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COMMENTARY

Gap junctional intercellular communication and carcinogenesis

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Introduction

While individual cells have their independent apparatus to maintain their own functions, the growth and behaviour of individual cells do depend on other neighbouring cells. In other words, cell–cell interaction is essential for the maintenance of tissue homeostasis and cellular society. Contrary to normal cells, cancer cells clearly behave as rebels of this ordered cellular society and thus neglect the homeostatic controls of neighbouring tissue. It is, therefore, likely that cell–cell interaction with surrounding normal cells is altered during multistage carcinogenesis. In fact, there are several lines of evidence which suggest that derangement of intercellular communication (IC*) facilitates the clonal growth of potential cancer cells, and that intact IC can work as a tumour suppressive element (1–3).

Most cells have two different ways to communicate with other cells. One is the growth factor or hormone-mediated communication, which does not need direct cell contact. The other is cell contact-mediated IC. Since the former type of IC needs extracellular factors for communication and since such factors are relatively easy to identify, the study of this type of IC has advanced rapidly and it became clear that alteration of this IC is important in the process of carcinogenesis. This is best shown by the fact that many oncogenes encode growth factors or growth factor receptors (4). Cell contact-mediated IC is the type whereby neighbouring cells whisper to each other and it is therefore difficult to know which kind of conversation they are making. However, owing to rapid progress in the knowledge and techniques of molecular biology, the study of this type of IC is now advancing very rapidly, especially by molecular cloning of cDNA which encodes communicating apparatus. Among cell contact-mediated IC, gap junctional intercellular communication (GJIC) is considered to play the pivotal role in the maintenance of tissue homeostasis (5,6). Since the gap junction is the only known structure whereby the interiors of contacting cells can be connected, it is believed that certain factors important for the maintenance of growth and differentiation are being exchanged to maintain the other cells in check (5,6). Because of the apparent importance of the gap junction in the maintenance of cellular society, the modulation of GJIC has long been proposed to be involved in carcinogenesis (5). Such a hypothesis was reinforced when the groups of Murray (7) and Trosko (8) discovered that certain tumour promoting agents can inhibit GJIC and proposed that such an inhibition may be involved in the clonal expansion of initiated cells by releasing them from suppressive control exerted by surrounding normal cells via GJIC.

*Abbreviations: IC, intercellular communication; GJIC, gap junctional intercellular communication; GJ, gap junction.

For those who are not very familiar with GJIC, it may be useful to review here the essential features and more recent advanced knowledge of gap junctions (GJs). It is generally accepted that GJs mediate the direct intercellular flow of molecules whose mol. wt is <1000 daltons, which includes anions, cations and uncharged molecules. So far, various molecules including cAMP, calcium and inositol triphosphate have been shown to be GJ permeants (6,9,10). GJs in different tissues are not always structurally identical. However, they all share a basic structure similarity; six subunit (connexin) proteins form a GJ hemi-channel (connexon) in each plasma membrane which docks with the connexon from apposing cells to form complete channels. These six column monomers are aligned with axis tilted slightly to normal in the membrane and enclose a central hole. It has been suggested that such a conduit may be closed under certain circumstances by twisting of the six subunits (11). The complete cDNAs corresponding to at least three different connexin proteins have been cloned; two liver GJ cDNAs code for a 32 kd protein, termed connexin 32 (12,13), and for a 26 kd protein, termed connexin 26 (14), and a heart cDNA codes for a 43 kd protein, connexin 43 (15). These connexins have both conserved and variable sequences. Using monoclonal antibodies, connexin membrane topology has recently been proposed (14,16,17). While amino termini and carboxy termini are located inside the cytoplasm, the protein is folded into the membrane twice, i.e. four membrane traverses to form an M shape with two extracellular regions and three cytoplasmic regions. The conserved sequences correspond to the putative membrane spanning and extracellular regions of the connexins, while the cytoplasmic regions are more variable among different connexins. The cytoplasmic regions contain sequences which can be phosphorylated by tyrosine kinases, cAMP-dependent kinase and protein kinase C (18). A schematic view of GJ structure is shown in Figure 1.

GJIC can be measured by various methods in culture; these methods can be divided into three groups, i.e. metabolic cooperation assay, electrophysiological method and dye-transfer assay (5). Each assay has its advantages and disadvantages and therefore the most appropriate method has to be chosen according to the purpose of each study. In addition to these functional assays for GJs, we now can analyse the level of GJ mRNA and the level and localization of these proteins using available cDNAs and antibodies. Use of such molecular probes in cancer research has just begun and considerable data are expected to come soon.

GJIC can be modulated at various points. This is quite relevant when we think about its involvement in carcinogenesis. It appears that GJIC can be modulated by different chemicals or by different physiological conditions which may be involved in the complex nature of carcinogenesis mechanisms; various types of tumour promoting agents may act at different points of GJIC regulation (18). In addition to the usual regulation at the level of transcription, mRNA stability, translation and post-translational processing, GJ functions may be regulated at the level of hemi-channel alignment and gating of mature channels. For the control of hemi-channel alignment, there is evidence that the

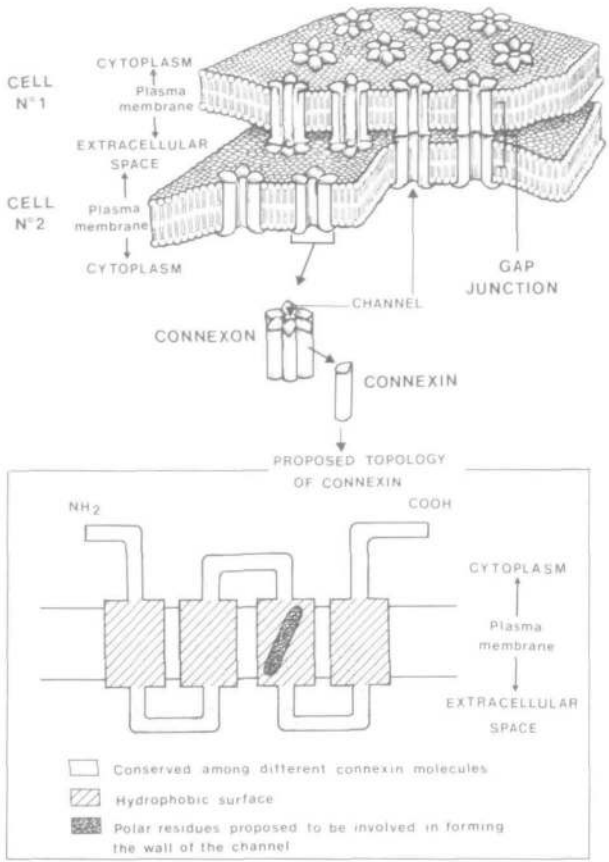

Fig. 1. Schematic view of gap junctions in membrane lipid bilayers and topology of connexins. While the structure and topology are generally considered common to various connexin molecules, there are important differences among them which may be related to the regulation of their function (see text and references cited for details).

expression of cellular recognition proteins such as cadherin molecules is essential for the function of GJIC (19). As to the gating of mature channels, it is proposed that under certain circumstances, the conduit of the channel can be twisted and can be closed (11). When genomic DNA of GJ proteins are sequenced more completely, we may know other different points of regulation such as *cis*-element and *trans*-acting factors.

Since this article is a commentary rather than a review of the subject, the following text is necessarily subjective and does not cover all aspects related to the role of GJIC in carcinogenesis.

Evidence for and against the involvement of GJIC in carcinogenesis

Possible involvement of GJIC in carcinogenesis is conceptually well accepted, but it is still a controversial issue. While many lines of evidence do suggest that altered GJIC is involved in carcinogenesis, there are also data which do not support such an involvement. It may be useful to summarize both stories.

The most supportive evidence for the involvement of GJIC in carcinogenesis is the finding that many tumour promoting agents can block this type of communication (1,7,8,20). The evidence has mostly come from *in vitro* studies. However, certain evidence has also come from *in vivo* studies (21,22). Furthermore, phorbol ester tumour promoter-mediated inhibition of GJIC can be antagonized by various mouse skin anti-promoting agents such as retinoic acid, glucocorticoids and cAMP (23). In addition, there are several lines of evidence that tumour promoter-mediated inhibition of GJIC is associated with enhancement of cell transformation. For example, phorbol ester-induced BALB/c3T3 cell transformation was associated with decreased GJIC (24), while the above anti-promoting agents inhibited complete or two-stage cell transformation of BALB/c3T3 cells (25). Furthermore, variant BALB/c3T3 cells which have higher susceptibility to cell transformation decreased their GJIC capacity when they reached confluence, whereas transformation-resistant cell lines did not show such a decrease, indicating that decreased GJIC may have acted as an endogenous tumour promoting stimulus (26). Also, TPA inhibited GJIC of Syrian hamster embryo cells which were sensitive to TPA-mediated enhancement of cell transformation, but not of those which were resistant to such transformation (27). Recent studies also suggest that Syrian hamster embryo cells can be more readily transformed at lower pH where GJIC is lower (28; R.A.LeBoeuf, personal communication). In molecular studies, analysis of mRNA and protein expression level of connexins during rat liver carcinogenesis indicates that decreased connexin expression occurs in preneoplastic lesions as well as in hepatocellular carcinomas (29,30). These results support the idea that decreased GJIC plays a role during carcinogenesis, rather than that such a decrease is the result of carcinogenesis.

In considering non-supportive evidence, phorbol ester tumour promoters did not inhibit GJIC of mouse epidermal cells *in vivo* when mice were painted with TPA (31). In culture, however, TPA did inhibit GJIC of mouse epidermal cells (32). In addition, compounds such as okadaic acid, transforming growth factor β (TGF-β) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) failed to inhibit GJIC while they did enhance transformation of BALB/c3T3 cells or C3H10T1/2 cells (33–35).

This apparent contradictory evidence suggests either that different tumour promoting agents operate via different mechanisms or that GJIC is indeed always inhibited by tumour promoting agents but sometimes only around so-called initiated cells. In the latter case, it is impossible to detect with our existing methods such localized action. While our evidence does not favour either of these two possibilities, in considering the diversity of tumour promoting chemicals it is conceivable that multiple mechanisms are involved in tumour promotion and that GJIC inhibition is not the sole mechanism of tumour promotion evoked by various chemicals.

GJIC in cancer cells; homologous versus heterologous communication

While the involvement of GJIC in the promotion stage of carcinogenesis is not yet clarified, it appears that altered GJ or GJIC is a common feature in many, if not all, cancer cells. It had once been considered that all cancer cells have no or decreased GJIC. This was due to the dogmatic view that since cancer cells show aberrant growth control, their GJIC capacities should be diminished. However, subsequently it has been shown that certain tumours or transformed cells have normal levels of GJs or GJIC capacity suggesting that GJIC decrease is not a *fait accompli* for cancer cells (36,37).

As a unifying concept, we have recently proposed that what is important for the maintenance of tumour or transformed cell phenotypes is a lack of heterologous communication, i.e. a lack of GJIC between tumours and surrounding normal cells (38). If we look at tumour cell communication capability from this point of view, it becomes clear that essentially all tumour cells do have altered heterologous communication, that is, they have less or

no GJIC with surrounding normal cells. We further propose that loss of heterologous communication between tumour and normal cells can be attained by one of the following two mechanisms. (i) Loss of homologous GJIC among tumour cells. It is proposed that if tumour cells do not communicate amongst themselves, it is likely that they do not communicate with surrounding normal cells. An example for such a change was first demonstrated by Loewenstein and his colleagues using liver cancer cells (39,40). Numerous subsequent studies support this type of loss of GJIC. The examples include cells from human stomach cancer, human mesothelioma, human breast cancer, human and mouse skin cancer, and rat liver tumour (41–46). (ii) Selective lack of GJIC between transformed and surrounding normal cells; while transformed cells maintain their own GJIC capacity, they do not communicate with surrounding normal cells. This type of lack of heterologous communication was first demonstrated using the BALB/c3T3 cell transformation system. Regardless of carcinogen used, cells within induced transformed foci communicated amongst themselves, but they did not communicate with surrounding normal cells (33,37,47). Similar selective GJIC has also been shown to occur in the combination of tumorigenic and non-tumorigenic rat liver epithelial cell lines (48). In both cases, normal and transformed cells express the same type of connexin genes (M.Mesnil, F.Katoh and H.Yamasaki, unpublished observations). Since both cell types have the same GJ proteins, selective lack of communication may be due to lack of recognition between transformed and normal cells. It is important to emphasize that this selective compartmentalization of transformed and normal cells in terms of GJIC is very rigid. Therefore, when one introduces into a transformed cell a cytotoxic compound which is not membrane-diffusible but is GJ-diffusible, this chemical can be spread only among transformed cells and does not enter surrounding normal cells. This idea has been successfully applied in an *in vitro* chemotherapy model (49). Using the antibodies against GJ proteins (connexins) or connexin cDNAs, recent studies revealed that GJ protein and/or gene expression are altered in liver tumours taken directly from animals or humans. A reduced number of GJs and reduced levels of connexin 32 mRNA were found in rat hepatocellular carcinomas as well as in preneoplastic lesions (29,50,51). On the other hand, when human hepatocellular carcinomas were analysed, there was no decrease of connexin 32 mRNA level in these tumours, in comparison with surrounding normal cells. There was, however, appearance or increase of connexin 43 gene expression in tumours (52). Although we do not know whether the connexin 43 and connexin 32 can form functional GJs in the liver, it is possible that the appearance of connexin 43 in tumours may disturb the communication with surrounding normal cells. However, at least in a *Xenopus* oocyte model system, it has been suggested that connexin 32 and connexin 43 can form functional GJs (53).

These results do suggest that many tumour cells do not communicate with surrounding normal cells. We propose that this is a key event for tumour cells to maintain their transformed phenotypes. If tumour cells do communicate with surrounding normal cells, there will probably be transfer of growth controlling factors from normal cells to transformed cells, possibly resulting in tumour suppression, i.e. transformed phenotypes may disappear. This is an idea common to that of tumour suppression mechanisms proposed through cell hybridization experiments (54,55), e.g. when tumour and normal cells are hybridized, usually hybrid cells do behave as normal cells, suggesting that normal cell phenotypes are dominant over tumour cells. Although we do not know what kind of growth controlling factors may be operating in GJIC-mediated control of cell proliferation, it is possible that GJIC may work as a kind of mini-hybridization of two types of cells. On the other hand, we cannot exclude the possibility that growth stimulating factors which are abundant in tumour cells are transferred to surrounding normal cells via GJIC so that the level of such factors in tumour cells is decreased by dilution into normal cells (5).

Role of IC in tumour suppression

If we extend our hypothesis that lack of GJIC between tumour cells and surrounding normal cells is essential for the maintenance of transformed phenotypes, it is also possible to postulate that resumption of IC between tumour cells and normal cells may act as a tumour suppressor and thereby eliminate transformed phenotypes. In fact, in the existing literature there are reports that cell–cell contact of tumour cells and normal cells can suppress transformed phenotypes.

The first description that transformed cells do not outgrow when they are in direct contact with an excess number of normal counterparts came from Stoker and his colleagues (56,57); when a small number of polyoma virus-transformed BHK21 cells was cocultured with non-transformed mouse fibroblasts, there was suppression of growth of transformed cells. Direct cell contact was necessary to attain such suppression. Subsequent studies have shown that there was indeed passage of molecules from surrounding normal cells to transformed cells via GJIC (50). Similar suppression was observed in a coculture of polyoma virus-transformed bovine fibroblasts and normal fibroblasts (58). In other studies, Sivak and van Duuren have shown the suppression of the outgrowth of SV40-transformed 3T3 cells in a coculture with a vast excess of normal cells (59). They further found that the addition of croton oil rescued the growth of SV40-transformed cells in coculture. Since croton oil contains TPA and other phorbol esters which are potent inhibitors of GJIC, it is possible that GJIC between transformed and non-transformed cells was a cause of the suppression of these transformed phenotypes. More recently, Herschman and Brankow (60) have shown that C3H10T1/2 cells cloned from transformed foci induced by UV radiation and TPA appeared transformed in homologous culture. However, when cocultured with normal cells, these transformed cells were unable to form foci. In the presence of TPA, the transformed cells formed foci in these mixed cultures, while retinoic acid could block this action of TPA. Since TPA is a GJIC blocker (7,8), and retinoic acid can antagonize such a TPA effect (24), these results again suggest that appearance of transformed phenotypes depends on heterologous GJIC between transformed and normal cells.

Suppression of transformed cells by close contact with normal cells was also observed after *in vivo* inoculation of mixed cell populations. Terzaghi-Howe (61) inoculated cell mixtures containing normal and neoplastic rat tracheal epithelial cells into the lumen of denuded tracheas, then transplanted these tracheas s.c. into syngeneic hosts where intact tracheal mucosa was regenerated from the inoculated cells within 2 to 3 weeks. If a reasonable number of normal cells was employed in tracheal repopulation there was no tumour formation over a 16 week period. However, in denuded tracheas containing neoplastic cells alone, or in impartially denuded tracheas containing comparable numbers of neoplastic cells covering a contiguous area of sub-mucosa, tumours developed 2 to 5 weeks after cell inoculation and transplantation. These results suggest that a certain degree of cell–cell contact between normal and tumour cells will suppress the outgrowth of neoplastic cells in this system.

Similarly, the suppression of tumorigenicity of transformed mouse epidermal cells by normal fibroblasts was demonstrated in the skin grafting method (62). Mouse keratinocytes malignantly transformed by infection with Harvey sarcoma virus can produce carcinomas when skin grafted onto syngeneic animals. However, the expression of their malignant phenotype was inhibited when the cells were grafted with normal dermal fibroblasts. These results suggest that interaction of transformed keratinocytes and dermal fibroblasts is important for suppression of expression of transformed phenotypes. Earlier studies of Hennings *et al.* (63) suggested that interaction with normal keratinocytes and not fibroblasts was important for growth suppression of tumorigenic keratinocytes.

Several studies examined the relationship between direct cell contact-mediated suppression of transformed phenotypes and GJIC *per se*. For example, Mehta *et al.* (64) used chemically and virally transformed C3H10T1/2 cells in coculture with normal counterparts. In cell combinations where heterologous communication was weak or absent, there was no detectable growth inhibition, but growth inhibition appeared when GJIC was induced by cAMP-dependent phosphorylation. Inhibition was also apparent in cell combinations where heterologous communication was strong. Such a growth inhibition was absent when GJIC was blocked by retinoids (retinoids stimulate GJIC of other cell types, see below). Another line of evidence for the involvement of GJIC in suppression of transformed phenotypes has come from the use of agents which can modulate GJIC. As described earlier, when BALB/c3T3 cells are transformed by different carcinogens, these cells did not communicate with surrounding normal cells although they communicated among themselves. When, however, cultures containing transformed foci were treated with upregulators of GJIC such as retinoic acid, cAMP or glucocorticoids, there was gradual resumption of GJIC between transformed and normal cells. In the continuous presence of these chemicals, there occurred a marked decrease of the number of transformed foci. In fact, attentive observation confirmed the actual gradual disappearance of transformed phenotypes. However, when transformed cells were isolated and cultured at clonal density in the presence of retinoic acid, cAMP or glucocorticoids, there was little decrease in the number of transformed colonies, suggesting further that it was the GJIC with normal cells that accounted for transformed cell suppression (25).

As described above, the relationship between heterologous GJIC and expression of transformed phenotypes was studied using either an *in situ* transformation system or cocultures of transformed and non-transformed cells. While the results from *in situ* transformation studies can be interpreted in a relatively straightforward manner, those from coculture studies are more difficult to interpret. In coculture (or re-constructed *in vivo*) systems, already-transformed cells are placed with non-transformed counterparts and transformed phenotypes are sometimes suppressed. In such cases, this implies that transformed cells are indistinguishable from surrounding non-transformed cells and, thus, poses the question of how such transformed cells could have clonally expanded in order to be isolated, i.e. why transformed cells could express their phenotypes in the original culture from which these cells were isolated. One reasonable explanation is that there is a critical population size beyond which the transformed cells could neglect the suppressive pressure from surrounding non-transformed cells and clonally expand with their own phenotypes (61,65). In the coculture, on the other hand, single transformed cells are surrounded by a vast number of non-transformed counterparts and thus their growth and phenotypic expression may be suppressed.

Regulation of oncogene expression by GJIC

In considering molecular mechanisms by which IC can modulate cell transformation, it is reasonable to suspect the involvement of oncogene expression. There are indeed several lines of evidence which suggest that cell–cell interaction, including GJIC, can influence the expression of oncogenes. For example, there are several studies which show that v-*myc* or N-*myc* transfection of mouse fibroblasts produce morphologically transformed foci only when normal cells are eliminated by a selective marker such as neomycin or by plating transfected cells at sparse cell density so that no contact with surrounding normal cells occurs (66,67).

A close correlation between GJIC and expression of oncogenes was reported using NIH3T3 cells which were infected or transfected with different oncogenes (68). For example, when NIH3T3 cells containing v-*myc* or v-*fos* genes were cocultured with normal cells, they communicated heterologously and did not form distinct foci on the monolayer of the normal cells. When a GJIC blocker, namely a phorbol ester, was added to the coculture, transformed foci appeared (68). On the other hand, *ras*- or *src*-containing cells did form distinct foci over a monolayer of normal cells and did not show heterologous communication with surrounding normal cells. These results clearly suggest that GJIC between normal cells and oncogene-containing cells can influence oncogene-mediated expression of transformed phenotypes.

Conversely, there are several reports that certain oncogenes can modulate GJIC in various cell types. A summary of this type of work is presented in Table I. Among various oncogenes, the effect of the *src* gene was most extensively studied on GJIC (68–72). All studies except one (68) reported reduced GJIC communication in cells with v-*src* or overexpressed c-*src* gene. The study of Bignami *et al.* (68) did not find consistent reduction of GJIC in v-*src*-transfected NIH3T3 cells but, as described above, showed the lack of heterologous GJIC with cocultured non-transfected NIH3T3 cells. The reason for this apparent discrepancy is not known, but it may be related to the method employed by GJIC determination; Bignami *et al.* (68) employed microinjection-dye transfer assay and the steady-state level of dye transfer/microinjected cells was measured. Such a measurement could be more insensitive than that of Azarnia *et al.* (70,72) who measured the dye transfer to only first-order neighbouring cells, which may reflect 'initial velocity' rather than 'steady-state' GJIC. While there is an indication that many other oncogenes can decrease GJIC of host cells, it is not always reproducibly confirmed. This is an important research area since such work will reveal molecular mechanisms by which a signal of cell–cell interaction (membrane phenomenon) can be transmitted to the nucleus to control the expression of genes which are critically involved in carcinogenesis. I would again emphasize the need to study heterologous GJIC here, since in the process of carcinogenesis, it is likely that interaction of an activated oncogene-containing cell with surrounding normal cells plays a critical role.

Discussion of future directions

Available data do suggest that GJIC plays an important role in carcinogenesis. There are also several lines of evidence indicating

Table I. Effect of oncogenes on homologous and heterologous gap junctional intercellular communication

Oncogene	Cells	Homologous communication[a]	Heterologous communication[b]	References
v-*src*	NRK	↓	NT	69
	NIH3T3	↓	NT	71
	Quail and chick embryo fibroblasts	↓	NT	70
	NIH3T3	–	–	68
c-*src*	NIH3T3	↓	NT	72
v-*ras*	NIH3T3	–	–	68
EJ-*ras*^{H}	BALB/c3T3	–	–	47
	Rat liver epith. cell line IAR20	–	+	81
	Rat liver epith. cell line	↓	NT	82
v-*myc*	NIH3T3	–	+	68
v-*fos*	NIH3T3	–	+	*
v-*mos*	C3H10T1/2	– or ↓	NT	83
PyMT	Rat F cells	↓	NT	84
	NIH3T3	–	–	*
PyLt	NIH3T3	–	+	*
SV40T	Human hepatocytes	↓	NT	*
	Human keratinocytes	↓	NT	*
	Human fibroblasts	↓	NT	85

[a]Homologous communication is the communication among oncogene-containing cells and their communication capacity was compared with that of normal counterparts.
[b]Heterologous communication is the presence (+) or absence (–) of communication between oncogene-containing cells and normal cells measured in coculture of these two types of cells.
↓, decreased; –, no change; ↑, enhanced; NT, not tested.
*Unpublished results.

that restoration of GJIC plays an important role in tumour suppression. However, it is important to emphasize that all such evidence is only circumstantial, and does not prove a causal relationship between GJIC alterations and carcinogenesis. One of the most important reasons why we know so little about a possible involvement of GJIC in carcinogenesis is due to the fact that there is not enough information about the kinds of factors traversing GJs. If we know candidate molecules which are going through GJs and which are important for the maintenance of cell growth and differentiation as well as tissue homeostasis, this opens approaches whereby we can test the hypothesis whether such molecules passaged from one cell to another through GJs are indeed important for carcinogenesis. Since the discovery of GJs, this problem has been raised and asked repeatedly. However, we all appreciate that there is an intrinsic difficulty in approaching this problem. Probably, the amount of such factors which goes through GJs is miniscule since candidate molecules are mostly signal transducing factors or second messengers. In fact, molecules which were reported to go through GJs include calcium, cAMP and inositol triphosphate, which play an important role in two major signal transduction pathways, i.e. cAMP-dependent kinase and protein kinase C-dependent pathways (9,10). While the approach is difficult, it is imperative for us to identify and know more about molecules going through GJs.

A possible new approach to discern the role of GJIC in carcinogenesis more directly is the use of molecular probes which have recently become available. For example, one can test whether the hypothesis that the restoration of GJIC in cancer cells can restore the normal phenotype by introducing connexin gene expression vectors. In fact, there is a preliminary result which suggests that introduction of connexin expression vectors can restore GJIC of otherwise communication deficient cancer cells (73). Along a similar line, improved knowledge of how GJIC is regulated is also essential to advance our hypothesis on the role of GJIC in carcinogenesis. In particular, the selective lack of GJIC between transformed and surrounding non-transformed BALB/c3T3 cells clearly indicates that two types of cells which are both communication-competent, may not necessarily communicate with each other even when expressing the same connexin. These results indicate control mechanisms of GJIC which are specific for heterologous cell types. Supporting this, recent studies indeed suggest that cell adhesion molecules are directly regulating the function of GJs; transfection of an expression vector for E-cadherin, a calcium-dependent cell adhesion molecule, greatly enhanced calcium-dependent GJIC in poorly communicating cells (74). Thus, the defect of GJIC in these cells is not due to the absence of GJ gene expression, but rather to the absence of cell adhesion molecule which controls the function of GJIC.

Most of the information related to GJIC and carcinogenesis has come from cell culture work. Although *in vitro* model systems are useful, it is always important to accumulate evidence from *in vivo* work and to verify whether hypotheses generated from *in vitro* systems are applicable *in vivo*. It is also important to reiterate that IC is a study of cell society; the use of *in vitro* models for this purpose is limited since interaction of different cell types can only be properly studied *in vivo*. At present, gene expression and distribution of proteins of GJs can be readily studied in specimens directly taken from *in vivo* materials. However, it is still difficult to examine the functions of GJs in tissues *in vivo*, although there are certain approaches which appear to be successful. We need further effort in this area of research.

While the study on the relationship between GJIC and carcinogenesis is mostly being studied within the context of

epigenetic (non-genotoxic) carcinogenesis, it is also possible that GJ genes themselves are the targets of carcinogens. For example, perhaps mutation of connexin genes (structure or regulatory sequences) can modulate subsequently the function of GJs. Another possible genetic change is GJ gene loss, as occurs in cases of certain tumour suppressor genes, such as retinoblastoma (75) and P53 (76). It is possible that connexin genes are lost and/or mutated in both alleles; therefore, gap junctional communication is lost. The idea is consistent with the hypothesis that GJIC works as a tumour suppressor element. With available molecular probes it should be possible to localize connexin genes on chromosomes and to see whether the loss or mutation of such genes in the chromosomes coincide with certain tumours in terms of their deletion sites and/or fragile sites.

When this manuscript was almost completed, a paper which strongly supports the idea described above appeared. Allelic deletions involving chromosome 18q were known to occur in >70% of human colorectal cancers (77). The gene which resides in this region has been cloned and the predicted amino acid sequence of the cDNA showed a sequence similarity to neural cell adhesion molecules (78). Since cell adhesion molecules are apparently involved in the direct regulation of GJIC (19,74), the loss of genes on chromosome 18q may lead to decreased GJIC.

While such information on the relationship between GJIC and carcinogenesis is yet to come, available data already suggest that the idea of the critical role of GJIC in carcinogenesis may be developed for practical purposes. For example, if many tumour promoting agents can block GJIC, this is one way to detect tumour promoting activity of environmental chemicals (79, 80). Considering the total absence of tests whch can detect non-genotoxic carcinogens, this endpoint should be validated internationally. Another possible application is to develop new chemotherapeutic methods. While most available chemotherapeutic methods are being developed towards killing tumour cells, if we induce IC links between normal and tumour cells, it should be possible to recruit tumour cells into normal IC cell society. By this way, tumour cells may coexist with normal cells, thus providing a milder therapeutic method (25). Alternatively, using our finding that certain transformed cells communicate among themselves while they do not communicate with normal cells, we have introduced a GJ-diffusible cytotoxic agent into transformed cells which were then selectively killed (49). This kind of approach may be used in future for *in vivo* purposes. The concept of cell society regulation by GJIC described here can also be applied to cancer chemoprevention and intervention; endogenous and exogenous factors which modulate GJIC may be efficiently used to prevent potential cancer cells deviating from normal cell society and homeostasis.

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