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ORIGINAL ARTICLE

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The effect of laminin and its peptide SIKVAV on a human salivary gland adenoid cystic carcinoma cell line

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Abstract We have previously demonstrated that laminin modulates the expression of adhesion molecules in an adenoid cystic carcinoma cell line (CAC2 cells). We are currently studying whether laminin can induce modifications in the overall morphology of CAC2 cells. These cells were grown in a three-dimensional preparation of laminin-1. Phenotype differences were assessed by light and transmission electron microscopy. CAC2 cells grown inside laminin-1 formed ductlike and pseudocystic structures. Based on our findings we suggest that laminin is a key regulator of tubular and pseudocystic patterns of adenoid cystic carcinoma. We also analyzed the effect of a molecular domain of laminin-1, the peptide SIKVAV (Ser-Ile-Lys-Val-Ala-Val) on CAC2 cells. This peptide was chosen because it is effective in cell proliferation and differentiation, and because it has never been tested before in salivary gland neoplasms. When CAC2 cells were grown inside SIKVAV-enriched laminin-1, only pseudocystic structures were observed. Since no ductlike structures were observed in samples treated with SIKVAV, we may assume that this peptide is at least one of the molecular domains of laminin responsible for the pseudocystic pattern observed in adenoid cystic carcinoma. Function disturbing experiments strongly suggested that the integrin $\alpha 3\beta 1$ play a role in the effect of laminin on CAC2 cells.

Keywords Salivary gland neoplasms · Adenoid cystic carcinoma · Extracellular matrix · Laminin · Cell culture · Integrins

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Introduction

Adenoid cystic carcinoma is a frequent malignant salivary gland neoplasm [7, 9, 10, 26]. It shows insidious and slow growth with high level of recurrence and distant metastasis long time after treatment. A prominent feature of adenoid cystic carcinoma is its affinity for basement membrane rich tissues, such as nerves and blood vessels [7, 9, 10, 26].

This neoplasm is characterized histologically by a sheet or islandlike proliferation of round or cuboidal epithelial cells, with scant cytoplasm and hyperchromatic large oval nucleus [7]. Growth patterns are solid, tubular, and pseudocystic. Pseudocysts are formed by neoplastic cells of either myoepithelial or epithelial phenotype and are filled with extracellular matrix components. Perineural invasion is a common histological finding of adenoid cystic carcinoma [7, 26].

Electron microscopy of adenoid cystic carcinoma shows both luminal and myoepithelial cells [7]. These cells are often separated by extracellular material, such as pools of basal lamina, collagen fibers, elastin, and glycosaminoglycans [5, 6, 7, 20, 27]. A conspicuous finding in the cribriform variant of adenoid cystic carcinoma is a thickened band of extensively reduplicated basement membrane [7]. Immunohistochemical studies have also demonstrated the presence of basement membrane proteins in this neoplasm [5, 6, 20].

It has been suggested that the extracellular matrix plays an important role as regulatory factor of phenotypic differences among salivary gland neoplasms [12, 14, 15, 16, 23]. We have previously demonstrated that laminin modulates the expression of adhesion molecules in an adenoid cystic carcinoma cell line (CAC2 cells) [9, 9]. We are currently studying whether laminin can induce modifications in the overall morphology of CAC2 cells. We have previously established a cell line (CAC2 cells), derived from human salivary gland adenoid cystic carcinoma [8, 9, 10]. CAC2 cells were grown in contact with laminin-1, in a three-dimensional preparation. Phenotype differences were assessed by light and transmis-

sion electron microscopy. We also studied the effect of a molecular domain of laminin, the peptide SIKVAV (Ser-Ile-Lys-Val-Ala-Val) on CAC2 cells. This peptide was chosen because it is effective in cell proliferation and differentiation, and because it has never been tested before in salivary gland neoplasms. In addition, we searched for integrins involved in the effect of laminin on CAC2 cells.

Materials and methods

Cell culture

CAC2 cells were derived from a human adenoid cystic carcinoma [8, 9, 10]. These cells were cultured in high glucose Dulbecco's modified Eagle's medium (Sigma, St. Louis, Mo., USA) supplemented by 10% fetal bovine serum (Cultilab, Campinas, SP, Brazil) and 1% antibiotic-antimycotic solution (Sigma). The cells were maintained in 25 cm^2 flasks in a humidified atmosphere of 5% CO_2 at 37°C.

Morphology of CAC2 cells was analyzed by phase contrast. Immunolocalization of smooth-muscle actin was used for phenotype characterization, as previously described [15]. We also searched for basement membrane proteins, such as type IV collagen and laminin.

Immunofluorescence

For smooth-muscle actin immunostaining, CAC2 cells grown on coverslips were fixed in paraformaldehyde 1% in phosphate-buffered saline (PBS) for 10 min, rinsed in PBS, and permeabilized with 0.5% Triton X-100 (Sigma) in PBS for 15 min. Cells were then subjected to an immunofluorescence protocol [13] using a mouse monoclonal antibody against smooth-muscle actin (Biogenex Laboratory, San Ramon, Calif., USA), diluted 1:100 in PBS. An anti-mouse fluorescein isothiocyanate conjugated (Amersham, Arlington Heights, Ill., USA) was used as secondary antibody. All incubations were carried out for 60 min at room temperature. The mounting medium was Pro Long (Molecular Probes, Eugene, Ore, USA). Replacement of the primary antibody by PBS was used as negative control [17].

For laminin and type IV collagen immunostaining of CAC2 cells we used the same protocol as described above, but with no permeabilization step. Laminin antibody (Sigma) was diluted 1:100, and type IV collagen antibody (Biogenex) was diluted 1:20 in PBS. Cy5 conjugates (Zymed Laboratories, San Francisco, Calif., USA) were used as secondary antibodies.

Three-dimensional preparation of laminin-1

We used a laminin-1 gel in Dulbecco's modified Eagle's medium (1 mg/ml, kindly provided by Dr. Matthew Hoffman NIDCR, NIH, Bethesda, Md., USA). CAC2 cells were then harvested from the culture flasks, resuspended inside laminin-1, and placed in Eppendorf tubes. Cells were then incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. CAC2 cells were grown within this three-dimensional preparation of laminin-1 for 48 h. CAC2 cells grown inside agarose served as controls. Three-dimensional preparations of CAC 2 cells (treated and control samples) were studied by light and transmission electron microscopy.

Light microscopy of CAC2 cells grown in three-dimensional preparations

CAC2 cells growing within laminin-1 were fixed in 10% formalin for 24 h. Even after fixation the laminin-1 gel used in our preparation is too soft to be embedded directly in paraffin. To circumvent this problem we dehydrated and embedded the samples in Histogel (Perk Scientific, Devon, Pa., USA). Heating the Histogel to 50°C converts the gel into a liquid state, which allows infiltration of the samples. After the infiltration the Histogel converts back into a solid as it cools. Final hardening is achieved at room temperature. Since Histogel is in aqueous media, the samples were then dehydrated again, paraffin-embedded and stained by hematoxylin-eosin. The same procedure was carried out for the controls (CAC2 cells grown inside agarose).

Transmission electron microscopy of CAC2 cells grown in three-dimensional preparations

For transmission electron microscopy pellets of CAC2 cells grown either within laminin-1 or inside agarose were fixed by immersion in 2.0% glutaraldehyde in 0.1 M sodium cacodylate buffer solution at pH 7.4 for 2 h and postfixed in 1% osmium tetroxide in the same buffer for 45 min. Then samples were washed in distilled water, stained en bloc with 0.5% uranyl acetate, rinsed, and dehydrated in graded ethanol. After immersion in propylene oxide samples were embedded in epoxy resin (Epon 812, Ted Pella, Redding, Calif., USA) and polymerized for 72 h at 60°C. Semithin sections (1 μm) were cut and stained with a mixture of 1% azure II, 2% methylene blue, and 2% borax in distilled water. Ultrathin sections were stained with lead citrate and uranyl acetate and examined in a JEOL 1010 transmission electron microscope (Jeol, Peabody, Mass., USA).

Culture of CAC2 cells in laminin-1 enriched with either the SIKVAV hexapeptide or with the SIKVAV-containing fragment of laminin

The SIKVAV hexapeptide (Calbiochem-Novabiochem, La Jolla, Calif., USA) and the SIKVAV-containing fragment of laminin (Sigma) were used in our study. The SIKVAV hexapeptide was diluted in 0.1% acetic acid, to reach a final concentration of 1 mg/ml. The SIKVAV-containing fragment of laminin (Cys laminin α-chain, amino acids 2091–2108, cat. # C-6171, Sigma) was diluted in milli-Q water to reach a final concentration of 1 mg/ml. The SIKVAV hexapeptide and the SIKVAV-containing fragment of laminin were diluted in the gel of laminin-1. Concentrations of the peptides in the gel ranged from 25 to 100 μg/ml. The best results were obtained with concentrations of 25 and 50 μg/ml. Bicarbonate was used to obtain a suitable pH. CAC2 cells were then harvested from the culture flasks, resuspended inside SIKVAV-enriched laminin-1, and placed in Eppendorf tubes. Cells were then incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. CAC2 cells were grown within these three-dimensional preparations of SIKVAV-enriched laminin-1 (either with the SIKVAV hexapeptide or with the SIKVAV-containing fragment of laminin) for 2 and 5 days. CAC2 cells grown inside laminin-1 with no synthetic peptides served as controls. Three-dimensional preparations of CAC2 cells (treated and control samples) were studied by light microscopy.

Integrins involved in the effect of laminin-1 on CAC2 cells

To address the identities of integrins that are involved in the effect of laminin-1 on CAC2 cells we used antibodies that specifically block integrin function. These antibodies were raised against α3, β1, and β4 integrins (Chemicon, Temecula Calif., USA). All antibodies were azide-free, to avoid cytotoxicity. CAC2 cells were harvested from the flasks by 0.02% EDTA in PBS (Versene solution) and incubated with antibodies to α3, β1, and β4 (1/20 in PBS) for 1 h at 37°C. CAC2 cells incubated with anti-human IgG (azide-free, Chemicon) served as controls. After blocking integrin function with specific antibodies, we carried out a cell adhesion assay. For this assay laminin-1 (50 μg/ml in PBS) was coated onto

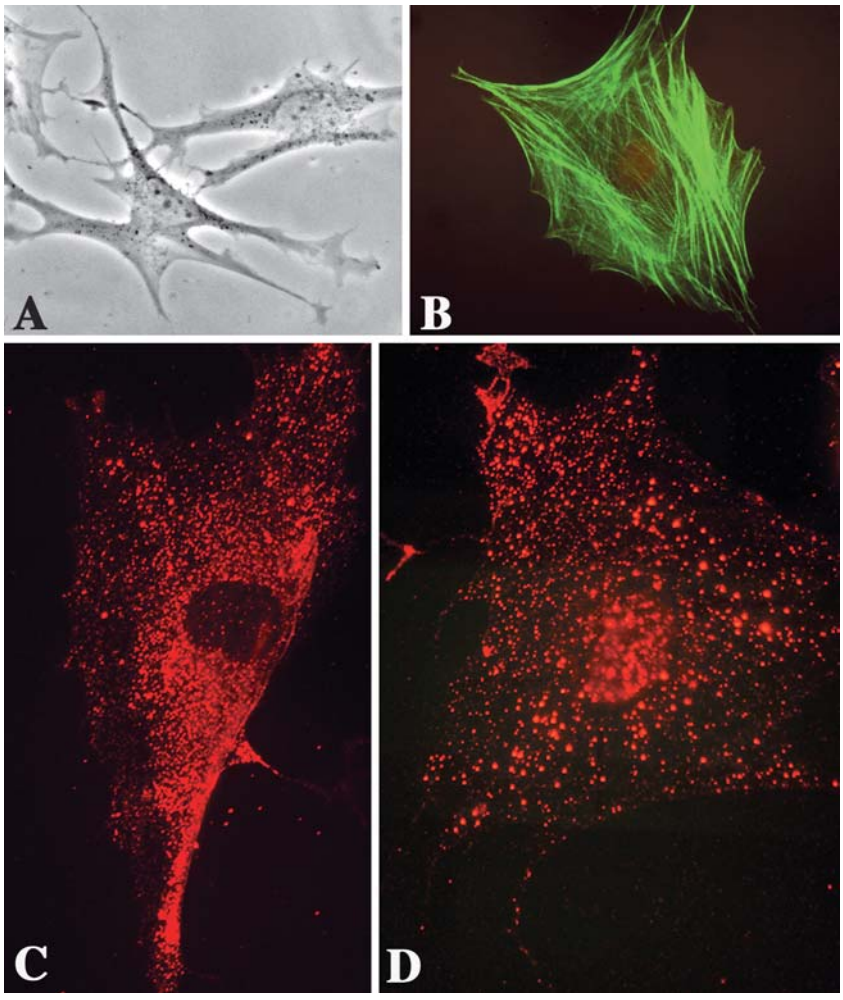

Fig. 1 **A** Phase contrast microscopy of CAC2 cells, exhibiting dendritic cells. **B** Immunofluorescence shows smooth-muscle actin, mostly as stress fibers. **C**, **D** Type IV collagen (**C**) and laminin (**D**) are also observed, forming multiple dots throughout the cell surface. **A** ×400x; **B**–**D** ×630

round-bottomed 96-well plates (Cultilab) by drying overnight at 4°C. The wells were blocked with 1% denatured BSA in PBS for 30 min at 37°C and washed twice with PBS. A number of 3.5×10^4 CAC2 cells in 100 µl PBS was added per well for 45 min at 37°C. After that the medium with nonadherent cells was gently removed from the wells. Attached CAC2 cells were fixed and stained with a solution of 0.2% crystal violet in 20% methanol for 12 min. After washing twice with water the cells were lysed with 50 µl 10% sodium dodecyl sulfate (Sigma) and the optical density (600 nm) measured in a spectrophotometer. Assays were carried out in triplicate at least three times. Counts of adherent cells were expressed as percentage of the control (mean ±standard error of the mean). IgG control was set at 100%.

We also carried out experiments blocking integrins as described above, and growing CAC2 cells within three-dimensional preparations of laminin-1. The rationale of this assay was to observe whether blockage of integrins prevents the effect of laminin-1 on CAC2 cells. Cells with integrins blocked by antibodies were grown inside laminin-1 for 2 days. We used two controls: (a) cells incubated with anti-human IgG and (b) cells with no treatment with anti-integrin antibodies. Control CAC 2 cells were then grown inside laminin-1. Treated and control samples were processed as described previously, and studied by light microscopy.

Results

Characterization of CAC2 cells

Phase contrast showed spindle shaped and dendritic cells (Fig. 1A). Immunofluorescence detected smooth-muscle actin (Fig. 1B), type IV collagen (Fig. 1C) and laminin (Fig. 1D). These markers are characteristic of myoepithelial differentiation.

Light microscopy of CAC2 cells grown within three-dimensional preparations

Control CAC2 cells grown inside agarose showed round and noncohesive cells with no particular arrangement (Fig. 1A). CAC2 cell line when cultured within laminin-1 assumed a new overall morphology (Fig. 2B–E), with cells delineating luminal spaces forming a ductlike pattern (Fig. 2B). We also observed compact aggregates of mostly polyhedral cells (Fig. 2C). In these aggregates spindle-shaped cells surrounded pseudocysticlike spaces (Fig. 2C, D). Few cells were either stellate or round-

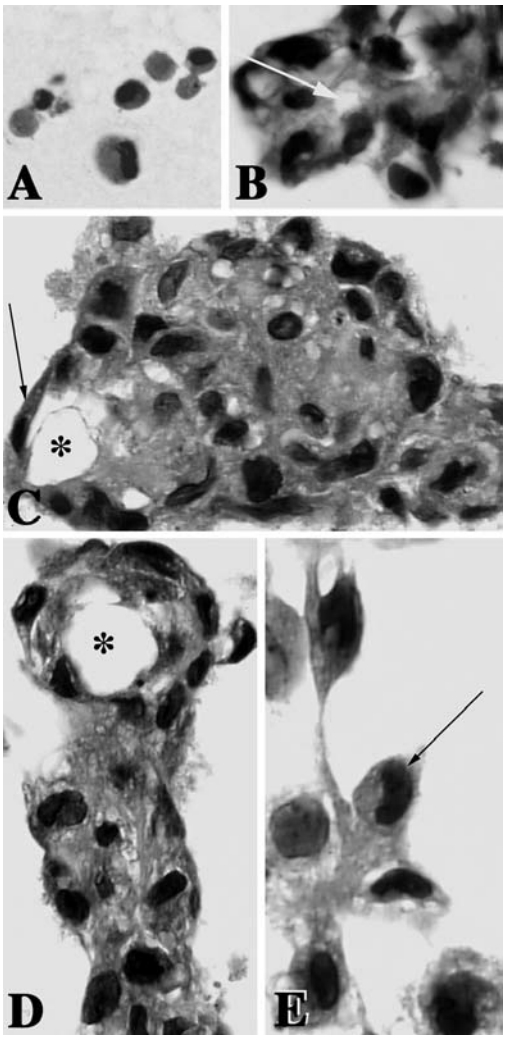

Fig. 2 **A** Control CAC2 cells grown inside agarose show round and noncohesive cell. **B**–**E** Morphology of CAC2 cells when grown inside a three-dimensional preparation of laminin. A new overall arrangement is observed, with cells delineating luminal spaces, forming a ductlike pattern (**B**, *arrow*). **C**, **D** Aggregates of mostly polyhedral cells are present (**C**), with spindle-shaped cells (**C**, *arrow*) surrounding a pseudocystic space (**C**, **D** *asterisks*). **E** Few cells are either stellate or round-shaped (*arrow*). Stellate cells show thin, long, and intercommunicating processes. **A**, **B**, **D**, **E** ×630; **C** ×400

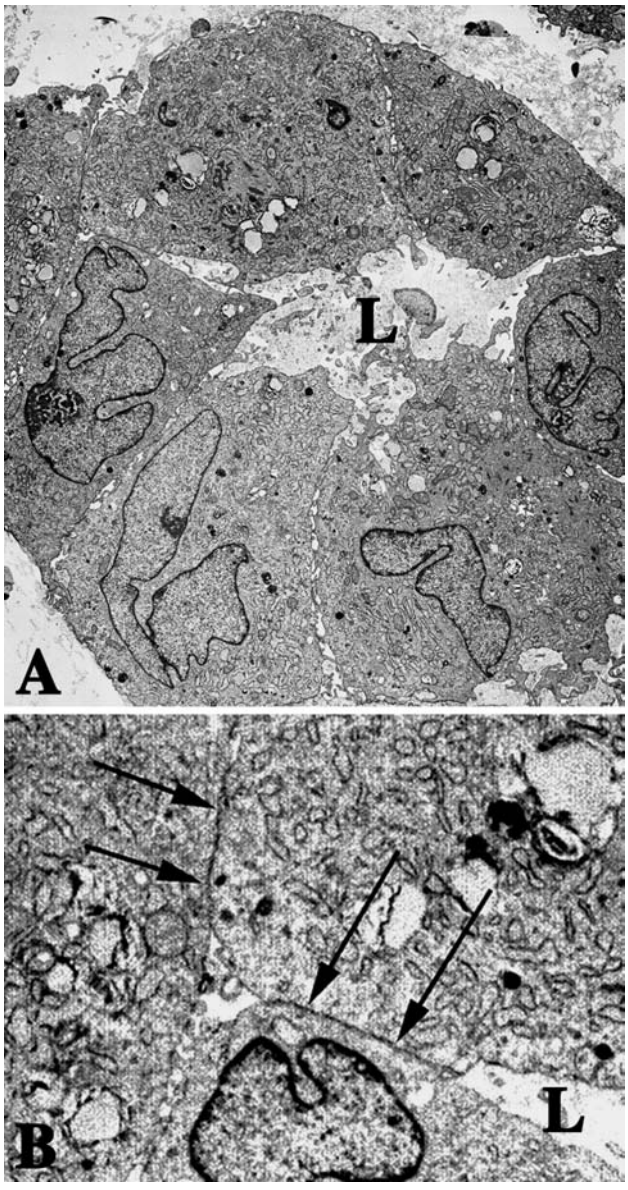

Fig. 3A,B Transmission electron microscopy of CAC2 cells grown within laminin. **A** We observed cuboidal cells delimiting luminal spaces. These cells are slightly polarized, with microvilli at apical plasmalemma. **B** Rudimentary junctions are observed (*arrows*). *L* Luminal space. **A** ×1200; **B** ×8000

shaped (Fig. 2E). Pseudocystic structures were observed in 30–50% of CAC2 cells grown within laminin-1. Pseudocysts diameter was 107,599±11,405 μm (n=20), measured by the Image J software (public domain software developed by Wayne Rasband, NIMH, NIH, USA).

Transmission electron microscopy of CAC2 cells grown within three-dimensional preparations

Transmission electron microscopy was used to further study the ductlike structures formed by CAC2 cells grown within laminin-1. We observed cuboidal cells delimiting luminal spaces (Fig. 3A). These cells were slightly polarized, with microvilli at apical plasmalemma. Rudimentary junctions were observed (Fig. 3B).

Culture of CAC2 cells in laminin-1 enriched with either the SIKVAV hexapeptide or with the SIKVAV-containing fragment of laminin

Light microscopy study of CAC2 cells grown for 2 days within a three-dimensional preparation of laminin-1 enriched with the SIKVAV-containing fragment of laminin (Cys laminin α-chain, amino acids 2091–2108) showed

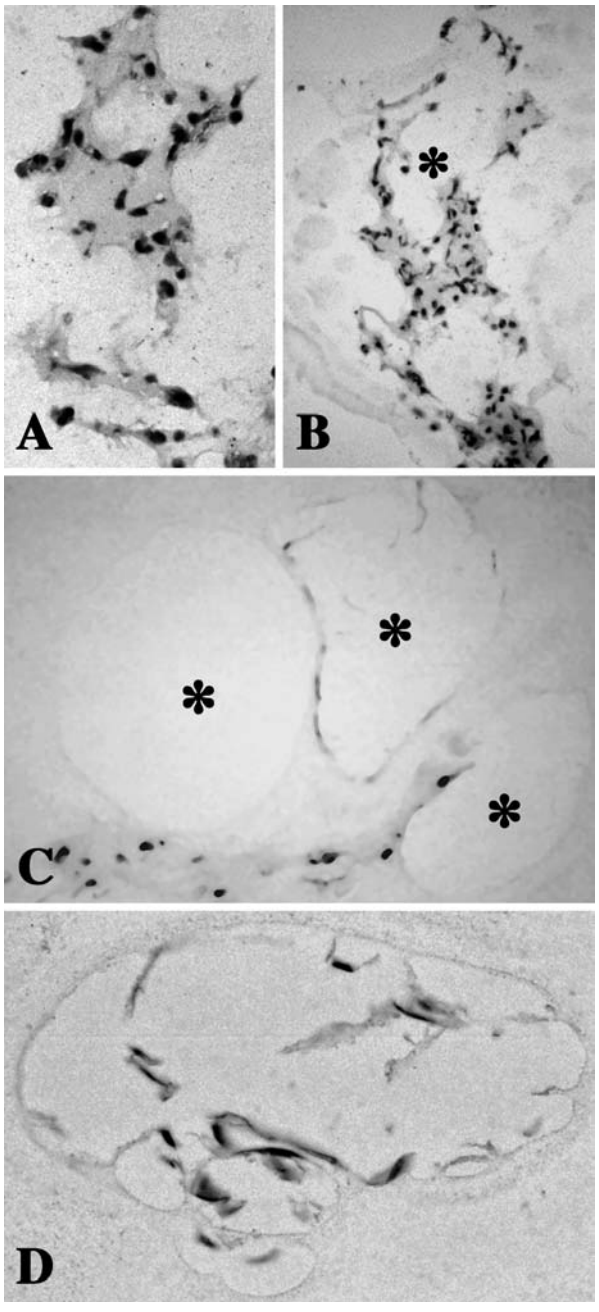

Fig. 4A–D Light microscopy study of CAC2 cells grown for 2 days within a three-dimensional preparation of laminin enriched with SIKVAV. **A**, **B** Cultures treated with the SIKVAV-containing fragment of laminin (Cys laminin α-chain, amino acids 2091–2108) show signs of matrix digestion (**B**, *asterisk*). **C**, **D** Cultures treated with laminin enriched with the hexapeptide SIKVAV exhibit large spaces between the cells (**C**, *asterisk*). These spaces are underlined by a layer of spindle-shaped cells. These features resemble the pseudocystic organization of adenoid cystic carcinoma in vivo (**D**). ×200

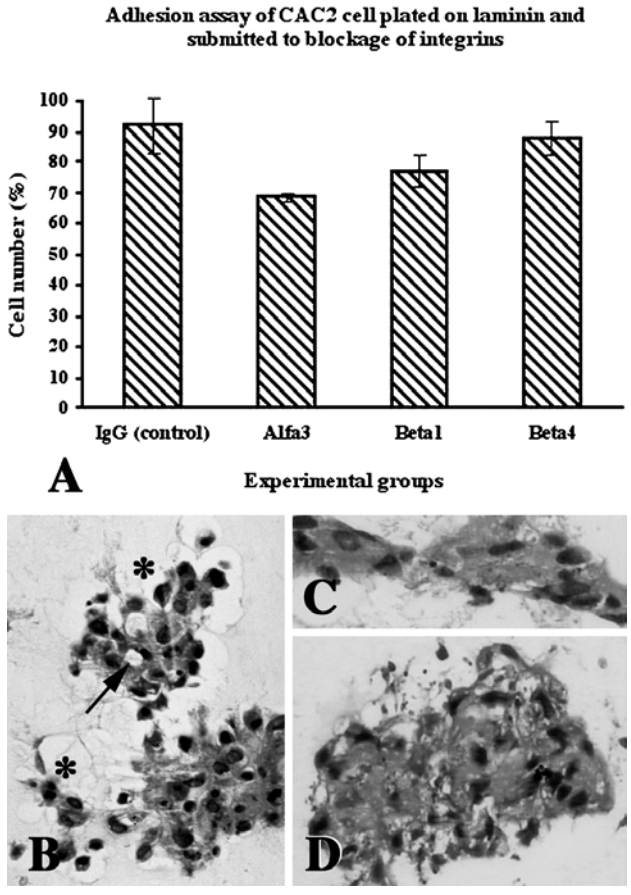

Fig. 5 **A** Cell adhesion assay shows that either α3 or β1 decreased the attachment of CAC2 cells to laminin-1. Three-dimensional culture of CAC2 cells also shows the effect of either α3 or β1. **B** Control cells, either incubated with anti-human IgG (not shown) or with no treatment with anti-integrin antibodies grown inside laminin-1 form solid patterns, ductlike structures (*arrow*), and pseudocysts (*asterisk*). **C**, **D** Cells treated by either anti-α3 integrin (**C**) or anti-β1 integrin (**D**) display a random distribution, with no particular configuration. ×200

signs of matrix digestion, with formation of large spaces between the cells (Fig. 4A, B).

The results observed with laminin-1 enriched with the SIKVAV-containing fragment of laminin (Cys laminin α-chain, amino acids 2091–2108) were also observed in preparations enriched with the SIKVAV hexapeptide. We observed spaces underlined by a layer of spindle-shaped cells (Fig. 4C). These features resemble the pseudocystic organization of adenoid cystic carcinoma in vivo (Fig. 4D). Pseudocystic structures were observed in 70–90% of CAC2 cells grown within laminin-1 enriched with SIKVAV. Pseudocysts diameter was 211.939± 44.776 μm (n=20), measured by the Image J software. Thus the pseudocysts created by laminin-1 enriched with the peptide SIKVAV were twice the size of the pseudocystic spaces induced by laminin-1 with no peptides.

CAC2 cells grown for 5 days within laminin-1 enriched either with the SIKVAV hexapeptide or with the SIKVAV-containing fragment of laminin showed the same features described above.

It is important to emphasize that both three-dimensional assays using SIKVAV-enriched laminin-1 (either with the SIKVAV hexapeptide or with the SIKVAV-containing fragment of laminin) created only pseudocystic-

like spaces. Neither ductlike structures nor solid arrangements were found.

Integrins involved in the effect of laminin-1 on CAC2 cells

Cell adhesion assay showed that blockage of either α3 or β1 integrins by specific antibodies decreased the attachment of CAC2 cells to laminin-1 (Fig. 5A). These antibodies also inhibited the effect of laminin-1 in the overall morphology of these cells. Control CAC2 cells, either incubated with anti-human IgG or with no treatment with anti-integrin antibodies, grown inside laminin-1 formed solid patterns, ductlike structures, and pseudocysts (Fig. 5B). These features were not observed in CAC2 cells subjected to integrin blockage. Cells treated by either anti-α3 integrin (Fig. 5C) or anti-β1 integrin (Fig. 5D) displayed a random distribution, with no particular configuration. Thus we infer that α3β1 integrin is an important receptor for the effect of laminin on CAC2 cells.

Discussion

Our results showed that laminin-1 regulates the morphology of cells derived from human adenoid cystic carcinoma (CAC2 cells). Growth of CAC2 cells within laminin-1 created ductlike and pseudocystic structures similar to those occurring in the neoplasm in vivo. When CAC2 were grown inside SIKVAV-enriched laminin-1, only pseudocystic structures were observed. We also found that the α3β1 integrin play a role in the effect of laminin on CAC2 cells.

Morphogenetic studies of normal and neoplastic salivary glands have been carried out by culturing cells in three-dimensional matrix environment [1, 4, 11, 12, 14, 23, 30, 31]. We have been studying tumors derived from intercalated duct, such as pleomorphic adenoma, myoepithelioma, and adenoid cystic carcinoma through this three-dimensional assay [14, 23]. We first demonstrated that a reconstituted basement membrane (Matrigel) regulates the morphology of cell lines derived from these neoplasms. We are currently investigating the role played by individual basement membrane molecules on the morphology of adenoid cystic carcinoma. We have chosen laminin because this molecule is abundant in basement membranes and has in its composition bioactive peptides, which modulate cell growth and differentiation [2, 3, 17, 18, 19, 21, 24, 29, 32]. Moreover, the vast majority of studies reporting phenotypic modifications induced by extracellular matrix on neoplastic salivary cell lines involve culture inside collagen gel [1, 4, 11, 30, 31]. Our report appears to be the first description of phenotypic modifications induced by a three-dimensional preparation of laminin-1. Thus we believe that culturing CAC2 cells inside laminin would provide important information on adenoid cystic carcinoma biology.

The laminins are structurally related glycoproteins found predominantly in basement membranes [21]. They exist as a cruciformlike structure formed by three chains, designated α, β, and γ, that are structurally homologous but have little similarity in the level of their amino acid sequences [21]. Eleven laminin isoforms have been described to date. Since laminin can bind many of the other components of the basement membrane, including collagen IV, perlecan, and entactin, as well as binding to itself, it is likely to play a role in organizing and possibly initiating the formation of the basement membrane matrix [2, 3, 18, 19, 21, 32]. However, laminins are more than a mere glue and surface over which cells move. There is emerging evidence that these molecules can regulate cell proliferation and have the capability of specifying cell and tissue development, differentiation, and function through their interaction with cell surface molecules [2, 3, 18, 19, 21, 22, 32]. Among these cell surface molecules the integrins are largely responsible for mediating the profound effects of the laminins on cell behavior. Integrins are a large family of adhesion receptors composed of two transmembrane glycoprotein subunits designated α and β [22]. They are bona fide receptors because they can both bind laminin, as well as many other ligands, and modulate intracellular signaling pathways in response to this binding [22].

Our results clearly demonstrated the effects of laminin-1 in the differentiation of an adenoid cystic carcinoma cell line (CAC2). Light and electron microscopy showed ductlike structures formed by polarized cuboidal cells delimiting a luminal space. Transmission electron microscopy revealed rudimentary junctions in these ductlike structures. The role of laminin in determining canalicular structures has previously been demonstrated in vascular endothelium as well as in glandular tissue. In endothelium laminin induces cell migration, proliferation, and realignment to form a new capillary structure [25]. This molecule also induces formation of ducto acinar units in cell lines derived from the submandibular gland [12]. Growth of CAC2 cells within laminin also created pseudocysts, similar to those occurring in the neoplasm in vivo. Pseudocyst formation could be regarded as one of the markers of terminal differentiation of adenoid cystic carcinomas [7].

Our peptide assay provided further information on the effect of laminin-1 on the morphology of CAC2 cells. Laminin is biologically active, as demonstrated by in vivo and in vitro studies [2, 3, 18, 19, 21, 32]. This biological effect is mostly related to peptides located in different domains of the molecule. Several active sites on laminin have been previously identified. YIGSR (Tyr-Ile-Gly-Ser-Arg) on β chain promotes cell adhesion and migration [21, 29]. On the other hand, SIKVAV, located on the carboxy-terminal end of the long arm of α-chain, is involved in cell adhesion, growth, neurite outgrowth, tumor growth, angiogenesis, and protease activity [17, 21, 24]. The fact that the effect of SIKVAV has never been assessed in salivary gland neoplasms has prompted us to study the modulation of CAC2 cells morphology by this bioactive peptide.

Light microscopy study of CAC2 cells grown within three-dimensional preparation of SIKVAV-enriched laminin-1 showed signs of matrix digestion, with formation of large spaces between the cells. These spaces could represent the expression of protease activity by CAC2 cells since SIKVAV can induce this kind of biological activity [17, 24]. These spaces were underlined by a layer of spindle-shaped cells. These features resemble the pseudocystic organization of adenoid cystic carcinoma in vivo. Since no ductlike structures were observed in samples treated with SIKVAV, we may assume that this peptide is at least one of the molecular domains of laminin-1 responsible for the pseudocystic pattern observed in adenoid cystic carcinoma. We should emphasize that the effect of SIKVAV has been assessed in different cells lines. However, there is no study on the expression of this peptide in human neoplasms. This is an interesting to point, to be addressed in future investigation.

Our results strongly suggested that $\alpha 3\beta 1$ integrin plays a role in the molecular effect of laminin on CAC2 cells. We tested this particular receptor because it is expressed in adenoid cystic carcinoma in vivo [20]. Adhesion assay showed that blockage of either $\alpha 3$ or $\beta 1$ subunits inhibits the attachment of CAC2 cells to laminin. This inhibition was only partial, suggesting that other integrins would be probably involved as receptors, such as $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 6\beta 1$, $\alpha 6\beta 4$, and $\alpha 7\beta 1$. These integrins also bind laminin-1 in different cells and tissues [22]. However, the experiment of growing CAC2 cells with the integrins $\alpha 3$ and $\beta 1$ blocked showed a striking result, with cells distributed at random, and absence of ductlike structures and pseudocysts. Thus we may infer that $\alpha 3\beta 1$ is part of the mechanism underlying the regulation of the morphological changes induced by laminin in CAC2 cells. We may also speculate that this integrin is related to the putative proteolytic effect of SIKVAV, since $\alpha 3\beta 1$ induces proteolysis [28].

This in vitro study demonstrated the effect of laminin-1 in a cell line derived from human adenoid cystic carcinoma (CAC2 cells). The comparison between our in vitro findings with the adenoid cystic carcinoma in vivo provides more information on effect of laminin-1. Morphological examination of this neoplasm in vivo shows large amounts of basement membrane molecules surrounding tumor islands [5, 6, 7, 20, 27]. Our in vitro assay mimicked this situation by growing CAC2 cells inside laminin-1. If we assume that the basement membrane observed in the neoplasm in vivo is secreted by the tumor cells themselves, we may speculate that laminin-1 acts as autocrine factor determining the morphogenetic changes in CAC2 cells. Moreover, we have more in vitro evidence on this putative autocrine effect, since immunofluorescence showed that CAC2 cells grown on plain coverslips secrete basement membrane molecules, such as laminin and type IV collagen. Our study went further, showing that a molecular domain of laminin, the peptide SIKVAV, enhances the formation of pseudocystic spaces on CAC2 cells. Further experiments analyzing the effects of other biologically active peptides on CAC2 cells would be relevant to determine more putative domains of laminin underlying the regulation of adenoid cystic carcinoma phenotype.

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