that Cx43 GJIC is required for suppression of epithelial-to-mesenchymal transition (EMT) genes by osteosarcoma cells when cultured with osteoblasts, suggesting that heterocellular GJIC between these two cells types restrains metastatic potential [183]. Thus, additional work is required to

resolve these conflicting results and clarify the role of GJIC in tumor invasion and metastasis, ideally through the use of an in vivo model system to ensure the presence of a tumor microenvironment. In vivo studies of cancer cell migration and invasion would particularly benefit from the development of new tools to target GJIC, including inhibitors with increased specificity or approaches to engineer tissue-specific expression of connexin mutants.

2.5.2. Connexin Hemichannels

While it may be difficult at times to experimentally distinguish between communication and adhesion mediated by GJs in metastasis and invasion, reported roles for connexin hemichannels would suggest that cells can receive pro- and/or anti-metastatic signals from the microenvironment in addition to signals from other cells. Several studies have linked connexin hemichannels and migration of non-transformed cells. In the context of the normal brain, Cx43 hemichannels are necessary—but not sufficient—for neuron-induced astrocyte migration [184,185], and calcium waves released through hemichannels have been proposed to play a role in neuronal proliferation and migration during development [186]. There are only a few studies that document a clear role for connexin hemichannels during invasion and metastasis. The first showed a microenvironmental role for hemichannels rather than a tumor cell-intrinsic role: open hemichannels on osteocytes create high ATP concentrations in the extracellular space, which inhibits the metastasis of breast cancer cells to the bone [53]. This leads to the question of whether hemichannels are then active on osteocytes in a non-tumor context or whether the presence of tumor cells modifies the behavior of microenvironmental bone cells, which would have implications for the assumption that hemichannels open primarily in disease states. Subsequent studies have also shown pro-metastatic roles for hemichannels on cancer cells. Recent work showed that breast cancer cell collective invasion occurred via hemichannel-released adenosine nucleosides/nucleotides, which activated purinergic receptors in an autocrine fashion to promote leader cell function [187]. Finally, a fascinating report suggested that, in addition to the passage of small molecules, hemichannels can also function for adhesion. The expression of high levels of Cx43 in glioma cells stimulated adhesion to cells with low Cx43 expression [188]. While this study did not rule out alterations in other cell adhesion markers or mechanisms in response to Cx43 expression, the idea that connexin hemichannels may play a role in cell adhesion may deserve additional study in metastasis.

2.5.3. Non-Junctional Roles

Compared to other cancer phenotypes, there is comparatively more information available regarding a non-junctional role for connexins in cancer cell invasion and metastasis, with both pro- and anti-metastatic functions described depending on context. In glioma cells, the C-terminal tail of Cx43 was sufficient to stimulate motility in a GJIC-independent manner (although the channel domain alone also stimulated migration, suggesting that Cx43 may induce glioma cell invasion through multiple mechanisms [189,190]). This tail-mediated mechanism might involve cytoskeletal rearrangement driven by interaction with cellular communication network factor 3 (CCN3), an integrin ligand involved in adhesion, as was shown for breast cancer cells [191]. Non-junctional pro-metastatic roles for Cx43 have also been described in prostate cancer [179,192], ovarian cancer [193], and cervical cancer [194], with additional non-junctional functions reported for Cx32 in hepatoma cells [195]. Studies on non-junctional, anti-metastatic connexin functions have been pioneered by the Laird laboratory, particularly for breast cancer. Cx26 inhibited breast cancer cell invasion through Matrigel by reducing matrix metalloproteinase (MMP) activity and integrin β1 levels even when localized intracellularly [64]. Similarly, Cx43 suppressed the invasion of transformed keratinocytes in a GJIC-independent manner via C-terminal interaction with caveolin-1 [196]. Suppression of invasion has also been shown for Cx32 in renal cell carcinoma cells, via a SRC-STAT3-vascular endothelial growth factor (VEGF) pathway [197], and for Cx43 in prostate cancer cells when localized intracellularly [179]. Translocation of the truncated GJA1-20k to the nucleus also stimulates N-cadherin expression and drives collective cell migration [198].

As for GJIC, there is still much work to do to understand non-junctional roles for connexins in tumor metastasis and invasion, but it is apparent that connexins play a substantial role in these processes.

3. Mechanistic Roles of Connexins in More Recently Appreciated Cancer Phenotypes

In their updated "Hallmarks of Cancer: The Next Generation," Hanahan and Weinberg added what they termed "emerging" or "enabling" characteristics of cancers [2]. Connexins play a role in each of these four cancer phenotypes, which include altered energy metabolism, avoiding the immune system and inducing inflammation, and genetic instability, and, to this point, the majority of functions that have been described appear to be channel dependent (with the exception of a few examples for genomic instability, as noted below). Particularly for metabolism, it is easy to see how the transfer of small metabolites through GJs is likely to play a role in cancer. The potential for non-junctional roles of connexins in these cancer phenotypes represents an opportunity for further discovery.

3.1. Alterations in Energy Metabolism

3.1.1. GJIC

Cancer cells require high amounts of energy to support increased proliferation, and they typically take up high amounts of glucose to provide this energy. However, rather than utilizing oxidative phosphorylation to produce ATP from this glucose, cancer cells often divert their glucose metabolism to an aerobic glycolytic pathway, which produces lower amounts of ATP more quickly and also less reactive oxygen species (ROS), along with more lactate [199]. As described above, molecules such as glucose, as well as ATP, AMP, guanosine triphosphate (GTP), cAMP, and other small energy carriers, are able to pass through GJs. In fact, in glioma cells, ATP and ADP made up more than 6% of the total material derived from labeled glucose that passed through GJs [200], and the passage of ATP through GJs has been implicated in many of the abovementioned cancer phenotypes.

For this reason, it is clear that altered expression of connexins or altered function of GJIC or hemichannels on cancer cells is likely to affect the energy resources of both tumor cells and the microenvironment. This has been well described in the context of normal astrocytes and transformation into malignant astrocytomas such as gliomas [201]. The loss of Cx43 in normal astrocytes, which presumably decreases glucose influx and thus the total amount of glucose available to the cell, drives an increase in membrane localization of the GLUT-1 and GLUT-3 glucose transporters [202], increasing glucose uptake [203]. This is a tempting model for alterations in glucose import in response to transformation of normal astrocytes into astrocytomas/gliomas driven by a loss of Cx43, particularly as gliomas primarily obtain glucose through high expression of GLUT1/3 [204]. However, the situation does not seem to be so simple, as restoring Cx43 function to rat C6 glioma cells did not alter GLUT1/3 localization or expression [205]. Instead, increased GJIC caused a release of hexokinase from the mitochondrial membrane, which results in decreased enzymatic activity and lowered ability to phosphorylate glucose, the first step of glycolysis [205]. Thus, an absence of GJIC is likely to play an important role in the ability of cancer cells to maintain glycolysis, although the mechanism of how GJIC alters hexokinase localization remains to be determined.

Tumors are heterogeneous and contain perivascular (oxygen- and nutrient-rich) areas and hypoxic (oxygen- and nutrient-poor) areas, in addition to a variety of cancer and non-transformed cell types. In conditions of hypoxia, cancer cells primarily produce energy through glycolysis, which leads to a buildup of acidic byproducts [206]. Coupling between normoxic and hypoxic tumor cells via GJs allows the passage of HCO_3^- ions from normoxic to hypoxic cells, which helps neutralize H^+ ions [206]. In this way, Cx43 GJs may help maintain hypoxic tumor cells. Cancer-associated fibroblasts (CAFs), which are found in the tumor microenvironment, form GJs with non-small cell lung cancer (NSCLC) cells, which drives aerobic glycolysis in the CAFs and increased oxidative phosphorylation in the cancer cells [178]. This leads to increased activation of the phosphatidylinositol 3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK)/ERK pathways and cancer cell invasion by inducing EMT.

Together, these studies all support a model where Cx43-mediated GJIC affects tumor cell metabolism, although some studies show that GJIC promotes tumor properties and some show that GJIC suppresses tumor properties. This again may be context dependent, perhaps a situation where heterocellular GJIC aids in tumor growth, while homocellular GJIC between tumor cells suppresses it.

3.1.2. Connexin Hemichannels

Connexin hemichannels have been shown to release NAD^+, a co-enzyme involved in many metabolic pathways [21,207], suggesting that hemichannels may work to support high levels of glycolysis in surrounding cells while potentially decreasing glycolysis in cancer cells themselves. Additionally, while it remains to be seen whether this is also the case in cancer cells, Cx43 has been shown to localize to mitochondria, in particular the inner mitochondrial membrane [208,209], in cardiomyocytes. It appears likely that the protein exists as part of a functional hemichannel structure there, as cross-linking studies suggest the presence of connexin hexamers that are capable of transfer of Lucifer yellow and can be inhibited by CBX and heptanol [208]. Treatment with 18-GA showed that these hemichannels appear to be important for mitochondrial K^+ flux, the inhibition of which is important for the maintenance of cancer cells, with restoration of normal flux leading to apoptosis [210]. K^+ flux is also involved in mitochondrial respiration [211,212], and, consistently, inhibition of mitochondrial hemichannel Cx43 reduces mitochondrial ATP production via respiration [213]. Furthermore, Cx43 has also been shown to regulate Ca^{2+} entry into the mitochondria in the heart, potentially leading to cell death [214]. Thus, connexin hemichannels release and take up molecules that play roles in metabolic pathways, and, although connexin localization in the mitochondrial membrane has not been shown in cancer, this represents an intriguing possibility to directly affect the metabolic state of cancer cells.

3.2. Inflammation and Immune Evasion

3.2.1. GJIC

Over the past two decades, an appreciation has developed for the role of GJs in immune function and inflammation in cancer, with primarily anti-tumorigenic roles reported (reviewed in [215–217]). Connexins are expressed on most types of immune cells, and GJIC is important for a variety of immune cell functions, including hematopoiesis, antigen presentation, phagocytosis, migration, and inflammation, among others [215]. Combination treatment with the pro-inflammatory cytokines TNFα and interferon gamma (IFNγ) upregulates Cx43 expression on monocytes/macrophages [218] and microglia [219] and leads to a transient homocellular GJ coupling. Inhibition of this newly established GJIC via 18-GA decreases transmigration of monocytes across a model blood–brain barrier, suggesting that immune cell GJIC stimulated by inflammation may play a role in the recruitment of immune cells to tumor sites (a potential anti-tumor role).

GJIC also plays a role upstream of TNFα and IFNγ production: the transfer of the second messenger cGAMP from tumor cells to astrocytes, macrophages, and dendritic cells (DCs) via GJIC induces expression of pro-inflammatory cytokines [166,220,221] and leads to activation of CD8+ T cells that target cancer cells [220]. However, cGAMP transfer can also be pro-tumorigenic by leading to the proliferation of tumor cells [166], indicating that inflammation serves multiple roles in the context of cancer. GJIC is also involved in antigen presentation through direct coupling between monocytes and DCs [222], between DCs [223], between tumor cells and endothelial cells [224], and at the immune synapses formed between melanoma cells and CD4+ T cells [225], melanoma cells and natural killer (NK) cells [226], and DCs and CD4+ T cells [227]. Moreover, GJIC between tumor cells and NK cells inhibited tumor cell targeting by NK cells [226]. Coupling between macrophages and tumor cells has also been shown to transfer tumor-suppressive miRNAs to the cancer cells [3]. These examples suggest that the ability of the immune system to target cancer cells is increased by: (1) heterocellular GJIC between tumor cells and immune cells; (2) heterocellular GJIC between different types of immune cells; and (3) homocellular GJIC within a population of immune cells, further supporting an anti-tumorigenic

role for connexins, with variable effects of pro-inflammatory cytokines. As immunotherapies are further tested and developed as anti-cancer therapies, the role of GJs and the potential of treatments to increase GJIC should be considered.

3.2.2. Connexin Hemichannels

It is clear that, in addition to GJs, hemichannels also localize to the immune synapse [228]. However, their function there is less understood. The release of ATP by hemichannels on activates purinergic receptors on T cells in an autocrine manner, which is necessary for T cell activation [229]. However, while connexin hemichannels do release ATP [230], pannexins appear to be more important for ATP-mediated activation at the immune synapse, as preferential inhibition of pannexin channels had a much greater effect on T cell activation compared to connexin hemichannel inhibition [229,231]. It has also been reported that Cx43 hemichannels on T cells are important for T cell proliferation [232]. A role for pannexins was not excluded here, but the use of a blocking antibody (Gap7M) that binds to the extracellular domain of several connexins would suggest specificity for connexin hemichannels. Thus, while connexin hemichannels have the potential to drive T cell activation, in practice, it seems that they may have a more established role in T cell proliferation. Furthermore, release of NAD^+ through hemichannels also has the potential to drive an anti-tumor immune response (reviewed in [21]). NAD^+ has a variety of roles in immune cells (reviewed in [233]), including determining T cell fate.

However, there has also been a documented role for connexin hemichannels in promoting inflammation (reviewed in [234]), potentially through the release of ATP to activate cytokine release by macrophages. Connexin mimetic peptides that block hemichannel function, including aCT1 and JM2, decrease inflammation during wound healing [235,236] and in response to CNS injuries [237,238]. When these observations are taken together, connexin hemichannels seem to have divergent functions, as they are important for the function of the immune system to activate an anti-tumor response but also drive inflammation that promotes tumor growth.

3.3. Genomic Instability

3.3.1. GJIC

Connexins impact genomic stability in a variety of ways, some direct and some indirect. Inflammation, as detailed above, can lead to DNA damage and genomic instability, suggesting that connexin functions that promote inflammation might then affect DNA stability [239]. GJIC plays a major role in the bystander effect, whereby irradiated cells can spread DNA damage-causing molecules to non-irradiated cells via GJIC [240], likely through the generation of reactive oxygen species (ROS) in the irradiated cell and, through the bystander effect, surrounding cells (reviewed in [241]). This phenomenon has been termed the "kiss of death," whereby cells share their toxic signals, including ROS molecules themselves [242], with surrounding cells [243]. In addition to irradiation, ROS can be generated through multiple mechanisms in which GJIC plays a role, including tumor cell metabolism and inflammation, and, through this role in DNA damage, can be pro-transformative (reviewed in [244]). However, GJIC can also provide a "kiss of life" [243], where the presence of functional GJs protects cells against oxidative stress and thus genomic instability by allowing the outward diffusion of pro-death signals; for example, GJIC protected human retinal pigment epithelial cells from death induced by a chemical oxidant [245] and astrocytes from ROS-induced oxidative stress [246]. The situation becomes even more complex when considering that antioxidants such as glutathione can also pass through GJs [247], suggesting that the local cellular concentrations of ROS and antioxidants will determine whether GJIC promotes or inhibits ROS-mediated DNA damage. Similarly, the degree to which ROS damage DNA and the ability of a cell to repair this damage will affect whether the DNA damage promotes transformation or is lethal to the cell.

3.3.2. Connexin Hemichannels

Most of the roles of GJIC in genomic instability also apply to hemichannels. Even the bystander effect, which is often thought of as requiring a direct coupling between cells, can be mediated by soluble factors in conditioned media, potentially through release and uptake by hemichannels [248]. This effect was recently shown to be inhibited by the hemichannel function-specific Cx43 mimetic peptide Gap19, suggesting that this effect is in fact due to hemichannels [120,249]. Both DNA damage-inducing ROS [120,246,249] and the antioxidant glutathione [250,251] are also able to pass through hemichannels. NAD^+, which can be released and taken up by hemichannels [252], also plays a role in genomic stability through the activity of sirtuin deacetylases [253,254]. The enzymatic activity of sirtuins requires NAD^+ [255], and SIRT2 and SIRT6 confer resistance to DNA damage by deactylating histones and inhibiting DNA recombination [253,254]. Cx43 has been proposed to act as a histone deacetylase inhibitor in a manner involving soluble factors [256], and this indirect effect on sirtuins mediated by NAD^+ release may be the underlying mechanism for this observation. Thus, as for GJs, the effects of connexin hemichannel activity on DNA damage and genomic instability likely depend on context, with the intra- and extracellular concentrations of molecules determining whether they are pro- or anti-tumorigenic.
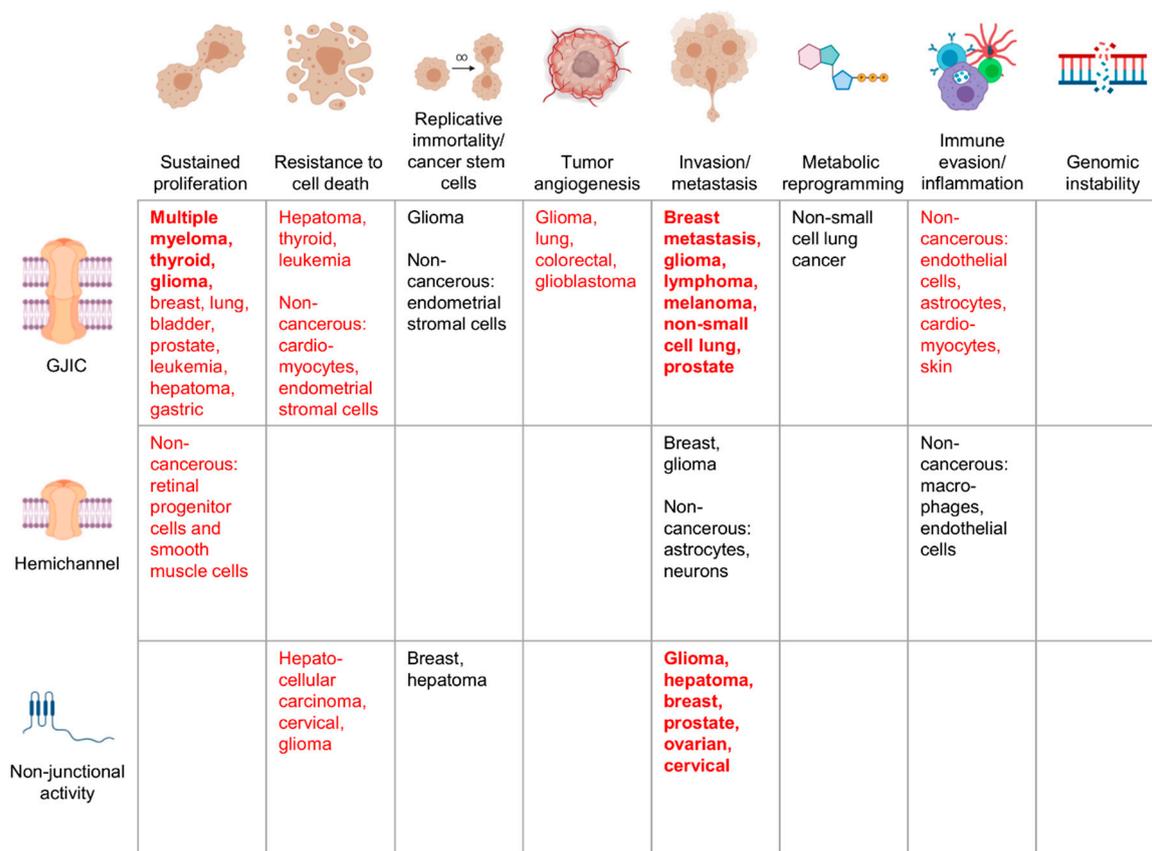
3.3.3. Non-Junctional Roles

One interesting study showed a non-channel role for Cx43 in genomic instability. Transfection of HeLa cells with Cx43 cDNA decreased genomic instability, but this effect was not lost upon treatment with 18-GA [257]. This suggests a fundamental role for Cx43 in maintaining genomic stability that is not mediated by the transfer of small molecules but rather by the protein itself, although the mechanisms of this remain to be determined. Less directly, expression of the GJA1-20k truncation form of Cx43, which contains part of the fourth transmembrane domain and the C-terminal tail (see [12]), in the heart decreased cellular ROS [258]. However, GJA1-20k acts as a type of chaperone to recruit full-length Cx43 to the plasma membrane, suggesting that it may also impact its channel activity [79]. Thus, it is not clear whether this effect is due to a channel-dependent or a channel-independent function of the protein. Regardless, the potential for non-channel functions of connexins to maintain genomic stability is fascinating and deserves further study.

4. Conclusions and Future Perspectives

Over the past 60 years, the field has advanced from the observation that cancer cells often lose communication and metabolic coupling to an understanding of many of the molecular and functional mechanisms underlying the role of connexins in cancer (Figures 1 and 2). As our knowledge continues to expand, it becomes clear that, despite the variety of connexin proteins and functions, many of the roles executed by connexins in cancer cells are tightly interconnected. The transfer and uptake/release of small molecules including ATP and NAD^+ are upstream of most—if not all—cancer phenotypes, supporting the hypothesis that altered connexin activity may be a unifying hallmark of cancer [259]. Remaining questions include the identity of essential molecules being transferred, the functional effects of connexin mislocalization, and the differential selectivity of different combinations of connexins. There is also a lot still to be learned about non-junctional roles for connexin proteins in cancer.

When evaluating connexin function in cancer, it is important to note that the role of a connexin in a given cancer cell will depend on the context, including the cell and tumor type, the connexin isoforms expressed in the cell, and the specific microenvironment of the cell (including the concentration of extracellular molecules and the surrounding cells available for GJ coupling), as well as the mutational and expression profiles of the cell. While mutations in connexin genes typically occur with only a low frequency in cancers [14], these alterations in connexin sequence likely affect function in some cases. The importance of GJIC between cancer cells (homocellular communication) may be very different from the necessity of communication between a tumor cell and a non-transformed cell in the
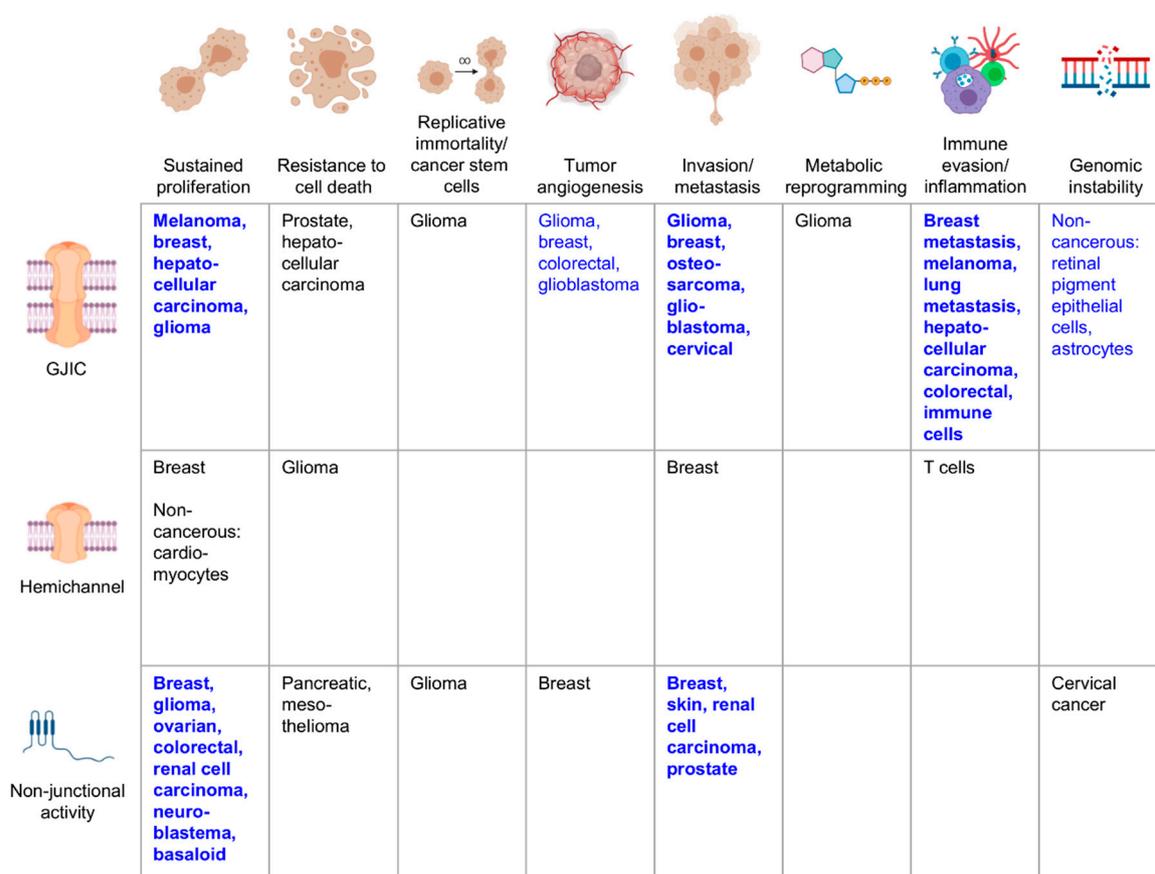
microenvironment (heterocellular communication). Differences in context likely underlie the many contradicting reports of connexin functions in cancer cells. Furthermore, relevant to the discussion presented here, multiple cancer phenotypes likely lie downstream of one connexin function, for example, the multiple roles of ATP, NAD$^+$, and ROS transfer through GJs and uptake and release by hemichannels.

	Sustained proliferation	Resistance to cell death	Replicative immortality/ cancer stem cells	Tumor angiogenesis	Invasion/ metastasis	Metabolic reprogramming	Immune evasion/ inflammation	Genomic instability
GJIC	**Multiple myeloma, thyroid, glioma,** breast, lung, bladder, prostate, leukemia, hepatoma, gastric	Hepatoma, thyroid, leukemia Non-cancerous: cardio-myocytes, endometrial stromal cells	Glioma Non-cancerous: endometrial stromal cells	Glioma, lung, colorectal, glioblastoma	**Breast metastasis, glioma, lymphoma, melanoma, non-small cell lung, prostate**	Non-small cell lung cancer	Non-cancerous: endothelial cells, astrocytes, cardio-myocytes, skin	
Hemichannel	Non-cancerous: retinal progenitor cells and smooth muscle cells				Breast, glioma Non-cancerous: astrocytes, neurons		Non-cancerous: macro-phages, endothelial cells	
Non-junctional activity		Hepato-cellular carcinoma, cervical, glioma	Breast, hepatoma		**Glioma, hepatoma, breast, prostate, ovarian, cervical**			

Figure 1. Cancers in which specific connexin functions have been described to be pro-tumorigenic. Schematic showing examples of the cancer types in which pro-tumorigenic properties have been attributed to a specific connexin function, whether gap junction intercellular communication (GJIC), connexin hemichannels, or a non-junctional function. Bold red text indicates strong evidence from many cancer types tying the connexin function to the corresponding cancer hallmark, with red text indicating a less robust link and black text indicating emerging evidence. Where mechanistic studies in cancer cells are not available, certain non-transformed cell populations are indicated. References for the indicated cancers are provided in the text. Figure illustrations generated using BioRender.com.

It has been challenging to determine whether alterations in connexin expression observed in tumor tissues have a direct or indirect effect on cancer cell phenotypes. The use of knockdown/knockout approaches has facilitated an understanding of these effects in animal models and cultured cells, but these techniques still do not shed light on the mechanisms underlying the effect of alterations in connexin expression. Furthermore, cells may compensate for removal of one connexin by upregulating another family member, further complicating the situation. One challenge of the field is the small number of truly specific inhibitors that target a given function of a given connexin. The development of better inhibitors is complicated by the high degree of similarly among connexins and the complex interplay among connexin functions. Non-specific inhibitors of GJs, including CBX, oleamide, mefloquine, octanol, 18-GA, and others [260], have been used and have contributed valuable knowledge to the field. However, these types of inhibitors are not ideal due to their off-target or non-specific effects and

their lack of selectivity for specific connexin proteins and specific connexin functions [260]. Work on Cx43 has been dramatically aided by the development of the Cx43 mimetic peptides that target one or more connexin function, although these peptides also modulate the functions of other connexins to varying degrees [10]. While the most mechanistic work has been performed for Cx43, it is clear that other connexins also have roles in cancer phenotypes. For other connexins, the only options to isolate the role of GJ vs. hemichannel vs. non-channel functions remain either nonspecific inhibitors or mutation of the connexin. Connexin point mutations bring about their own set of challenges, including requiring either altering the levels of protein expressed via overexpression or genome editing, as well as a detailed understanding of the structure–function relationships within the molecule. However, connexin point mutations may still affect more than one connexin function concurrently (for example, GJ and hemichannel activity or protein–protein binding and channel function), confounding the interpretation of results.

	Sustained proliferation	Resistance to cell death	Replicative immortality/ cancer stem cells	Tumor angiogenesis	Invasion/ metastasis	Metabolic reprogramming	Immune evasion/ inflammation	Genomic instability
GJIC	**Melanoma, breast, hepato-cellular carcinoma, glioma**	Prostate, hepato-cellular carcinoma	Glioma	Glioma, breast, colorectal, glioblastoma	**Glioma, breast, osteo-sarcoma, glio-blastoma, cervical**	Glioma	**Breast metastasis, melanoma, lung metastasis, hepato-cellular carcinoma, colorectal, immune cells**	Non-cancerous: retinal pigment epithelial cells, astrocytes
Hemichannel	Breast Non-cancerous: cardio-myocytes	Glioma			Breast		T cells	
Non-junctional activity	**Breast, glioma, ovarian, colorectal, renal cell carcinoma, neuro-blastema, basaloid**	Pancreatic, meso-thelioma	Glioma	Breast	**Breast, skin, renal cell carcinoma, prostate**			Cervical cancer

Figure 2. Cancers in which specific connexin functions have been described to be tumor suppressive. Schematic showing examples of the cancer types in which tumor suppressive properties have been attributed to a specific connexin function, whether gap junction intercellular communication (GJIC), connexin hemichannels, or a non-junctional function. Bold blue text indicates strong evidence from many cancer types tying the connexin function to the corresponding cancer hallmark, with blue text indicating a less robust link and black text indicating emerging evidence. Where mechanistic studies in cancer cells are not available, certain non-transformed cell populations are indicated. References for the indicated cancers are provided in the text. Figure illustrations generated using BioRender.com.

Although the existence of connexin hemichannels has become more accepted in the field, their study continues to be complicated by the presence of pannexin hemichannels, which are sensitive to many of the same inhibitors as connexin channels [10]. The development of approaches to target the functions of connexins other than Cx43 without disrupting pannexin function will greatly aid

our understanding of connexins in cancer and provides an immense opportunity to gain insight into the importance of connexin activity in both cancer and normal physiology. These types of inhibitors will also have a high therapeutic potential to treat the multitude of pathological conditions, including cancer, in which connexins are involved.

Author Contributions: Conceptualization, E.E.M.-H. and J.D.L.; Funding acquisition, O.R. and J.D.L.; Investigation, E.E.M.-H.; Supervision, J.D.L.; Visualization, E.E.M.-H. and J.D.L.; Writing—original draft, E.E.M.-H.; Writing—review & editing, all authors. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Loewenstein, W.R.; Kanno, Y. Intercellular communication and the control of tissue growth: Lack of communication between cancer cells. *Nature* **1966**, *209*, 1248–1249. [CrossRef]
2. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646–674. [CrossRef] [PubMed]
3. Aucher, A.; Rudnicka, D.; Davis, D.M. MicroRNAs transfer from human macrophages to hepato-carcinoma cells and inhibit proliferation. *J. Immunol.* **2013**, *191*, 6250–6260. [CrossRef] [PubMed]
4. Suzhi, Z.; Liang, T.; Yuexia, P.; Lucy, L.; Xiaoting, H.; Yuan, Z.; Qin, W. Gap Junctions Enhance the Antiproliferative Effect of MicroRNA-124-3p in Glioblastoma Cells. *J. Cell. Physiol.* **2015**, *230*, 2476–2488. [CrossRef] [PubMed]
5. Peng, Y.; Wang, X.; Guo, Y.; Peng, F.; Zheng, N.; He, B.; Ge, H.; Tao, L.; Wang, Q. Pattern of cell-to-cell transfer of microRNA by gap junction and its effect on the proliferation of glioma cells. *Cancer Sci.* **2019**, *110*, 1947–1958. [CrossRef]
6. Matsuuchi, L.; Naus, C.C. Gap junction proteins on the move: Connexins, the cytoskeleton and migration. *Biochim. Biophys. Acta* **2013**, *1828*, 94–108. [CrossRef]
7. Saez, J.C.; Leybaert, L. Hunting for connexin hemichannels. *FEBS Lett.* **2014**, *588*, 1205–1211. [CrossRef]
8. Rozas-Villanueva, M.F.; Casanello, P.; Retamal, M.A. Role of ROS/RNS in Preeclampsia: Are Connexins the Missing Piece? *Int. J. Mol. Sci.* **2020**, *21*, 4698. [CrossRef]
9. Varela-Vazquez, A.; Guitian-Caamano, A.; Carpintero-Fernandez, P.; Fonseca, E.; Sayedyahossein, S.; Aasen, T.; Penuela, S.; Mayan, M.D. Emerging functions and clinical prospects of connexins and pannexins in melanoma. *Biochim. Biophys. Acta Rev. Cancer* **2020**, *1874*, 188380. [CrossRef]
10. Willebrords, J.; Maes, M.; Crespo Yanguas, S.; Vinken, M. Inhibitors of connexin and pannexin channels as potential therapeutics. *Pharmacol. Ther.* **2017**, *180*, 144–160. [CrossRef]
11. Mesnil, M.; Krutovskikh, V.; Piccoli, C.; Elfgang, C.; Traub, O.; Willecke, K.; Yamasaki, H. Negative growth control of HeLa cells by connexin genes: Connexin species specificity. *Cancer Res.* **1995**, *55*, 629–639. [PubMed]
12. Basheer, W.; Shaw, R. The "tail" of Connexin43: An unexpected journey from alternative translation to trafficking. *Biochim. Biophys. Acta* **2016**, *1863*, 1848–1856. [CrossRef] [PubMed]
13. Aasen, T.; Leithe, E.; Graham, S.V.; Kameritsch, P.; Mayan, M.D.; Mesnil, M.; Pogoda, K.; Tabernero, A. Connexins in cancer: Bridging the gap to the clinic. *Oncogene* **2019**, *38*, 4429–4451. [CrossRef] [PubMed]
14. Aasen, T.; Mesnil, M.; Naus, C.C.; Lampe, P.D.; Laird, D.W. Gap junctions and cancer: Communicating for 50 years. *Nat. Rev. Cancer* **2016**, *16*, 775–788. [CrossRef] [PubMed]
15. Aasen, T. Connexins: Junctional and non-junctional modulators of proliferation. *Cell Tissue Res.* **2015**, *360*, 685–699. [CrossRef]
16. Delmar, M.; Laird, D.W.; Naus, C.C.; Nielsen, M.S.; Verselis, V.K.; White, T.W. Connexins and Disease. *Cold Spring Harb. Perspect. Biol.* **2018**, *10*, a029348. [CrossRef]

17. Graham, S.V.; Jiang, J.X.; Mesnil, M. Connexins and Pannexins: Important Players in Tumorigenesis, Metastasis and Potential Therapeutics. *Int. J. Mol. Sci.* **2018**, *19*, 1645. [CrossRef]

18. Mesnil, M. Connexins and cancer. *Biol. Cell* **2002**, *94*, 493–500. [CrossRef]

19. Mesnil, M.; Crespin, S.; Avanzo, J.L.; Zaidan-Dagli, M.L. Defective gap junctional intercellular communication in the carcinogenic process. *Biochim. Biophys. Acta* **2005**, *1719*, 125–145. [CrossRef]

20. Naus, C.C.; Laird, D.W. Implications and challenges of connexin connections to cancer. *Nat. Rev. Cancer* **2010**, *10*, 435–441. [CrossRef]

21. Schalper, K.A.; Carvajal-Hausdorf, D.; Oyarzo, M.P. Possible role of hemichannels in cancer. *Front. Physiol.* **2014**, *5*, 237. [CrossRef] [PubMed]

22. Sinyuk, M.; Mulkearns-Hubert, E.E.; Reizes, O.; Lathia, J. Cancer Connectors: Connexins, Gap Junctions, and Communication. *Front. Oncol.* **2018**, *8*, 646. [CrossRef] [PubMed]

23. Vinken, M.; Vanhaecke, T.; Papeleu, P.; Snykers, S.; Henkens, T.; Rogiers, V. Connexins and their channels in cell growth and cell death. *Cell. Signal.* **2006**, *18*, 592–600. [CrossRef] [PubMed]

24. Wu, J.I.; Wang, L.H. Emerging roles of gap junction proteins connexins in cancer metastasis, chemoresistance and clinical application. *J. Biomed. Sci.* **2019**, *26*, 8. [CrossRef] [PubMed]

25. Yamasaki, H.; Naus, C.C. Role of connexin genes in growth control. *Carcinogenesis* **1996**, *17*, 1199–1213. [CrossRef] [PubMed]

26. Dominiak, A.; Chelstowska, B.; Olejarz, W.; Nowicka, G. Communication in the Cancer Microenvironment as a Target for Therapeutic Interventions. *Cancers* **2020**, *12*, 1232. [CrossRef] [PubMed]

27. Asencio-Barria, C.; Defamie, N.; Saez, J.C.; Mesnil, M.; Godoy, A.S. Direct Intercellular Communications and Cancer: A Snapshot of the Biological Roles of Connexins in Prostate Cancer. *Cancers* **2019**, *11*, 1370. [CrossRef]

28. Pinto, G.; Brou, C.; Zurzolo, C. Tunneling Nanotubes: The Fuel of Tumor Progression? *Trends Cancer* **2020**, *6*, 874–888. [CrossRef]

29. Ariazi, J.; Benowitz, A.; De Biasi, V.; Den Boer, M.L.; Cherqui, S.; Cui, H.; Douillet, N.; Eugenin, E.A.; Favre, D.; Goodman, S.; et al. Tunneling Nanotubes and Gap Junctions-Their Role in Long-Range Intercellular Communication during Development, Health, and Disease Conditions. *Front. Mol. Neurosci.* **2017**, *10*, 333. [CrossRef]

30. Nawaz, M.; Fatima, F. Extracellular Vesicles, Tunneling Nanotubes, and Cellular Interplay: Synergies and Missing Links. *Front. Mol. Biosci.* **2017**, *4*, 50. [CrossRef]

31. Ribeiro-Rodrigues, T.M.; Martins-Marques, T.; Morel, S.; Kwak, B.R.; Girao, H. Role of connexin 43 in different forms of intercellular communication—Gap junctions, extracellular vesicles and tunnelling nanotubes. *J. Cell Sci.* **2017**, *130*, 3619–3630. [CrossRef] [PubMed]

32. Valdebenito, S.; Lou, E.; Baldoni, J.; Okafo, G.; Eugenin, E. The Novel Roles of Connexin Channels and Tunneling Nanotubes in Cancer Pathogenesis. *Int. J. Mol. Sci.* **2018**, *19*, 1270. [CrossRef]

33. Hitomi, M.; Deleyrolle, L.P.; Mulkearns-Hubert, E.E.; Jarrar, A.; Li, M.; Sinyuk, M.; Otvos, B.; Brunet, S.; Flavahan, W.A.; Hubert, C.G.; et al. Differential connexin function enhances self-renewal in glioblastoma. *Cell Rep.* **2015**, *11*, 1031–1042. [CrossRef] [PubMed]

34. Huang, Y.C.; Kuo, C.L.; Lu, K.W.; Lin, J.J.; Yang, J.L.; Wu, R.S.; Wu, P.P.; Chung, J.G. 18alpha-Glycyrrhetinic Acid Induces Apoptosis of HL-60 Human Leukemia Cells through Caspases- and Mitochondria-Dependent Signaling Pathways. *Molecules* **2016**, *21*, 872. [CrossRef] [PubMed]

35. Moosavi, M.A.; Moasses Ghafary, S.; Asvadi-Kermani, I.; Hamzeiy, H.; Rahmati, M.; Ahmadi, A.H.; Nikanfar, A.; Sanaat, Z.; Asadi-Khiavi, M. Carbenoxolone induces apoptosis and inhibits survivin and survivin-DeltaEx3 genes expression in human leukemia K562 cells. *Daru* **2011**, *19*, 455–461.

36. Hasan, S.K.; Siddiqi, A.; Nafees, S.; Ali, N.; Rashid, S.; Ali, R.; Shahid, A.; Sultana, S. Chemopreventive effect of 18beta-glycyrrhetinic acid via modulation of inflammatory markers and induction of apoptosis in human hepatoma cell line (HepG2). *Mol. Cell Biochem.* **2016**, *416*, 169–177. [CrossRef]

37. Jensen, K.; Patel, A.; Klubo-Gwiezdzinska, J.; Bauer, A.; Vasko, V. Inhibition of gap junction transfer sensitizes thyroid cancer cells to anoikis. *Endocr. Relat Cancer* **2011**, *18*, 613–626. [CrossRef]

38. Cao, D.; Jia, Z.; You, L.; Wu, Y.; Hou, Z.; Suo, Y.; Zhang, H.; Wen, S.; Tsukamoto, T.; Oshima, M.; et al. 18beta-glycyrrhetinic acid suppresses gastric cancer by activation of miR-149-3p-Wnt-1 signaling. *Oncotarget* **2016**, *7*, 71960–71973. [CrossRef]

39. Cao, D.; Wu, Y.; Jia, Z.; Zhao, D.; Zhang, Y.; Zhou, T.; Wu, M.; Zhang, H.; Tsukamoto, T.; Oshima, M.; et al. 18beta-glycyrrhetinic acid inhibited mitochondrial energy metabolism and gastric carcinogenesis through methylation-regulated TLR2 signaling pathway. *Carcinogenesis* **2019**, *40*, 234–245. [CrossRef]

40. Huang, R.Y.; Chu, Y.L.; Huang, Q.C.; Chen, X.M.; Jiang, Z.B.; Zhang, X.; Zeng, X. 18beta-Glycyrrhetinic acid suppresses cell proliferation through inhibiting thromboxane synthase in non-small cell lung cancer. *PLoS ONE* **2014**, *9*, e93690. [CrossRef]

41. Lin, K.W.; Huang, A.M.; Hour, T.C.; Yang, S.C.; Pu, Y.S.; Lin, C.N. 18beta-Glycyrrhetinic acid derivatives induced mitochondrial-mediated apoptosis through reactive oxygen species-mediated p53 activation in NTUB1 cells. *Bioorg. Med. Chem.* **2011**, *19*, 4274–4285. [CrossRef] [PubMed]

42. Shetty, A.V.; Thirugnanam, S.; Dakshinamoorthy, G.; Samykutty, A.; Zheng, G.; Chen, A.; Bosland, M.C.; Kajdacsy-Balla, A.; Gnanasekar, M. 18alpha-glycyrrhetinic acid targets prostate cancer cells by down-regulating inflammation-related genes. *Int. J. Oncol.* **2011**, *39*, 635–640. [CrossRef]

43. Sharma, G.; Kar, S.; Palit, S.; Das, P.K. 18beta-glycyrrhetinic acid induces apoptosis through modulation of Akt/FOXO3a/Bim pathway in human breast cancer MCF-7 cells. *J. Cell. Physiol.* **2012**, *227*, 1923–1931. [CrossRef] [PubMed]

44. Sathya, S.; Sudhagar, S.; Sarathkumar, B.; Lakshmi, B.S. EGFR inhibition by pentacyclic triterpenes exhibit cell cycle and growth arrest in breast cancer cells. *Life Sci.* **2014**, *95*, 53–62. [CrossRef] [PubMed]

45. Fu, J. Cx43 expressed on bone marrow stromal cells plays an essential role in multiple myeloma cell survival and drug resistance. *Arch. Med. Sci.* **2017**, *13*, 236–245. [CrossRef] [PubMed]

46. Zhang, Z.Q.; Hu, Y.; Wang, B.J.; Lin, Z.X.; Naus, C.C.; Nicholson, B.J. Effective asymmetry in gap junctional intercellular communication between populations of human normal lung fibroblasts and lung carcinoma cells. *Carcinogenesis* **2004**, *25*, 473–482. [CrossRef] [PubMed]

47. Yamasaki, H.; Krutovskikh, V.; Mesnil, M.; Tanaka, T.; Zaidan-Dagli, M.L.; Omori, Y. Role of connexin (gap junction) genes in cell growth control and carcinogenesis. *C. R. L'Acad. Sci. Ser. III* **1999**, *322*, 151–159. [CrossRef]

48. Grek, C.L.; Rhett, J.M.; Bruce, J.S.; Abt, M.A.; Ghatnekar, G.S.; Yeh, E.S. Targeting connexin 43 with alpha-connexin carboxyl-terminal (ACT1) peptide enhances the activity of the targeted inhibitors, tamoxifen and lapatinib, in breast cancer: Clinical implication for ACT1. *BMC Cancer* **2015**, *15*, 296. [CrossRef]

49. Tittarelli, A.; Guerrero, I.; Tempio, F.; Gleisner, M.A.; Avalos, I.; Sabanegh, S.; Ortiz, C.; Michea, L.; Lopez, M.N.; Mendoza-Naranjo, A.; et al. Overexpression of connexin 43 reduces melanoma proliferative and metastatic capacity. *Br. J. Cancer* **2015**, *113*, 259–267. [CrossRef]

50. Chandrasekhar, A.; Kalmykov, E.A.; Polusani, S.R.; Mathis, S.A.; Zucker, S.N.; Nicholson, B.J. Intercellular redistribution of cAMP underlies selective suppression of cancer cell growth by connexin26. *PLoS ONE* **2013**, *8*, e82335. [CrossRef]

51. Lim, P.K.; Bliss, S.A.; Patel, S.A.; Taborga, M.; Dave, M.A.; Gregory, L.A.; Greco, S.J.; Bryan, M.; Patel, P.S.; Rameshwar, P. Gap junction-mediated import of microRNA from bone marrow stromal cells can elicit cell cycle quiescence in breast cancer cells. *Cancer Res.* **2011**, *71*, 1550–1560. [CrossRef] [PubMed]

52. Zong, L.; Zhu, Y.; Liang, R.; Zhao, H.B. Gap junction mediated miRNA intercellular transfer and gene regulation: A novel mechanism for intercellular genetic communication. *Sci. Rep.* **2016**, *6*, 19884. [CrossRef] [PubMed]

53. Zhou, J.Z.; Riquelme, M.A.; Gu, S.; Kar, R.; Gao, X.; Sun, L.; Jiang, J.X. Osteocytic connexin hemichannels suppress breast cancer growth and bone metastasis. *Oncogene* **2016**, *35*, 5597–5607. [CrossRef] [PubMed]

54. Pearson, R.A.; Dale, N.; Llaudet, E.; Mobbs, P. ATP released via gap junction hemichannels from the pigment epithelium regulates neural retinal progenitor proliferation. *Neuron* **2005**, *46*, 731–744. [CrossRef] [PubMed]

55. Song, D.; Liu, X.; Liu, R.; Yang, L.; Zuo, J.; Liu, W. Connexin 43 hemichannel regulates H9c2 cell proliferation by modulating intracellular ATP and [Ca^{2+}]. *Acta Biochim. Biophys. Sin.* **2010**, *42*, 472–482. [CrossRef] [PubMed]

56. Song, M.; Yu, X.; Cui, X.; Zhu, G.; Zhao, G.; Chen, J.; Huang, L. Blockade of connexin 43 hemichannels reduces neointima formation after vascular injury by inhibiting proliferation and phenotypic modulation of smooth muscle cells. *Exp. Biol. Med.* **2009**, *234*, 1192–1200. [CrossRef] [PubMed]

57. Leithe, E.; Mesnil, M.; Aasen, T. The connexin 43 C-terminus: A tail of many tales. *Biochim. Biophys. Acta Biomem. Br.* **2018**, *1860*, 48–64. [CrossRef]

58. Nelson, T.K.; Sorgen, P.L.; Burt, J.M. Carboxy terminus and pore-forming domain properties specific to Cx37 are necessary for Cx37-mediated suppression of insulinoma cell proliferation. *Am. J. Physiol. Cell Physiol.* **2013**, *305*, C1246–C1256. [CrossRef]

59. Good, M.E.; Nelson, T.K.; Simon, A.M.; Burt, J.M. A functional channel is necessary for growth suppression by Cx37. *J. Cell Sci.* **2011**, *124*, 2448–2456. [CrossRef]

60. Jacobsen, N.L.; Pontifex, T.K.; Li, H.; Solan, J.L.; Lampe, P.D.; Sorgen, P.L.; Burt, J.M. Regulation of Cx37 channel and growth-suppressive properties by phosphorylation. *J. Cell Sci.* **2017**, *130*, 3308–3321. [CrossRef]

61. Jacobsen, N.L.; Pontifex, T.K.; Langlais, P.R.; Burt, J.M. Phosphorylation-Dependent Intra-Domain Interaction of the Cx37 Carboxyl-Terminus Controls Cell Survival. *Cancers* **2019**, *11*, 188. [CrossRef] [PubMed]

62. Lampe, P.D.; TenBroek, E.M.; Burt, J.M.; Kurata, W.E.; Johnson, R.G.; Lau, A.F. Phosphorylation of connexin43 on serine368 by protein kinase C regulates gap junctional communication. *J. Cell Biol.* **2000**, *149*, 1503–1512. [CrossRef] [PubMed]

63. Qin, H.; Shao, Q.; Curtis, H.; Galipeau, J.; Belliveau, D.J.; Wang, T.; Alaoui-Jamali, M.A.; Laird, D.W. Retroviral delivery of connexin genes to human breast tumor cells inhibits in vivo tumor growth by a mechanism that is independent of significant gap junctional intercellular communication. *J. Biol. Chem.* **2002**, *277*, 29132–29138. [CrossRef]

64. Kalra, J.; Shao, Q.; Qin, H.; Thomas, T.; Alaoui-Jamali, M.A.; Laird, D.W. Cx26 inhibits breast MDA-MB-435 cell tumorigenic properties by a gap junctional intercellular communication-independent mechanism. *Carcinogenesis* **2006**, *27*, 2528–2537. [CrossRef] [PubMed]

65. Dang, X.; Doble, B.W.; Kardami, E. The carboxy-tail of connexin-43 localizes to the nucleus and inhibits cell growth. *Mol. Cell. Biochem.* **2003**, *242*, 35–38. [CrossRef] [PubMed]

66. Shima, K.; Muramatsu, T.; Abiko, Y.; Yamaoka, Y.; Sasaki, H.; Shimono, M. Connexin 43 transfection in basaloid squamous cell carcinoma cells. *Oncol. Rep.* **2006**, *16*, 285–288. [CrossRef] [PubMed]

67. Sirnes, S.; Bruun, J.; Kolberg, M.; Kjenseth, A.; Lind, G.E.; Svindland, A.; Brech, A.; Nesbakken, A.; Lothe, R.A.; Leithe, E.; et al. Connexin43 acts as a colorectal cancer tumor suppressor and predicts disease outcome. *Int. J. Cancer* **2012**, *131*, 570–581. [CrossRef] [PubMed]

68. Herrero-Gonzalez, S.; Gangoso, E.; Giaume, C.; Naus, C.C.; Medina, J.M.; Tabernero, A. Connexin43 inhibits the oncogenic activity of c-Src in C6 glioma cells. *Oncogene* **2010**, *29*, 5712–5723. [CrossRef]

69. Giepmans, B.N.; Hengeveld, T.; Postma, F.R.; Moolenaar, W.H. Interaction of c-Src with gap junction protein connexin-43. Role in the regulation of cell-cell communication. *J. Biol. Chem.* **2001**, *276*, 8544–8549. [CrossRef]

70. Qiu, X.; Cheng, J.C.; Klausen, C.; Chang, H.M.; Fan, Q.; Leung, P.C. EGF-Induced Connexin43 Negatively Regulates Cell Proliferation in Human Ovarian Cancer. *J. Cell. Physiol.* **2016**, *231*, 111–119. [CrossRef]

71. Moorby, C.; Patel, M. Dual functions for connexins: Cx43 regulates growth independently of gap junction formation. *Exp. Cell Res.* **2001**, *271*, 238–248. [CrossRef] [PubMed]

72. Hou, X.; Khan, M.R.A.; Turmaine, M.; Thrasivoulou, C.; Becker, D.L.; Ahmed, A. Wnt signaling regulates cytosolic translocation of connexin 43. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2019**, *317*, R248–R261. [CrossRef] [PubMed]

73. Huang, R.P.; Fan, Y.; Hossain, M.Z.; Peng, A.; Zeng, Z.L.; Boynton, A.L. Reversion of the neoplastic phenotype of human glioblastoma cells by connexin 43 (cx43). *Cancer Res.* **1998**, *58*, 5089–5096. [PubMed]

74. De Feijter, A.W.; Matesic, D.F.; Ruch, R.J.; Guan, X.; Chang, C.C.; Trosko, J.E. Localization and function of the connexin 43 gap-junction protein in normal and various oncogene-expressing rat liver epithelial cells. *Mol. Carcinog* **1996**, *16*, 203–212. [CrossRef]

75. Fitzgerald, D.J.; Fusenig, N.E.; Boukamp, P.; Piccoli, C.; Mesnil, M.; Yamasaki, H. Expression and function of connexin in normal and transformed human keratinocytes in culture. *Carcinogenesis* **1994**, *15*, 1859–1865. [CrossRef] [PubMed]

76. Thiagarajan, P.S.; Sinyuk, M.; Turaga, S.M.; Mulkearns-Hubert, E.E.; Hale, J.S.; Rao, V.; Demelash, A.; Saygin, C.; China, A.; Alban, T.J.; et al. Cx26 drives self-renewal in triple-negative breast cancer via interaction with NANOG and focal adhesion kinase. *Nat. Commun.* **2018**, *9*, 578. [CrossRef] [PubMed]

77. Krutovskikh, V.; Mazzoleni, G.; Mironov, N.; Omori, Y.; Aguelon, A.M.; Mesnil, M.; Berger, F.; Partensky, C.; Yamasaki, H. Altered homologous and heterologous gap-junctional intercellular communication in primary human liver tumors associated with aberrant protein localization but not gene mutation of connexin 32. *Int. J. Cancer* **1994**, *56*, 87–94. [CrossRef]

78. Joshi-Mukherjee, R.; Coombs, W.; Burrer, C.; de Mora, I.A.; Delmar, M.; Taffet, S.M. Evidence for the presence of a free C-terminal fragment of cx43 in cultured cells. *Cell Commun. Adhes.* **2007**, *14*, 75–84. [CrossRef]

79. Smyth, J.W.; Shaw, R.M. Autoregulation of connexin43 gap junction formation by internally translated isoforms. *Cell Rep.* **2013**, *5*, 611–618. [CrossRef]

80. Epifantseva, I.; Xiao, S.; Baum, R.E.; Kleber, A.G.; Hong, T.; Shaw, R.M. An Alternatively Translated Connexin 43 Isoform, GJA1-11k, Localizes to the Nucleus and Can Inhibit Cell Cycle Progression. *Biomolecules* **2020**, *10*, 473. [CrossRef]

81. Fujimoto, E.; Satoh, H.; Negishi, E.; Ueno, K.; Nagashima, Y.; Hagiwara, K.; Yamasaki, H.; Yano, T. Negative growth control of renal cell carcinoma cell by connexin 32: Possible involvement of Her-2. *Mol. Carcinog.* **2004**, *40*, 135–142. [CrossRef] [PubMed]

82. Rivedal, E.; Witz, G. Metabolites of benzene are potent inhibitors of gap-junction intercellular communication. *Arch. Toxicol.* **2005**, *79*, 303–311. [CrossRef] [PubMed]

83. Tekpli, X.; Rivedal, E.; Gorria, M.; Landvik, N.E.; Rissel, M.; Dimanche-Boitrel, M.T.; Baffet, G.; Holme, J.A.; Lagadic-Gossmann, D. The B[a]P-increased intercellular communication via translocation of connexin-43 into gap junctions reduces apoptosis. *Toxicol. Appl. Pharmacol.* **2010**, *242*, 231–240. [CrossRef] [PubMed]

84. Du, Z.J.; Cui, G.Q.; Zhang, J.; Liu, X.M.; Zhang, Z.H.; Jia, Q.; Ng, J.C.; Peng, C.; Bo, C.X.; Shao, H. Inhibition of gap junction intercellular communication is involved in silica nanoparticles-induced H9c2 cardiomyocytes apoptosis via the mitochondrial pathway. *Int. J. Nanomed.* **2017**, *12*, 2179–2188. [CrossRef]

85. Yu, J.; Berga, S.L.; Zou, W.; Sun, H.Y.; Johnston-MacAnanny, E.; Yalcinkaya, T.; Sidell, N.; Bagchi, I.C.; Bagchi, M.K.; Taylor, R.N. Gap junction blockade induces apoptosis in human endometrial stromal cells. *Mol. Reprod. Dev.* **2014**, *81*, 666–675. [CrossRef]

86. Worsdorfer, P.; Maxeiner, S.; Markopoulos, C.; Kirfel, G.; Wulf, V.; Auth, T.; Urschel, S.; von Maltzahn, J.; Willecke, K. Connexin expression and functional analysis of gap junctional communication in mouse embryonic stem cells. *Stem Cells* **2008**, *26*, 431–439. [CrossRef]

87. Sinyuk, M.; Alvarado, A.G.; Nesmiyanov, P.; Shaw, J.; Mulkearns-Hubert, E.E.; Eurich, J.T.; Hale, J.S.; Bogdanova, A.; Hitomi, M.; Maciejewski, J.; et al. Cx25 contributes to leukemia cell communication and chemosensitivity. *Oncotarget* **2015**, *6*, 31508–31521. [CrossRef]

88. Mesnil, M.; Piccoli, C.; Tiraby, G.; Willecke, K.; Yamasaki, H. Bystander killing of cancer cells by herpes simplex virus thymidine kinase gene is mediated by connexins. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 1831–1835. [CrossRef]

89. Yang, L.; Chiang, Y.; Lenz, H.J.; Danenberg, K.D.; Spears, C.P.; Gordon, E.M.; Anderson, W.F.; Parekh, D. Intercellular communication mediates the bystander effect during herpes simplex thymidine kinase/ganciclovir-based gene therapy of human gastrointestinal tumor cells. *Hum. Gene Ther.* **1998**, *9*, 719–728. [CrossRef]

90. Burrows, F.J.; Gore, M.; Smiley, W.R.; Kanemitsu, M.Y.; Jolly, D.J.; Read, S.B.; Nicholas, T.; Kruse, C.A. Purified herpes simplex virus thymidine kinase retroviral particles: III. Characterization of bystander killing mechanisms in transfected tumor cells. *Cancer Gene Ther.* **2002**, *9*, 87–95. [CrossRef]

91. Krutovskikh, V.A.; Piccoli, C.; Yamasaki, H. Gap junction intercellular communication propagates cell death in cancerous cells. *Oncogene* **2002**, *21*, 1989–1999. [CrossRef] [PubMed]

92. Kameritsch, P.; Khandoga, N.; Pohl, U.; Pogoda, K. Gap junctional communication promotes apoptosis in a connexin-type-dependent manner. *Cell Death Dis.* **2013**, *4*, e584. [CrossRef] [PubMed]

93. Asamoto, M.; Hokaiwado, N.; Murasaki, T.; Shirai, T. Connexin 32 dominant-negative mutant transgenic rats are resistant to hepatic damage by chemicals. *Hepatology* **2004**, *40*, 205–210. [CrossRef] [PubMed]

94. Decrock, E.; Krysko, D.V.; Vinken, M.; Kaczmarek, A.; Crispino, G.; Bol, M.; Wang, N.; De Bock, M.; De Vuyst, E.; Naus, C.C.; et al. Transfer of IP(3) through gap junctions is critical, but not sufficient, for the spread of apoptosis. *Cell Death Differ.* **2012**, *19*, 947–957. [CrossRef] [PubMed]

95. Decrock, E.; De Bock, M.; Wang, N.; Gadicherla, A.K.; Bol, M.; Delvaeye, T.; Vandenabeele, P.; Vinken, M.; Bultynck, G.; Krysko, D.V.; et al. IP3, a small molecule with a powerful message. *Biochim. Biophys. Acta* **2013**, *1833*, 1772–1786. [CrossRef]

96. Wang, M.; Berthoud, V.M.; Beyer, E.C. Connexin43 increases the sensitivity of prostate cancer cells to TNFalpha-induced apoptosis. *J. Cell Sci.* **2007**, *120*, 320–329. [CrossRef]

97. Yang, Y.; Zhu, J.; Zhang, N.; Zhao, Y.; Li, W.Y.; Zhao, F.Y.; Ou, Y.R.; Qin, S.K.; Wu, Q. Impaired gap junctions in human hepatocellular carcinoma limit intrinsic oxaliplatin chemosensitivity: A key role of connexin 26. *Int. J. Oncol.* **2016**, *48*, 703–713. [CrossRef]

98. Decrock, E.; De Vuyst, E.; Vinken, M.; Van Moorhem, M.; Vranckx, K.; Wang, N.; Van Laeken, L.; De Bock, M.; D'Herde, K.; Lai, C.P.; et al. Connexin 43 hemichannels contribute to the propagation of apoptotic cell death in a rat C6 glioma cell model. *Cell Death Differ.* **2009**, *16*, 151–163. [CrossRef]

99. Evans, W.H.; De Vuyst, E.; Leybaert, L. The gap junction cellular internet: Connexin hemichannels enter the signalling limelight. *Biochem. J.* **2006**, *397*, 1–14. [CrossRef]

100. Nicotera, P.; Leist, M.; Ferrando-May, E. Intracellular ATP, a switch in the decision between apoptosis and necrosis. *Toxicol. Lett.* **1998**, *102–103*, 139–142. [CrossRef]

101. Zhao, Y.; Lai, Y.; Ge, H.; Guo, Y.; Feng, X.; Song, J.; Wang, Q.; Fan, L.; Peng, Y.; Cao, M.; et al. Non-junctional Cx32 mediates anti-apoptotic and pro-tumor effects via epidermal growth factor receptor in human cervical cancer cells. *Cell Death Dis.* **2017**, *8*, e2773. [CrossRef] [PubMed]

102. Xiang, Y.; Wang, Q.; Guo, Y.; Ge, H.; Fu, Y.; Wang, X.; Tao, L. Cx32 exerts anti-apoptotic and pro-tumor effects via the epidermal growth factor receptor pathway in hepatocellular carcinoma. *J. Exp. Clin. Cancer Res.* **2019**, *38*, 145. [CrossRef] [PubMed]

103. Lai, Y.; Fan, L.; Zhao, Y.; Ge, H.; Feng, X.; Wang, Q.; Zhang, X.; Peng, Y.; Wang, X.; Tao, L. Cx32 suppresses extrinsic apoptosis in human cervical cancer cells via the NFkappaB signalling pathway. *Int. J. Oncol.* **2017**, *51*, 1159–1168. [CrossRef]

104. Uzu, M.; Sato, H.; Shimizu, A.; Shibata, Y.; Ueno, K.; Hisaka, A. Connexin 43 enhances Bax activation via JNK activation in sunitinib-induced apoptosis in mesothelioma cells. *J. Pharmacol. Sci.* **2017**, *134*, 101–107. [CrossRef] [PubMed]

105. Sun, Y.; Zhao, X.; Yao, Y.; Qi, X.; Yuan, Y.; Hu, Y. Connexin 43 interacts with Bax to regulate apoptosis of pancreatic cancer through a gap junction-independent pathway. *Int. J. Oncol.* **2012**, *41*, 941–948. [CrossRef]

106. Giardina, S.F.; Mikami, M.; Goubaeva, F.; Yang, J. Connexin 43 confers resistance to hydrogen peroxide-mediated apoptosis. *Biochem. Biophys. Res. Commun.* **2007**, *362*, 747–752. [CrossRef] [PubMed]

107. Plotkin, L.I.; Manolagas, S.C.; Bellido, T. Transduction of cell survival signals by connexin-43 hemichannels. *J. Biol. Chem.* **2002**, *277*, 8648–8657. [CrossRef] [PubMed]

108. Wu, R.; Xu, C.; Jin, F.; Tan, Z.; Gu, B.; Chen, L.; Yao, X.; Zhang, M. Derivation, characterization and differentiation of a new human embryonic stem cell line from a Chinese hatched blastocyst assisted by a non-contact laser system. *Hum. Cell* **2010**, *23*, 89–102. [CrossRef]

109. De Kock, J.; Vanhaecke, T.; Biernaskie, J.; Rogiers, V.; Snykers, S. Characterization and hepatic differentiation of skin-derived precursors from adult foreskin by sequential exposure to hepatogenic cytokines and growth factors reflecting liver development. *Toxicol. In Vitro* **2009**, *23*, 1522–1527. [CrossRef]

110. Thi, M.M.; Urban-Maldonado, M.; Spray, D.C.; Suadicani, S.O. Characterization of hTERT-immortalized osteoblast cell lines generated from wild-type and connexin43-null mouse calvaria. *Am. J. Physiol. Cell Physiol.* **2010**, *299*, C994–C1006. [CrossRef]

111. Xie, H.Q.; Hu, V.W. Modulation of gap junctions in senescent endothelial cells. *Exp. Cell Res.* **1994**, *214*, 172–176. [CrossRef] [PubMed]

112. Yeh, H.I.; Chang, H.M.; Lu, W.W.; Lee, Y.N.; Ko, Y.S.; Severs, N.J.; Tsai, C.H. Age-related alteration of gap junction distribution and connexin expression in rat aortic endothelium. *J. HistoChem. CytoChem.* **2000**, *48*, 1377–1389. [CrossRef] [PubMed]

113. Zhao, W.; Lin, Z.X.; Zhang, Z.Q. Cisplatin-induced premature senescence with concomitant reduction of gap junctions in human fibroblasts. *Cell Res.* **2004**, *14*, 60–66. [CrossRef] [PubMed]

114. Statuto, M.; Bianchi, C.; Perego, R.; Del Monte, U. Drop of connexin 43 in replicative senescence of human fibroblasts HEL-299 as a possible biomarker of senescence. *Exp. Gerontol.* **2002**, *37*, 1113–1120. [CrossRef]

115. Pakula, M.; Witucka, A.; Uruski, P.; Radziemski, A.; Moszynski, R.; Szpurek, D.; Maksin, K.; Wozniak, A.; Sajdak, S.; Tykarski, A.; et al. Senescence-related deterioration of intercellular junctions in the peritoneal mesothelium promotes the transmesothelial invasion of ovarian cancer cells. *Sci. Rep.* **2019**, *9*, 7587. [CrossRef]

116. Zhang, X.; Chen, X.; Wu, D.; Liu, W.; Wang, J.; Feng, Z.; Cai, G.; Fu, B.; Hong, Q.; Du, J. Downregulation of connexin 43 expression by high glucose induces senescence in glomerular mesangial cells. *J. Am. Soc. Nephrol.* **2006**, *17*, 1532–1542. [CrossRef]

117. Michishita, E.; McCord, R.A.; Berber, E.; Kioi, M.; Padilla-Nash, H.; Damian, M.; Cheung, P.; Kusumoto, R.; Kawahara, T.L.; Barrett, J.C.; et al. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature* **2008**, *452*, 492–496. [CrossRef]

118. Ponsaerts, R.; De Vuyst, E.; Retamal, M.; D'Hondt, C.; Vermeire, D.; Wang, N.; De Smedt, H.; Zimmermann, P.; Himpens, B.; Vereecke, J.; et al. Intramolecular loop/tail interactions are essential for connexin 43-hemichannel activity. *FASEB J.* **2010**, *24*, 4378–4395. [CrossRef]

119. Iyyathurai, J.; Wang, N.; D'Hondt, C.; Jiang, J.X.; Leybaert, L.; Bultynck, G. The SH3-binding domain of Cx43 participates in loop/tail interactions critical for Cx43-hemichannel activity. *Cell. Mol. Life Sci.* **2018**, *75*, 2059–2073. [CrossRef]

120. Ramadan, R.; Vromans, E.; Anang, D.C.; Goetschalckx, I.; Hoorelbeke, D.; Decrock, E.; Baatout, S.; Leybaert, L.; Aerts, A. Connexin43 Hemichannel Targeting with TAT-Gap19 Alleviates Radiation-Induced Endothelial Cell Damage. *Front. Pharmacol.* **2020**, *11*, 212. [CrossRef]

121. Goldberg, G.S.; Bechberger, J.F.; Tajima, Y.; Merritt, M.; Omori, Y.; Gawinowicz, M.A.; Narayanan, R.; Tan, Y.; Sanai, Y.; Yamasaki, H.; et al. Connexin43 suppresses MFG-E8 while inducing contact growth inhibition of glioma cells. *Cancer Res.* **2000**, *60*, 6018–6026. [PubMed]

122. Lathia, J.D.; Mack, S.C.; Mulkearns-Hubert, E.E.; Valentim, C.L.; Rich, J.N. Cancer stem cells in glioblastoma. *Genes Dev.* **2015**, *29*, 1203–1217. [CrossRef] [PubMed]

123. Trosko, J.E.; Chang, C.C. Isolation and characterization of normal adult human epithelial pluripotent stem cells. *Oncol. Res.* **2003**, *13*, 353–357. [CrossRef] [PubMed]

124. Trosko, J.E. From adult stem cells to cancer stem cells: Oct-4 Gene, cell-cell communication, and hormones during tumor promotion. *Ann. N. Y. Acad. Sci.* **2006**, *1089*, 36–58. [CrossRef]

125. Trosko, J.E.; Chang, C.C.; Upham, B.L.; Tai, M.H. Ignored hallmarks of carcinogenesis: Stem cells and cell-cell communication. *Ann. N. Y. Acad. Sci.* **2004**, *1028*, 192–201. [CrossRef]

126. Trosko, J.E. Cancer Prevention and Therapy of Two Types of Gap Junctional Intercellular Communication(-)Deficient "Cancer Stem Cell". *Cancers* **2019**, *11*, 87. [CrossRef]

127. Yu, S.C.; Xiao, H.L.; Jiang, X.F.; Wang, Q.L.; Li, Y.; Yang, X.J.; Ping, Y.F.; Duan, J.J.; Jiang, J.Y.; Ye, X.Z.; et al. Connexin 43 reverses malignant phenotypes of glioma stem cells by modulating E-cadherin. *Stem Cells* **2012**, *30*, 108–120. [CrossRef]

128. Arun, S.; Ravisankar, S.; Vanisree, A.J. Implication of connexin30 on the stemness of glioma: Connexin30 reverses the malignant phenotype of glioma by modulating IGF-1R, CD133 and cMyc. *J. Neuro-Oncol.* **2017**, *135*, 473–485. [CrossRef]

129. Hsiao, P.J.; Jao, J.C.; Tsai, J.L.; Chang, W.T.; Jeng, K.S.; Kuo, K.K. Inorganic arsenic trioxide induces gap junction loss in association with the downregulation of connexin43 and E-cadherin in rat hepatic "stem-like" cells. *Kaohsiung J. Med. Sci.* **2014**, *30*, 57–67. [CrossRef]

130. Shen, Y.; Li, Y.; Ma, X.; Wan, Q.; Jiang, Z.; Liu, Y.; Zhang, D.; Liu, X.; Wu, W. Connexin 43 SUMOylation improves gap junction functions between liver cancer stem cells and enhances their sensitivity to HSVtk/GCV. *Int. J. Oncol.* **2018**, *52*, 872–880. [CrossRef]

131. Liu, L.; Li, H.; Guo, Z.; Ma, X.; Cao, N.; Zheng, Y.; Geng, S.; Duan, Y.; Han, G.; Du, G. The Combination of Three Natural Compounds Effectively Prevented Lung Carcinogenesis by Optimal Wound Healing. *PLoS ONE* **2015**, *10*, e0143438. [CrossRef] [PubMed]

132. Ruch, R.J. Connexin43 Suppresses Lung Cancer Stem Cells. *Cancers* **2019**, *11*, 175. [CrossRef] [PubMed]

133. Ishibashi, K.; Ishii, K.; Sugiyama, G.; Sumida, T.; Sugiura, T.; Kamata, Y.U.; Seki, K.; Fujinaga, T.; Kumamaru, W.; Kobayashi, Y.; et al. Deregulation of Nicotinamide N-Methyltransferase and Gap Junction Protein Alpha-1 Causes Metastasis in Adenoid Cystic Carcinoma. *Anticancer Res.* **2018**, *38*, 187–197. [CrossRef] [PubMed]

134. Mulkearns-Hubert, E.E.; Torre-Healy, L.A.; Silver, D.J.; Eurich, J.T.; Bayik, D.; Serbinowski, E.; Hitomi, M.; Zhou, J.; Przychodzen, B.; Zhang, R.; et al. Development of a Cx46 Targeting Strategy for Cancer Stem Cells. *Cell Rep.* **2019**, *27*, 1062–1072.e5. [CrossRef]

135. Yang, Y.C.; Wang, S.W.; Hung, H.Y.; Chang, C.C.; Wu, I.C.; Huang, Y.L.; Lin, T.M.; Tsai, J.L.; Chen, A.; Kuo, F.C.; et al. Isolation and characterization of human gastric cell lines with stem cell phenotypes. *J. Gastroenterol. Hepatol.* **2007**, *22*, 1460–1468. [CrossRef]

136. Kuramoto, K.; Yamamoto, M.; Suzuki, S.; Sanomachi, T.; Togashi, K.; Seino, S.; Kitanaka, C.; Okada, M. AS602801, an Anti-Cancer Stem Cell Drug Candidate, Suppresses Gap-junction Communication Between Lung Cancer Stem Cells and Astrocytes. *Anticancer Res.* **2018**, *38*, 5093–5099. [CrossRef]

137. Patel, S.A.; Ramkissoon, S.H.; Bryan, M.; Pliner, L.F.; Dontu, G.; Patel, P.S.; Amiri, S.; Pine, S.R.; Rameshwar, P. Delineation of breast cancer cell hierarchy identifies the subset responsible for dormancy. *Sci. Rep.* **2012**, *2*, 906. [CrossRef]

138. Walker, N.D.; Elias, M.; Guiro, K.; Bhatia, R.; Greco, S.J.; Bryan, M.; Gergues, M.; Sandiford, O.A.; Ponzio, N.M.; Leibovich, S.J.; et al. Exosomes from differentially activated macrophages influence dormancy or resurgence of breast cancer cells within bone marrow stroma. *Cell Death Dis.* **2019**, *10*, 59. [CrossRef]

139. Gangoso, E.; Thirant, C.; Chneiweiss, H.; Medina, J.M.; Tabernero, A. A cell-penetrating peptide based on the interaction between c-Src and connexin43 reverses glioma stem cell phenotype. *Cell Death Dis.* **2014**, *5*, e1023. [CrossRef]

140. Kawasaki, Y.; Omori, Y.; Li, Q.; Nishikawa, Y.; Yoshioka, T.; Yoshida, M.; Ishikawa, K.; Enomoto, K. Cytoplasmic accumulation of connexin32 expands cancer stem cell population in human HuH7 hepatoma cells by enhancing its self-renewal. *Int. J. Cancer* **2011**, *128*, 51–62. [CrossRef]

141. Gartner, C.; Ziegelhoffer, B.; Kostelka, M.; Stepan, H.; Mohr, F.W.; Dhein, S. Knock-down of endothelial connexins impairs angiogenesis. *Pharmacol. Res.* **2012**, *65*, 347–357. [CrossRef] [PubMed]

142. Fang, J.S.; Angelov, S.N.; Simon, A.M.; Burt, J.M. Cx37 deletion enhances vascular growth and facilitates ischemic limb recovery. *Am. J. Physiol. Heart Circ. Physiol.* **2011**, *301*, H1872–H1881. [CrossRef] [PubMed]

143. Alonso, F.; Domingos-Pereira, S.; Le Gal, L.; Derre, L.; Meda, P.; Jichlinski, P.; Nardelli-Haefliger, D.; Haefliger, J.A. Targeting endothelial connexin40 inhibits tumor growth by reducing angiogenesis and improving vessel perfusion. *Oncotarget* **2016**, *7*, 14015–14028. [CrossRef] [PubMed]

144. Walker, D.L.; Vacha, S.J.; Kirby, M.L.; Lo, C.W. Connexin43 deficiency causes dysregulation of coronary vasculogenesis. *Dev. Biol.* **2005**, *284*, 479–498. [CrossRef]

145. Zhang, W.; DeMattia, J.A.; Song, H.; Couldwell, W.T. Communication between malignant glioma cells and vascular endothelial cells through gap junctions. *J. Neurosurg.* **2003**, *98*, 846–853. [CrossRef]

146. Kikuchi-Taura, A.; Okinaka, Y.; Takeuchi, Y.; Ogawa, Y.; Maeda, M.; Kataoka, Y.; Yasui, T.; Kimura, T.; Gul, S.; Claussen, C.; et al. Bone Marrow Mononuclear Cells Activate Angiogenesis via Gap Junction-Mediated Cell-Cell Interaction. *Stroke* **2020**, *51*, 1279–1289. [CrossRef]

147. Strale, P.O.; Clarhaut, J.; Lamiche, C.; Cronier, L.; Mesnil, M.; Defamie, N. Down-regulation of Connexin43 expression reveals the involvement of caveolin-1 containing lipid rafts in human U251 glioblastoma cell invasion. *Mol. Carcinog.* **2012**, *51*, 845–860. [CrossRef]

148. Choudhary, M.; Naczki, C.; Chen, W.; Barlow, K.D.; Case, L.D.; Metheny-Barlow, L.J. Tumor-induced loss of mural Connexin 43 gap junction activity promotes endothelial proliferation. *BMC Cancer* **2015**, *15*, 427. [CrossRef]

149. Thuringer, D.; Jego, G.; Berthenet, K.; Hammann, A.; Solary, E.; Garrido, C. Gap junction-mediated transfer of miR-145-5p from microvascular endothelial cells to colon cancer cells inhibits angiogenesis. *Oncotarget* **2016**, *7*, 28160–28168. [CrossRef]

150. Thuringer, D.; Boucher, J.; Jego, G.; Pernet, N.; Cronier, L.; Hammann, A.; Solary, E.; Garrido, C. Transfer of functional microRNAs between glioblastoma and microvascular endothelial cells through gap junctions. *Oncotarget* **2016**, *7*, 73925–73934. [CrossRef]

151. Jin, H.; Eun, S.Y.; Lee, J.S.; Park, S.W.; Lee, J.H.; Chang, K.C.; Kim, H.J. P2Y2 receptor activation by nucleotides released from highly metastatic breast cancer cells increases tumor growth and invasion via crosstalk with endothelial cells. *Breast Cancer Res.* **2014**, *16*, R77. [CrossRef] [PubMed]

152. Muhleder, S.; Fuchs, C.; Basilio, J.; Szwarc, D.; Pill, K.; Labuda, K.; Slezak, P.; Siehs, C.; Proll, J.; Priglinger, E.; et al. Purinergic P2Y2 receptors modulate endothelial sprouting. *Cell. Mol. Life Sci.* **2020**, *77*, 885–901. [CrossRef] [PubMed]

153. Kohn, E.C.; Alessandro, R.; Spoonster, J.; Wersto, R.P.; Liotta, L.A. Angiogenesis: Role of calcium-mediated signal transduction. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 1307–1311. [CrossRef] [PubMed]

154. Moccia, F.; Negri, S.; Shekha, M.; Faris, P.; Guerra, G. Endothelial Ca(2+) Signaling, Angiogenesis and Vasculogenesis: Just What It Takes to Make a Blood Vessel. *Int. J. Mol. Sci.* **2019**, *20*, 3962. [CrossRef] [PubMed]

155. Szkudlarek, M.; Bosio, R.M.; Wu, Q.; Chin, K.V. Inhibition of angiogenesis by extracellular protein kinase A. *Cancer Lett.* **2009**, *283*, 68–73. [CrossRef]

156. Shao, Q.; Wang, H.; McLachlan, E.; Veitch, G.I.; Laird, D.W. Down-regulation of Cx43 by retroviral delivery of small interfering RNA promotes an aggressive breast cancer cell phenotype. *Cancer Res.* **2005**, *65*, 2705–2711. [CrossRef]

157. McLachlan, E.; Shao, Q.; Wang, H.L.; Langlois, S.; Laird, D.W. Connexins act as tumor suppressors in three-dimensional mammary cell organoids by regulating differentiation and angiogenesis. *Cancer Res.* **2006**, *66*, 9886–9894. [CrossRef]

158. Wang, W.K.; Chen, M.C.; Leong, H.F.; Kuo, Y.L.; Kuo, C.Y.; Lee, C.H. Connexin 43 suppresses tumor angiogenesis by down-regulation of vascular endothelial growth factor via hypoxic-induced factor-1alpha. *Int. J. Mol. Sci.* **2014**, *16*, 439–451. [CrossRef]

159. Qin, H.; Shao, Q.; Thomas, T.; Kalra, J.; Alaoui-Jamali, M.A.; Laird, D.W. Connexin26 regulates the expression of angiogenesis-related genes in human breast tumor cells by both GJIC-dependent and -independent mechanisms. *Cell Commun. Adhes.* **2003**, *10*, 387–393. [CrossRef]

160. Czyz, J.; Szpak, K.; Madeja, Z. The role of connexins in prostate cancer promotion and progression. *Nat. Rev. Urol.* **2012**, *9*, 274–282. [CrossRef]

161. McLachlan, E.; Shao, Q.; Laird, D.W. Connexins and gap junctions in mammary gland development and breast cancer progression. *J. Mem. Br. Biol.* **2007**, *218*, 107–121. [CrossRef] [PubMed]

162. Uzu, M.; Sin, W.C.; Shimizu, A.; Sato, H. Conflicting Roles of Connexin43 in Tumor Invasion and Growth in the Central Nervous System. *Int. J. Mol. Sci.* **2018**, *19*, 1159. [CrossRef] [PubMed]

163. Boucher, J.; Monvoisin, A.; Vix, J.; Mesnil, M.; Thuringer, D.; Debiais, F.; Cronier, L. Connexins, important players in the dissemination of prostate cancer cells. *Biochim. Biophys. Acta Biomem. Br.* **2018**, *1860*, 202–215. [CrossRef] [PubMed]

164. Naus, C.C.; Aftab, Q.; Sin, W.C. Common mechanisms linking connexin43 to neural progenitor cell migration and glioma invasion. *Semin. Cell Dev. Biol.* **2016**, *50*, 59–66. [CrossRef] [PubMed]

165. Mao, X.Y.; Li, Q.Q.; Gao, Y.F.; Zhou, H.H.; Liu, Z.Q.; Jin, W.L. Gap junction as an intercellular glue: Emerging roles in cancer EMT and metastasis. *Cancer Lett.* **2016**, *381*, 133–137. [CrossRef]

166. Chen, Q.; Boire, A.; Jin, X.; Valiente, M.; Er, E.E.; Lopez-Soto, A.; Jacob, L.; Patwa, R.; Shah, H.; Xu, K.; et al. Carcinoma-astrocyte gap junctions promote brain metastasis by cGAMP transfer. *Nature* **2016**, *533*, 493–498. [CrossRef]

167. Hong, X.; Sin, W.C.; Harris, A.L.; Naus, C.C. Gap junctions modulate glioma invasion by direct transfer of microRNA. *Oncotarget* **2015**, *6*, 15566–15577. [CrossRef]

168. Sin, W.C.; Aftab, Q.; Bechberger, J.F.; Leung, J.H.; Chen, H.; Naus, C.C. Astrocytes promote glioma invasion via the gap junction protein connexin43. *Oncogene* **2016**, *35*, 1504–1516. [CrossRef]

169. Pollmann, M.A.; Shao, Q.; Laird, D.W.; Sandig, M. Connexin 43 mediated gap junctional communication enhances breast tumor cell diapedesis in culture. *Breast Cancer Res.* **2005**, *7*, R522–R534. [CrossRef]

170. Zibara, K.; Awada, Z.; Dib, L.; El-Saghir, J.; Al-Ghadban, S.; Ibrik, A.; El-Zein, N.; El-Sabban, M. Anti-angiogenesis therapy and gap junction inhibition reduce MDA-MB-231 breast cancer cell invasion and metastasis in vitro and in vivo. *Sci. Rep.* **2015**, *5*, 12598. [CrossRef]

171. Haddad, L.; El Hajj, H.; Abou-Merhi, R.; Kfoury, Y.; Mahieux, R.; El-Sabban, M.; Bazarbachi, A. KSHV-transformed primary effusion lymphoma cells induce a VEGF-dependent angiogenesis and establish functional gap junctions with endothelial cells. *Leukemia* **2008**, *22*, 826–834. [CrossRef] [PubMed]

172. Stoletov, K.; Strnadel, J.; Zardouzian, E.; Momiyama, M.; Park, F.D.; Kelber, J.A.; Pizzo, D.P.; Hoffman, R.; VandenBerg, S.R.; Klemke, R.L. Role of connexins in metastatic breast cancer and melanoma brain colonization. *J. Cell Sci.* **2013**, *126*, 904–913. [CrossRef] [PubMed]

173. Ito, A.; Katoh, F.; Kataoka, T.R.; Okada, M.; Tsubota, N.; Asada, H.; Yoshikawa, K.; Maeda, S.; Kitamura, Y.; Yamasaki, H.; et al. A role for heterologous gap junctions between melanoma and endothelial cells in metastasis. *J. Clin. Investig.* **2000**, *105*, 1189–1197. [CrossRef] [PubMed]

174. Nojima, H.; Ohba, Y.; Kita, Y. Oleamide derivatives are prototypical anti-metastasis drugs that act by inhibiting Connexin 26. *Curr. Drug Saf.* **2007**, *2*, 204–211. [CrossRef]

175. Elzarrad, M.K.; Haroon, A.; Willecke, K.; Dobrowolski, R.; Gillespie, M.N.; Al-Mehdi, A.B. Connexin-43 upregulation in micrometastases and tumor vasculature and its role in tumor cell attachment to pulmonary endothelium. *BMC Med.* **2008**, *6*, 20. [CrossRef]

176. Lin, J.H.; Takano, T.; Cotrina, M.L.; Arcuino, G.; Kang, J.; Liu, S.; Gao, Q.; Jiang, L.; Li, F.; Lichtenberg-Frate, H.; et al. Connexin 43 enhances the adhesivity and mediates the invasion of malignant glioma cells. *J. NeuroSci.* **2002**, *22*, 4302–4311. [CrossRef]

177. Oliveira, R.; Christov, C.; Guillamo, J.S.; de Bouard, S.; Palfi, S.; Venance, L.; Tardy, M.; Peschanski, M. Contribution of gap junctional communication between tumor cells and astroglia to the invasion of the brain parenchyma by human glioblastomas. *BMC Cell Biol.* **2005**, *6*, 7. [CrossRef]

178. Luo, M.; Luo, Y.; Mao, N.; Huang, G.; Teng, C.; Wang, H.; Wu, J.; Liao, X.; Yang, J. Cancer-Associated Fibroblasts Accelerate Malignant Progression of Non-Small Cell Lung Cancer via Connexin 43-Formed Unidirectional Gap Junctional Intercellular Communication. *Cell Physiol. Biochem.* **2018**, *51*, 315–336. [CrossRef]

179. Lamiche, C.; Clarhaut, J.; Strale, P.O.; Crespin, S.; Pedretti, N.; Bernard, F.X.; Naus, C.C.; Chen, V.C.; Foster, L.J.; Defamie, N.; et al. The gap junction protein Cx43 is involved in the bone-targeted metastatic behaviour of human prostate cancer cells. *Clin. Exp. Metastasis* **2012**, *29*, 111–122. [CrossRef]

180. Aftab, Q.; Sin, W.C.; Naus, C.C. Reduction in gap junction intercellular communication promotes glioma migration. *Oncotarget* **2015**, *6*, 11447–11464. [CrossRef] [PubMed]

181. Plante, I.; Stewart, M.K.; Barr, K.; Allan, A.L.; Laird, D.W. Cx43 suppresses mammary tumor metastasis to the lung in a Cx43 mutant mouse model of human disease. *Oncogene* **2011**, *30*, 1681–1692. [CrossRef]

182. Yang, J.; Liu, B.; Wang, Q.; Yuan, D.; Hong, X.; Yang, Y.; Tao, L. Connexin 32 and its derived homotypic gap junctional intercellular communication inhibit the migration and invasion of transfected HeLa cells via enhancement of intercellular adhesion. *Mol. Med. Rep.* **2011**, *4*, 971–979. [CrossRef]

183. Fukuda, S.; Akiyama, M.; Harada, H.; Nakahama, K.I. Effect of gap junction-mediated intercellular communication on TGF-beta induced epithelial-to-mesenchymal transition. *Biochem. Biophys. Res. Commun.* **2019**, *508*, 928–933. [CrossRef]

184. Alvarez, A.; Lagos-Cabre, R.; Kong, M.; Cardenas, A.; Burgos-Bravo, F.; Schneider, P.; Quest, A.F.; Leyton, L. Integrin-mediated transactivation of P2 × 7R via hemichannel-dependent ATP release stimulates astrocyte migration. *Biochim. Biophys. Acta* **2016**, *1863*, 2175–2188. [CrossRef]

185. Lagos-Cabre, R.; Brenet, M.; Diaz, J.; Perez, R.D.; Perez, L.A.; Herrera-Molina, R.; Quest, A.F.G.; Leyton, L. Intracellular Ca(2+) Increases and Connexin 43 Hemichannel Opening Are Necessary but Not Sufficient for Thy-1-Induced Astrocyte Migration. *Int. J. Mol. Sci.* **2018**, *19*, 2179. [CrossRef] [PubMed]

186. Weissman, T.A.; Riquelme, P.A.; Ivic, L.; Flint, A.C.; Kriegstein, A.R. Calcium waves propagate through radial glial cells and modulate proliferation in the developing neocortex. *Neuron* **2004**, *43*, 647–661. [CrossRef] [PubMed]

187. Khalil, A.A.; Ilina, O.; Vasaturo, A.; Venhuizen, J.H.; Vullings, M.; Venhuizen, V.; Bilos, A.; Figdor, C.G.; Span, P.N.; Friedl, P. Collective invasion induced by an autocrine purinergic loop through connexin-43 hemichannels. *J. Cell Biol.* **2020**, *219*, e201911120. [CrossRef] [PubMed]

188. Cotrina, M.L.; Lin, J.H.; Nedergaard, M. Adhesive properties of connexin hemichannels. *Glia* **2008**, *56*, 1791–1798. [CrossRef]

189. Bates, D.C.; Sin, W.C.; Aftab, Q.; Naus, C.C. Connexin43 enhances glioma invasion by a mechanism involving the carboxy terminus. *Glia* **2007**, *55*, 1554–1564. [CrossRef] [PubMed]

190. Crespin, S.; Bechberger, J.; Mesnil, M.; Naus, C.C.; Sin, W.C. The carboxy-terminal tail of connexin43 gap junction protein is sufficient to mediate cytoskeleton changes in human glioma cells. *J. Cell. Biochem.* **2010**, *110*, 589–597. [CrossRef]

191. Sin, W.C.; Tse, M.; Planque, N.; Perbal, B.; Lampe, P.D.; Naus, C.C. Matricellular protein CCN3 (NOV) regulates actin cytoskeleton reorganization. *J. Biol. Chem.* **2009**, *284*, 29935–29944. [CrossRef] [PubMed]

192. Zhang, A.; Hitomi, M.; Bar-Shain, N.; Dalimov, Z.; Ellis, L.; Velpula, K.K.; Fraizer, G.C.; Gourdie, R.G.; Lathia, J.D. Connexin 43 expression is associated with increased malignancy in prostate cancer cell lines and functions to promote migration. *Oncotarget* **2015**, *6*, 11640–11651. [CrossRef] [PubMed]

193. Zhao, J.; Klausen, C.; Yi, Y.; Cheng, J.C.; Chang, H.M.; Leung, P.C.K. Betacellulin enhances ovarian cancer cell migration by up-regulating Connexin43 via MEK-ERK signaling. *Cell. Signal.* **2020**, *65*, 109439. [CrossRef] [PubMed]

194. Behrens, J.; Kameritsch, P.; Wallner, S.; Pohl, U.; Pogoda, K. The carboxyl tail of Cx43 augments p38 mediated cell migration in a gap junction-independent manner. *Eur. J. Cell Biol.* **2010**, *89*, 828–838. [CrossRef] [PubMed]

195. Li, Q.; Omori, Y.; Nishikawa, Y.; Yoshioka, T.; Yamamoto, Y.; Enomoto, K. Cytoplasmic accumulation of connexin32 protein enhances motility and metastatic ability of human hepatoma cells in vitro and in vivo. *Int. J. Cancer* **2007**, *121*, 536–546. [CrossRef]

196. Langlois, S.; Cowan, K.N.; Shao, Q.; Cowan, B.J.; Laird, D.W. The tumor-suppressive function of Connexin43 in keratinocytes is mediated in part via interaction with caveolin-1. *Cancer Res.* **2010**, *70*, 4222–4232. [CrossRef]

197. Fujimoto, E.; Sato, H.; Shirai, S.; Nagashima, Y.; Fukumoto, K.; Hagiwara, H.; Negishi, E.; Ueno, K.; Omori, Y.; Yamasaki, H.; et al. Connexin32 as a tumor suppressor gene in a metastatic renal cell carcinoma cell line. *Oncogene* **2005**, *24*, 3684–3690. [CrossRef]

198. Kotini, M.; Barriga, E.H.; Leslie, J.; Gentzel, M.; Rauschenberger, V.; Schambony, A.; Mayor, R. Gap junction protein Connexin-43 is a direct transcriptional regulator of N-cadherin in vivo. *Nat. Commun.* **2018**, *9*, 3846. [CrossRef]

199. Lu, J.; Tan, M.; Cai, Q. The Warburg effect in tumor progression: Mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Lett.* **2015**, *356*, 156–164. [CrossRef]

200. Goldberg, G.S.; Lampe, P.D.; Sheedy, D.; Stewart, C.C.; Nicholson, B.J.; Naus, C.C. Direct isolation and analysis of endogenous transjunctional ADP from Cx43 transfected C6 glioma cells. *Exp. Cell Res.* **1998**, *239*, 82–92. [CrossRef]

201. Tabernero, A.; Medina, J.M.; Giaume, C. Glucose metabolism and proliferation in glia: Role of astrocytic gap junctions. *J. Neurochem.* **2006**, *99*, 1049–1061. [CrossRef] [PubMed]

202. Sanchez-Alvarez, R.; Tabernero, A.; Medina, J.M. Endothelin-1 stimulates the translocation and upregulation of both glucose transporter and hexokinase in astrocytes: Relationship with gap junctional communication. *J. Neurochem.* **2004**, *89*, 703–714. [CrossRef] [PubMed]

203. Tabernero, A.; Giaume, C.; Medina, J.M. Endothelin-1 regulates glucose utilization in cultured astrocytes by controlling intercellular communication through gap junctions. *Glia* **1996**, *16*, 187–195. [CrossRef]

204. Nishioka, T.; Oda, Y.; Seino, Y.; Yamamoto, T.; Inagaki, N.; Yano, H.; Imura, H.; Shigemoto, R.; Kikuchi, H. Distribution of the glucose transporters in human brain tumors. *Cancer Res.* **1992**, *52*, 3972–3979.

205. Sanchez-Alvarez, R.; Tabernero, A.; Medina, J.M. The increase in gap junctional communication decreases the rate of glucose uptake in C6 glioma cells by releasing hexokinase from mitochondria. *Brain Res.* **2005**, *1039*, 189–198. [CrossRef]

206. Dovmark, T.H.; Hulikova, A.; Niederer, S.A.; Vaughan-Jones, R.D.; Swietach, P. Normoxic cells remotely regulate the acid-base balance of cells at the hypoxic core of connexin-coupled tumor growths. *FASEB J.* **2018**, *32*, 83–96. [CrossRef]

207. Yaku, K.; Okabe, K.; Hikosaka, K.; Nakagawa, T. NAD Metabolism in Cancer Therapeutics. *Front. Oncol.* **2018**, *8*, 622. [CrossRef]

208. Miro-Casas, E.; Ruiz-Meana, M.; Agullo, E.; Stahlhofen, S.; Rodriguez-Sinovas, A.; Cabestrero, A.; Jorge, I.; Torre, I.; Vazquez, J.; Boengler, K.; et al. Connexin43 in cardiomyocyte mitochondria contributes to mitochondrial potassium uptake. *Cardiovasc. Res.* **2009**, *83*, 747–756. [CrossRef]

209. Rodriguez-Sinovas, A.; Boengler, K.; Cabestrero, A.; Gres, P.; Morente, M.; Ruiz-Meana, M.; Konietzka, I.; Miro, E.; Totzeck, A.; Heusch, G.; et al. Translocation of connexin 43 to the inner mitochondrial membrane of cardiomyocytes through the heat shock protein 90-dependent TOM pathway and its importance for cardioprotection. *Circ. Res.* **2006**, *99*, 93–101. [CrossRef]

210. Bonnet, S.; Archer, S.L.; Allalunis-Turner, J.; Haromy, A.; Beaulieu, C.; Thompson, R.; Lee, C.T.; Lopaschuk, G.D.; Puttagunta, L.; Bonnet, S.; et al. A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell* **2007**, *11*, 37–51. [CrossRef]

211. Korge, P.; Honda, H.M.; Weiss, J.N. K+-dependent regulation of matrix volume improves mitochondrial function under conditions mimicking ischemia-reperfusion. *Am. J. Physiol. Heart Circ. Physiol.* **2005**, *289*, H66–H77. [CrossRef] [PubMed]

212. Heinen, A.; Camara, A.K.; Aldakkak, M.; Rhodes, S.S.; Riess, M.L.; Stowe, D.F. Mitochondrial Ca^{2+}-induced K^+ influx increases respiration and enhances ROS production while maintaining membrane potential. *Am. J. Physiol. Cell Physiol.* **2007**, *292*, C148–C156. [CrossRef] [PubMed]

213. Boengler, K.; Ruiz-Meana, M.; Gent, S.; Ungefug, E.; Soetkamp, D.; Miro-Casas, E.; Cabestrero, A.; Fernandez-Sanz, C.; Semenzato, M.; Di Lisa, F.; et al. Mitochondrial connexin 43 impacts on respiratory complex I activity and mitochondrial oxygen consumption. *J. Cell. Mol. Med.* **2012**, *16*, 1649–1655. [CrossRef] [PubMed]

214. Gadicherla, A.K.; Wang, N.; Bulic, M.; Agullo-Pascual, E.; Lissoni, A.; De Smet, M.; Delmar, M.; Bultynck, G.; Krysko, D.V.; Camara, A.; et al. Mitochondrial Cx43 hemichannels contribute to mitochondrial calcium entry and cell death in the heart. *Basic Res. Cardiol.* **2017**, *112*, 27. [CrossRef] [PubMed]

215. Gleisner, M.A.; Navarrete, M.; Hofmann, F.; Salazar-Onfray, F.; Tittarelli, A. Mind the Gaps in Tumor Immunity: Impact of Connexin-Mediated Intercellular Connections. *Front. Immunol.* **2017**, *8*, 1067. [CrossRef] [PubMed]

216. Glass, A.M.; Snyder, E.G.; Taffet, S.M. Connexins and pannexins in the immune system and lymphatic organs. *Cell. Mol. Life Sci.* **2015**, *72*, 2899–2910. [CrossRef] [PubMed]

217. Neijssen, J.; Pang, B.; Neefjes, J. Gap junction-mediated intercellular communication in the immune system. *Prog. Biophys. Mol. Biol.* **2007**, *94*, 207–218. [CrossRef]

218. Eugenin, E.A.; Branes, M.C.; Berman, J.W.; Saez, J.C. TNF-alpha plus IFN-gamma induce connexin43 expression and formation of gap junctions between human monocytes/macrophages that enhance physiological responses. *J. Immunol.* **2003**, *170*, 1320–1328. [CrossRef]

219. Eugenin, E.A.; Eckardt, D.; Theis, M.; Willecke, K.; Bennett, M.V.; Saez, J.C. Microglia at brain stab wounds express connexin 43 and in vitro form functional gap junctions after treatment with interferon-gamma and tumor necrosis factor-alpha. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4190–4195. [CrossRef]

220. Schadt, L.; Sparano, C.; Schweiger, N.A.; Silina, K.; Cecconi, V.; Lucchiari, G.; Yagita, H.; Guggisberg, E.; Saba, S.; Nascakova, Z.; et al. Cancer-Cell-Intrinsic cGAS Expression Mediates Tumor Immunogenicity. *Cell Rep.* **2019**, *29*, 1236–1248.e7. [CrossRef]

221. Ablasser, A.; Schmid-Burgk, J.L.; Hemmerling, I.; Horvath, G.L.; Schmidt, T.; Latz, E.; Hornung, V. Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP. *Nature* **2013**, *503*, 530–534. [CrossRef]

222. Huang, M.N.; Nicholson, L.T.; Batich, K.A.; Swartz, A.M.; Kopin, D.; Wellford, S.; Prabhakar, V.K.; Woroniecka, K.; Nair, S.K.; Fecci, P.E.; et al. Antigen-loaded monocyte administration induces potent therapeutic antitumor T cell responses. *J. Clin. Investig.* **2020**, *130*, 774–788. [CrossRef] [PubMed]

223. Mendoza-Naranjo, A.; Saez, P.J.; Johansson, C.C.; Ramirez, M.; Mandakovic, D.; Pereda, C.; Lopez, M.N.; Kiessling, R.; Saez, J.C.; Salazar-Onfray, F. Functional gap junctions facilitate melanoma antigen transfer and cross-presentation between human dendritic cells. *J. Immunol.* **2007**, *178*, 6949–6957. [CrossRef] [PubMed]

224. Benlalam, H.; Jalil, A.; Hasmim, M.; Pang, B.; Tamouza, R.; Mitterrand, M.; Godet, Y.; Lamerant, N.; Robert, C.; Avril, M.F.; et al. Gap junction communication between autologous endothelial and tumor cells induce cross-recognition and elimination by specific CTL. *J. Immunol.* **2009**, *182*, 2654–2664. [CrossRef] [PubMed]

225. Hofmann, F.; Navarrete, M.; Alvarez, J.; Guerrero, I.; Gleisner, M.A.; Tittarelli, A.; Salazar-Onfray, F. Cx43-Gap Junctions Accumulate at the Cytotoxic Immunological Synapse Enabling Cytotoxic T Lymphocyte Melanoma Cell Killing. *Int. J. Mol. Sci.* **2019**, *20*, 4509. [CrossRef] [PubMed]

226. Tittarelli, A.; Mendoza-Naranjo, A.; Farias, M.; Guerrero, I.; Ihara, F.; Wennerberg, E.; Riquelme, S.; Gleisner, A.; Kalergis, A.; Lundqvist, A.; et al. Gap junction intercellular communications regulate NK cell activation and modulate NK cytotoxic capacity. *J. Immunol.* **2014**, *192*, 1313–1319. [CrossRef] [PubMed]

227. Elgueta, R.; Tobar, J.A.; Shoji, K.F.; De Calisto, J.; Kalergis, A.M.; Bono, M.R.; Rosemblatt, M.; Saez, J.C. Gap junctions at the dendritic cell-T cell interface are key elements for antigen-dependent T cell activation. *J. Immunol.* **2009**, *183*, 277–284. [CrossRef]

228. Mendoza-Naranjo, A.; Bouma, G.; Pereda, C.; Ramirez, M.; Webb, K.F.; Tittarelli, A.; Lopez, M.N.; Kalergis, A.M.; Thrasher, A.J.; Becker, D.L.; et al. Functional gap junctions accumulate at the immunological synapse and contribute to T cell activation. *J. Immunol.* **2011**, *187*, 3121–3132. [CrossRef]

229. Schenk, U.; Westendorf, A.M.; Radaelli, E.; Casati, A.; Ferro, M.; Fumagalli, M.; Verderio, C.; Buer, J.; Scanziani, E.; Grassi, F. Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels. *Sci. Signal.* **2008**, *1*, ra6. [CrossRef]

230. Kang, J.; Kang, N.; Lovatt, D.; Torres, A.; Zhao, Z.; Lin, J.; Nedergaard, M. Connexin 43 hemichannels are permeable to ATP. *J. Neurosci.* **2008**, *28*, 4702–4711. [CrossRef]

231. Manohar, M.; Hirsh, M.I.; Chen, Y.; Woehrle, T.; Karande, A.A.; Junger, W.G. ATP release and autocrine signaling through P2 × 4 receptors regulate gammadelta T cell activation. *J. Leukoc. Biol.* **2012**, *92*, 787–794. [CrossRef] [PubMed]

232. Oviedo-Orta, E.; Perreau, M.; Evans, W.H.; Potolicchio, I. Control of the proliferation of activated CD4+ T cells by connexins. *J. Leukoc. Biol.* **2010**, *88*, 79–86. [CrossRef] [PubMed]

233. Audrito, V.; Manago, A.; Gaudino, F.; Sorci, L.; Messana, V.G.; Raffaelli, N.; Deaglio, S. NAD-Biosynthetic and Consuming Enzymes as Central Players of Metabolic Regulation of Innate and Adaptive Immune Responses in Cancer. *Front. Immunol.* **2019**, *10*, 1720. [CrossRef] [PubMed]

234. Rhett, J.M.; Yeh, E.S. The Potential for Connexin Hemichannels to Drive Breast Cancer Progression through Regulation of the Inflammatory Response. *Int. J. Mol. Sci.* **2018**, *19*, 1043. [CrossRef] [PubMed]

235. Ghatnekar, G.S.; O'Quinn, M.P.; Jourdan, L.J.; Gurjarpadhye, A.A.; Draughn, R.L.; Gourdie, R.G. Connexin43 carboxyl-terminal peptides reduce scar progenitor and promote regenerative healing following skin wounding. *Regen. Med.* **2009**, *4*, 205–223. [CrossRef] [PubMed]

236. Soder, B.L.; Propst, J.T.; Brooks, T.M.; Goodwin, R.L.; Friedman, H.I.; Yost, M.J.; Gourdie, R.G. The connexin43 carboxyl-terminal peptide ACT1 modulates the biological response to silicone implants. *Plast. Reconstr. Surg.* **2009**, *123*, 1440–1451. [CrossRef]

237. Calder, B.W.; Matthew Rhett, J.; Bainbridge, H.; Fann, S.A.; Gourdie, R.G.; Yost, M.J. Inhibition of connexin 43 hemichannel-mediated ATP release attenuates early inflammation during the foreign body response. *Tissue Eng. Part A* **2015**, *21*, 1752–1762. [CrossRef]

238. Danesh-Meyer, H.V.; Kerr, N.M.; Zhang, J.; Eady, E.K.; O'Carroll, S.J.; Nicholson, L.F.; Johnson, C.S.; Green, C.R. Connexin43 mimetic peptide reduces vascular leak and retinal ganglion cell death following retinal ischaemia. *Brain* **2012**, *135*, 506–520. [CrossRef]

239. Colotta, F.; Allavena, P.; Sica, A.; Garlanda, C.; Mantovani, A. Cancer-related inflammation, the seventh hallmark of cancer: Links to genetic instability. *Carcinogenesis* **2009**, *30*, 1073–1081. [CrossRef]

240. Nagasawa, H.; Little, J.B. Induction of sister chromatid exchanges by extremely low doses of alpha-particles. *Cancer Res.* **1992**, *52*, 6394–6396.

241. Klammer, H.; Mladenov, E.; Li, F.; Iliakis, G. Bystander effects as manifestation of intercellular communication of DNA damage and of the cellular oxidative status. *Cancer Lett.* **2015**, *356*, 58–71. [CrossRef] [PubMed]

242. Feine, I.; Pinkas, I.; Salomon, Y.; Scherz, A. Local oxidative stress expansion through endothelial cells—A key role for gap junction intercellular communication. *PLoS ONE* **2012**, *7*, e41633. [CrossRef] [PubMed]

243. Andrade-Rozental, A.F.; Rozental, R.; Hopperstad, M.G.; Wu, J.K.; Vrionis, F.D.; Spray, D.C. Gap junctions: The "kiss of death" and the "kiss of life". *Brain Res. Brain Res. Rev.* **2000**, *32*, 308–315. [CrossRef]

244. Fiaschi, T.; Chiarugi, P. Oxidative stress, tumor microenvironment, and metabolic reprogramming: A diabolic liaison. *Int. J. Cell Biol.* **2012**, *2012*, 762825. [CrossRef]

245. Hutnik, C.M.; Pocrnich, C.E.; Liu, H.; Laird, D.W.; Shao, Q. The protective effect of functional connexin43 channels on a human epithelial cell line exposed to oxidative stress. *Investig. Ophthal. Mol. Vis. Sci.* **2008**, *49*, 800–806. [CrossRef]

246. Le, H.T.; Sin, W.C.; Lozinsky, S.; Bechberger, J.; Vega, J.L.; Guo, X.Q.; Saez, J.C.; Naus, C.C. Gap junction intercellular communication mediated by connexin43 in astrocytes is essential for their resistance to oxidative stress. *J. Biol. Chem.* **2014**, *289*, 1345–1354. [CrossRef]

247. Nakamura, T.Y.; Yamamoto, I.; Kanno, Y.; Shiba, Y.; Goshima, K. Metabolic coupling of glutathione between mouse and quail cardiac myocytes and its protective role against oxidative stress. *Circ. Res.* **1994**, *74*, 806–816. [CrossRef]

248. Desai, S.; Kumar, A.; Laskar, S.; Pandey, B.N. Cytokine profile of conditioned medium from human tumor cell lines after acute and fractionated doses of gamma radiation and its effect on survival of bystander tumor cells. *Cytokine* **2013**, *61*, 54–62. [CrossRef]

249. Hoorelbeke, D.; Decrock, E.; De Smet, M.; De Bock, M.; Descamps, B.; Van Haver, V.; Delvaeye, T.; Krysko, D.V.; Vanhove, C.; Bultynck, G.; et al. Cx43 channels and signaling via IP3/Ca(2+), ATP, and ROS/NO propagate radiation-induced DNA damage to non-irradiated brain microvascular endothelial cells. *Cell Death Dis.* **2020**, *11*, 194. [CrossRef]

250. Tong, X.; Lopez, W.; Ramachandran, J.; Ayad, W.A.; Liu, Y.; Lopez-Rodriguez, A.; Harris, A.L.; Contreras, J.E. Glutathione release through connexin hemichannels: Implications for chemical modification of pores permeable to large molecules. *J. Gen. Physiol.* **2015**, *146*, 245–254. [CrossRef]

251. Shi, W.; Riquelme, M.A.; Gu, S.; Jiang, J.X. Connexin hemichannels mediate glutathione transport and protect lens fiber cells from oxidative stress. *J. Cell Sci.* **2018**, *131*. [CrossRef] [PubMed]

252. Bruzzone, S.; Guida, L.; Zocchi, E.; Franco, L.; De Flora, A. Connexin 43 hemi channels mediate Ca2+-regulated transmembrane NAD+ fluxes in intact cells. *FASEB J.* **2001**, *15*, 10–12. [CrossRef]

253. Yang, B.; Zwaans, B.M.; Eckersdorff, M.; Lombard, D.B. The sirtuin SIRT6 deacetylates H3 K56Ac in vivo to promote genomic stability. *Cell Cycle* **2009**, *8*, 2662–2663. [CrossRef] [PubMed]

254. Mostoslavsky, R.; Chua, K.F.; Lombard, D.B.; Pang, W.W.; Fischer, M.R.; Gellon, L.; Liu, P.; Mostoslavsky, G.; Franco, S.; Murphy, M.M.; et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* **2006**, *124*, 315–329. [CrossRef] [PubMed]

255. Imai, S.; Armstrong, C.M.; Kaeberlein, M.; Guarente, L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **2000**, *403*, 795–800. [CrossRef] [PubMed]

256. Zhao, W.; Han, H.B.; Zhang, Z.Q. Suppression of lung cancer cell invasion and metastasis by connexin43 involves the secretion of follistatin-like 1 mediated via histone acetylation. *Int. J. Biochem. Cell Biol.* **2011**, *43*, 1459–1468. [CrossRef] [PubMed]

257. Zhu, W.; Mironov, N.; Yamasaki, H. Increased genetic stability of HeLa cells after connexin 43 gene transfection. *Cancer Res.* **1997**, *57*, 2148–2150.

258. Basheer, W.A.; Fu, Y.; Shimura, D.; Xiao, S.; Agvanian, S.; Hernandez, D.M.; Hitzeman, T.C.; Hong, T.; Shaw, R.M. Stress response protein GJA1-20k promotes mitochondrial biogenesis, metabolic quiescence, and cardioprotection against ischemia/reperfusion injury. *JCI Insight* **2018**, *3*. [CrossRef]

259. Sin, W.C.; Crespin, S.; Mesnil, M. Opposing roles of connexin43 in glioma progression. *Biochim. Biophys. Acta* **2012**, *1818*, 2058–2067. [CrossRef]

260. Manjarrez-Marmolejo, J.; Franco-Perez, J. Gap Junction Blockers: An Overview of their Effects on Induced Seizures in Animal Models. *Curr. Neuropharmacol.* **2016**, *14*, 759–771. [CrossRef]