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Review

Connexin-based signaling in acute myelogenous leukemia (AML)

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ABSTRACT

Normal and malignant hematopoiesis are regulated by intercellular communication in the hematopoietic microenvironments, and both soluble mediators as well as direct cell–cell contact play important functional roles. Gap junctions are complex membrane structures that transfer molecules between neighboring cells and thereby alter intracellular signaling and metabolism. The gap junction building blocks, the connexins, are also involved in gap junction-independent intercellular communication by forming hemichannels that transfer substances between the intra- and extracellular spaces. Connexins are furthermore involved in cell regulation as single molecules by modulating intracellular pathways and possibly gene transcription. The role of connexins in leukemogenesis and leukemic cell functions are not well characterized. In this review, we describe the known effects of gap junctions and connexins in acute myelogenous leukemia and the diverse potential of connexins in acute myelogenous leukemia chemosensitivity, intracellular signaling and cell death regulation.

Contents

1. Introduction

The hematopoietic stem cell niche is the microenvironment of stem cells, their surrounding stromal cells, i.e. fibroblasts, adipocytes, endothelial and osteoblastic cells and extracellular matrix where the hematopoiesis takes place. In these niches, the normal hematopoiesis is regulated by a wide range of soluble mediators and direct cell–cell interactions. Stromal cells participate through both these mechanisms, and endothelial and osteoblastic cells are the main components

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of the vascular and endosteal niches, respectively. The niches and the stromal cells might therefore be active participants in the development of leukemic hematopoiesis as well [1].

Previous in vitro studies have clearly shown that soluble mediators alter acute myelogenous leukemic (AML) cell regulation of proliferation and apoptosis both when the leukemic cells are cultured alone [2] and co-cultured with fibroblasts [3], endothelial cells [4] and osteoblasts [5]. In addition, direct cell–cell interactions between AML cells and other neighboring cells, including osteoblasts and endothelial cells, will also alter AML cell functions [6,7].

Even though the function of gap junctions and their connexin building blocks affects the regulations of several cellular functions, including growth and apoptosis of normal and malignant cells, and also seems to be important for chemosensitivity, little is known about their role in leukemogenesis and leukemic cell functions. The aim of this review is therefore to summarize recent results and describe the potential roles of connexins in human AML.

2. Gap junctions, hemichannels and connexins

The gap junction (GJ) is one of several structures involved in intercellular communication. GJs form small channels between neighboring cells (Fig. 1) that transport molecules and ions with molecular weights up to approximately 1 kDa, e.g. Ca^{2+}, cyclic adenosine monophosphate (cAMP) and inositol triphosphate (IP_3) [8,9]. Each of the two communicating cells contributes to these GJs by their own connexin hemichannels (CxHcs) also named connexons (Figs. 1 and 2), and each CxHc consists of six transmembrane proteins called connexins (Cx) (Fig. 2). Twenty-one different human Cxs have been detected and they are generally named according to their approximate molecular mass in kDa, e.g. Cx32, Cx43 and Cx45 [8]. Each Cx shows tissue- or cell-specific expression, and most organs and many cell types express more than one Cx [8]. Due to the fact that Cxs can oligomerize with other Cxs, a wide range of different CxHcs can be formed [9]. The large number of possible Cx combinations in each CxHc results in a structural basis for functional heterogeneity of GJs that will probably be the basis for a functional heterogeneity as well.

Normal neutrophils, monocytes, macrophages and hematopoietic stem cells express Cx43 and usually additional Cxs (see [10], and references therein). Bone marrow stromal cells also express different Cxs including Cx43 and Cx45 [11,12]. GJs and Cxs are therefore likely to participate in the regulation of normal hematopoiesis [13]. The functional importance of GJs and Cxs in carcinogenesis is also illustrated by the detection of abnormal or defective GJ communication in various solid tumors including liver, bladder, breast and prostate cancers [14] (Table 1). Even though their role in carcinogenesis is not clear, it is possible that Cxs and GJs are involved in both tumor growth regulations, tumor cell migration and metastasis as thoroughly described in a recent review [15].

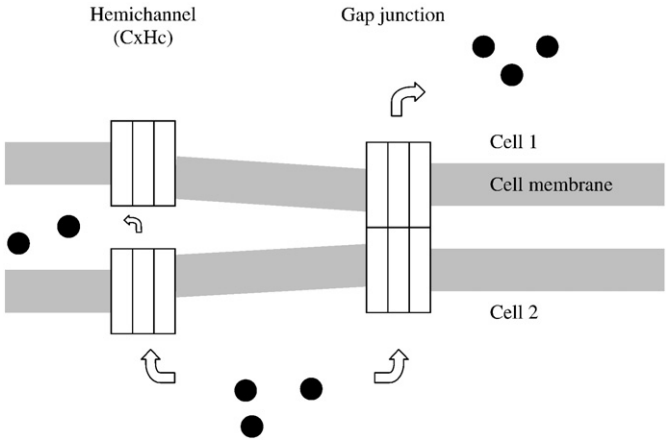

Fig. 1. The figure illustrates two adjoining cells connected by one gap junction transferring small signaling molecules, e.g. Ca^{2+}, cAMP and IP_3 (●). The figure also shows one CxHc in each cell membrane that can either connect and thereby create another gap junction, or function as a communication channel with the intercellular space. Both these mechanisms are involved in the regulation of neighboring cells, including apoptosis and cell growth.

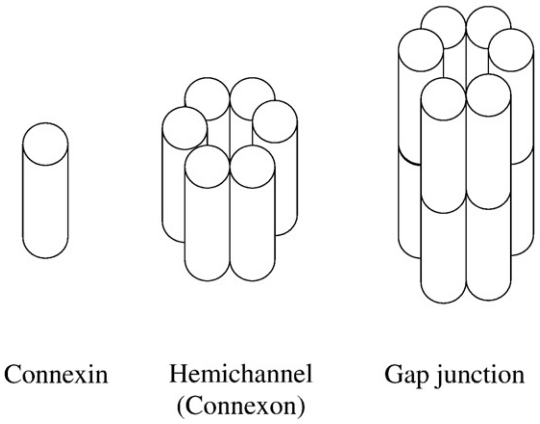

Fig. 2. The figure illustrates the structural relation between a connexin, a CxHc and a gap junction. Each single CxHc is formed by six connexins. These connexins may be of the same subtype, e.g. Cx43, or a combination of different subtypes, e.g. Cx43 and Cx45. Each CxHc of a gap junction is anchored to adjoining cells (see Fig. 1). The two CxHcs of a single gap junction can be of different types, i.e. one CxHc can be made of one type of connexins, whereas the other CxHc can be made of another type of connexins.

3. The roles of connexins in AML

Leukemia is an aggressive malignancy where both disease development and chemosensitivity seem to be regulated by neighboring stromal cells, i.e. the cells that form the hematopoietic stem cell niche [1]. As will be described below, it seems likely that GJs are involved in the communication between AML and stromal cells during disease development.

3.1. The functional role of Cxs in AML cells

Studies of both the HL-60 and PLB-985 AML cell lines have shown that direct contact with KM-102 bone marrow stromal cells inhibits AML cell differentiation [16] (Table 2), and dye transfer studies suggest that this effect was associated with the formation of functional GJs [16]. Studies of both normal and leukemic bone marrow cells have demonstrated the formation of Cx43 GJs between stromal and hematopoietic cells, and Cx43 GJs were upregulated in AML bone marrows [17]. Formation of functional GJs was also detected during coculture of the murine stromal cell S17 and the lymphoblastic leukemic cell line CCRF-CEM [18]. These studies showed that the GJ coupling between the stromal and leukemic

Table 1
Aberrant connexin expression/function in various tumors.

Disease	Cx function
Liver cancer	Cx32 – mostly located to the cytoplasm Cx43 – aberrant expression
Bladder cancer	Cx26 – loss Cx43 – variable expression
Prostate cancer	Cx32 – decreased expression Cx43 – decreased expression
Breast tumor	Cx26 – upregulated Cx43 – heterogeneous expression
Lung cancer	Cx43 – decreased expression Low dye-transfer

The table presents a short overview of aberrant connexin expression and function in malignant diseases. The table is based on [14].

Table 2
Effects of Cx/GJ in AML.

Cells/Animal	Cx/GJ	Function		Reference
AML BM	Cx43 + GJ	Expression	↑	16
U937	Cx43 mRNA	AML1-ETO induced expression	↑	18
Primary cells	Cx43, Cx45	Membrane expression	↑	np
HL-60	GJ	Differentiation	↓	15
HL-60, KG1	GJ	Cell growth	↓	17
U937	Cx43	Cell growth	↓	18
Mice	Cx32-KO	Increased risk of leukemic incidence		20

The table presents an overview of functional interactions between connexins and AML cells. BM, bone marrow; KO, knock-out; np, not published.

cells inhibited proliferation by retaining the leukemic cells in their G_0-phase of the cell cycle. This effect was abrogated by carbenoxolone that inhibits GJ-communication. Importantly, these anti-proliferative effects of GJ coupling were also detected for the AML cell lines HL-60 and KG1 [18]. In contrast to these results, functional GJ was not found between the murine S17 stromal cells and murine B-lineage cells harvested from BALB/cAn mice bone marrows [11], suggesting that the GJ communication between stromal and leukemic cells depends on the stromal and/or leukemic cell phenotype.

The t(8;21)(q22;q22) translocation, which encodes the AML1-ETO hybrid protein, is detected in 12% of patients with *de novo* AML [19]. Induced expression of the AML1-ETO fusion gene in the AML U937 cell line upregulated Cx43 mRNA levels [19]. The upregulation was possibly caused by altered signaling through the c-Jun N-terminal kinase pathway [20], and increased Cx43 expression directly inhibited the leukemic cell growth whereas suppression of Cx43 with small interfering RNA overcomes the growth-inhibitory effect of AML1-ETO in leukemic cells [19]. This growth-inhibitory effect of Cx43 may increase chemosensitivity and contribute to the good prognosis with high AML-free survival after chemotherapy for patients with t(8;21). Nonetheless, the small interfering RNA will also inhibit the expression of CxHcs leaving open the question whether the growth-inhibitory effect of AML1-ETO in leukemic cells is mediated by Cx43 alone, only CxHcs or functional GJs. A role of Cxs in leukemogenesis is further supported by studies in knock-out animal models; Cx32 knock-out mice show an increased incidence of hematopoietic malignancies after treatment with the genotoxic chemical methylnitrosourea compared to wild-type mice [21].

We have analyzed the mRNA levels and membrane molecule expression of different Cxs in primary human AML blasts. The mRNA levels of Cx32 and Cx43 showed low expression for a large majority of patients, but a subset of patients expressed detectable surface levels of Cx32, Cx37, Cx43 and Cx45 (unpublished data). Taken together, these observations suggest that Cxs are important for disease development in AML, possibly by mediating growth-regulatory signaling from neighboring stromal cells (Table 2).

3.2. Connexins and chemosensitivity

Very few studies have investigated the possible importance of Cxs and GJ communications for chemosensitivity of malignant cells (Table 3). Cx43 expression increased the sensitivity of human glioblastoma cells to etoposide, paclitaxel and the anthracyclin doxorubicin, and this increased chemosensitivity was associated with decreased expression of anti-apoptotic Bcl-2 [22]. This observation, together with the results from AML1-ETO induced Cx43 expression in AML cells (above) [19], suggests that expression of this particular Cx decreases proliferation and increases chemosensitivity.

Doxorubicin has also been associated with decreased GJ-communication in WB-F344 rat-liver epithelial cells, in which the Cx43 subtype dominates [23]. This effect of doxorubicin was mediated via the epidermal growth factor (EGF) receptor that activated the extracellular signal-regulated kinase (ERK)-1 and ERK-2 [23]. Doxorubicin treatment of the breast cancer cell line MCF-7 was associated with increased apoptosis and decreased Cx43 expression, particularly when doxorubicin was added in combination with docetaxel [24]. Functional GJs also protect the lymphoblastic leukemic cell line CCRF-CEM against methotrexate induced cell death when co-cultured with the stromal cell line S17 [18]. Thus, there seem to be an association between Cx43 expression and regulation of apoptosis, but the molecular explanations are not known.

Table 3
Functional interactions between cytotoxic drugs and Cx/GJ.

Drug	Cx/GJ	Function		Reference
Methotrexate	GJ	Cell viability	↑	17
	GJ	Apoptosis	↓	17
Doxorubicin	Cx43	Bcl-2 expression	↓	21
	GJ	Intercellular communication	↓	22
	Cx43	Expression	↓	23
Arsenic trioxide	GJ	Intercellular communication	↓	24
Retinoic acid	Cx43	Expression	↑	25,26
	GJ	Intercellular communication	↑	25,26
	Cx32	Cx phosphorylation	↑	25

The table presents an overview of functional interactions between connexins and different chemotherapeutic drugs commonly used in AML therapy.

Arsenic trioxide, which is currently approved for the treatment of acute promyelocytic leukemia (APL), seems to inhibit GJ-communication between vascular endothelial cells [25]. On the other hand, retinoic acid can increase the Cx43 membrane expression and GJ intercellular communication in the human Hep G2 and murine 3T3 cells [26,27]. The Cx32 membrane expression was not altered, but Cx32 phosphorylation was increased after in vitro retinoic acid treatment [26]. Taken together, these observations suggest that retinoic acid can regulate GJ communication at different levels, and increased Cx43 expression may be important for the anti-leukemic effect of all-trans retinoic acid (ATRA) in combination with conventional chemotherapies in APL.

Although few, these observations show that chemotherapeutic drugs commonly used in AML treatment can affect Cxs and GJs. Most studies suggest that Cx43 expression increases chemosensitivity and this seems to be true both for anthracyclins and ATRA. Cx32 may have a similar effect. In contrast, other studies suggest that decreased communication through GJs may be a part of the anticancer effect for several chemotherapeutics. The most likely explanation for these apparently conflicting results is that the effect of GJs on cell growth

Table 4
Functional interactions between intracellular pathways and Cx/GJ.

Pathway	Cx/GJ	Function		Reference
Src	Cx43	Cell survival	↑	29
	Cx43 + GJ	Opening	↓	30,31
	Cx32	Apoptosis	↓	32
MAPK	Cx43	Expression	↑	38
	Cx43 + GJ	Intercellular communication Cx phosphorylation	↓	39,40
	Cx43	Endocytosis	↓	41
	Cx43 + GJ	Uncoupling	↓	42
Wnt/β-catenin	Cx43	Expression	↑	52,53
	Cx43	Protein levels (by G-CSF)	↑	55
PI3K/AKT	Cx32	mRNA expression	↓	62
	Cx40	Expression	↑	63
	Cx43	Expression	↓	63
PKA	GJ	Intercellular communication, Assembly of new GJs	↑	28
	Cx40	Expression	↑	63
	Cx43	Expression	↓	63
PKC	Cx43	Intercellular communication, Cx phosphorylation	↓	28
	Cx43 + GJ	Formation	↓	28

The table presents an overview of functional connections between connexins and different signaling pathways that may connect connexins to leukemogenesis and chemosensitivity in AML.

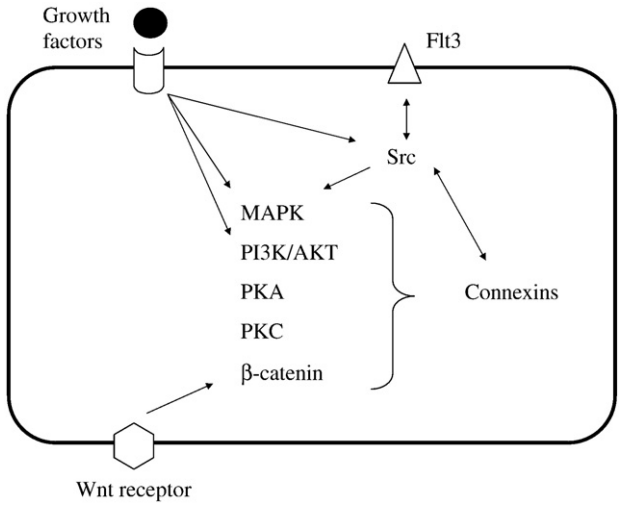

Fig. 3. The figure gives an overview of possible interactions between various intercellular pathways and connexins. Based on the literature (see text), connexin regulation is clearly affected at least by the Src, MAPK, PI3K/AKT, PKA, PKC and Wnt/β-catenin pathways in different cell types. These pathways are under control of various hematopoietic growth factors including the Flt3- and Wnt-ligands. Connexins may also alter the activity of intercellular pathways via its interaction with Src.

and apoptosis regulation differs between various Cxs. Another explanation could be cell-specific differences, but at least for Cx43 this seems less likely because the chemosensitizing/growth-inhibitory effect was detected in different cells.

4. Connexins and intracellular pathways in AML

Several intracellular signaling pathways are involved in the regulation of Cx expression and function in leukemic cells (overviewed in Table 4 and Fig. 3). Here we show that different signaling pathways may connect Cx to leukemogenesis and possibly also chemosensitivity in AML.

4.1. Src pathways

Src is an intracellular non-receptor family of kinases that regulates intracellular signaling [28]. Src can promote neoplastic transformation and seems to be involved in the progression of diverse malignancies including leukemias, lymphomas and myeloma [28,29]. As recently reviewed [29], Src can alter the activation of Cx43 both directly as well as indirectly via several other kinases including mitogen activated protein kinase (MAPK). Interestingly, previous studies have demonstrated that Cx43 CxHcs can interact with Src and promote cell survival by mechanisms involving MAPK [30]. The Src pathway can also inhibit Cx43-based GJ communication [31,32]. All together, these observations suggest that there seem to be a bidirectional crosstalk between Cx43 and Src (Fig. 3) at least in certain cells, but it is not known whether this can be seen in AML. The Src inhibitor PP1 potentiated Cx32-dependent cell growth suppression in Cx32-tranfected renal cells and apoptosis was then induced through reduction of anti-apoptotic Bcl-2 and Bcl-xL [33]. Thus, Src seems to be involved in the regulation of various Cxs. However, it is important to note that the role of Src in the regulation of Cx43 and Cx32 might not be comparable due to their different structures, biochemistry, e.g. their phosphorylation status, and the fact that the Src regulation of different Cxs may be cell specific and involve different signaling pathways.

The Src-Cx/GJ-interactions may be involved in leukemogenesis via the Fms-like tyrosine kinase-3 (Flt-3). Flt3 is a hematopoietic growth factor receptor that promotes survival and proliferation of myeloid precursors, and mutations of Flt-3 have been detected in approximately 30% of AML patients [34]. Flt-3-internal tandem duplications (ITD) are common, and these mutations are associated with an adverse prognosis [34]. Different Src subtypes interfere with the maturation process of Flt-3 by lowering the expression of the glycosylated high molecular weight form of Flt-3 and increasing the low molecular weight form; this is a kinase-dependent process [35]. On the other hand, Flt-3 ligation can activate the Src kinase [36]. The Flt-3 pathway may thereby affect Cx-mediated communication indirectly through its crosstalk with Src (Fig. 3).

Finally, ATRA-induced differentiation of the leukemic cell lines NB-4, HL-60 and U937 and primary AML cells is inhibited by Src [37]. This observation suggests that Src mediates chemoresistance, but it is not known whether this Src-mediated effect also involves inhibition of retinoic acid-induced Cx43 expression (see above).

4.2. MAPK/MEK/ERK pathways

The MAPK and MEK/ERK pathways are a highly complex cascade reaction transmitting signals from different ligands [38]. As already mentioned, Src is connected to MAPK that can phosphorylate Cx43 [29]. Several observations suggest that there exist direct functional interactions between Cxs and the MAPK/MEK/ERK pathways. Studies in various cell types have demonstrated that these pathways are involved in Cx43 upregulation by Angiotensin II [39], Cx43 phosphorylation with decreased GJ communication [40,41], Cx43 endocytosis induced by the carcinogen Lindane [42] and Cx43 GJ uncoupling induced by EGF [43]. The ERK pathway also seems to be involved in GJ-independent survival signaling by Src [30].

The relevance of these pathways in AML has been shown in several reports describing that a subset of AML cells show activated MAPK [44,45], suggesting that MAPK may be involved in leukemogenesis [44]. MEK and ERK are also activated in AML cells [46], and the MEK activation blocker PD98059 decreases both growth and survival of AML cell lines as well as primary cells [47]. The ERK pathway seems to cooperate with Flt-3 in regulation of cell survival [45]. Taken together, the available observations suggest that MAPK and MEK/ERK pathways are involved in downregulation of Cx expression and/or GJ communication in AML.

4.3. Wnt/β-catenin pathway

The Wnt/β-catenin pathway is one of at least three pathways activated upon Wnt receptor ligation and initiates complex intracellular events involving a wide range of mediators [48]. Wnt signaling is involved in control of self-renewal and is thus an important regulator of hematopoietic stem cells [48]. The pathway is also activated in AML and this aberrant activation may contribute to the malignant phenotype [49]. β-catenin is expressed by primary AML cells for a majority of patients, and the level correlates with the capacity of clonogenic proliferation and cellular self-renewal [50]. Primary AML blasts with Flt-3-ITD express high levels of β-catenin, and the leukemogenic effects of Flt-3-ITD seem to be mediated in part by the Wnt signaling pathway [51]. This pathway is in addition involved in AML chemosensitivity regulation [52].

The Wnt/β-catenin pathway interferes with Cx43 that acts as a functional target of Wnt1 signaling [53]. Cx43 expression is increased by Wnt overexpression [53,54] probably through regulation at the transcriptional level [53,55]. In cardiomyocytes, granulocyte-colony stimulating factor (G-CSF) increased both the β-catenin (possibly via Wnt activation) and Cx43 protein levels [56], suggesting a coordinated regulation of at least this particular Cx and components in the Wnt/β-catenin pathway. In addition, a direct association between Cx43 and β-catenin is demonstrated in rat cardiomyocytes where β-catenin seems to be required for Cx43 transport to the cell membrane [57].

In the Wnt/calcium pathway, Wnt5a increases the intracellular calcium levels and thereby modulates the calcium-sensitive proteins, including calcium/calmodulin-dependent protein kinase II and protein kinase C (PKC) [58]. GJs are able to coordinate intercellular calcium propagation [59] and Cx32 is identified with calmodulin-binding domains [60] and may therefore represent a functional connection of Cxs to this pathway.

Taken together, these observations show that the Wnt/β-catenin pathway is important in AML. This pathway can interfere with Cx expression, Cx43 in particular, and these interactions may thus alter functional characteristics of AML cells.

4.4. PI3K/AKT, PKA and PKC

Phosphoinositide 3-kinase (PI3K) is an important signaling component downstream to several growth factor receptor tyrosine kinases that act via the second messenger PIP3 to activate protein kinase B (AKT) [61]. The PI3K/AKT pathway is involved in regulation of proliferation and survival for many cells [61], including clonogenic AML cell growth [62] and AML cell survival [63,64], and the AKT pathway seems to cooperate with Flt-3 in cell survival regulation in some AML cells [45]. Interestingly, the Cx32 mRNA levels are downregulated in AKT transfected rat hepatocytes by a mechanism involving the integrin-linked kinase pathway [65]. The PI3K/AKT pathway is also involved in the regulation of Cx40 and Cx43 expression in adenovirus vector E4 infected endothelial cells [66]. In these cells, Cx40 expression was increased, whereas Cx43 expression was suppressed by the adenovirus infection, and both these effects were abrogated both by the PI3K inhibitor LY294002 and the protein kinase A (PKA) inhibitor H89 [66]. PKA can increase GJ communication and facilitate the assembly of new GJs [29]. The role of PKA is not well characterized in AML, but its activity is upregulated in ATRA treated NB-4 AML cells [67] and it is important for the anti-apoptotic effect of the Flt3-ITD [68].

As reviewed elsewhere [29], Src can directly or via diacylglycerol and mobilized calcium activate protein kinase C (PKC). Activated PKC can phosphorylate Cx43 and, in most experiments, reduce GJ communication. PKC-γ can reduce Cx43 GJ formation in lens epithelial cells, PKC-α and β can inhibit fibroblasts coupling and PKC-ε can associate with Cx43 in cardiomyocytes (see [29] and references therein). PKC is detected in a subset of primary AML cells and PKC activation is associated with poor survival [69]. Interestingly, ingenol 3-angelate (PEP005), which is a PKC agonist, induces apoptosis in AML cells [70].

Altogether, the PI3K/AKT, PKA and PKC signaling pathways can interact with regulations of Cx expression, Cx40 and Cx43 in particular, and GJ communication. These pathways represent possible therapeutic targets in AML, but it is not known whether modulation of Cx/GJ is important for the anti-leukemic effects of this therapeutic strategy.

4.5. Growth factor initiated signaling

Intracellular pathways are usually directly or indirectly controlled by a wide range of mediators including growth factors. Diverse hematopoietic growth factors activate the Src, PI3K and MAPK/ERK systems (Fig. 3) including erythropoietin, thrombopoietin, stem cell factor, G-CSF, granulocyte-macrophage-CSF (GM-CSF), interleukin (IL)-3, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), EGF, insulin-like growth factor (IGF)-1, tumor necrosis factor (TNF)-α and fibroblastic growth factor (FGF) [28,71–74]. These cytokines, and possibly others as well, may thereby be involved in the regulation of Cx and GJ intercellular communication. This has been demonstrated for some of them: (i) PDGF inhibits GJ communication in the fibroblast T3T A31 cell line [75] and mesangial cells [74], (ii) G-CSF increases Cx43 expression and sustain GJ functions in cardiomyocytes [56], (iii) FGF-1 can increase membrane permeability through CxHcs in HeLa cells that express Cx43 or Cx45 [76], (iv) TNFα together with interferon-γ (INFγ) increases Cx43 expression and GJ communication in human monocytes [77] and (v) IL-1 inhibits GJ communication in murine stromal cells expressing Cx43 [11]. Obviously, a wide range of hematopoietic growth factors are potential regulators of Cx and GJ functions that are possibly important both for normal and neoplastic hematopoiesis.

The PKC activator PEP005 (see over) can increase the release of several cytokines both by AML cells and by neighboring non-leukemic cells, e.g. INFγ and TNFα release by immunocompetent cells [78]. Several of the Cx-affecting growth factors are available in the AML microenvironment and affect proliferation and constitutive cytokine secretion by primary human AML cells [79–81]. Autocrine and paracrine mechanisms may therefore interact with Cxs in AML. This suggest, as recently reviewed for growth factors [82], that Cxs may participate in mechanisms determining leukemic cell chemosensitivity and possibly the risk of relapse from minimal residual disease.

5. Gap junction- and Hemichannel-independent effects of connexins

As reviewed elsewhere, Cxs can alter cellular functions independently of both GJs and CxHcs [83,84]. To the best of our knowledge, these effects have not been studied in AML, although they may be involved in leukemogenesis or other AML functions.

5.1. Communication through CxHcs

CxHcs can affect neighboring cells by their release of different substances, e.g. adenosine triphosphate (ATP) and nicotinamid dinucleotid (NAD^+), into the extracellular space [83]. ATP can then bind its P2 purinergic receptors and thereby induce the production of IP_3 that in turn raises the intracellular Ca^{2+} levels and initiate Ca^{2+} waves [83]. Extracellular ATP suppresses the growth and induces cell differentiation of the HL-60 AML cell line, the latter partly by P2 receptor ligation and PKA activation [85]. Released NAD^+ can be converted into cyclic ADP-ribose by the ectoenzyme CD38 and then reenter neighboring cells and trigger Ca^{2+} release from the endoplasmatic reticulum [83]. However, leukemic stem cells are generally CD38 negative, so this CxHc effect might therefore be less important in AML.

CxHcs seem to have pro-apoptotic effects that are mediated trough several mechanisms including prolonged opening of the hemichannels leading to imbalance of ionic gradients [86]. However, CxHcs may also mediate anti-apoptotic effects [86]. This is observed in osteocytic and osteoblastic cells where opening of their Cx43 CxHcs by biphosphonates exerts anti-apoptotic effects that are mediated by Src kinase and ERKs [87]. Thus, cells localized to the endosteal stem cell niche, including leukemic cells, may interact via CxHcs and thereby alter the physiology of the niche.

5.2. CxHc-independent effects of connexins

The cytoplasmic carboxyl domain of Cx43 can suppress the growth of both the Neuro 2a cell line as well as the human osteosarcoma U2OS cell line [75,88]. In transfected HeLa cells, the carboxyterminal tail of Cx43 inhibits cell proliferation, suggesting that membrane localization of the protein is not required for its antiproliferative effects [89]. Interestingly, these C-terminals were localized to the nucleus both in HeLa cells and cardiomyocytes, suggesting that these parts of Cx43 may be involved in regulation of gene expression. However, the significance of these experiments implies that the C-terminals are physiological active, which remains to be determined. Studies of Cx45.6 in chick eye lens further suggest that Cxs may be involved GJ-independent differentiation [90]. Although none of these studies are

linked to AML, they suggest that Cxs may function as intracellular regulators of both proliferation and possibly differentiation.

6. Connexins may affect AML cell death mechanisms

As previously reviewed by Bruserud et al. [91], several studies suggest that the regulation of apoptosis is important for outcome after intensive chemotherapy in AML. There are several indications that Cx are involved in AML (Table 2), and apoptosis represents one candidate mechanism that may be affected. Diverse functionality for Cxs in cell death control has been demonstrated or suggested, including apoptosis-related gene expression that may occur independently of GJ activity [92,93]. Cx43 is the predominant isoform in many tissues and has been particularly studied in this context, but other Cxs such as Cx26 and Cx32 may also be involved [94].

Cxs can interfere with cellular homeostasis, injury responses and survival processes via several mechanisms. As building blocks of GJs, they facilitate transfer of cell fate signals. Such an explanation has been used to describe the clustered cell death in some cancer cell populations [95] and spread of cell death in ischemia [96]. As far as we know, it remains unclear if local transfer of death signals occurs between adjacent cells of various types, e.g. between leukemic and stromal cells in bone marrow. There are many candidate modulators of survival and death that can pass through GJs, including apoptosis effectors, second messengers, nutrients and toxins. Whether this eventually will influence cell viability seems to be determined by the type and physiological status of the participating cells. Ca^{2+}, IP_3 and cAMP have all been suggested as potential death messengers when transferred via gap junctions [93]. Ca^{2+} contributes to the regulation of apoptotic cascades and mitochondrial permeability [97], whereas IP_3 can trigger the release of Ca^{2+} and thereby have pro-apoptotic effects [98]. Finally, effects of cAMP are mediated via cAMP-dependent kinase, and cAMP analogs can induce cell death in leukemic cells [99]. In contrast to the transfer of death signals, it has also been suggested that gap junctions promote survival of adjacent cells either by providing nutrients, ATP or antioxidants, or by restricting the flux of harmful compounds such as toxins and reactive oxygen species (ROS) [86]. In total, the physiological outcome of the transport of cell fate modulators through GJs seems to depend on the cellular context and the balance between different signaling pathways.

The roles of Cxs in cell death/survival do not necessarily involve GJ activity. Several reports suggest that Cxs mediate autonomous regulation. Cx26 was found to regulate gene expression in breast tumor cells via unknown GJ-dependent as well as -independent mechanisms [100]. Phosphorylation of serine 262 in Cx43 has been demonstrated to regulate DNA synthesis in cardiomyocytes apparently independent of GJ formation [101]. Cx43 has also been localized to the nucleus and the mitochondria where it obviously serves other roles than GJ formation [92], but it remains an open question whether or not it forms hemichannels in the mitochondrial membrane [102]. The mitochondria link processes of cellular metabolism and cell death pathways, and a large number of reports have implicated these organelles in the process of carcinogenesis [103].

7. Conclusion

The role of Cxs in AML is not well characterized. Still, it seems evident that different Cxs, at least Cx32 and Cx43, are expressed by AML cells and can mediate growth regulation possibly via interactions with stromal cells. Different chemotherapeutic drugs commonly used in AML therapy can alter Cx expression and GJ function of various cells. Cxs might thus be involved in leukemogenesis and chemosensitivity in AML.

Various hematopoietic growth factors and intracellular pathways important in the regulations of leukemic cell functions and development are able to alter Cx regulations in various cells. GJs, CxHcs and Cxs are in addition active participants in apoptosis regulation. Therefore, Cxs are clearly having the potential to interfere with diverse signaling pathways of clinical relevance in human AML.

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