# Cell adhesion receptors and cancer

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**Abstract** Cell-to-cell and cell-to-extracellular matrix (ECM) interactions in the functions of cell adhesion and signal transduction are important in global control of cell phenotypes and cell behavior and are crucial for maintenance of homeostasis and structural/functional stabilization of tissues and organs. Cell adhesion receptors are recognized as the molecular basis of cell adhesion. Cadherin and Integrin are widely expressed adhesion receptors in most tissues. They are transmembrane glycoproteins which, through their cytoplasmic domain, bind to many proteins at the inner surface of cell membrane to form molecule-linkage complexes and then connect with the cytoskeleton. Through cell adhesion receptors a network functioning as cell adhesion and signal transduction is organized between tissue cells and cell-ECM. In this regard cell adhesion receptors play an important role in regulation of morphogenesis, cell-cell recognition, cell migration, cell sorting and the determination of cell's fate in development. They mediate cell functions and their fault expression is intimately correlated with development of disorders like cancer. Several isoforms of Integrin were found to have tumor suppressor effect. Some components in the molecule-linkage of focal contact are actin-binding proteins as well as substrates of kinase in the Integrin initiated signal pathway to play a role as signal transducer. Some of these molecules exhibited tumor suppressor effect too. Decreased expression of E-Cadherin has been demonstrated in many epithelium originated carcinomas. Cadherin associated membrane adhesion plaque molecule β-Catenin is also involved in the oncogene Wnt signal pathway. Both E-Cadherin and β-Catenin were proved respectively with tumor suppressor effect against invasiveness and metastasis. That Cadherin is important for the posttranslationally functional expression of Connexin has been supported by evidence from developmental biology and cancer cell differentiation studies to suggest that some sort of interrelation feedback control exists between the two signal pathways.

**Keywords:** **adhesion receptors, integrin, cadherin, protein complex, cell adhesion and signal transduction, cell phenotype and behavior.**

A new era of human proteome research in life science as a milestone of the coming century is emerging.

This new field is based on the knowledge of nucleic acid to answer the functions and changes of proteins in human life. Exploration of life science is in the process of reaching the essence of life and approaching the fundamental basis of diseases[1].

Expanding studies on cell adhesion receptors have formed one of the most active areas in protein molecular biology and cell biology in the past 20 years. This area has been interrelated with the advances in cancer research. Cell adhesion receptors are the major architectural molecules of cell junctions where they bind to the inner membrane cytoplasmic adhesive proteins and then anchor the cytoskeleton system. The molecular architecture of cell junctions and their functions in cell adhesion and signal transduction are representative of the structure/function characters of cellular proteins in *in vivo* conditions. These characters include the formation of multiprotein complex as the structural/functional unit, the dual role of a protein and the multiple pathways in protein regulation. Advances in this area have benefited by the development of new approaches on molecular, cellular and intact individual levels, such as tracing studies by microinjection of fluorochrome labeled proteins, especially the green fluorescent protein (GFP), the fluorescence recovery after photobleach (FRAP) techniques combined with computer analysis, DNA truncation mutation and cell transfection/expression studies of tagged vectors with inserted target gene cDNA, and the dominant-negative inhibition experiments at the *in vivo* cellular and intact individual levels. Application of the new techniques has provided convincing evidence for unveiling the central role of adhesion receptors in cell activities and in development. The achievements in this area facilitate cancer research at many levels and draw promising clues in applied studies against cancer.

## 1 Multiple mechanisms of cell adhesion

Multiple forms of adhesion structures are assembled at the cell-cell and cell-ECM (including basement membranes) contact. They are membranous junctions that connect with intracellular cytoskeletons. Cells are organized into specific tissues through cell junctions which are determinant to the overall integral tissue types and cell behavior such as cell-cell recognition, cell migration, cell sorting and ultimately the cell fate in development. In this regard, cell adhesion systems could be considered as the mechanisms in helping translation of the basic genetic information into 3-D architectural forms of cells in tissue[2].

## 2 Functional unit of cell adhesion

As a specialized contact between cell-cell and cell-ECM, cell adhesions comprise standard multiprotein complexes, including 2 major groups of proteins: the adhesion receptors and receptor associated adhesion plaque proteins.

(ⅰ) Adhesion receptors. These molecules are transmembrane glycoproteins which mediate the specific recognition and binding activities between cell surfaces and cell surface to ligands of ECM. To date 5 superfamilies of adhesion receptors have been demonstrated. They are integrins, cadherins, Immunoglobins, Selectins and Proteoglycans[2,3].

(ⅱ) Adhesion plaque (cytoplasmic plaque) proteins. Cytoplasmic plaque consists of many adhesive or binding proteins at the inner membrane of cell junctions where adhesion receptors are connected. The adhesion plaque binds outward to the intracellular domain of adhesion receptor molecules while inward it anchors the cytoskeletal filaments. Adhesion plaques are multiprotein complexes generally composed of Vinculin, α-Actinin, Zyxin and Tensin. The adhesion plaque at the inner membrane of cell-cell adhesion junctions contains specifically α-Catenin, β-Catenin and Plakoglobin while at the inner membrane of cell-ECM contact, the focal contact, it contains specifically Talin, Paxillin and FAK[4,5].

## 3 Integrin receptor and cell adhesion

Focal contact is the major membrane adhesion structure that is conducted by integrin receptors between cell and ECM or basement membranes. Integrin is a heterodimer of α- and β-subunits. Many different α- and β-subunits have been identified. Differential combination between different α- and β-subunits brings about different integrin heterodimers of which more than 20 isoforms have been demonstrated[6]. Several isoforms of integrin can be expressed on the surface of one cell type. Overlapping of binding affinity between different integrins with the same ligand also can happen. Modulation of molecular interactions

and binding affinity between receptors and ligands regulates cell adhesion, migration, motility and hence cell behavior. Therefore, focal contact should be regarded as the regulatory/coordinate point between cell adhesion and movement. Actively moving cells usually lack focal contacts because their focal contacts are unstable, transient or too small to be observed. Adhesive cells always spread well and form many focal contacts to the substrate. These focal contacts are completely assembled, representative of highly ordered molecular architecture of adhesion protein complex.

( | ) Dynamic regulation of integrin mediated cell adhesion. Currently, it is accepted that regulation mechanisms of integrin mediated cell adhesion could happen at different levels, mainly (a) modulation on binding affinity between receptors and ligands such as in the activation process of platelet aggregation and leukocyte adhesion; (b) assembly state of adhesion protein complexes. These events can be induced by intracellular signals or can result from binding of cells with the extracellular ligands, or both.

(1) Regulation on binding affinity of integrin to ligands. Platelet integrin $\alpha_{IIb}\beta_3$ showed conformational changes and increase of binding affinity to fibrinogen or to von Willebrand factors after platelet activation[6,7]. This mechanism of regulation is important in prevention of blood platelet aggregation under stimulation of unrelated factors. This affinity affective changes of molecular conformation could propagate quite a distance along the molecular domains; for example, after binding of activating antibodies to the rod-like domain of the near membrane region of integrin molecule, signals could be propagated to the extracellular headlike end of the molecule and induces conformational changes and binding affinity of the latter. Accumulating evidence suggests that the cytoplasmic tails of both the platelet integrin $\alpha_{IIb}\beta_3$ and the leukocyte integrin $\beta_2$ indeed can influence the binding affinity and adhesion condition of the whole molecule. Albeit serine/threonine kinase has been suggested to trigger the above activation effect, direct phosphorylation of the C-terminal of the integrin appears to be dispensable for this process. Accordingly, it is assumed that some unknown molecules with binding specificity to the C-terminal of the integrin should be responsible.

Conversion of several different conditions of cells is probably involved in the regulation of integrin binding affinity. For example, leukocytes interacted with the endothelial cells of the blood vessel wall in several different conditions of cell adhesion during homing or in the infiltration at the inflammation region. They form unstable adhesion (tether) with the blood vessel surface in normal circulating conditions and thus display rolling along the blood vessel wall. When subjected to local signal (e.g. cytokines) stimulation, the leukocytes form firm adhesion with the blood vessel wall, then penetrate through the endothelium. It is now known that even a single type of integrin such as $\alpha_4\beta_1$ could complete the whole process, they interacted with the vascular cell adhesion molecule-1 (VCAM-1), an endothelial integrin ligand belonging to the Immunoglobin superfamily, to accomplish the process of tethering and rolling functions[8].

(2) Assembly condition of focal contact protein complex. It is now known that the assembly process of adhesion protein complex at focal contact is under control of the binding affinity between integrin and the ligand and of the intracellular signal events. Integrin molecules bind to ligands first, then form clusters on the membrane (clustering). The synergistic response triggered by ligand-occupancy of integrin molecules and their clustering is an assembly of adhesion plaque protein complexes at the inner surface of the membrane and reorganization of the local cytoskeleton and the activation of the local signal pathways. Another response is activation of focal contact kinase (FAK)[9,10], the unique component of focal contact associated adhesion plaque. FAK is a non-receptor tyrosine kinase. Activated FAK can induce tyrosine phosphorylation of other adhesion plaque components such as Paxillin and Tensin[10,11]. Many reports recently have indicated that GTP-binding protein Rho family is involved in regulation of the formation of focal contact and the interaction between membrane adhesion plaque and actin. Members of Rho family such as Rac, Rho and $cdc^{42}$ have been suggested to act in the same pathway sequentially in adjusting alteration of actin-membrane interactions and adhesion conditions to stimulate the assembly of filopodia, ruffling membranes, stress fibers and associated focal contacts. It has also been suggested that integrin regulates the level of the second messenger phosphatidylinositol 4,5-biphosphate (PIP2) to stimulate the Rho-dependent signal pathways. Interactions between PIP2 and the actin-binding proteins have been demonstrated to play roles in promoting polymerization of F-actins[2,9,12,13].

(ii) Integrin-induced signal transduction through adhesion. The involvement of integrin-mediated adhesion in regulatory signal pathways for cell adhesion and movement, cell growth, apoptosis and expression of special genes is an important field in biology. Integrin-conducted major signal transductions are in 2 lines: (1) The first line is the local control of signal events in the adhesion area per se, where the signals can induce changes of anchorage and assembly of the local cytoskeleton and secretory activities thus to control the local adhesion conditions. The regulation process of platelet aggregation is regarded as a good example because platelet is a cell fragment without nucleus. Accordingly no cell cycle regulation activities exist in platelet. Yet, the major known integrin-associated phosphorylation events including the kinases and substrates and systems for the generation of second messengers all happen and exist in the platelet[2]. All these signaling reactions take part in the regulation of post-aggregation processes of platelet, including platelet spreading (the same as cell spreading), platelet contraction (to induce clot retraction), secretory activities of platelet granule exocytosis. The transduction of these signals is dependent on integrin($\alpha_{IIb}\beta_3$)-mediated adhesion because obviously these signals are needed and produced only after platelet aggregation. (2) The second line of integrin-conducted signal events includes signals for cell growth and differentiation, especially the signals that influence control of cell cycle and gene transcription activities. For example, after adhesion of fibroblast to the Fibronectin (FN) of ECM the intracellular cell-cycle related pathway Ras/MAP kinase (MAPK) is activated. This is the molecular signal basis of anchorage-dependent cell proliferation. Integrin also may activate the MAPK signal pathway through stimulation of FAK at adhesion plaque and its interaction with signal proteins $Grb_2$ and SOS in Ras pathway. This type of adhesion conducted growth signals cooperates with growth factor triggered signal events in harmony in regulation of cell growth. The activation of MAPK pathway may induce phosphorylation and activation of certain transcription factors. Therefore, integrin-induced activation of Ras/MAPK pathway is probably correlated with the control of gene expression by integrin[14].

(iii) Tumor suppressor effect of integrin and prospect in applied studies against cancer. It has been found from early studies that decreased expression of integrin correlates with cell transformation and tumor metastasis. Recently, further evidence showed that the expression level of individual member of integrins differ in the tumor cell, some decreased, some increased or unchanged[15]. However, it has been exciting about the demonstrations that some integrin molecules do exhibit tumor suppressor effect because this will provide incipient clues and promising prospect in applied studies against cancer. It has been demonstrated that the tumor suppressing integrin $\alpha_5\beta_1$ (receptor of FN) induces expression of specific growth-arrest gene gas-1 and inhibit transcription of immediate genes in cell growth[16]. Transformed cells expressed reduced level of $\alpha_5\beta_1$ and the transformed phenotypes were suppressed after restoration of $\alpha_5\beta_1$ by gene transfection. Integrin $\alpha_2\beta_1$ expression displayed inhibitory effect on malignant phenotypes of breast cancer. However, $\alpha_2\beta_1$ showed stimulation effect on metastasis of human rhabdomyosarcoma cells. $\alpha_V\beta_5$ promoted the terminal differentiation of squamous cell carcinoma and inhibited malignant cell growth. Integrin $\alpha_3\beta_1$ is a tumor suppressor gene in human rhabdomyosarcoma. $\alpha_6$ is the tumor suppressor gene of mammary epithelial cells, but its expression level increased in hepatocarcinma cells. $\alpha_V\beta_3$ has been demonstrated to be related with angiogenesis of tumors. Antagonizing to the angiogenetic effect of $\alpha_V\beta_3$ may induce apoptosis of angiogenic blood vessel cells and cause tumor regression[15]. However, the same molecule can cooperate with metal proteinase of ECM to stimulate tumor cell invasiveness and metastasis[17].

(iv) Dual role of focal contact associated adhesion plaque proteins. The sequential assembly of the molecular linkage of adhesion plaque proteins (outside→inside) determines the stability of membrane adhesion plaque structure and the integrin receptor initiated signal transduction. Adhesion plaque proteins such as α-actinin and actin perform dual function as the key molecule of adhesion plaque and the initiator in assembly of actin cytoskeleton. In developing skeletal muscle myotubes, Actin and α-Actinin formed complexes of I-Z-I bodies at the growth tips[18]. They not only are precursors of sarcomeric Z line but also initiate the assembly of nascent sarcomeres at the termini of myofibrils in connection with sarcolema. Another adhesion plaque protein Tensin is an actin-binding protein. Tensin by itself is also the specific phosphorylation substrate of FAK after activation by integrin receptor initiated signals and thus plays a role in signal transduction.

(1) Molecular mutation of adhesion plaque proteins correlates with development of diseases. Taking sarcomeric-α-Actinin (s-α-Actinin) as an example, deletion of amino acid sequence from the C-terminal induced expression of hypertrophied Z line disorder phenotypes in skeletal muscle myotubes (Nemaline myopathy)[19]. Expression of the C-terminal truncated s-α-Actinin in non-muscle cells induced disassembly of stress fibers, disruption of adhesion structures, cells detachment and cell death[20].

(2) Adhesion plaque proteins exhibit tumor suppressor effect. Reduced expression of α-Actinin and Vinculin was found in some transformed cells and cancer cells which exhibited high capacity of metastasis. Other experiments using dominant-negative inhibition against Vinculin expression in 3T3 cells resulted in loss of anchorage-dependent cell growth and induction of malignant transformation. Transfection of Vinculin or α-Actinin cDNA into cancer cells or transformed cells which showed the reduced expression level of the respective protein resulted in reversal of malignant phenotypes and inhibition of *in vivo* tumorigenicity[15]. The mechanisms involved in the above process are unclear; however, tumor suppressing effect of these adhesion plaque proteins and the important roles they play in regulation of cell proliferation/differentiation through conduction of cell adhesion/signal transduction cannot be ignored.

## 4 Cadherin receptor mediated cell-cell adhesion

Cadherin and the assembled adherent junctions (AJ) and desmosome junctions (DJ) exist in most solid tissues. Cell adhesion conducted by these junctions is crucial for maintenance of stability of solid tissues.

(ⅰ) Molecular properties of cadherin. Cadherin adhesion receptor is a transmembrane glycoprotein which is $Ca^{2+}$-dependent and has homophillic character. It interacts and binds with each other between cell surface to cell surface by their extracellular (EC) domains. Similarly, its C-terminal and the intracellular domains bind to adhesion plaque proteins and through them anchor the cytoskeletons. E-cadherin, N-cadherin and P-cadherin are the 3 members that were the earliest-reported and are still categorized as classic cadherins. Consequently, it was found that adhesion receptors in DJ junctions share highly homologous sequences with cadherin, hence they belong to the same superfamily of cadherin. The 3 classic cadherins only comprise a subfamily of cadherin superfamily. Among them E-cadherin has been most studied and best understood. Cadherin-mediated cell-cell adhesion interactions have been reported to play an important role in cell recognition, migration, sorting, in morphogenesis and polarity determination of developing embryos. On the other hand, cadherins are expressed continuously in most mature tissues. E-cadherin is necessary for epithilial tissue differentiation. Cells suppressed in E-cadherin expression cannot keep stable and tight adhesion albeit they still express other kinds of adhesive and junction molecules. Cadherin knock-out experiments in embryonic stem cells have demonstrated that E-cadherin and N-cadherin regulate gene expression, morphogenesis and determine the fate and behavior of embryonic cells[21,22].

(ⅱ) Cadherin receptor-associated adhesion plaques. Cadherins mediate cell-cell adhesion through formation of AJ junctions at the membrane. Inside the AJ membrane, cadherin molecules link the membrane adhesion plaque proteins, Catenins (α-,β-,γ-) with their intracellular domain and then anchor the termini of Actin cytoskeleton. Inside the DJ membrane, the DJ adhesion receptors link the membrane adhesion plaque molecule Plakoglobin then anchor the intermediate filament (IF) cytoskeleton. It is now known that the transmembrane adhesion receptors of DJ, Desmocollin and Desmoglein are homologous molecules of cadherin and belong to the superfamily of cadherin. The DJ-associated inner membrane adhesion plaque protein Plakoglobin is homologous to β-Catenin and belongs to the same superfamily of Catenin. Therefore, this form of adhesion junctions is considered junctions of the cadherin/Catenin type. Without expression of Catenin, cadherin molecule by itself cannot accomplish adhesion and signal transduction functions. Special attention has been drawn to β-Catenin which binds directly to cadherin and α-Catenin thus as an intermediate linker between the 2 molecules. Furthermore, β-Catenin plays a role as a key molecule in the whole molecular linkage of cadherin/Catenin type junctions[4]. β-Catenin is also regulated by signals from eqidermal growth factor receptors (EGFR). β-Catenin interacts with tumor suppressor gene Adenomatous Polyposis Coli (APC) product and with tight junction cytoplasmic plaque protein ZO-1 too. β-Catenin is such a molecule as connects with multiple signal pathways. Reports from experimental embryonic studies demonstrated that β-Catenin is highly homologous to the Drosophila segment

polarity gene Armadillo and thus indicated that β-Catenin is an evolutionally conserved important molecule in regulation of cell growth and differentiation. In fact, it has been shown that β-Catenin is a component in the Wingless/Wnt signal pathway. Wingless/Wnt belongs to Wnt oncogene superfamily and plays a role in control of development and cell transformation. The above information suggests strongly that cadherin/Catenin/cytoskeleton system-conducted dual function of cell adhesion and signal transduction is important for control of cell growth, cell differentiation, cell movement and apoptosis[23—25].

However, the above evidence was mainly focused on E-cadherin and basically came from the *in vitro* cell free systems or from developmental studies of the lower vertebrates like amphibians. Less is known about the working model of cadherin-conducted adhesion and signal transduction in animals of higher class and human beings. Likewise, less is known about the roles of cadherins in addition to E-cadherin. We have recently studied expression of cadherins in human normal lung cells and lung carcinoma PG cells in culture. Normal lung cells expressed N-cadherin. Binding of N-cadherin at the AJ with membrane adhesion plaque proteins α-, β-Catenins and the anchorage of the termini of Actin stress fibers to AJ adhesion plaques was demonstrated by co-localization of these molecules at AJ in immunofluorescent double-stainings. These cultured normal lung cells exhibited anchorage-dependent growth control. They did not form foci in soft agar. In contrast, in highly metastatic human lung carcinoma PG cells, N-cadherin expression level was obviously lower than in normal. The distribution of N-cadherin immunofluorescence was not observed on the membrane. The Actin cytoskeleton was disassembled and cell adhesion structure was poorly organized in PG cells. These results indicated that cadherin/Catenin/Actin protein complex which is important in control of cell growth and differentiation of normal lung cells[26] was disordered in lung carcinoma PG cells.

( ||| ) Cadherin/Catenin and cancer.

(1) Reduced level of E-cadherin expression in cancer cells. Reduction of E-cadherin expression in epithelium-originated cancers has been reported including cancers of head, neck, esophagus, skin, thyroid, lung, mammary gland, stomach, liver, kidney, pancreas, colon, bladder, prostate and female genital tract. Reduction of E-cadherin expression is possibly correlated with the malignant grade of cancer cells and lower survival rates of cancer patients. Transfection of E-cadherin cDNA into cancer cells could increase the expression level of E-cadherin, inhibit invasiveness and metastasis of cancer cells; even reduce protease secretion by some cancer cells[15].

(2) Mutation of E-cadherin correlates with carcinogenesis. Recent reports demonstrated that there is point mutation at the $Ca^{2+}$ binding site of E-cadherin in diffuse-type gastric carcinoma. Deleted mutation at the EC domain of E-cadherin was demonstrated in infiltrate lobular breast carcinoma. Hypermethylation at the promoter region of E-cadherin gene was responsible for reduction of its expression level in cancer cells. Inhibition of E-cadherin expression was also found in Erb-$B_2$ receptor cDNA transfected mammary carcinoma cells. Erb-$B_2$ receptor was assumed to be related with malignant metastasis capacity of cancer cells[15,27,28].

(3) The anti-invasiveness and anti-metastasis tumor suppressor effect of E-cadherin and Catenin. Decreased expression of E-cadherin was demonstrated in highly metastasic cancer cells while after restoration of E-cadherin expression by gene transfection, these cancer cells were inhibited in the capacity of invasiveness and metastasis. Normal kidney epithelial cell MDCK line expressed high level of E-cadherin and displayed tight cell-cell adhesion. When cells were treated by inhibitory antibody, E-cadherin was inhibited, and cells became metastatic. Some cancer cells showed the normal level of E-cadherin but inhibited level of β-Catenin. In prostate cancer cells α-Catenin was inhibited. After recovery of expression level of α-Catenin through transfection, cell adhesion function was improved and their *in vivo* tumorigenicity was inhibited[15].

## 5 Adherens junction and cell-cell communication

( | ) Expression of gap junction gene connexin and gap junctional intercellular communication (GJIC). Connexin gene encoded protein of gap junction is a transmembrane protein. 6 subunits of connexin assemble into a membrane channel of gap junction (GJ). Connection of GJ channels at the juxtaposed membrane of the neighboring cells forms intercellular communication channels which allow transfer

of small molecules with MW< 1000 u. Cytoplasmic components such as $Ca^{2+}$, cAMP, IP3, amino acids and nucleotides can pass through GJ channels. GJ is the only membrane channel structure known to allow direct exchange of cytoplasmic components between cells; therefore it plays an important role in metabolic cooperation and direct intercellular signaling between tissue cells in the maintenance of homeostasis in organisms. Expression of GJ structures and GJIC function in early development and their temporal/spatial difference in development strongly suggest that GJIC is important in regulation of cell growth and differentiation. GJIC is also demonstrated in most mature tissue cells. It is involved in control of cell secretion, entraining synchronous activity between excitable cells such as GJIC in chemical-synapse and cardiac intercalate discs, nerve/muscle junctions. GJIC is often reduced or even deficient in actively proliferating cells of regenerating tissue and in uncontrolled growing tumor cells. These cells even lost GJ structures or/and their connexin gene was transcriptionally inhibited. Increasing evidence suggests that changes of expression of connexin gene and the GJIC function correlate with development of diseases and tumors. Therefore, this field of basic and applied research has drawn growing attention in the recent 10 years[29,30].

(ii) Connexin (Cx) family and cellular internet. The existence of the Connexin family has been demonstrated in the early 1990s; however, what a specific role each Cx member plays in the development process, in GJIC function and in cell activities is still not clear. More than 12 Cx members were demonstrated in mammalians. The expression of these Cx members is tissue specific and overlapping between tissues as well. Each cell type expresses at least more than 2 Cxs. Recently, it has been reported that the selectivity of molecular charges (+, −), molecular size and gating directions (uni- and bidirections) is characteristic of different Cx protein assembled GJ channels. Thus, different organization of each Cx members in Gj is assumed to provide special signals and "dialect" in cellular internet. The above advances have profound influence on the understanding of GJIC function and promote further progress in this field. Gating of GJ is not overall bidirection, uniform in molecular charge and molecular size adaption but displays selectivity of molecular charge, size and transport directions. This may explain why a Cx protein expressed in normal cells was lost in tumor and why some Cx protein not expressed in normal cells unexpectedly appeared in tumors[29—31].

(iii) Interrelation and cooperation of cell adhesion with GJIC. It has been assumed from some research lines that cell-cell recognition and adhesion is needed for establishment of GJIC and the cadherin-mediated cell adhesion is involved. In Novikoff cells, when the extracellular domain of N-cadherin was inhibited by $F_{ab}$ fragments of antibodies, these cells' GJ disappeared from the membrane and GJIC was inhibited. Phosphorylation form of Connexin43 (Cx43) protein (Cx43-$P_2$) is necessary for cytoplasmic transportation of this protein after translation to form GJs on the membrane; the level of Cx43-$P_2$ is suggested to be controlled by expression of cadherin (L-CAM). Dominant-negative inhibition experiment data from another group demonstrated that a similar type of developmental defects was induced by inhibition either on cadherin expression or on expression of Connexin. It is thus indicated that a certain kind of feedback regulation mechanisms for *in vivo* homeostasis exists between signal pathways conducted by these 2 molecules[29,30]. Less is known about this function in mammalian cells.

We have reported recently that human normal lung cells communicated efficiently, formed well-defined cell-cell adhesions and expressed high level of Cx43 and N-cadherin proteins respectively. On the contrary, the highly metastatic human lung carcinoma PG cells were defective of GJIC; neither was Cx43 expressed. Level of N-cadherin protein in PG was obviously lower than in normal. PG cells contacted loosely. In the Cx43 positively transfected PG subclones, one clone that expressed high level of both Cx43 and N-cadherin proteins showed suppressed cell malignancy and reduced tumorigenicity in nude mice. This subclone exhibited strong cell-cell adhesion. Their Cx43 protein immunofluorescence was distributed on the membrane GJ sites[31]. It appeared that N-cadherin protein expression is necessary for cytoplasmic transportation of Cx43 protein to the membrane and then the assembly of membrane GJ structures in human lung cells.

## 6 Prospect in applied studies against cancer

All malignant tumors share the common property of uncontrolled growth, invasiveness and metastasis which are dead threatening to life. Prosperous achievements have been conducted from tumor suppressor

studies. These results were contributive to investigation of mechanisms of cancer development and have provided molecular markers for diagnoses and prognoses of cancer, some markers have been in the process of clinic trials. These findings improved the ratio of early diagnoses of malignant tumors, hence they provided more opportunities for radical cure. In addition, these findings provided clues for therapeutic studies against cancer. Studies on adhesion receptors have shown broad prospect for applied research against cancer. One can expect that the more we know about architecture/function of the cadherin/Catenin/Actin protein complexes, the more we could see their central roles in converging of the intra-, extracellular signals and how they regulate cell activities through internet of various signal pathways. Combination of molecular cellular and intact individual studies will throw new light on this field.

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