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Forum Review Article

Gap Junctions and Cancer: New Functions for an Old Story

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Abstract

Cancer was one of the first pathologies to be associated with gap-junction defect. Despite the evidence accumulated over the last 40-year period, the molecular involvement of gap junctions and their structural proteins (connexins) in cancer has not been elucidated. The lack of a satisfying explanation may come from the complexity of the disease, evolving through various stages during tumor progression, with cancer cells exhibiting different phenotypes. Here, the question of the involvement of gap junctions has been readdressed by considering the connexin expression/function level at different fundamental stages of carcinogenesis (cell proliferation, cell invasion, and cancer cell dissemination). By performing this analysis, it becomes clear that gap junctions are probably differently involved, depending on the stage of the cancer progression considered. In particular, the most recent data suggest that connexins may act on cell growth by controlling gene expression through a variety of processes (independent of or dependent on the gap-junctional communication capacity). During invasion, connexins have been demonstrated to enhance adherence of cancer cells to the stroma, migration, and probably their dissemination by establishing communication with the endothelial barrier. All these data present a complex picture of connexins in various functions, depending on the cell phenotype. *Antioxid. Redox Signal*. 11, 323–338.

Introduction

Cancer was the first human pathology to be associated with gap-junction defects. From the first observations linking gap-junction function to cell growth control, ~40 years ago, a tremendous accumulation of results reported a loss, or at least, a diminished coupling capacity among cancer cells or between cancer cells and their surrounding normal counterparts. In a previous review, we mentioned the complexity and the variety of models used for establishing the idea that gap-junctional intercellular communication (GJIC) is actively involved in carcinogenesis (70). An interesting point of this research was that, despite the variety of models (cells freshly isolated from tumors or established cancer cell lines from animal or human origins, the wide range of organs and tissues, *etc*.), the conclusions were globally similar. A first conclusion was that despite a series of underlying molecular mechanisms, the cancer phenotype is associated with a loss of coupling (Fig. 1). A second was that phenotypic normalization is a consequence of the reinduction of GJIC (Fig. 1). However, despite this unity of data acquired for decades, the real involvement of gap junctions in cancer is still an open question.

Actually, the lack of satisfying explanations may be because the question itself was not correctly formulated. Indeed, cancer is a complex and evolving disease. Through the years, a phenotypically heterogeneous population of cells generates, forming a tumor. Some of these cells become able to invade the surrounding tissues, passing across the endothelial barrier, spreading through the vascular system, and colonizing distant organs. Each step of this evolution of the disease is the consequence of new capacities, which are acquired by some cancer cells through this long process called tumor progression or carcinogenesis. Looking back at this evolving reality of cancer, we may wonder whether the question is still valid or appropriate. Probably the pioneer of this field of research, Werner Loewenstein (64), was right when he associated GJIC with, and only with, cell-growth control. Later, the loss of growth control (and thus, loss of gap junctions) was rapidly and globally identified by others with cancer, forgetting all the fundamental steps of the disease, of their progression, and of their particular cellular character-

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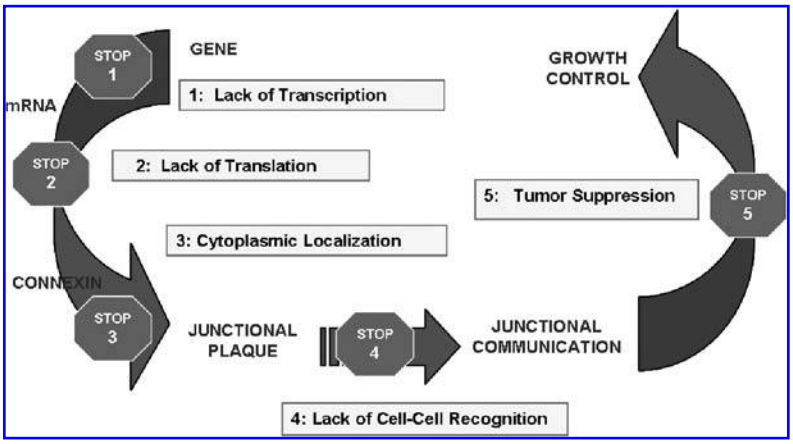

FIG. 1. Possible mechanisms leading to an inhibition of gap-junctional intercellular communication (GJIC) capacity in cancer. Various experimental data have shown that each step leading to the establishment of GJIC can be altered and induce the loss of junctional communication. These accumulated data revealed that four major events (STOPS) can be the cause of a lack of junctional communication (lack of transcription of connexin genes; lack of translation of connexin mRNA; lack of membrane addressing leading to an accumulation of connexin proteins in the cytoplasm; and lack of cell–cell recognition preventing the establishment of junctional intercellular communication). More recent data have shown that the reestablishment of GJIC by connexin cDNAs may be not sufficient for inducing cell growth control in cancer cells (STOP 5). In this last case, the expression of specific connexins seems to be a more critical event than the recovery of junctional communication. In other words, connexins could control cell growth independently of cell-to-cell communication.

istics. Progressively, the Loewenstein hypothesis attributing a role for gap junctions in growth control became a dogma, asserting that "gap junctions are lost in carcinogenesis."

Consequently, despite such a claim, intriguing exceptions to the dogma have been observed. For instance, the recovery of GJIC was not always able to normalize cell growth, but the connexin type that was reexpressed was decisive (69) (Fig. 1). In other cases, the reexpression of connexins was able to normalize cell growth but without reestablishing GJIC (35). Even if these observations were obtained from simple cell lines, from a model that is far from the heterogenous complexity of the solid tumors, such observations led to the idea that transfected connexins may directly act on growth control, independently of GJIC, probably by modifying the pattern of gene expression of cancer cells. This speculation was supported, more recently, by the discovery of a more-complex behaviour of connexins inside the cells: aberrant nonmembranous localizations (inside the cytoplasm or even in the nucleus) having the capacity to interact with a variety of internal proteins. These observations may support a role of connexins as proteins able to modify growth by modulating signalling pathways, and in turn, gene expression. These observations were, at least, in favor of a multiplicity of connexin roles. Connexins progressively became not only the fundamental structural pieces of the gap-junction plaque. Besides connexin effects associated with localizations incompatible with coupling activity, it also appeared that they could be involved in the establishment of communication between the extracellular and intracellular domains, by forming hemichannels. Considering these various behaviors of connexins, we may ask whether they are involved only in growth control, as W. Loewenstein was assuming, or if they act differently in the various steps of the cancer process. If this last assumption is correct, it could explain other exceptions to the dogma, such as the capacity of some cancer cells to exhibit a large amount of connexins and a large GJIC capacity.

During the last decade, new observations were made within this research field. As mentioned, some of these observations were apparently contradicting the "dogma." They accompanied the discovery of a wider range of behaviors of connexins, which might fit better with the complexity of the evolving cancer process. In this article, we review the different behaviors and/or functions of connexins that were observed for specific stages of cancer progression. From such a "stage-by-stage" analysis, it could be possible to have a more precise picture of the real involvement of connexins in carcinogenesis.

Positioning the Problem

Before initiating such an analysis, it is necessary to define the model to be studied. If gap junctions play a role in cancer, we may assume that solid tumors are the best model to demonstrate it, and hematologic malignancies can be excluded from our analysis (we do not mean that gap junctions or connexins do not play any role in such types of cancers). Then, reconsidering the progression of solid tumors, it can be schematically separated into three fundamental stages: growth of the primary tumor, invasion, and dissemination (Fig. 2). After dissemination, a few cancer cells may eventually form distant colonies, such as metastases. Because some solid tumors do not metastasize (brain tumors) and because this very late stage is similar to a new cycle of tumor progression, it is not considered in our study. Indeed, the cell expansion of metastases seems to start as the beginning of another cancer progression: growth of a new (secondary and distant) tumor able eventually to undergo other dissemination cycles.

These three very fundamental stages of solid tumor progression are representative of three very different phenotypes of cancer cells: deregulated growth (tumor formation), motility (invasive tumor), and interaction with both the endothelial barrier of blood or lymphatic vessels (cancer cell dissemination) and possibly with the cells of the colonized organs. Already, by considering these different phenotypes, it is possible to predict that the junctional behavior of cancer cells should be different at every stage. For instance, the motility capacity may appear logically as the consequence of the disruption of intercellular junctions to permit cell detachment. Conversely, for dissemination, the interactivity with the endothelial barrier would logically need the estab-

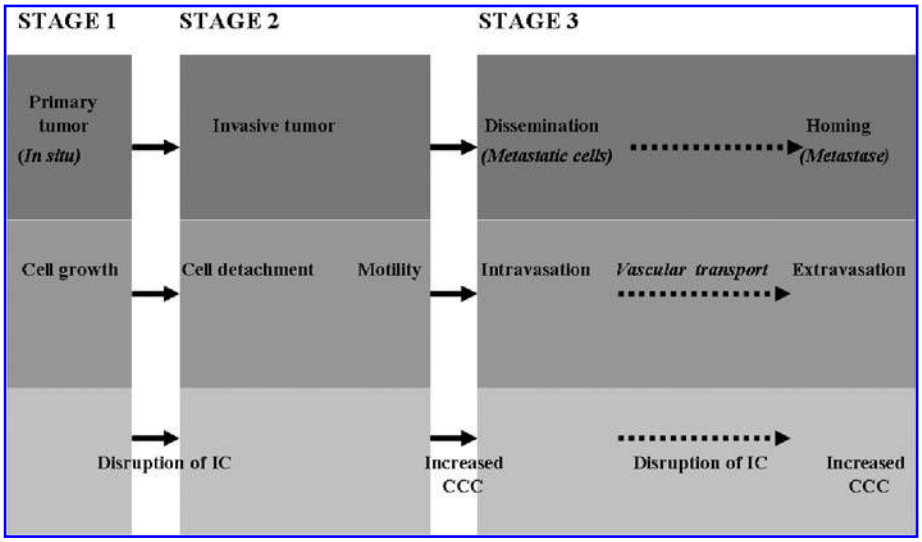

FIG. 2. Positioning the problem. Progression of solid tumors can be schematically separated into three fundamental stages: Stage 1 (growth of the primary tumor), Stage 2 (invasion), and Stage 3 (dissemination). After dissemination, cancer cells may form metastases (homing). These three histologic stages (*upper layer*) are representative of three different phenotypes of the cancer cells (*middle layer*): deregulated growth (tumor formation), motility (invasive tumor), and interaction with the endothelial barrier of blood or lymphatic vessels (intravasation and extravasation). By considering these different phenotypes, it is possible to predict that the junctional behavior of the cancer cells is different at every stage (*lower layer*). The motility capacity is probably the consequence of a disruption of intercellular junctions (IC) to permit cell detachment. Conversely, for dissemination, the interaction with the endothelial barrier would need the establishment of cell–cell contact (CCC) between cancer cells and endothelial cells.

lishment of cell–cell contact between cancer cells and endothelial cells. Thus, if gap junctions do play a role in carcinogenesis, this role might be different depending on the cancer stage that is being considered (Fig. 2).

Stage 1: Gap Junctions and Tumor Growth

The link between gap junctions and cell growth was established more than 40 years ago (64). Growth regulation was one of the first physiologic roles attributed to gap junctions and GJIC. From that time, a considerable amount of work has confirmed this early assumption in a wide range of models (95, 106, 107, 115). This link was not just an antiparallel association observed between increased cell growth and loss of gap junctions (and/or coupling). It was apparently confirmed by more "active" investigations, such as inhibiting GJIC by carcinogens (mostly tumor-promoting agents) or decreasing growth by reinducing GJIC (by using connexin cDNA transfection or chemical treatment). It was also confirmed *in vivo* by using transgenic mice, which exhibit higher tumor susceptibility when defective for specific connexins: liver tumors in Cx32-deficient mice and lung neoplasms in Cx43-deficient mice (2, 105). However, despite this tremendous number of observations, the real impact of gap junctions in cell-growth regulation is not yet understood, even if some molecular processes can be drawn (Fig. 3). The most accomplished approaches in this domain are some molecular studies showing that cell proliferation is regulated more by connexin expression than by the extent to which coupling occurs, probably by modulating gene expression.

The control of gene expression by connexins

Precursor studies in this field were obtained mostly by transfecting Cx43 cDNA in various cell systems (Fig. 4). One of the first of these studies was made by using a transformed dog kidney epithelial cell line. Once transfected by Cx43, GJIC was restored, and the cell morphology became flatter. Interestingly, the proliferation of the transfected cells, which was mostly dependent on cell density, was associated with a decreased expression of cell cycle–regulatory genes, such as cyclin A, D1, D2, and cyclin-dependent kinases (*CDK5* and *CDK6*). Because these genes are known to be critical to cell-cycle progression, this could explain the increased G_1- and S-phase duration that was observed in the transfected cells (8). This work did not prove that Cx43 directly acts on specific gene expression, but it determined for the first time a link between the establishment of GJIC and a pattern of gene expression. Other studies confirmed this preliminary work. For instance, a longer G_1 phase also was observed after Cx43 transfection in osteosarcoma cells, probably because of less degradation of p27. This leads to a higher amount of p27, at the posttranslational level, which inhibits the enzymatic activity of CDK, and, in turn, accumulates the hypophosphorylated form of Rb, which characterizes the G_1 phase (117). A similar effect has been observed after connexin transfection in liver (Cx32) and lung (Cx43) carcinoma cells. In those cells, the G_1-phase prolongation was similarly linked to p27 accumulation, but also to a decreased amount of cyclin D1 (55). The increased amount of p27 and the decrease of Skp2 (S-phase kinase–associated protein-2) were also the consequence of Cx43 expression in mouse embryo fibroblasts (117–119).

Because it is expected, as well as in the preceding, that any change in cell proliferation is accompanied by a related change in cell-cycle gene expression, other similar strategies revealed that transfection of connexin could influence more-particular gene expressions. This was shown in C6 rat glioma cells. In response to Cx43 transfection, both increased (osteopontin, KC, Cyr61, pecanex, 2c9 gene) and decreased (RPL19) gene expressions were revealed by the differential display approach (75–77). The decreased expression of the ribosomal protein L19 (RPL19) is an interesting point, because its increased expression is associated with advanced colorectal tumors (39) and has even been estimated to be predictive of shorter survival for prostate and breast cancer patients (4, 31). Even if the real involvement of RPL19 in the

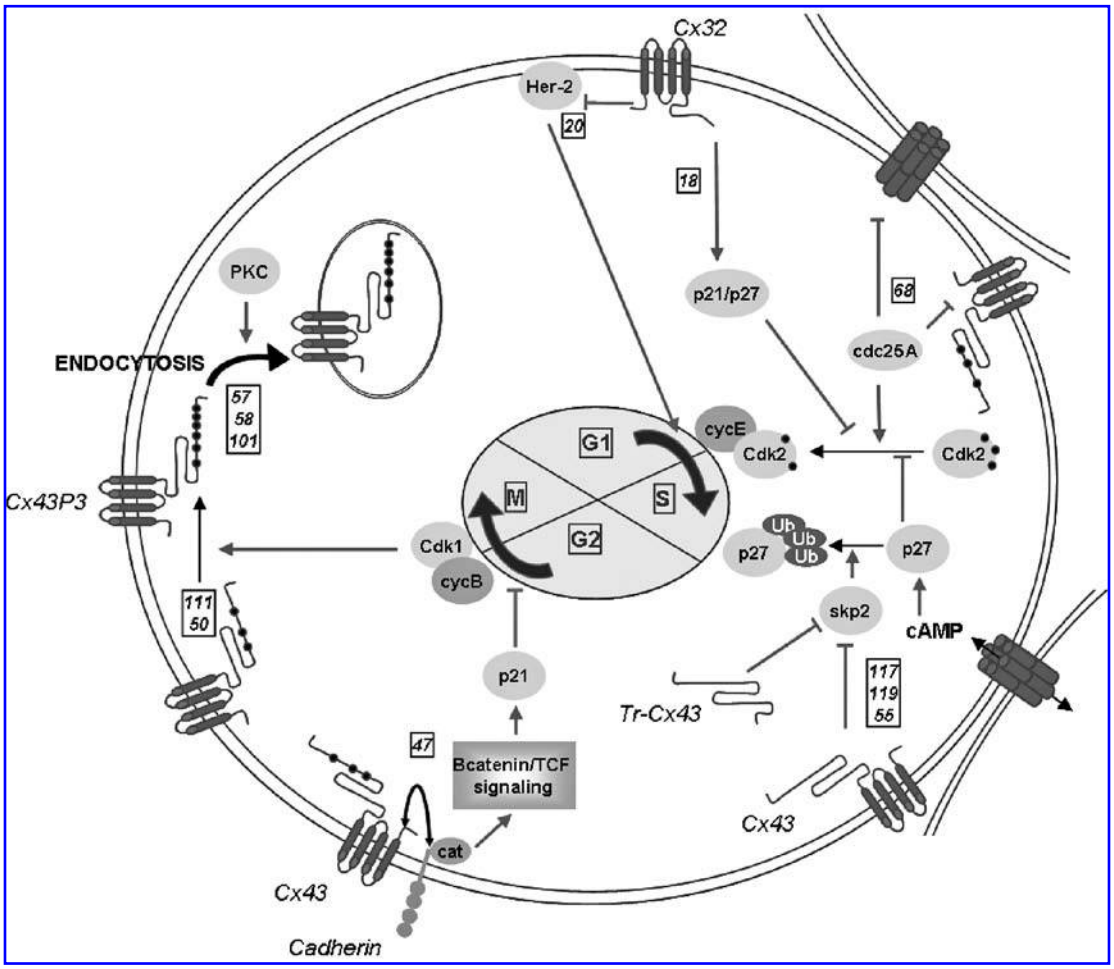

FIG. 3. Connexins and cell-growth regulation. In this cell, molecular events associating connexins and regulation of cell growth are shown. Cell-cycle phases are noted from G1 to M at the center of the cell. The molecular processes are described in the references noted as numbers in the white squares. •: Phosphorylated sites of the carboxy-terminal part of Cx43. cAMP, cyclic adenosine monophosphate; cdc25A, cell-division cycle 25 homolog A; Cdk1, cyclin-dependent kinase 1; Cdk2, cyclin-dependent kinase 2; Cx32, connexin32; Cx43, connexin43; Cx43P3, hyperphosphorylated form of Cx43; cycB, cyclin B; cycE, cyclin E; Her-2, human epidermal growth factor receptor-2; p21, cyclin-dependent kinase inhibitor p21; p27, cyclin-dependent kinase inhibitor p27; PKC, protein kinase C; skp2, S-phase kinase-associated protein 2; TCF, T-cell factor; Tr-Cx43, truncated carboxy-terminal part of Cx43; Ub, ubiquitination of p27. Modified from (ref. 9).

cancer phenotype is not understood, such an unexpected observation suggests that Cx43 expression exerts not only growth control on rat C6 glioma cells, but also a kind of normalization of the phenotype. In these cells, Cx43 transfection, which restores more contact inhibition than it decreases growth, is also correlated to the decreased secretion of MFG-E8 (milk fat globule epidermal growth factor 8), a factor involved in cell anchorage (25). Such a result is similar to that obtained in a previous study reporting that Cx43 modulates expression of other extracellular proteins, such as insulin-like growth factor–binding protein 4 (6), or diffusible factors such as the monocyte chemotactic protein (MCP-1, a protein believed to play an important role in tumor formation, which is decreased in human glioblastoma cells transfected by Cx43) (38). More-powerful strategies, such as cDNA array studies, now allow detecting a large number of genes that are differentially expressed, depending on the Cx43 level of expression. Such strategies, by using astrocytes cultured from Cx43-deficient mice, have detected >250 genes with altered expression, including those related to apoptosis, cell growth, and transcription factors that may act on a secondary level on gene-expression patterns (40, 41). It would be interesting to determine to what extent these genes are still differently expressed in Cx43 heterozygous mice and how these differences can explain, for instance, the susceptibility of these mice to lung neoplasms (2).

GJIC-mediated control of gene expression

As mentioned earlier, Cx43 was shown to reduce the mRNA amount and secretion of MFG-E8 in C6 rat glioma cells (25). By extending this association between Cx43 and MFG-E8 to other cell types, it is possible to claim that if the suppression of MFG-E8 is related to Cx43 growth suppression, HeLa cells would not be susceptible because they do not produce significant levels of MFG-E8 (59). Interestingly, mammary carcinoma cells do express high levels of MFG-E8 (84) and, similar to C6 glioma cells, are normalized by Cx43

expression (33). In other words, breast carcinoma and glioma cells display decreased levels of Cx43 and increased MFG-E8 levels (25).

Without altering either telomerase activity or MAPK level, this effect on MFG-E8 expression is specific to Cx43 transfection. Cx32 is also capable of restoring dye coupling in C6 cells, but does not act on MFG-E8 expression. In this case, it seems that GJIC mediated by Cx43 does control MFG-E8 expression (mRNA level) and its level of secretion. Establishing GJIC by other connexins, such as Cx32, in these cells (or preventing Cx43-mediated GJIC by using dominant-negative Cx43 mutants) prevents this phenotypic effect (25). It has been long known that Cx43, but not Cx32, suppressed C6 glioma cell growth (5). How Cx43-mediated GJIC would specifically affect MFG-E8 expression is still unknown. Paradoxically, Cx32 transfection resulted in extensive dye transfer, contrary to Cx43 transfection. This result means that dye-transfer capacity may not be related to growth suppression. Sharing endogenous metabolites would be more significant, because, in contrast to fluorescent dyes, the Cx43 transfectants permitted the exchange of such metabolites more efficiently than Cx32 transfectants (25). In support of this, it was shown that ATP and glutamate diffuse much more efficiently (160 and 29 times, respectively) through Cx43 channels than through Cx32 channels (24). Interestingly, this permeability difference may have something to do with the phosphorylation status of some intracellular metabolites. For instance, adenosine passed ~12-fold better through channels formed by Cx32. In contrast, AMP and ADP passed about eightfold better, and ATP >300-fold better, through channels formed by Cx43. Thus, addition of phosphate to adenosine appears to shift its relative permeability from Cx32 channels to Cx43 channels (26).

These data are consistent with the idea that GJIC mediated by specific connexins is able to modify gene expression and cell growth. Actually, it has been reported that the channel diameter would allow the determination of distinct biologic end points by filtering the passage of intercellular signals according to their size (108). The control of gene expression by Cx43-mediated GJIC can also be extended, at least, to another cell system. Transfection of Cx43 was shown to differentiate rhabdomyosarcoma cells by decreasing their growth rate, inducing cell fusion and myosin expression (88). Interestingly, when GJIC of myoblasts was inhibited by octanol or γ-glycyrrhetinic acid, myogenic regulatory factor gene expression (such as myogenin and MRF4) was prevented, as well as cell fusion (89). This result also supports the hypothesis of a direct control of gene expression by Cx43-mediated GJIC (77). Another similar example comes from the osteoblastic model in which a connexin-responsive element (CxRE), with a CT-rich region, has been identified in the promoter of osteocalcin and collagen I α-1 genes (102). In this model, extracellular growth stimulation induces the synthesis of second messengers that transit through gap junctions to activate ERK and PI3/Akt pathways. The translocation of ERK into the nucleus activates transcription factors that recognize CxRE and induce the osteocalcin and collagen I α-1 transcriptions (Fig. 4).

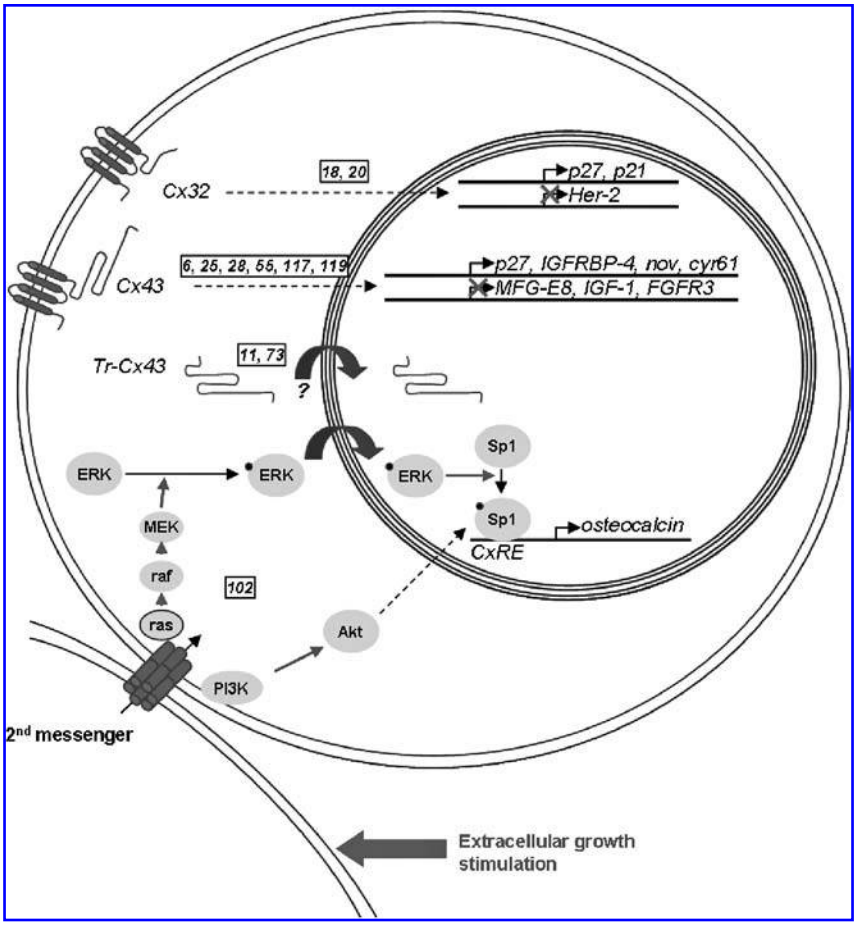

FIG. 4. Connexins and regulation of gene expressions. In this cell, signalling pathways associating connexins and particular gene expressions are shown. The molecular processes are described in the references noted as numbers in the white squares. •: phosphorylated sites. AKT, v-akt murine thymoma viral oncogene homologue; Col I, collagen I alpha-1; Cx32, connexin32; Cx43, connexin43; CxRE, connexin-responsive element; Cyr61, cysteine-rich, angiogenic inducer, 61; ERK, extracellular regulated MAP kinase; FGFR3, fibroblast growth factor receptor 3; Her-2, human epidermal growth factor receptor-2; IGF-1, insulin-like growth factor 1; IGFRBP-4, insulin-like growth factor binding protein 4; MEK, MAP kinase-ERK kinase; MFG-E8, milk fat globule-EGF factor 8; nov, nephroblastoma overexpressed gene; p21, cyclin-dependent kinase inhibitor p21; p27, cyclin-dependent kinase inhibitor p27; PI3K, phosphatidylinositol 3-kinase; Sp1, Sp1 transcription factor; Sp3, Sp3 transcription factor; Tr-Cx43, truncated carboxy-terminal part of Cx43. Modified from ref. 9.

Despite such examples, it is still difficult to figure out how the exchange of particular metabolites, permitted by a selective permeability of specific connexins (for instance, Cx43 in C6 glioma cells), could regulate a gene-expression pattern acting on cell growth in a cloned cell population. It is possible that connexin-mediated cell growth is more complicated, as it seems to be for Cx43-transfected cells. In this cell system, protein interaction may complete GJIC-mediated cell growth, which was previously observed, because a protein (CCN3) was found to interact specifically with the C-terminal part of the transfected Cx43 in C6 glioma cells (19). Because this interaction does not occur with other connexins, such as Cx40 or Cx32, which do not control the *in vitro* growth of C6 cells, it is expected that this close interaction between Cx43 and CCN3 is also part (with Cx43-mediated GJIC) of the molecular process controlling cell growth.

GJIC, connexin specificity, tissue-specific expression, specific protein interactions; toward a unifying theory?

Such a close interaction between CCN3 and Cx43 brings another piece of information for our reflection. It is known that each member of the large connexin family (~20 members in humans) exhibits a specific cytoplasmic part in terms of amino-acid sequence and length (110). Because it is also known that these cytoplasmic sequences permit or exclude particular interactions with proteins inside the cells (32), it is possible that such interactions control intercellular permeability, signalling pathways, and thus cell growth.

Moreover, because connexins are specifically distributed in tissues, we may argue that they play specific roles in the tissues in which they are expressed. One of these roles could be growth control. If this is true, the tissue-specific pattern of expression of the connexins could be related to their specific growth effect, depending on the responding cell type in which they are transfected (5, 69) (Table 1). In other words, the responding cells could "offer" the appropriate molecular environment (cytoplasmic interacting proteins), which could act on cell growth. Similarly, if the cytoplasmic domains of the transfected connexin do not coincide with the interacting protein environment (wrong connexin), no effect on cell growth would occur. The same effect would happen if, for a transfected connexin, the appropriate interacting protein environment is not present (wrong cell type). This would be the case for Cx32, Cx43, and Cx40 in HeLa cells or Cx32 in C6 glioma cells (5, 69).

These specific interactions, dependent on the connexin type, help to make a link, for some recent cases, between the presence of connexins and the cytoplasmic localization of putative transcription factors. Once the connexin is absent, the cytoplasmic protein is translocated inside the nucleus and could act as a transcription factor able to regulate cell growth. This "translocation pattern" has been observed for Cx32 with Discs Large homolog 1 in hepatocytes (14) and for Cx43 with CCN3 (19, 22). Several examples support this emerging hypothesis. Among them, in cardiomyocytes, β-catenin was shown to interact directly with Cx43 and to translocate to the nucleus when activated by Wnt-1 signalling. β-Catenin transactivates Cx43 transcription, providing a mechanism whereby Cx43 binding to another molecule, independent of its channel function, could regulate its own transcription (1). As mentioned earlier, in C6 glioma cells, when Cx43 is down-regulated, CCN3 translocates to the nucleus, producing a transformed phenotype with increased growth rate (19, 22). Similarly, the transcription factor ZO-1–associated nucleic acid–binding protein (ZONAB) is colocalized and interacts with ZO-1 and connexins in astrocytes and oligodendrocytes (83). Nuclear localization of members of the MAGUK family of proteins has been reported for both ZO-1 and CASK (calcium/calmodulin-dependent serine kinase) with nuclear translocation, causing alterations of cell proliferation, activation of the TCF/LEF family of transcription factors, and changes in the levels of cell-cycle regulators, such as cyclin D and c-Myc (34). Similarly, a close interaction has been shown between Cx26 and the protein YAF2 (YY1-associated factor 2), which modulates the activity of transcription factors such as YY1 and N-myc (personal communication by V. Krutovskikh).

Table 1. Tumor Suppression and Specific Expression of Connexins

	Connexin		
Cell type	*Endogenous*	*Transfected*	*Tumorigenicity*
Prostate[a]	Cx32/Cx43	Cx32/Cx43	Decreased (71)
Fibroblasts[b]	Cx43	Cx43	Decreased (94)
HeLa cells[a]	Cx26	Cx26	Decreased (69)
	Cx43	Cx43[d]	Decreased (69)
		Cx32	No change (69)
		Cx40	No change (69)
Glioma[c]	Cx43 (astrocytes)	Cx43	Decreased (122)
	Cx30 (astrocytes)	Cx30[e]	Decreased (87)
		Cx32[f]	Decreased (5)

The transfection of connexin cDNAs does not systematically inhibit the tumorigenicity of cancer cells. In some cases, this inhibition is correlated to the reexpression of the connexins, which are originally expressed in the native normal tissue (endogenous connexins), contrary to the other connexins (underlined).
[a]Human cells.
[b]Mouse cells.
[c]Rat cells.
[d]The effect was not observed for all clones tested.
[e]Only proliferation *in vitro* was tested.
[f]The effect was observed *in vivo* but not *in vitro*.

In such scenarios, specific connexins would then be able to "stabilize" the cytoplasmic localization of putative transcription factors. Such a mechanism would establish a direct link between two apparently contradictory situations: the membrane localization of the connexin and the connexin-mediated control of gene expression in the nucleus.

Gap junction-independent control of cell growth through aberrant localizations of connexins

So far, we have reviewed processes that seem to confirm the role of GJIC in cell growth with the specific expression of connexins and their ability to interact with intracellular proteins. Unfortunately, this way of thinking is not absolute and cannot be extended to observations that clearly reported connexin-mediated growth control independent of gap-junction formation.

One of the first observations came again from glioma cells. The transfection of Cx43 in human glioblastoma cell lines

(U251 and T98G cells) was shown to induce flatter morphology, increased doubling time (in log phase; increased G_1 phase), decreased saturation density, colony-forming activity in soft agar (anchorage dependency), and tumorigenicity in nude mice (35). These phenotypic changes were similar to what was observed in tumor cell lines after connexin transfection, but what was less classic was the lack of GJIC induction; all these effects were related to an accumulation of the unphosphorylated form of Cx43 in the cytoplasm and even in the nucleus (35). "Normalizing" the cell phenotype by Cx43 transfection without inducing any obvious change of GJIC also was observed in immortalized cells from embryonic Cx43 KO mice (66). More generally, the lack of a clear correlation between phenotypic changes and GJIC capacity may be observed. For instance, transfection of HeLa cells expressing an exogenous Cx26 (Cx26-HeLa cells) with mutated forms of Cx26 (P87L or R143W) enhanced their tumorigenicity without any change in dye coupling (15). Moreover, in the same model, the transfection of the C60F Cx26 mutant did not modify the growth of Cx26-HeLa cells, even if it reduced dye-coupling (15). Such observations tend to demonstrate that, in some circumstances, the phenotypic action of connexins in transfected cells may not be related to their GJIC abilities.

This is probably the case when the transfected connexins are not localized in the plasma membrane and do not form any gap-junction plaque (35). An assumption reinforced by several studies indicates that some connexin mutants that are unable to be inserted into the plasma membrane can still downregulate the cell proliferation (56, 80). Interestingly, a GJIC-independent mechanism of tumor suppression has been observed for Cx26 and Cx43 overexpressed in MDA-MB-231 breast cancer cells only *in vivo* (or in three-dimensional culture), but not in classic two-dimensional cultures. This work demonstrates that the culture procedure may affect the connexin-mediated growth control, which is dependent on a three-dimensional environment (67). More generally, the sensitivity of cultured cells to any culture parameter reinforces the necessity to be cautious about *in vitro* data.

These studies tend to demonstrate that connexins, and mostly Cx43, bear an independent capacity to inhibit cell proliferation, apart from their ability to form junctional plaques. No clear hypothesis explains how a cytoplasmic connexin can regulate cell growth. Furthermore, it is difficult to conciliate the cytoplasmic localization of transfected connexins, which may have some tumor-suppressive effect, with the same localization of endogenous connexins, which has been frequently observed in tumor tissues and cancer cells (70). More intriguing is cell-growth control associated with the nuclear localization of transfected Cx43 (8, 35). Even if it was not related to growth inhibition, a nuclear signal was observed by confocal microscopy for the endogenous Cx43 in src- and neu-transformed rat liver epithelial cells (13). From such observations, it was even suggested that Cx43 could directly act on gene expression (13, 35). Again, it is hard to understand how an integral transmembrane protein like Cx43 could enter in the nucleus and interact with DNA. However, this assumption recently gained more importance by transfecting only the carboxy-terminal part of Cx43 (CT-Cx43). These approaches have shown that CT-Cx43 can decrease cell growth without modifying GJIC while it is detected inside the nucleus (11, 118). This would mean that CT-Cx43 can act directly on gene transcription, but this has not been proven. This negative effect of the carboxy-terminal part of the Cx43 on cell proliferation was also observed in Neuro2a cells (73). An intriguing point is that the entire Cx43 or its CT fragment exhibits similar effects (decreased cell proliferation) in U20S cells (118, 119). Does it mean that the entire transfected Cx43 can be fragmented into a CT-Cx43 piece, which may then act inside the nucleus and suppress Skp2 expression? CT-Cx43 was also found to be as effective as the complete Cx43 in suppressing the growth of Neuro2a cells (73), HeLa cells (11), and HEK293 cells (12). This seems to suggest that some particular events (or enzymatic process) can induce the appearance of a CT-Cx43 piece. Such an event seems to be the consequence of heart ischemia (53). In the cardiomyocytes, this phenomenon might induce p53 and PIDD (p53-induced protein with a death domain), which contribute to the growth-suppressive effects of CT-Cx43 and probably to cell death, promoting effects under conditions of ischemia (53). More studies are needed definitively to demonstrate that CT-Cx43 is not an artefact and can be physiologically produced under certain circumstances by some particular enzymatic processes, if it plays crucial roles in cell biology (growth regulation or apoptosis) and if it acts inside the nucleus as a transcriptional factor. This last observation would establish a solid link between Cx43 expression and control of gene expression.

Gap junction-independent control of cell growth through hemichannel function of connexins

To complete the panorama, in this part of the review we must also mention that connexins could act on cell growth even in the plasma membrane, through another gap-junction–independent process, by forming hemichannels. Hemichannels are known normally to be closed but may open in response to various stimuli, such as membrane depolarization, reactive oxygen species, low extracellular Ca^{2+}, and increased cytoplasmic Ca^{2+} (96). It has been suggested that they may function as a release pathway for small paracrine messengers such as ATP or glutamate. It has been observed that connexin expression by potentiating ATP release (and long-distance calcium waves) can decrease cell proliferation (36) and that calcium waves can modulate proliferation in the developing neocortex (109) by propagating through radial glial cells. More recently, it was shown that inflammatory conditions in astroglial cells could both reduce intercellular communication *via* gap junctions and enhance the cellular exchange with the extracellular milieu *via* Cx43 hemichannels (93). Both the reduction in GJIC and the increase in membrane permeability were mediated by a p38 mitogen–activated protein kinase–dependent pathway (93). So far, to our knowledge, no involvement of connexin hemichannels has been demonstrated as a cell-growth regulator in cancer cells.

Stage 2: Gap Junctions and Invasion

Originally, Cx43-knockout mice were made the better to understand what the physiologic implications of such a connexin are during development and in adult life. Actually, because Cx43 is expressed at the very first stages of embryogenesis, it was expected that the development of these mice would be prevented. It was surprising to see that, despite

the lack of Cx43, mice could follow an apparently normal pattern of cell differentiation, leading to apparently normal mice at birth (91). However, the Cx43-KO mice could not survive after birth because of a heart defect preventing blood reoxygenation. This first attempt for knocking down a connexin gene led to the important deduction that probably most of the Cx43 functions were replaced by other members of the connexin family, except for, at least, bone differentiation (60) and gonad development (45). It also suggested that particular Cx43 functions cannot be replaced, such as the heart defect that was a consequence of changes in neural crest cell migration. This observation was the first to reveal a close association between connexins and cell migration. Interestingly, this association was not directly related to cancer cell invasion (63). Thus, the involvement of Cx43 in cell migration was kept confined to the embryogenesis situation.

Another model, closer to the cancer situation, suggested a role for certain connexins in cell mobility. This came from HeLa cells transfected with various connexins (27). It appeared that Cx43-transfected HeLa cells were more invasive in precultured embryonic chicken heart fragments than Cx40-, Cx31-transfected, and nontransfected HeLa cells. This property might be specific to Cx43 expression, because it was not related to GJIC capacity (similar dye-coupling capacity between the different connexin-transfected clones) or to cell proliferation. Actually, this work was the extension of a previous observation demonstrating that gap-junctionally coupled tumor cells are invasive, whereas noncoupled tumor cells, like wild-type HeLa cells, are not (7).

The glioma model

If connexins are involved in cancer cell migration, this could explain why some cancer cells look like exceptions to the dogma and do express connexins. If this assumption is correct, then some tumor cells that are known to be particularly invasive, such as glioma cells, should express connexins. Some experimental approaches have shown that the level of Cx43 expression is related to the mobility capacity of some glioma cells. For instance, it has been observed that more glioma cells express Cx43, and they also migrate more, as deduced from wound healing and transwell assays (3). Any knocking down of the Cx43, for instance, by stable shRNA strategy confirmed the link existing between expression level and migration. This assumption came mostly from a series of experiments with variants of rat C6 glioma cells as a model.

Because Cx43 expression increases the ability of glioma cells to aggregate (62), it may help those cells to adhere and migrate. The use of antibodies directed against the extracellular domain of Cx43 reduced cell aggregation and suggests that connexin may have adhesive properties. These connexin-adhesive properties require the formation of gap-junction plaques but not gap-junctional coupling. However, for migration and dispersion within the brain parenchyma, the establishment of functional channels between host astrocytes and Cx43-transfected glioma cells was necessary (62). Cx32-transfected glioma cells do not exhibit such a high capacity for migration. This suggests that the molecular processes that are involved, either for adhesion or migration, are probably different. The role that Cx43-mediated GJIC could play in migration is not understood, but it may lead to another spectacular phenomenon; when Cx43-C6 glioma cells and astrocytes were cocultured, the astrocytic phenotype was modified (smaller size and lower level of the astrocytic marker GFAP). It was suggested that this phenotypic transformation of astrocytes may contribute to their susceptibility to glioma invasion (116). Actually, the presence of normal astrocytes seems to increase the migrating capacity of Cx43-transfected rat C6 glioma cells, compared with the nontransfected cells (120). The acquired migration capacity is related to the presence of a larger amount of matrix metalloproteinases (MMP-2 and MMP-9) in culture medium from Cx43-C6 glioma cells. The presence of MMP does play a role in this invasive process, because a specific inhibitor significantly inhibited this capacity.

The question, then, is how does Cx43 control MMP expression in the culture medium; is that process dependent or not on the GJIC establishment between astrocytes and glioma cells? Such a question is very similar to the question that was asked about connexin control on cell-cycle–related gene expression. And again, the real role of GJIC in migration capacity of glioma cells is still controversial, because recent data with truncated Cx43 are in favor of a role of the CT portion of Cx43 independently on GJIC capacity (3).

Comparison with non pathological situations

It would be a mistake to estimate that connexin-mediated cell migration is a pathologic process. Such a connexin-mediated process has also been described during development. For instance, in rodents, during neocortical brain development, connexins are involved in cell migration (16, 21). In rats, connexins (Cx26 and Cx43) are expressed in migrating neurons from the ventricular zone, along radial fibers extending to the pial surface. Cx26 and Cx43 are highly localized in neurons to the regions of contact with the radial fibers. The *in situ* knockdown of connexin expression by shRNA strategy led to the conclusion that connexin expression is necessary for migration. Similarly, the involvement of Cx43 in migration of neural crest cells in mice is necessary for their correct development. In this model, a reduction of GJIC was associated with a reduced rate of neural crest cell migration (36). Based on these observations, it was proposed that gap junctions may mediate the cell-to-cell movement of second messengers and other cell-signalling molecules involved in regulating cell locomotion (37, 63). However, if N-cadherin seemed to be important for mediating Cx43-mediated GJIC (113), recent data suggest that Cx43 would enhance motility more independent of GJIC capacity, by interacting with vinculin and other actin-binding proteins (IQGAP-1, drebrin, α-actinin). In other words, such data suggest that Cx43 would play an important role in the dynamic regulation of the actin cytoskeleton, which would be independent on β_1 integrin expression level and GJIC capacity (114). Such a complex molecular process would play a crucial role not only on the motility but also on the directionality of the migrating cells.

The involvement of GJIC in migration is also controversial in the developmental context. For instance, in rats, the channel function does not contribute to the role of gap junctions in neuronal migration, a process that is not mediated by hemichannel function (16) either. The migration process seems to be mostly dependent on the adhesive property of

gap junctions and on the interaction between Cx43 and the actin cytoskeleton. In some models, coexpressed connexins are thought to exert different functions, as in migrating neurons expressing Cx26 (dominant role in nuclear translocation) and Cx43 (dominant role in branch stabilization) (16). In cancer, most of the studies indicate an active role for GJIC in the invasive capacity; this has been demonstrated mostly in glioma models. Except for a major study (3), the establishment of heterocellular coupling between glioma cells and astrocytes seems to be more commonly found (62, 81). From these data, gap junctions would have two complementary roles in cancer invasion: cell adhesion (independent of GJIC) and migration (dependent on coupling).

Other cancer cases

If, in the brain, an interesting parallel can be drawn between pathologic (gliomas) and nonpathologic (brain development) conditions, in terms of GJIC and connexin involvement, no such parallel may be found in other tissues. In other organs, cell migration is mostly related to cancer, and connexins have also been suspected to play a role. As for growth regulation, connexin specificity seems to be implicated, regardless the tissue type. For instance, migration of skin cancer cells is also associated with gap junctions and Cx26 expression (44). Similarly, Cx26 expression seems to govern invasion, and migration in prostate cancer may be through a close interaction between Cx26 and focal adhesion kinase. Such a result tends to demonstrate a novel mechanism of Cx26 for adhesion regulation by a gap junction–independent phenomenon (104). In another *in vitro* cancer prostate model, the cell motility of low and high rat metastatic cell lines was increased when cocultured on a fibroblast-coated surface; a Cx43 expression–independent effect (72).

For each model, when connexin expression is found to be associated with cell motility, general agreement exists about the necessity of a membrane localization of the connexin, even if the molecular mechanism involved is connexin-type dependent (close interaction with the actin network or with focal adhesion kinase, *etc*.). This is indeed the case, except for Cx32, which enhances both motility and invasiveness of a human liver cancer cell line (HUH7 cells) when accumulated in the cytoplasm (61, 82). Accumulation of connexins in the cytoplasm has been frequently observed in cancer cells, independent of the connexin type expressed. Would it mean that such an accumulation could be an intermediate stage for tumor cells before becoming more invasive? For instance, Cx26 becomes mainly cytoplasmic in human colorectal adenocarcinomas (49). The fact that this cytoplasmic expression follows a focally decreased expression of Cx26 in adenomas with severe dysplasia led the authors to conclude that the cytoplasmic presence of Cx26 could indicate a different role of Cx26 in neoplastic cells (49). It would be interesting to study whether the cytoplasmic accumulation of connexins in late tumoral stages is predictive of invasive behavior.

The relation between connexin expression and cancer cell motility is not always clear. Apparently, this is the case for breast cancer cells. For instance, in breast cancer cells (Hs578T cells), silencing Cx43 leads to increased growth (which is in agreement with Cx43-mediated growth control in breast cancer cells), as well as an increase of the migration capacity (contrary to the Cx43-mediated cell-migration capacity observed in glioma cells) (99). The expression of Cx26 inhibits both proliferation and invasion of MDA-MB-435 cells through a GJIC-independent mechanism and reduces the expression of MMP-9 (46).

Stage 3: Gap Junctions and Metastasis

One of the first steps of metastasis implies that cancer cells closely interact with endothelial cells to pass through the vascular barrier and to disseminate into lymph or blood flow. Because connexin expression is frequently lost in primary tumors, it was thought for long that tumor cells would interact with endothelial cells only through a cell–cell recognition process. Such an assumption was in agreement with the initial dogma that cancer cells do not communicate through gap junctions and with data establishing that the metastatic capacity was inversely correlated to their GJIC capacity (29, 30, 78, 92, 98). This was also in agreement with observations showing that overexpression of antimetastatic genes such as *BRMS1* and *TIMP-1* could restore GJIC (98, 100). However, more-recent studies have shown that connexin expression may be present in later stages of carcinogenesis, favoring the migration capacity of invasive cells. This would suggest that invasive cells escaping from the primary tumors might also interact with endothelial cells through gap-junction channels. Actually, this phenomenon probably happens, because GJIC has been observed between vascular endothelial cells and malignant cells from various tumor types, such as glioma (121), melanoma (42, 97), and breast cancer cells (86). Such an observation suggests that connexins, which are frequently lost in the primary tumors, may appear at later stages of cancer progression. This was clearly observed during the progression of human breast cancer: Cx26- and Cx43-negative primary tumors were found to develop Cx26- and Cx43-positive metastases in lymph nodes (50). Similar results were obtained in mouse skin carcinogenesis; Cx26 expression, which is reduced at the early stages, is restored in tumor metastases in the lymph nodes (48).

The direct exchange of information between cancer cells and endothelial cells might then permit diapedesis and dissemination of cancer cells toward target organs in the body. The frequency of this phenomenon is augmented by neoangiogenesis.

Connexins and neo-angiogenesis

It is well known that neoangiogenesis is an important event favoring both tumor growth and spread of metastatic cells. By attracting to themselves newly formed vessels, hypoxic tumors dramatically increase the risk that invasive cancer cells might closely interact with endothelial cells. Some data even suggest that connexin expression could control this crucial and very preliminary step of the metastatic process (90).

These data support the hypothesis that connexin expression would have a protective effect against angiogenesis. Most of them were obtained by using breast cancer cells. The decreased expression of Cx43 in breast cancer cells (Hs578T cells) results in decreased levels of thrombospondin-1 (TSP-1; an antiangiogenesis molecule) and increased expression of VEGF (99). Cx43 or Cx26 overexpression in breast cancer cells leads to decreased fibroblast growth factor receptor-3 (90). Cx26 was also found to downregulate the expression of

connective tissue growth factor (CTGF), another angiogenesis-related gene, and to increase TSP-1 (90). These data confirm connexins as possible gene regulators and suggest that their level of expression can directly control angiogenesis. From these breast cancer cell models, connexins mostly appear as inhibitors of angiogenesis, because their overexpression may lead to the secretion of factors preventing endothelial cell tubulogenesis and migration (67). Interestingly, these regulations seem to be controlled by mechanisms that may not involve GJIC establishment (67, 90).

However, by considering other kinds of tumor cells, such as glioma cells, the picture appears to be completely different. Cx43 expression in human malignant glioma cells was determinant for significantly increasing tubulogenesis of cocultured human umbilical vascular endothelial cells (HUVECs). Interestingly, even if VEGF concentration was higher in the medium of Cx43-expressing glioma cells, direct Cx43-mediated GJIC between glioma cells and HUVECs may play a more-important role in tubulogenesis (121). No difference in tube formation was found in medium conditioned by either Cx43-expressing or Cx43-deficient glioma cells (121). Although they express a similar connexin, Cx43, glioma cells and breast cancer cells apparently induce opposite effects on angiogenesis. It is too early to conclude that these differences are the consequences of the variety of models; the data concerning breast cancer cells seem to be more consistent at this stage of the study, because they present a uniform set of results. More data are needed concerning the proangiogenic effect of Cx43 in glioma cells. Nevertheless, even if they are contradictory, these results are in favor of a complex interaction occurring between cancer cells and endothelial cells, in which connexins are expected to exert either a GJIC-dependent or -independent effect on angiogenesis. This complexity is illustrated by the fact that VEGF can also downregulate the expression of Cx43 (85) and block GJIC in endothelial cells (103).

Communication between cancer cells and endothelial cells

The close contact between invasive cancer cells and endothelial cells is a preliminary step for metastasis. This step is necessary for the eventual passage of cancer cells through the endothelial barrier. A complex process of recognition occurs between the two cell types during this crucial phase. Not all the molecular components involved in the recognition process and during the diapedesis step have been identified, but one of the most spectacular findings is the direct and transitory establishment of GJIC between cancer cells and endothelial cells (42, 86, 97, 121). Endothelial cells are known to express various types of connexins (Cx37, Cx40, Cx43), and thus, they can, in theory, communicate with cancer cells expressing compatible connexins. The precise role of connexins in endothelial cells has not been elucidated, but the close interaction of at least some of them (Cx40, Cx43) with tight-junction molecules (occluding, claudin-5, ZO-1) suggests that they might be required to maintain the endothelial barrier function (74). Another role would be the establishment of a direct and transitory GJIC with interacting cells, such as leukocytes and cancer cells. This is obviously the case for Cx43-expressing malignant glioma cells, which can form Cx43 gap-junction plaques with HUVECs (121). These two very different cell types were found to communicate efficiently through the Cx43 plaques, and calcium signalling was even shown to be transferred from glioma cells to endothelial cells (121).

Metastatic melanoma cells were also found to communicate directly with endothelial cells (42). Cx26 was involved in this phenomenon, and its level of expression was directly related to the metastatic capacity of the cells. Transfection of Cx26 in nonmetastatic melanoma cells rendered them highly communicating and metastatic. Conversely, transfection with a dominant-negative mutant of Cx26 in metastatic melanoma cells prevented GJIC and decreased their metastatic capacity (42). Interestingly, the invasive capacity of human melanoma cells was also related to their level of Cx26 expression, because it was noted that Cx26 expression is low in cells residing in the basal layer of the skin but upregulated in cells invading the dermis (42). The presence of Cx26 in squamous cell carcinoma cells has even been reported as a bad prognostic predictor (44). The close relation between metastatic capacity and Cx26 expression supported the use of a specific inhibitor of Cx26 for preventing the metastatic behavior of melanoma cells (43). Connexins typically expressed by endothelial cells are not functionally compatible with Cx26, but a recent study demonstrated the presence of Cx26 in endothelial cells of the small vessels surrounding the melanoma cells (97).

Connexins, diapedesis and interactions with colonized organs

The link between connexin expression and metastatic behavior suggests that heterocellular GJIC between tumor cells and endothelial cells may control tumor cell diapedesis. This is confirmed by the induction of Cx43 in a GJIC-deficient mammary tumor cell line, which increased twice the diapedesis efficiency (86). This was mediated by the presence of functional Cx43 plaques between the tumor cells and the endothelial cells. Any prevention of the heterocellular GJIC decreased diapedesis efficiency (86).

It is not known how heterocellular GJIC controls diapedesis, and GJIC appears to be an important component of a complex process in which adhesion probably occurs before the establishment of functional gap-junction channels between the arrested tumor cell and the endothelium. Endothelial cell adhesion molecules play a critical role in the adhesion process, and any interference with the adhesion phenomenon seems to decrease metastasis. This was demonstrated for one of them, the lung endothelial cell adhesion molecule-1 (Lu-ECAM-1), which plays a critical role in recognition and initial arrest of tumor cells (murine melanoma cells) in lung venules (23, 123). Such recognition facilitates extravasation of tumor cells (17).

Finally, once diapedesis has been performed, the last step of this third fundamental stage of metastasis implies interactions between metastatic cells and the cells of the colonized tissue (tissue targeting). Very little has been investigated about these possible interactions, but recently it was shown that gap junctions could also play a role in this process. As demonstrated for a human breast carcinoma cell line, which metastasizes to bones when injected intracardially in mice, it appears that heterotypic GJIC can occur with human osteoblastic cells (52). This heterotypic communication was even quantitatively greater than the homotypic communica-

tion in bone. Moreover, it was not significantly affected by BRMS1 transfection, contrary to the homotypic communication between breast cells. These results suggested that the degree of heterotypic GJIC between breast cancer cells and the osteoblastic cells of the target tissue correlates with the breast metastatic potential. From such results, it would be interesting to check whether the metastatic cells generally communicate with cells of their target organs and if these interactions play a role in the dormancy of metastasis. In other words, it would be interesting to understand whether the level of heterotypic GJIC with normal host cells plays a role in the growth control of metastasized cells.

Conclusion

In this last decade, the involvement of connexins in cancer became much more complex than it was 40 years ago (64). During this early period, an association was initially observed between the lack of GJIC and the cancer phenotype. Such an association allowed formulating the hypothesis that direct cell-to-cell coupling controls cell growth. This hypothesis was confirmed by other observations, such as the capacity of tumor-promoting agents to inhibit GJIC (70). Then, after the technical progress of biology, more molecular approaches became available, such as the connexin cDNA transfections, which appeared to "normalize" the cancer phenotype. Overexpressions of particular connexins were shown to diminish growth of the transfected tumor cells, confirming the initial assumption. Connexins appeared as putative tumor suppressors, but because their lack of function during carcinogenesis was not due to gene mutations (contrary to classic tumor suppressors), they were classified as class II of tumor suppressors. Moreover, even *in vivo* approaches partly confirmed the tumor "protector" effect of connexin expression. Depending on invalidated connexins, to a certain extent, transgenic mice appeared to be more susceptible to spontaneously or chemically induced neoplasm formation (2, 105).

However, starting with these molecular approaches, some observations suggested that the involvement of connexins in carcinogenesis was not so simple as expected. Downregulation of growth was observed after connexin transfection independently on GJIC establishment (35). It became clear that the tumor-suppressive role could depend on the connexin type in relation with the type of recipient cancer cells (5, 69) (Table 1). These last observations could establish a link between the specific tissue distribution of connexins and their specific "normalizing" capacity. All these findings suggested that connexins could regulate cell growth by various means, which could depend on the establishment of GJIC or not. They also suggested that connexins could regulate gene expression related to cell growth as a membrane protein, cytoplasmic protein, nuclear protein, interacting protein, or cytoplasmic (or nuclear) fragment of it (see stage 1 part of this review). Most of these last studies were carried out by using Cx43 as a model. The situation of connexin-mediated cell growth is confusing, and it seems that, depending on the cell models used, the picture may be different. A possibility would be that connexins control gene expression through specific interactions with cytoplasmic proteins. If this be the case, they should be able to control different signalling pathways, because their cytoplasmic sequences are unique for each type of connexin. Despite the amount of information, the question "how do connexins regulate cell growth?" is yet to be resolved. One way to answer that question could be by using transgenic mice to check which genes are differently expressed when a specific connexin is deficient. Such an approach is becoming more and more developed (40, 41).

Cancer is not only a deregulation of growth. It is also the appearance of an invasive phenotype and dissemination of metastatic cells (stages 2 and 3 of this review). Despite this evidence, most of the research about the involvement of connexins in carcinogenesis was restricted to growth control. When some investigations were pursued to see whether connexins play a role in later stages of carcinogenesis (invasion, metastasis), it probably appeared to be the case. If, in the initial stage of growth deregulation, connexins could be assimilated to tumor suppressors, in these later stages, they look like oncogenes. Globally, the expression of connexins seems to increase adherence and cell motility. Except for a few exceptions, this phenomenon seems to require the formation of gap-junction plaques. It is still controversial whether GJIC is needed, but it is interesting to note that recovery of connexin expression may occur in some invasive parts of the tumors and in the metastasizing cells in lymph nodes.

These observations imply other roles for connexins. They favor adherence and motility, and, in some cases, they permit the invasive cancer cells to communicate through heterocellular GJIC with the parenchymal cells of the invaded tissue and endothelial cells. This last capacity would be necessary for diapedesis and dissemination to other parts of the organism. An open question is whether they are also involved in the formation of metastasis by permitting heterocellular GJIC between metastatic cells and the cells of the colonized organs. Thus, in less than a decade, connexin went from friend (tumor suppressor) to foe, according to the cell models used, playing an important role in dissemination. Of course, more studies on appropriate models are needed definitively to reach conclusions about these new aspects of connexin biology and its multiplicity of roles, which make the connexins involved in various and very different cellular processes.

Why are connexins reexpressed in invasive cells? Is this phenomenon related to hypoxia or apoptosis? Is the tumor environment involved? If yes, any culture approach may then be useless.

Understanding the real involvement of connexins in cancer is now a very complex problem. At least, it seems that these molecules play different roles depending on the cancer stage considered. Based on the cell phenotype and/or the cellular environment, they could accumulate different functions. Actually, maybe these different functions are not completely pathologic; connexins are involved, at least, in cell migration during brain development, and probably they are also involved in diapedesis of macrophages. Such a succession of roles played by connexins in a nonpathologic process happens, for instance, during the formation of an autonomous and transient organ resulting from maternal/fetal interactions, the human placenta (65).

The human trophoblastic cell represents a pseudo-tumoral model with spatiotemporally controlled proliferation and invasion processes. At 7–8 days after conception, the blastocyst invades the uterus, and the formation of the placenta is

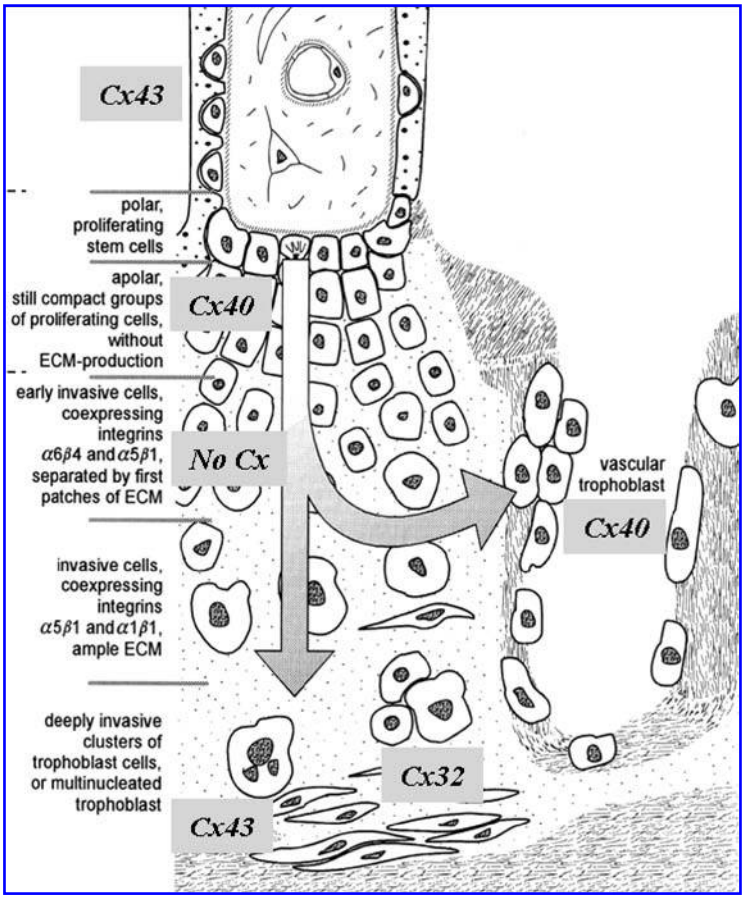

FIG. 5. Schematic presentation of connexin expression in the human trophoblast. Connexins are differentially expressed during human placentation. Cx40 expression is associated with proliferation of trophoblastic cells. Cx40 expression is then lost when cytotrophoblastic cells become invasive and leave the column. It is reexpressed afterward in perivascular or vascular cells. Cx43 and Cx32 are expressed in trophoblastic cell aggregates. VT, villous trophoblast; EVT, extravillous trophoblast; CC, cell columns. Modified from ref. 54.

the result of a complex series of interactions between fetal trophoblast and maternal cells in the decidua. During placentation, two differentiation pathways lead to the formation of two distinct trophoblastic cell populations: the villous phenotype and the extravillous phenotype (Fig. 5). In the extravillous phenotype, cytotrophoblastic cells proliferate, detach from the basement membrane, and aggregate into cell columns to attach to the uterine wall. From there, individual cells migrate into the decidua and the myometrium, with a capability of remodelling the endometrium and its vasculature. Some of the extravillous cytotrophoblastic cells invade the uterine arterioles, destroy the media, and replace the endothelial cells. In the case of an uncontrolled invasion process, choriocarcinomas (trophoblast origin) may appear during pregnancy and are characterized by early metastases.

In the proximal proliferative part of the cell column (Fig. 5), Cx40 is expressed (10, 111) as in malignant trophoblastic cells. However, no evidence of GJIC was detected in the cells when investigated with the gap-FRAP approach (10). When the cytotrophoblastic cells are leaving the column, Cx40 disappears and then is reexpressed in perivascular or vascular trophoblastic cells. Furthermore, Cx40 antisense treatment also results in the abolishment of extravillous cell proliferation. Based on these results, it was suggested that Cx40 channels are required for the proliferation of extravillous cells in cell columns and that a loss of GJIC contributes to differentiation to the invasive extravillous cell phenotype (79). Moreover, when deeply invasive trophoblastic cells aggregate and form multinucleated trophoblasts, they express Cx43 and Cx32. Interestingly, similar to other cell systems, such as myoblasts (88, 89) and osteoclasts (102), Cx43 expression and function are linked to a cell-fusion process that occurs mainly during villous differentiation (Fig. 5).

In consideration of the trophoblast model, it seems that connexins are differently involved during the first steps of the formation of the placenta, which is very similar to a "controlled carcinogenesis process." It seems that this model is a confirmation of the accumulation of connexin functions (growth control, invasion, dissemination in general circulation) we have mentioned in this review. It defends the assumption that connexins could control, by a variety of functions, crucial cellular (pathologic and nonpathologic) events.

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Abbreviations

ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; BRMS1, breast cancer metastasis suppressor 1; CASK, calcium/calmodulin-dependent serine kinase; CDK, cyclin-dependent kinase; CT-Cx43, carboxy-terminal part of connexin43; CTGF, connective tissue growth factor; Cx, connexin; CxRE, connexin-responsive element; Cyr61, cysteine-rich angiogenic inducer 61; ERK, extracellular signal-regulated kinase;

FRAP, fluorescence recovery after photobleaching; GJIC, gap-junctional intercellular communication; HUVECs, human umbilical vascular endothelial cells; Lu-ECAM, lung endothelial cell adhesion molecule 1; MAGUK, membrane-associated guanylate kinase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemotactic protein-1; MFG-E8, milk fat globule epidermal growth factor 8; MMP, matrix metalloproteinase; MRF4, muscle regulatory factor 4; PIDD, p53-induced protein with a death domain; RPL19, ribosomal protein L19; Skp2, S-phase kinase-associated protein-2; TIMP-1, tissue inhibitor of metalloproteinase 1; TSP-1, thrombospondin; VEGF, vascular endothelial growth factor; YAF2, YY1-associated factor 2; ZO-1, zonula occludens 1; ZONAB, ZO-1–associated nucleic acid–binding protein.

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