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Implication of the tetraspanin CD9 in the immune system and cancer

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IMPLICACIÓN DE LA TETRASPANINA CD9 EN EL SISTEMA INMUNE Y EN EL CÁNCER

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RESUMEN

Las tetraspaninas son moléculas de la superficie celular de amplia distribución en los organismos eucarióticos. Poseen como característica estructural peculiar cuatro dominios transmembranales, regiones N- y C-terminales intracitoplásmicas, y dos lazos extracelulares de distinto tamaño. También poseen un motivo de secuencia CCG en el lazo extracelular mayor, así como residuos polares conservados en los dominios transmembranales. Las células sanguíneas de los mamíferos expresan combinaciones peculiares de distintas tetraspaninas, incluyendo los antígenos de diferenciación CD9, CD37, CD53, CD81/TAPA-1, CD82, CD151/PETA-3 y CD231/TALLA1.

En este trabajo se resumen la estructura y las interacciones de sus regiones citoplásmicas con proteínas del citoesqueleto y señalizadoras, como la proteína cinasa C (PKC) o la Fosfatidil-Inositol 4-cinasa (PI4-K). Sus interacciones específicas con otras tetraspaninas, con integrinas, antígenos de histocompatibilidad, y miembros de la superfamilia de las inmunoglobulinas son también revisadas.

Las tetraspaninas son proteínas "adaptadoras" o "facilitadoras". Al formar parte de complejos moleculares, modulan funciones celulares clave que incluyen la fusión celular, la adhesión, la migración, la diferenciación y la transducción de señales. Las tetraspaninas se organizan en una red con distintos niveles de asociación, determinados por su resistencia a la solubilización por detergentes. En concreto, se analizan las tetraspaninas como reguladoras del Sistema Inmunitario gracias a sus interacciones con los receptores de antígeno de los linfocitos T y B, las moléculas de histocompatibilidad de clase I y clase II, y los co-receptores CD2, CD4, CD5, CD8 y CD19. Por último, se revisa detalladamente el papel de la tetraspanina CD9 en la función de las células linfoides y mieloides, su relevancia en infecciones como el HIV, y la importancia de su asociación con integrinas en la progresión cancerosa.

PALABRAS CLAVE: Tetraspaninas/ Integrinas/ HIV/ Cáncer/ CD9/ CD37/ CD53/ CD81/ TAPA-1/ CD82/ CD151/PETA-3/ CD231/TALLA1.

ABSTRACT

Tetraspanins are cell surface proteins widely distributed in eukaryotic organisms. They characteristically span four times the plasma membrane, have intracellular N and C terminal regions, and two extracellular loops of unequal size. Tetraspanins also possess a CCG motif in the large extracellular loop, and conserved polar residues in the transmembrane domains. Mammalian blood cells express different sets of tetraspanins including the differentiation antigens CD9, CD37, CD53, CD81/TAPA-1, CD82, CD151/PETA-3 and CD231/TALLA1.

Here, tetraspanin structure and their cytoplasmic tail interactions with cytoskeletal and signalling proteins like Protein kinase C (PKC) or Phosphatidyl Inositol 4-kinase (PI4-K) are briefly summarized. The specific interactions with other cell surface proteins, forming complexes with other tetraspanins and members of the integrin family, MHC histocompatibility antigens, or members of the immunoglobulin superfamily are also reviewed.

Tetraspanins are considered as "adapter" or "facilitating" proteins and, through their participation in complexes, they modulate key cellular functions like cell fusion, adhesion, migration, differentiation and signal transduction. The organization of the tetraspanin web, based on different association levels determined by their resistance to detergent solubilization, is described. In particular, tetraspanins participating in the regulation of the Immune System through interactions with the B- and T-cell receptors, the class I and class II MHC antigens, and co-receptors such as CD2, CD4, CD5, CD8, or CD19 are analyzed. At last, the role of CD9 in myeloid and lymphoid cell function, its relevance to HIV infection, and the importance of tetraspanin association with integrins to cancer progression are described in detail.

KEY WORDS: Tetraspanins/ Integrin/ HIV/ Cancer/ CD9/ CD37/ CD53/ CD81/TAPA-1/ CD82/ CD151/PETA-3/ CD231/TALLA1.

INTRODUCTION

Tetraspanins are a family of cell surface proteins that span four times the plasma membrane, and whose N and C terminal regions are both intracellular, delimiting two extracellular domains of unequal size termed SEL (Short Extracellular Loop) or EC1 (Extracellular Domain 1) and LEL (Large Extracellular Loop) or EC2 (Extracellular Domain 2). There are many types of proteins that contain four transmembrane domains, but in order to belong to the tetraspanin family, they must fulfil several additional structural requirements. These are the presence of 4-6 conserved cysteines including the CCG (Cysteine-Cysteine-Glycine) motif in the LEL domain, that allow for the formation of two, and in some cases three, intramolecular disulfide bonds in this domain[1], as well as several conserved polar residues in the transmembrane domains. This protein superfamily is widely distributed in eukaryotic organisms, and members of this family have been found in fungi, worms, insects and mammals. Mammalian tetraspanins comprise 32 members that include the differentiation antigens CD9, CD37, CD53, CD81/TAPA-1, CD82, CD151/PETA-3 and CD231/TALLA1 that are expressed by different lineages of blood cells[1-4]. All nucleated cells express on their surface a repertoire of different tetraspanins.

STRUCTURAL ASPECTS OF TETRASPANINS

The generic topology of a tetraspanin molecule has been established by different types of studies and is illustrated in Figure 1. From a structural point of view, the two (or three) disulfide bonds within the LEL subdivide this domain into two regions, a constant and a variable region. The constant region contains three differentiated segments of α-helix (termed A, B and E helices) that constitute a potential dimerization surface present in all tetraspanins[5,6]. The variable region contains all the sites so far identified that are involved in lateral interactions among different tetraspanin molecules as well as between tetraspanins and other membrane proteins.

The first, third and fourth transmembrane domains of tetraspanins contain conserved polar residues that can form strong hydrogen bonds among them. These domains stabilize each tetraspanin molecule during its biosynthesis and in addition favour the associations among tetraspanin molecules and with other proteins; these associations are crucial for the assembling and maintenance of the tetraspanin web.

The cysteine residues in the cytoplasmic domains represent palmitoylation sites, which contribute to the interactions tetraspanin-tetraspanin[7,8]. The N and C-terminal cytoplasmic tails contain potential binding sites for cytoskeletal or signalling proteins, like Protein kinase C (PKC)[9] or Phosphatidyl Inositol 4-kinase (PI4-K)[10-12].

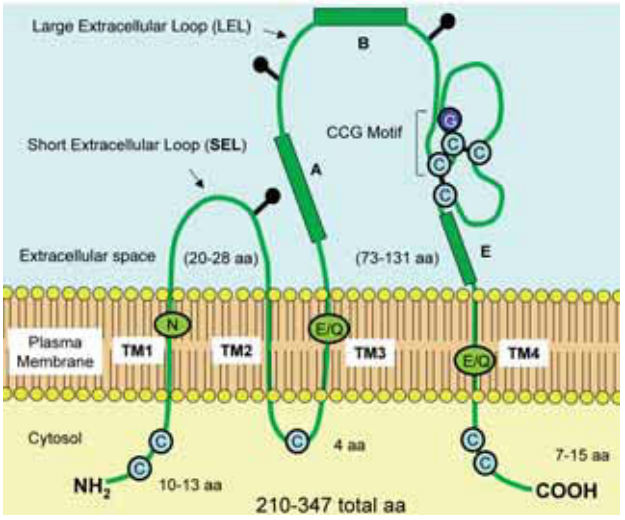

Figure 1. *Schematic general structure of tetraspanins. Tetraspanins present four transmembrane domains (TM) that contain conserved polar residues (green ovals) and delimit two extracellular loops of unequal size (Short Extracellular Loop SEL, and Large Extracellular Loop LEL, respectively). Both loops can contain one or several glycosylation sites (black rods), depending on the particular tetraspanin considered. The conserved cysteine residues are depicted by blue circles. Because of the formation of disulfide bonds (black lines) between the cysteines of the CCG motif and other conserved cysteines of the LEL, this domain folds and adopts a "mushroom" shape. The number of disulfide bonds ranges between 2 to 5 among the different tetraspanins. Several conserved cysteines are also present in the intracellular loop as well as in the N- and C-termini, which represent potential palmitoylation sites.*

DIFFERENT TYPES OF TETRASPANIN INTERACTIONS

One fundamental feature of tetraspanins is their high ability to establish specific interactions with other cell surface proteins, specially with other tetraspanins and with members of the integrin family, MHC histocompatibility antigens, and some members of the immunoglobulin superfamily[1,13,14]. Tetraspanins are accordingly considered as "adapter" or "facilitating" proteins characterized by their ability to organize networks of interactions among different cell surface proteins. These multiprotein complexes constitute authentic tetraspanin-enriched membrane microdomains, which have been termed "tetraspanin web". Through their participation in these complexes, tetraspanins have been implicated in key cellular functions like cell fusion, adhesion, migration, differentiation and signal transduction[1,2,4,15]. The organization of the tetraspanin web is based on different association levels (as illustrated in Figure 2). The first level comprises the primary or direct protein associations which are resistant to stringent detergents like Digitonin or Triton-X-100 and display high stoichiometry. Within this first level are included the interactions between a particular tetraspanin and one or few specific proteins, known as its "partners". A second level of organization includes the more abundant and indirect interactions, which

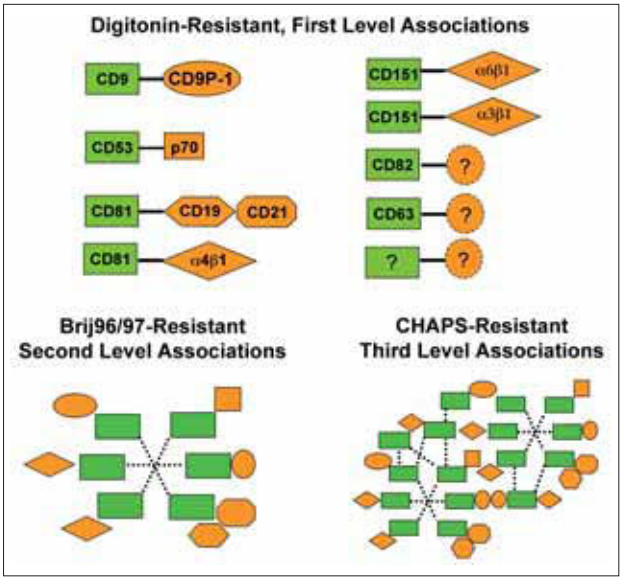

Figure 2. *Different levels of associations within the tetraspanin protein complexes. The first-level complexes (connected through solid lines) are resistant to solubilization in the stringent detergent Digitonin and contain a tetraspanin molecule (green rectangles) associated to its molecular partner (orange shapes). Among the tetraspanins partners, integrins (diamonds), the CD19 (hexagons) and CD21 (octagons) B cell antigens, the CD9-partner 1 (CD9P, ovals), the HLA-DR (triangles) and other yet unidentified molecules (circles) are represented. The second-level complexes (connected through broken lines) resist solubilization in milder detergents like Brij96/97 and include the tetraspanin-tetraspanin associations of first-level complexes. The third-level complexes are resistant to very weak detergents like CHAPS and comprise associations of several second-level complexes.*

are resistant to milder detergents like Brij 96/97. Within this second level are included the associations among different tetraspanins that bring together several primary complexes. The third level of organization of the tetraspanin web comprises large and weak complexes, resistant only to very mild detergents like Brij 99 or CHAPS, which display in some cases properties similar to those of lipid rafts.

TETRASPANINS AND LIPID RAFTS

A key question is whether the tetraspanin-enriched membrane microdomains are coincident or related with other types of membrane microdomains, like the lipid rafts, that provide sites for localization of receptors and molecules implicated in cell signalling. The fact that lipid rafts are highly dynamic structures and very heterogeneous with regard to their composition and properties complicates the answer about the coincidence of these structures and tetraspanin microdomains. Although some of the properties of lipid rafts, as their enrichment in cholesterol and glycosphingolipids, are shared with the tetraspanin-based domains, most studies indicate that both types of microdomains are distinct entities. While lipid rafts are defined as detergent-resistant microdomains enriched in cholesterol and glycosphingolipids that contain abundant GPI-anchored proteins, the tetraspanins can be found localizing both in the resistant and in the soluble fractions and, besides, no specific associations between tetraspanins and GPI-anchored proteins have been described[16].

TETRASPANINS IN THE IMMUNE SYSTEM

The tetraspanins interact with many proteins that play critical roles in the immune system, like the B cell receptor (BCR) and the T cell receptor (TCR), class I and class II MHC antigens, and co-receptors such as CD2, CD4, CD5, CD8 and CD19. Diverse studies have demonstrated the important role of tetraspanins in the lymphoid and myeloid cell function. Importantly, knock-out (KO) mice for CD37, CD81 and CD151 show major alterations in their immune system. CD81 KO mice have a lower expression of CD19 antigen on their B cells and accordingly show a lower Ca^{2+} mobilization following stimulation of CD19, a reduction of B1 lymphocytes in the peritoneum, and reduced production of IgG1 in a Th2 immune response[17-19]. CD37 KO mice display deficiencies in the T cell-dependent B cellular response[20]. The CD151 KO mice show, in addition to defects in the homeostasis and in αIIbβ3 integrin signalling[21], a hyper-proliferation of T lymphocytes in response to *in vitro* stimulation with mitogenic agents.

The tetraspanin CD9 is a 21-24 kDa protein that is abundantly expressed on the surface of endothelial cell, on some leukocytes, and on many types of tumoral cells[22,23]. This molecule was initially identified as a lymphohematopoietic marker[24] and received the name of "Motility-Regulatory Protein" (MRP), since monoclonal antibodies specific for this molecule inhibited the motility and migration of tumoral cells[25]. This molecule has been also implicated in the formation and maintenance of muscular myotubes[26,27], in nervous cells neurite outgrowth[27] and in the fusion between egg and sperm[28]. Functional interactions between CD9 and several members of the β1 and β3 integrin subfamilies has been described in many cell types; monoclonal antibodies specific against CD9 affect and exert regulatory roles on the adhesion, migration and signalling that are mediated by the associated integrins[29].

Recently, an important role has been reported for endothelial CD9 (as well as for CD151) in the regulation of the adhesive function of ICAM-1 (an important ligand for leukocyte integrin LFA-1) and VCAM-1 (a ligand for integrin α4β1)[30]. In this study, it is shown that both endothelial ICAM-1 and VCAM-1 localize to tetraspanin domains

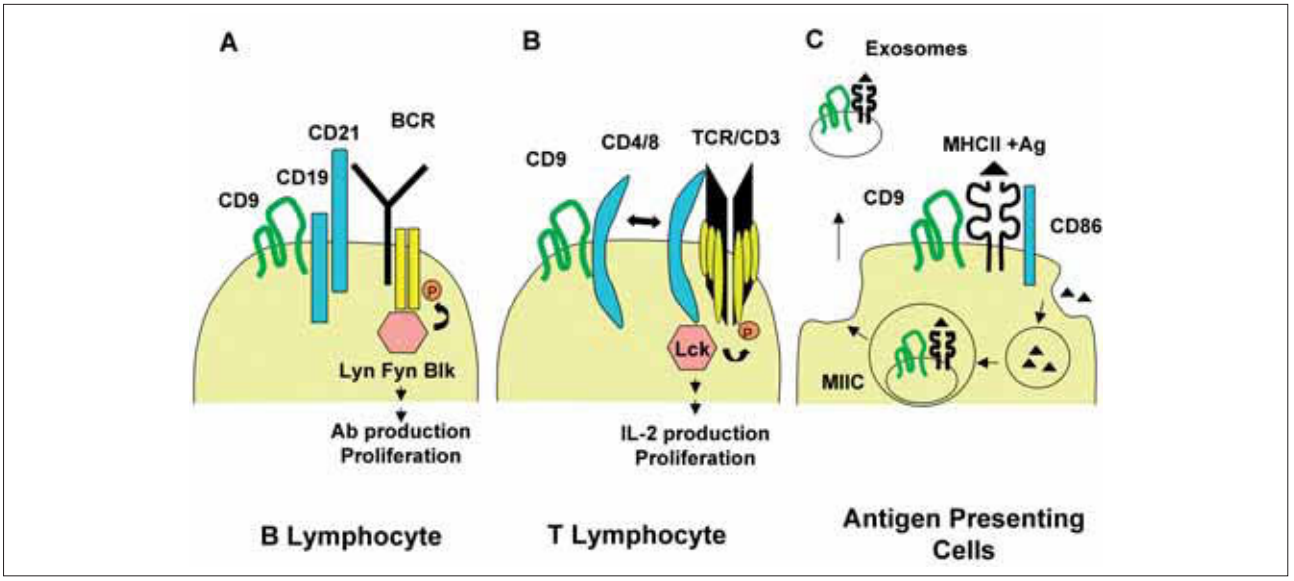

Figure 3. *Scheme of CD9 functions in B and T lymphocytes and in Antigen Presenting Cells. A) CD9 forms part of the CD19/CD21 complex in B cells, which facilitates the signalling following antigen binding to the BCR. B) CD9 sequesters CD4 and CD8, inhibiting the signalling from the TCR in T cells. The co-stimulatory molecules CD4 and CD8 are associated to the kinase Lck, which phosphorylates CD3, inititating a signalling cascade leading to activation, IL-2 release and proliferation of T cells. C) CD9 associates to MHC Class II and co-stimulatory molecules like CD86 on the plasma membrane of APCs and in the intracellular vesicles enriched in MHC class II that are later released (exosomes) from these cells. Antigens are endocytosed and processed before being loaded onto MHC class II in the intracellular vesicles that are also enriched in tetraspanins. The fusion of these vesicles with the plasma membrane results in location of MHC class II molecules loaded with antigenic peptides on the cell surface and in the release of exosomes, which are able to stimulate T cell activation and proliferation. CD9 (and other tetraspanins) favours the clustering of peptide-loaded MHC class II molecules with co-stimulatory molecules (CD86), facilitating antigen presentation and T cell activation.*

associated with CD9 and CD151, and that these associations are relevant in the adhesive function of these molecules and in the regulation of leukocyte transendothelial migration.

CD9 IN LYMPHOID CELLS

CD9 does not seem to be necessary for the normal development of B lymphocytes, since the CD9-KO mice have normal numbers of B cells. However, CD9 plays a role in the function of these cells as it potentiates their β1 integrin-dependent migration, and this is accompanied by increased tyrosine phosphorylation levels[31]. In addition, CD9 participates in the signalling triggered from the BCR, as this tetraspanin (as well as CD53 and CD82) becomes associated with the CD19/CD21 complex[32] which enhances the signals from the BCR and thus lowers the threshold for B cell activation (Fig. 3). Although the precise role of CD9 in the CD19/CD21 complex signalling has not been clarified, it has been ruled out that CD9 is necessary for stabilization of CD19, as CD9-deficient B cells express normal levels of CD19[33]. This association has been studied in more detail for CD81, that is directly associated with CD19 and whose presence is crucial for proper expression of CD19 and hence, for correct signalling from the BCR[18]. Tetraspanins therefore exert a facilitating role on the signals originated from the BCR, as they contribute to maintain the CD19/CD21 co-stimulatory complex.

Besides its implication in the BCR signalling in B lymphocytes, CD9 (among other tetraspanins like CD37, CD53, CD81, CD82 and CD151) also modulates the TCR signalling in T cells (Fig. 3). In this regard, the co-localization of CD9 with signalling components of the TCR complex has been reported[34]. CD9 has been found associated with CD3, CD4 and CD5[35] and co-engagement of CD3 and CD9 induces a potent stimulatory signal that is independent of CD28 and does not result in IL-2 production[36-38]. Translocation of CD9 and other co-stimulatory tetraspanins to lipid rafts in activated T cells has been also documented in these studies. On the other hand, besides its co-stimulatory role as a molecule associated to CD3, CD9 also modulates the signalling mediated by CD4. It has been postulated that the mechanism by which tetraspanins modulate CD4 signalling involves sequestration of both CD4 and CD8. These two co-stimulatory proteins are associated to Lck and therefore their tetraspanin-mediated sequestration would keep Lck away from the TCR, resulting in inhibition of signalling and IL-2 production and T cell proliferation[39-41].

Associations of integrins with tetraspanins are functionally relevant in B and T cell intracellular signalling. It has been reported that tetraspanins CD9, CD63, CD81, CD151 and CD231/TALLA-1 associate with isoforms of PKC and PI4-K, facilitating the formation of signalling complexes in the proximity of β1 integrins. Tetraspanins modulate integrin signalling and cytoskeletal rearrangements that precede cell spreading and migration. In this regard, ectopic expression of CD9 in B cells causes an induction of tyrosine phosphorylation during β1 integrin-mediated migration[31]. In T lymphocytes, CD9 brings conventional isoforms of PKC to the proximity of integrins α3β1 and α6β1, therefore facilitating the phosphorylation of these integrins and regulating T cell motility and morphology[9].

CD9 IN MYELOID CELLS

The role of tetraspanins in the function and signalling of neutrophils, monocytes and macrophages has not been studied in detail because monoclonal antibodies directed to specific tetraspanins bind at the same time to the different Fc receptors (FcR) present on the surface of these cells, complicating the functional effects observed. Despite these co-ligation effects, some evidences suggest a physical proximity between these molecules (tetraspanins and FcR). For instance, tetraspanin CD9 has been found to co-localize with FcγRIIb/III on macrophages and to be involved in the activation of these cells[42]. Another study shows a physical interaction between CD9 and FcγRI by immunoprecipitation[43]. These complexes are however unstable in highly hydrophobic detergents, indicating that FcR and CD9 must be associated through indirect interactions. Therefore, though several studies link CD9 with different signal transduction pathways in myeloid cells, the actual relevance of CD9 is dubious because of the co-ligation effect of antibodies used in these studies. Studies based on the analysis of myeloid cells from CD9-defficient mice will likely provide more precise information about the role of CD9 in these cells.

On the other hand, the expression of CD9 is induced in several myeloid cell lines (K562, THP-1) when they are differentiated with phorbol esters (PMA), suggesting a possible important functional role of CD9 in these differentiated myeloid cells[44,45]. However, this role has not been characterized yet.

CD9 IN ANTIGEN-PRESENTING CELLS

Tetraspanins are involved in antigen processing and presentation by Antigen-Presenting Cells (APC) (Fig. 3). For instance, CD9 and CD81 have been found associated with MHC class II on the membrane of dendritic cells, while CD63 and CD82 are found in intracellular vesicles[46]. Several mechanisms have been documented to explain the implication of tetraspanins in the regulation of antigen processing and presentation. Firstly, tetraspanins associate with MHC class II and co-stimulatory molecules in intracellular vesicles called exosomes[47], that are the main sites for peptide loading of MHC class II, and once these vesicles are released to the extracellular medium they stimulate T cell proliferation. Tetraspanins could play an important role in the assembly and stabilization of these MHC-peptide complexes and at the same time, they could regulate the traffic of these vesicles. In addition, the interactions between tetraspanins and PI4-K could be also important, since PI4-K is required for vesicular transport from the Golgi apparatus. Secondly, tetraspanins mediate the clustering of MHC molecules loaded with antigenic peptides together with co-stimulatory molecules like CD86, enhancing in this way MHC avidity and facilitating the activation of T cells[48].

CD9 AND INFECTIOUS DISEASE

CD9 has been implicated in the susceptibility to infection by feline immunodeficiency virus (FIV), which is similar to HIV and utilizes the chemokine receptor CXCR4. CD9 is not the co-receptor for this virus but rather participates at a stage subsequent to virus entry[49]. CD9 has also been implicated in the process of infection by the canine rabies virus as this tetraspanin is involved in cell-cell fusion process but not in viral-cell fusion[50,51]. CD9 also participates in the binding and endocytosis of the diphtheria toxin mediated by the transmembrane form of heparin-binding epidermal growth factor (pro-HB-EGF)[52]. Finally, it has been recently described that tetraspanins CD9 and CD81 can modulate membrane fusion during syncytia formation in HIV-infected cells[53]. Treatment with antibodies specific for these tetraspanins potentiates syncytia formation by human T lymphoblasts infected with HIV. In addition, inhibition of the expression of CD9 or CD81 results in enhanced syncytia formation and conversely, overexpression of these tetraspanins renders these cells less susceptible to syncytia formation. These data indicate that CD9 and CD81 exert an important role in membrane fusion during the process of syncytia formation.

TETRASPANIN ASSOCIATIONS WITH INTEGRINS AND THEIR RELEVANCE IN CANCER

Several biochemical studies have revealed that tetraspanins associate with specific members of the integrin family. These associations are especially relevant in cancer, as both tumor growth and metastasis are processes regulated by adhesion

receptors belonging to the integrin family and are also regulated by tetraspanins. The effects of tetraspanins on tumor growth and metastasis could be therefore partly explained through modulation of integrin-mediated adhesion. However, there is controversy as to whether tetraspanins exert their effects through regulation of the adhesive properties of integrin. In this regard, some studies show that tetraspanin specific antibodies potentiate integrin-dependent cellular adhesion, whereas in other studies tetraspanins only have a minor effect on integrin ligand binding or on integrin-mediated cell adhesion[29,54]. In any case, tetraspanins regulate events that take place after integrin ligand binding (post-adhesive events), such as adhesion strengthening that augments the resistance of cells to be detached from immobilized integrin ligands. The mechanisms by which tetraspanins strengthen integrin-dependent adhesion are poorly characterized. Since tetraspanins localize some isoforms of PKC in the proximity of integrins, it is assumed that they contribute to the regulation of the reorganization of actin cytoskeleton, which in turn is required for adhesion strengthening. The tetraspanin/integrin complexes have also relevance in cellular motility. In this regard, it has been observed that transfection of a B cell line with CD9 alters the motility of these cells that is dependent on integrins α6β1 and α4β1[31]. In addition, these complexes have been found to localize at the leading edge and filopodia, as well as in vesicles concentrated at the uropod, which are all structures implicated in cell migration.

The relationship between CD9 and tumoral metastasis has been widely studied. Different studies have proposed that CD9 works as a metastasis suppressor molecule because of the inverse correlation found between the level of expression of this tetraspanin and the malignancy grade, the occurrence of metastasis, and patient survival rate that has been established in many types of cancer, including melanomas, and lung, colon and breast carcinomas[55-57]. Moreover, the transfection of CD9 into melanoma cells reduces their metastatic potential[58]. The molecular mechanisms that are responsible for this metastasis-suppressor role of CD9 remain rather obscure, although some authors suggest that these effects of CD9 are mediated through the associated integrins, that would regulate the adhesive capacities of tumor cells as well as their migration and invasion[13]. In contrast with the abundantly documented relationship between the expression of CD9 and the metastatic capacity of different tumors, the possible implication of CD9 in the process of primary tumor formation (tumorigenesis) has been addressed in very few studies. In our laboratory, several monoclonal antibodies (mAbs) specific for human CD9 have been recently obtained and characterized. The use of these mAbs has allowed us to identify a functional epitope on the CD9 molecule whose expression depends on changes in the activation state of associated β1 integrins, particularly the α6β1 integrin, which is a receptor for laminin. This functional epitope has been named PAINS-13, and is located in the C-terminal half (variable region) of the CD9 LEL domain[59]. *In vitro* studies carried out in endothelial cells have revealed that mAb PAINS-13 exerts important functional effects that are mediated through ligation of CD9 when is associated to activated integrins; these effects include a significant inhibition of endothelial cell migration during cellular wound healing assays, and a specific enhancement of endothelial cell spreading onto laminin[59]. As a continuation of these *in vitro* studies with endothelial cells, we decided to address the study of the possible implication of CD9 in the tumorigenesis process *in vivo* in a model of athymic nude mice. For this purpose, we used several human colon carcinoma cell lines that were treated with several anti-CD9 mAbs, or in which CD9 was either expressed ectopically or overexpressed. We observed that CD9 expression and/or treatment with specific anti-CD9 mAbs induced a series of cellular effects that included an important increase in β1 integrin-mediated cellular adhesion, the induction of striking morphological changes, and a significant inhibition of the proliferative capacity of these carcinoma cells[60]. This reduction in human colon carcinoma cell proliferation correlated well with a decreased tumorigenic capacity observed *in vivo* in nude mice, as determined by the parameters of increased latency time and reduced tumor incidence and size[60]. We have ruled out that the mechanism by which expression of CD9 or treatment with anti-CD9 mAbs reduce the proliferative capacity of colon carcinoma cells is an induction of apoptosis or an arrest at a specific phase of cell cycle. We have observed that the transmembrane form of TNF-α is involved in the inhibition of the proliferative capacity, as its levels are elevated after treatment with anti-CD9 mAbs and, more importantly, the inhibiton in proliferation is reversed by agents that selectively block this cytokine. It is likely that the juxtacrine signalling that is triggered through the interaction of transmembrane TNF-α and TNFR-II on adjacent tumoral cells is responsible for the reduction observed in the proliferative capacity. We have not characterized yet the intracellular signals that could be implicated in the CD9-mediated induction of morphological changes and in the reduction of proliferation in human colon carcinoma cells.

A similar inhibition caused by anti-CD9 antibodies has also been described in other cell types[61,62]. The work of Murayama et al. describes that the mAb ALB6 inhibits the proliferation of different gastrointestinal tumor cell types, negatively modulating the activity of Erk1/2 and activating the p38 and JNK MAP kinases through the adapter protein Shc, triggering cellular apoptosis. These results with mAb ALB6 contrast with our data obtained with different anti-

CD9 mAbs, which as already mentioned, do not induce apoptosis in human colon carcinoma cells[60]. This suggests that the mechanisms implicated in the inhibition of proliferation can differ depending on the tumor cell types studied or the specific anti-CD9 mAbs employed.

On the other hand, similar anti-proliferative effects have also been observed with mAbs specific for CD81 in human lymphoma cells[63], indicating that the implication of tetraspanins in the regulation of cellular growth could be a more general process, not restricted to a particular tetraspanin.

DISCLOSURES

The authors declare no financial conflict of interest.

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