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Tenascin: Growth and Adhesion Modulation—Extracellular Matrix Degrading Function: an *In Vitro* Study

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Tenascin (TN), a recently characterised extracellular matrix protein, largely confined to the process with the development of embryo in areas of epithelial–mesenchymal interactions and in areas where there are morphogenetic movements and tissue patterning, has a highly restricted expression in adult tissues. The expression of TN is enhanced in a variety of human neoplastic lesions. However, function(s) and molecular mechanisms of enhanced expression in neoplastic lesions remain unclear. We employed human tongue carcinoma cells (SCCKN), human salivary gland adenocarcinoma cells (SGT-1), normal mouse embryonic fibroblasts (NIH3T3-3) and *K-ras*-2 transformed fibroblasts (Cle-H3) in an *in vitro* study to elucidate the biological roles of TN. In *in vitro* studies, all the cell lines examined had enhanced secretion of TN in the presence of transforming growth factor-beta in a dose-dependent manner and TN itself was found to possess a growth-enhancing activity. Moreover, studies on adhesion of the cell lines on coated substrates of fibronectin (FN), laminin (LN), tenascin (TN), TN/FN and TN/LN showed that all the cells adhere and spread well on FN and LN. However, on TN they attach poorly and remain rounded. The relative concentrations of TN and FN affected the cellular adhesion and morphology. In SCCKN and SGT-1, but not in NIH3T3 and Cle-He3 fibroblasts, a higher concentration of TN inhibited cellular adhesion on fibronectin, suggesting that cells attach poorly on TN, it may interfere with the action of fibronectin, and the relative concentrations of TN, FN or LN may affect cellular adhesion and morphology which may differ in different cell types. When TN was added in the growth medium of exponentially growing cells, the cells lost their cell to cell contact and were seen to be separating. The presence of these extracellular matrix proteins were further tested to determine whether they could modulate the secretion of proteolytic enzymes responsible for extracellular matrix degradation by tumour cells, when the neoplastic cells but not the non-neoplastic cells grown on FN/TN substrate showed positive immunofluorescence for collagenase. FN, LN or TN alone did not induce collagenase in the tumour cells. If the same is true *in vivo*, although a number of factors and interactions may implicate the ultimate outcome, the enhanced expression of TN in neoplastic lesions may have potential implications for tumour growth, differentiation, cellular adhesion, invasion and metastasis.

Keywords: tenascin, extracellular matrix, adhesion, invasion, metastasis

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INTRODUCTION

The dynamic adhesive interactions between tumour cells and extracellular matrix (ECM) components, as well as the enzymatic degradation of the ECM by tumour cells are key features in the process of tumour invasion and metastasis [1, 2]. A loss in cell–cell adhesion and an increase in cellular motility are clearly prerequisites in determining cell morphology and migration and the development of invasive tumours and metastasis. The disseminating tumour cells have to migrate through the extracellular matrix and as tumour extracellular matrix is believed to be different and contributes in the process of carcinogenesis, the matrix may contain increased levels of potentially anti-adhesive, adhesion modulating, or migration-stimulating components. Moreover, the cellular behaviour may be determined by components of the ECM, and tumour cells themselves may respond to the extracellular microenvironment in such a way that there may be aberrant changes in the cellular receptors and in their signal transduction mechanisms, as well as gene regulation. Adhesive

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and degradative functions have been shown by many studies to contribute to the metastatic properties of tumour cells [2, 3].

The invasiveness of neoplastic cells is believed to be mediated by a multitude of proteolytic enzymes secreted by tumour cells which act on degradation of the ECM components [4]. An enhanced tumour cell motility and proteolysis is a potential mechanism for cancer cells to penetrate the basement membrane and enter the interstitial stroma, where they may grow *in situ* or may metastasise to distant sites [5]. In addition, proteolysis is also believed to mediate the angiogenesis necessary for continued growth of solid tumours [6]. The metalloproteinases (MMPs), comprising collagenases and stromelysins, are ECM-degrading proteinases and the observations leading to inhibition of tumour invasion by inhibitors of MMPs and the expression of the MMP gene in several types of human neoplasia associated with increased local invasion suggest that MMPs may play a crucial role in tumour invasion and metastasis [4].

TN is a large molecular weight component of extracellular matrix protein that is expressed temporarily and in a site-restricted manner during embryogenesis and fetal development but with a restricted expression in the adult tissues, particularly at sites of cell proliferation, migration and ECM remodelling. It may have a crucial role in the condensing mesenchyme and epithelial mesenchymal interactions during organogenesis in fetal development [7–10]. The expression of TN in early embryo has been found to diminish cell adhesion, and, as a result participate in determining migratory pathways in early development, such as those of neural crest cells [5, 11–13]. In adult tissues, TN is present at sites of continuous cellular renewal, the expression of which is enhanced in the dermis during wound healing, suggesting its role in tissue remodelling [14, 15] and carcinogenesis [10, 16, 17].

The functions of TN in remodelling and affecting the behaviour of neoplastic cells in tumour tissues has not been clearly defined. Normal fibroblasts, epithelial cells, glia and neurons cultured in the presence of TN have a diminished cellular adhesion [7, 18]. However, whether similar interactions exist in neoplastic cells remains to be fully investigated, given such a large number of neoplastic cell types and their heterogeneous characteristics. The destabilisation of cellular adhesion to the ECM could enhance cell motility and alternatively, TN may signal cells to alter the cellular response in ECM remodelling [4, 5, 9, 19].

The present study uses *K-ras*-2 transformed mouse NIH3T3 fibroblasts and normal NIH3T3 as a model for analysing the adhesion modulating function of tenascin and the results are compared with human tongue carcinoma cell lines, (SCCKN) and human salivary gland adenocarcinoma (SGT-1) cell lines. The expression of tenascin is enhanced in a wide variety of neoplastic lesions including those of the oral mucosa [20] and salivary glands [21]. We investigated whether TN is produced by tumour cells and whether its production is under growth factor control. Numerous studies have documented the interaction of TN and FN molecules, and MMP expression is upregulated in response to stimuli that alter cellular adhesion or actin cytoskeleton, as in cells treated with antifibronectin receptor antibodies or 120 kDa chymotryptic fragments of FN [22–25]. Therefore, we compared the adhesive abilities of these cells to tenascin and to fibronectin and laminin, the other two RGDS-dependent, integrin binding proteins, which have a known adhesive function, and further investigated the promotion of growth and the interactions of these ECM proteins in the production of collagenase, an ECM-degrading metalloproteinase, in an *in vitro* model.

MATERIALS AND METHODS

Cells and cell cultures

Mouse embryo NIH 3T3 fibroblast cells, *K-ras* transformed NIH3T3 fibroblasts, Cle-H3 and human tongue carcinoma cells (SCCKN) were obtained from RIKEN Cell Bank at Tsukuba City, Tokyo, Japan. The primary cultures of salivary gland adenocarcinoma cells were a kind gift from Dr C.H. Lee, Dangkok University, Seoul, Korea where the primary cultures were obtained from a poorly differentiated adenocarcinoma of the human submandibular gland. The primary cultures were cloned using the colony cylinders technique and characterised in our laboratory. One of the clones, SGT-1 which was used in the present study has been found to express cytokeratin 8, vimentin, glial fibrillary acidic protein and epidermal growth factor receptor and at an ultrastructural level, these cells were found to possess desmosomal communication and occasional bundles of fibrils running parallel to the cytoplasmic membrane, closely resembling the intercalated and basal cells of salivary ducts (manuscript in preparation). All the cells were maintained in Dulbecco's minimal essential medium DMEM (Gibco, Burlington, Canada) supplemented with 10% fetal bovine serum (FBS) (Gibco). Cell suspensions for passaging and experiments were obtained by brief treatment with 0.05% trypsin and 0.5 mM EDTA.

Secretion of tenascin by epithelial tumour cells

Production of tenascin by epithelial tumour cells was assayed by Western blotting [26] and sandwich enzyme linked immunoassay that is able to detect tenascin in traces in the serum and the conditioned medium from the tumour cells [27]. In brief, TN molecules in the conditioned medium were concentrated by 40% ammonium sulphate precipitation and centrifuged at 10000 rpm for 30 min. The pellets were dissolved in 10 mM Tris–HCl containing 1 mM EDTA at pH 8, heat denatured and SDS–PAGE electrophoresis was performed with PhastGel gradient 8–25 separation method file 110 of PhastSystem (Pharmacia LKB, Sweden). The proteins were transferred to an Immobilin transfer membrane (Millipore, Tokyo, Japan) using the PhastTransfer development technique file No. 221 (Pharmacia LKB). The first antibody used was MAB 1927 anti-tenascin (Chemicon, U.S.A.) and the enzymatic activity was visualised by DAB.

The concentrated conditioned medium was used in a sandwich enzyme immunoassay for quantitative analysis of secretion of tenascin by the cells with or without TGF-β [27]. In brief, $F(ab)_2$, fragments of polycolonal anti-tenascin antibodies (Chemicon, U.S.A.) were immobilised on polystyrene balls (3.2 mm in diameter, Immunochemical Inc. Okayama, Japan) and incubated at 37°C for 3 h by shaking with various amounts of purified human tenascin (JIMRO, Japan) or concentrated medium in 96-well plates. The reaction medium was aspirated and washed with sodium phosphate buffer and treated with β-D-galactosidase labelled monoclonal antibody (Dr Kusakabe, Tsukuba Life Science Center, Tsukuba City, Japan) overnight at 4°C. The galactosidase activity bound to the polystyrene balls was assayed with 0.15 mM 4-methylumbeliferyl β-D-galactosidase (Sigma, St Louis, Missouri, U.S.A.) and the relative absorbance at 450 nm measured using the BIORAD enzyme immunoassay.

Cell adhesion assays

Cell attachment on coated substrates was measured using two adhesion assays, firstly by staining cells that had adhered and spread on the coated surface and counting cells under a microscope and secondly by metabolically labelling cells and measuring the adhered cells by scintillation counting. All experiments were conducted in triplicate and the two assays gave comparable results. In the first assay, exponentially growing cells were trypsinised and were resuspended in attachment medium, serum-free DMEM containing 2 mg/ml bovine serum albumin (heat inactivated fraction V, Sigma). Cells at 2×10^4/wells were added to 96-well polystyrene plates that had been precoated by fibronectin (FN), laminin (LN), tenascin (TN), fibronectin/tenascin (FN/TN) or tenascin/laminin (TN/LN) using coating procedures described by Chiquet-Ehrismann *et al.* [28]. Human FN was purchased from Becton-Dickinson, U.K., LN from Chemicon Inc., California, U.S.A. and TN from JIMRO, Japan. After seeding on the coated surface, the cells were allowed to adhere at 37°C for a specified period of time ranging from 30 min to 6 h. Non-adherent cells were removed by rinsing three times in Ca^{2+} and Mg^{2+} free phosphate buffered saline and adherent cells were fixed with 1% glutaraldehyde, stained with haematoxylin and counted under the microscope.

In an alternative adhesion assay, exponentially growing cells were labelled by incubation for 24 h with [methyl-^{3}H] thymidine 3 μCi/ml; Amersham, Japan). The labelled cells were trypsinised and added to coated plates as described previously. The adherent cells were lysed with 1% sodium docedyl sulphate and 0.5 M NaOH for 1 h and radioactivity was measured by scintillation counting, corrected for quenching and the percentage of adhesion was determined by comparison with the total amount of radioactivity associated with cells added to each well.

Synthetic peptides RGDS and GRGESP were obtained from Sigma and diluted as directed by the manufacturer. When used in adhesion assays, the peptides were added to the coated wells before addition of the cells.

Growth-enhancing activity of TN

Cells grown in DMEM containing 10% FBS at 37°C, 5% CO_2 until confluence were obtained by brief trypsinisation and seeded at 1×10^5 cells/well and incubated in DMEM containing 10% FBS for 24 h. Soluble TN was added and cultured for 12 h. [Methyl-^{3}H] thymidine 3 μCi/ml was then added, incubated for 30 min and the cells were then washed three times with Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS). The attached cells were lysed and the radioactivity measured by scintillation counting.

Secretion of collagenase by tumour cells on FN, LN, TN, TN/FN and TN/LN substrate

Cells at 1×10^5 suspended in DMEM and 10% FBS were seeded on chamber slides precoated with FN (20 nM), LN (10 nM), TN (10 nM)/FN (20 nM) or TN (10 nM)/LN (20 nM), and cultured for 6–18 h. Monoclonal anti-human collagenase, mouse IgG1 clone MAB 1902 which recognise all tissue derived collagenase (Chemicon Inc., U.S.A.) and FITC labelled IgG (Dako, Denmark) were used to detect the immunofluorescence of collagenase. Immunoblotting using human collagenase and anticollagenase MAB 1902 showed specific bands recognising tissue collagenase (MMP-1) and Mr 92 000 type IV collagenase (data not shown).

RESULTS

Production of tenascin by tumour cells

Epithelial tumour lines, which were not able to secrete tenascin under normal *in vitro* growth conditions were able to produce TN upon addition of TGF-β in the culture medium (Fig. 1) and the secretion was dose-dependent. Figure 2 shows the dose-dependent curve for TGF-β induced tenascin secretion in the cell lines determined by sandwich enzyme assay.

Growth-enhancing function of TN

Addition of TN to the culture medium enhanced the incorporation of radiolabelled thymidine in all the cell lines studied. The increased thymidine incorporation in cells upon addition of TN is shown in Fig. 3.

Cellular adhesion assay

Adhesion of cells to substrates coated with TN, FN and LN (*Figs* 4–6). We compared the ability of human tongue carcinoma cells (SCCKN), salivary gland adenocarcinoma cells (SGT-1), mouse embryo fibroblasts NIH3T3 and *K-ras* transformed Cle-H3 to adhere and spread on the polystyrene surface coated with TN, FN and LN, depending on the

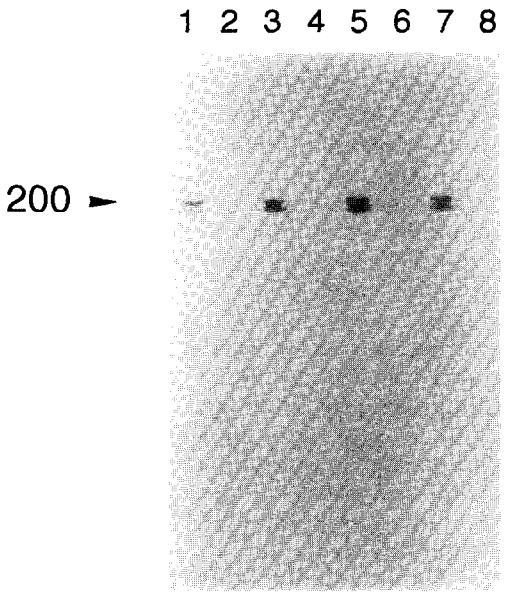

Fig. 1. Immunoblotting for TN. After SDS-PAGE electrophoresis of the conditioned medium using phastSystem (LKB Pharmacia), the proteins were transferred to nitrocellulose membrane and stained with monoclonal antibody antitenascin MAB 1927. Lanes 1, 3, 5, 7: conditioned medium from human tongue carcinoma cell line (SCCKN), salivary gland adenocarcinoma (SGT-1) cell line, NIH3T3 and Cle-H3 fibroblasts, respectively, upon addition of TGF-β (10 nM) for 48 h. Lanes 2, 4, 6 and 8: conditioned medium from the same cells without addition of TGF-β.

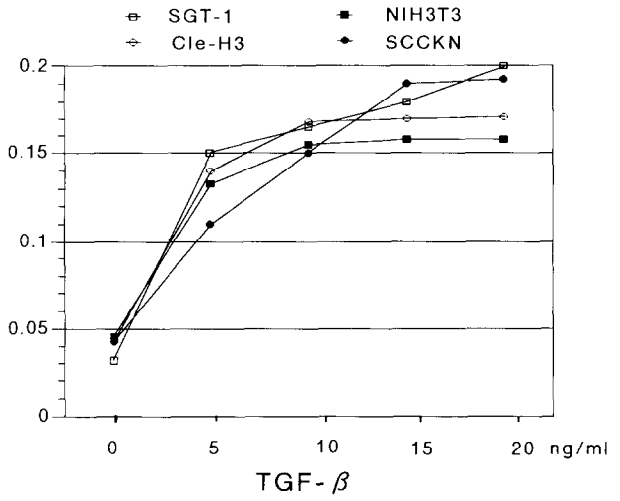

Fig. 2. Dose–response curve for TGF-β-induced TN secretion by SGT-1, Cle-H3, NIH3T3 and SCCKN: absorbance at 405 nm.

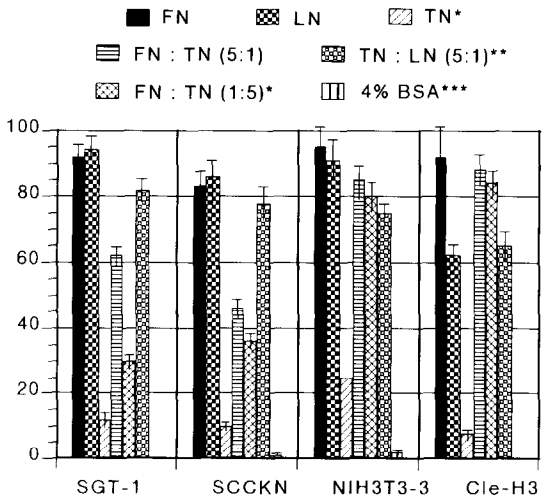

Fig. 4. Cellular adhesion assay: percentage of attached cells after 1 h (mean ± S.D.). * Cells remained rounded and failed to spread; ** a comparable result was obtained with tenascin and laminin at 1:5; * control experiment.**

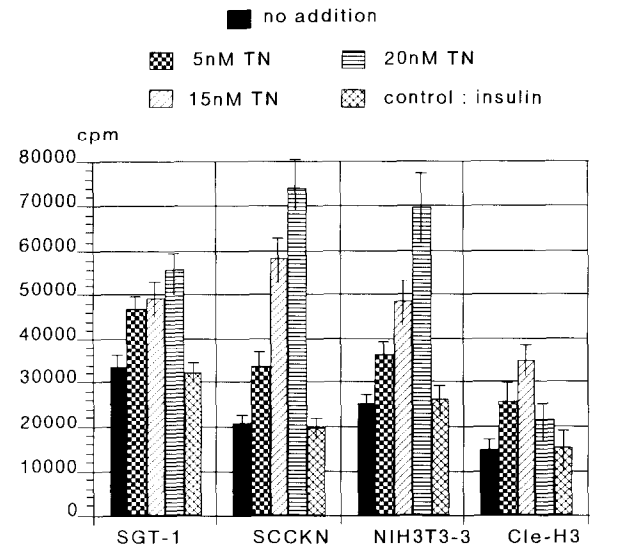

Fig. 3. Increased uptake of thymidine in cells upon addition of TN in the culture medium: [methyl-^{3}H] thymidine incorporation (cpm). The experiment was carried out in triplicate wells.

concentration of the coating substrates. At an optimum concentration (5–10 μg/ml for FN, 2–5 μg/ml of LN and 3–20 μg/ml of TN when a consistent and maximum cellular adhesion specific to the substrate on test was obtained), all the cell lines adhered well on FN. However, in contrast, Cle-H3 adhered poorly on laminin and the morphology of adhered cells was not uniform. On the TN coated surface, all the cells adhered poorly and remained rounded. The adhesion of these cells was transient on precoated surfaces treated with bovine serum albumin. Maximum adhesion was obtained at 30 min to 1 h, and after 180 min the adhesion was decreasing with the passage of time. The cells remained adhered when 10% FBS was added to the growth medium.

We also tested the cellular adhesion on combined substrate comprising FN, TN and LN in different proportions. On TN and FN, the presence of a higher concentration of TN was responsible for rounding of SCCKN and SGT-1 cells, whereas the presence of higher concentrations of FN showed adherent cell morphology similar to that on FN alone. TN did not interfere with the attachment of SCCKN and SGT-1 on LN. NIH3T3 and Cle-H3 adhered, albeit less, on FN/TN or TN/LN and were able to spread. Therefore, TN interfered with the cellular attachment of SCCKN and SGT-1 on fibronectin and the cellular morphology and adhesion efficiency were affected by the relative concentrations of TN and FN. On the other hand, TN did not interfere with the cellular attachment of NIH3T3 and Cle-H3 fibroblasts on FN and LN.

Adhesion of the cell lines to FN, LN and TN was incompletely inhibited (50–70%) by the addition of synthetic RGDS peptide at 75–100 μM but no effect was observed with the control GRGESP peptide. Soluble TN added in the exponentially growing cells, after 48 h of addition, resulted in a loss of cell to cell contacts and the cells were seen retracting from each other (Fig. 7).

The presence of TN and FN on the adhesion surface enhanced the expression of collagenase

Immunofluorescence for collagenase was observed in the neoplastic cells but not in the normal NIH3T3 fibroblasts when the cells were cultured on FN/TN coated substrate (Fig. 8). Substrates coated with FN, LN or TN alone were unable to induce collagenase in the tumour cells.

DISCUSSION

At least three different mechanisms by which the extracellular matrix can regulate cell behaviour have been suggested [29]: firstly, via the composition of the ECM proteins in a given tissue; secondly, through synergistic interactions between growth factors and the ECM molecules; and finally, through the cell surface receptors that mediate adhesion to the ECM components. In the present study, we have shown that TN can be secreted by both epithelial and mesenchymal cells in the presence or TGF-β. TN alone may have a growth factor-like activity and enhances cell proliferation as also

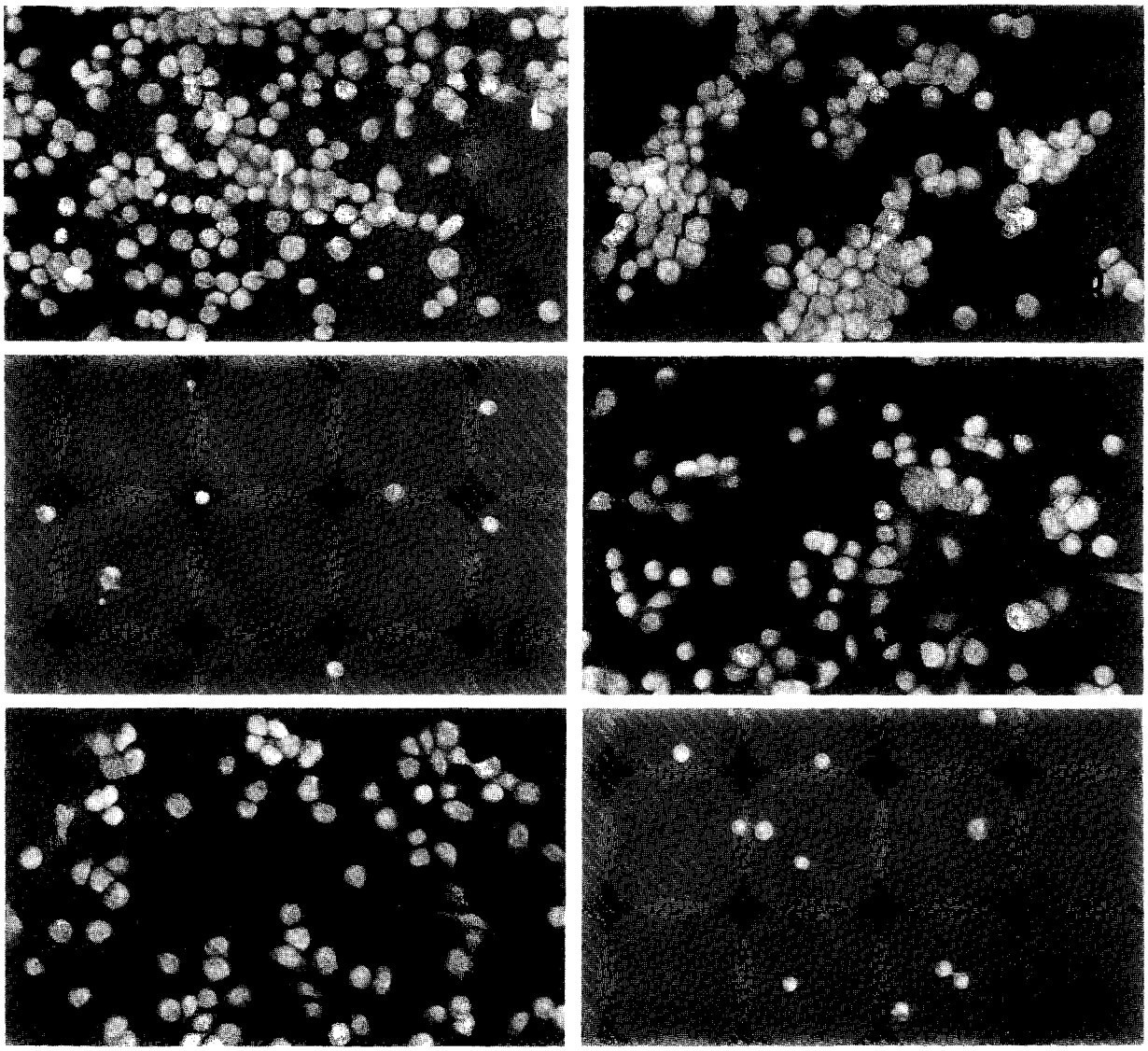

Fig. 5. Adhesion of salivary gland adenocarcinoma SGT-1 at 1 h on (A) fibronectin, (B) laminin, (C) tenascin, (D) tenascin and laminin, (E) fibronectin and tenascin 5:1 and (F) fibronectin and tenascin 1:5 coated substrate. The cells attached well on fibronectin and laminin. The adhesion was poor on tenascin and the cells remained rounded. Tenascin did not interfere with cellular adhesion on laminin and the relative concentration of tenascin and fibronectin affected the cellular adhesion and morphology. A presence of a higher concentration of tenascin interfered with cellular attachment on fibronectin.

suggested by End *et al.* [30]. A combination of TN and other growth factors and alterations in cell surface receptors of neoplastic cells may act synergistically to modulate the growth of different cell types and may affect the tumour progression, invasive behaviour and metastatic potential.

One of the potential functions of TN that has received much attention, in numerous studies, is that of adhesion modulating properties of neoplastic cells on ECM coated surfaces, but there are mixed results [28, 31–37]. In adhesion assays using normal and oncogene-transformed cells, the ability of neoplastic cells to adhere to the ECM proteins have been found to be either reduced, increased or unchanged [38, 39]. During tumour invasion and metastasis, existing contacts have to be broken and newer contacts formed between cell–cell or cell ECM. These interactions at the molecular level are indeed complex, as might be expected, for such a wide range of cell surface molecules and ligands that may interact in cellular adhesion, and TN has both cell adhesion and an anti-adhesion domain [40]. Our present study, employing human tongue and salivary gland carcinoma cells as well as normal and oncogene-transformed fibroblasts, was designed to evaluate the potential of modulating cellular adhesion and ECM degradation by TN in an *in vitro* model in combination with FN and LN, the two most widely studied extracellular matrix proteins in cellular adhesion.

In the present study, we were able to demonstrate that while the binding potential of normal and neoplastic cells with FN was similar, the *K-ras*-transformed fibroblasts adhered less to LN, which also affected the cellular morphology of the adhered neoplastic cells. TN, on the other hand, affected the cell adhesion and spread on the coated surface but its binding potential to all cells was albeit decreased when combined with FN and LN, and the relative concentration of TN in comparison with FN was most prominent in affecting the cellular binding and morphology of SCCKN and SGT-1. However, the presence of LN or FN in addition to TN

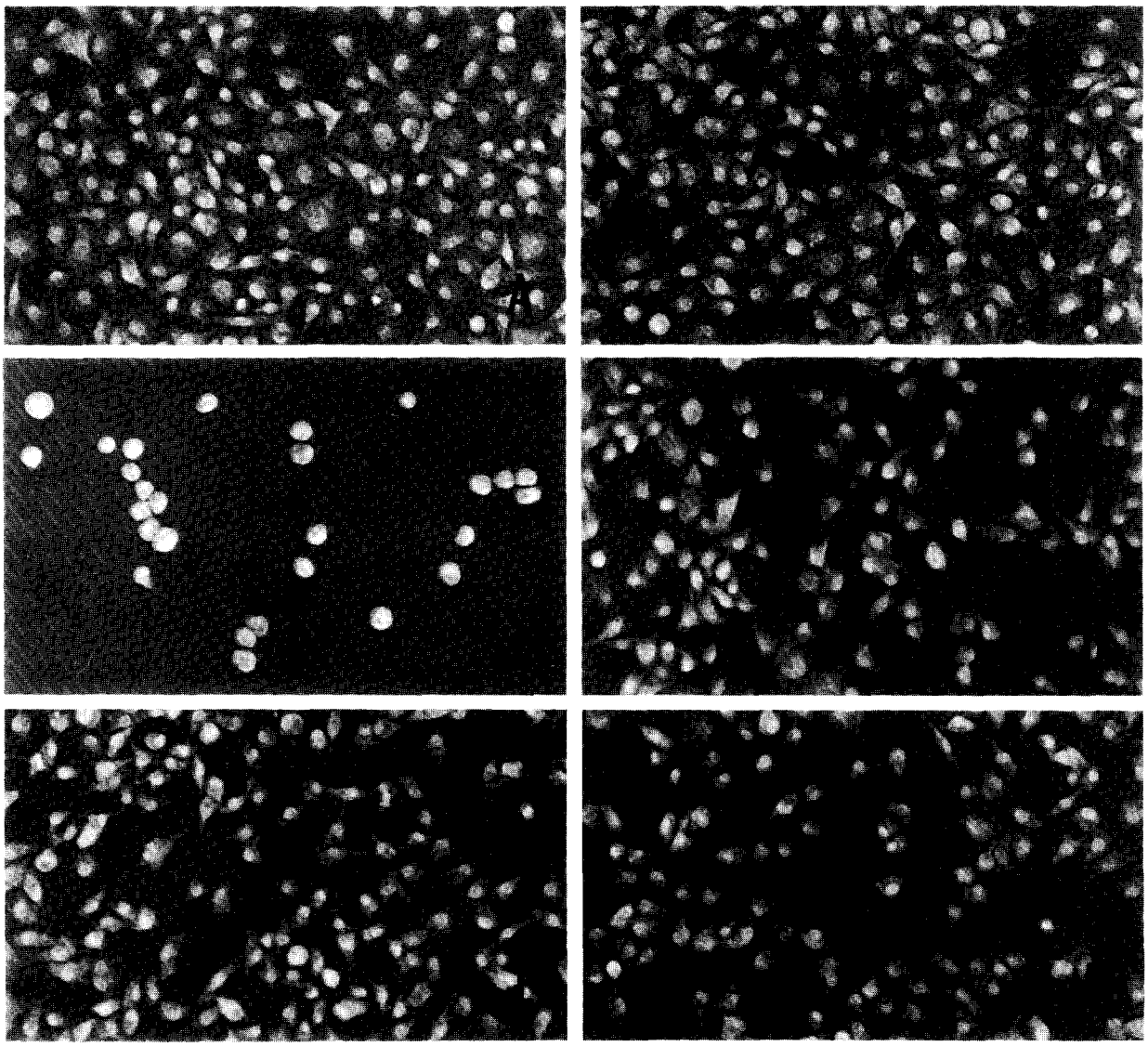

Fig. 6. Adhesion of NIH3T3 fibroblasts at 1 h on (A) fibronectin, (B) laminin, (C) tenascin, (D) tenascin and laminin, (E) fibronectin and tenascin 5:1 and (F) fibronectin and tenascin 1:5. The cells attached well on fibronectin and laminin. However, on tenascin the attachment was poor and the cells remained rounded. On fibronectin and tenascin and tenascin and laminin coated substrate, the cells attached, albeit less than on fibronectin or laminin alone.

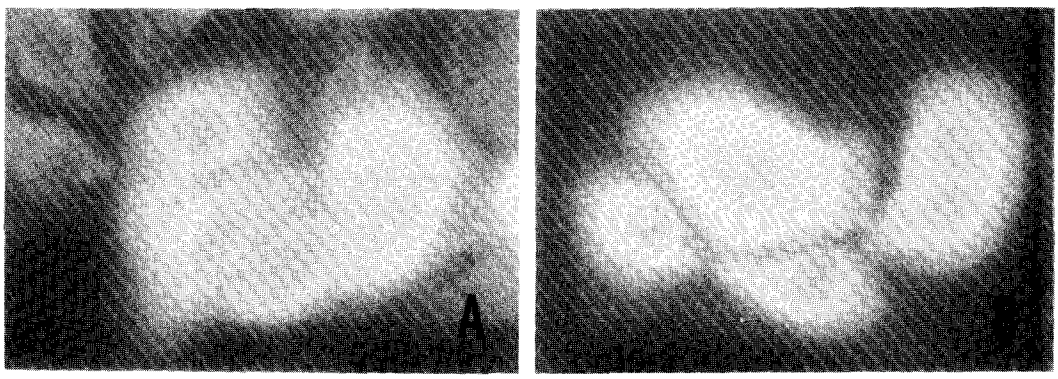

Fig. 7. Human tongue carcinoma cells, SCCKN—immunofluorescence for cytokeratin 8. The cells are growing under (A) normal growth medium and (B) 48 h after addition of tenascin where the cells are losing cell to cell contact and are separating.

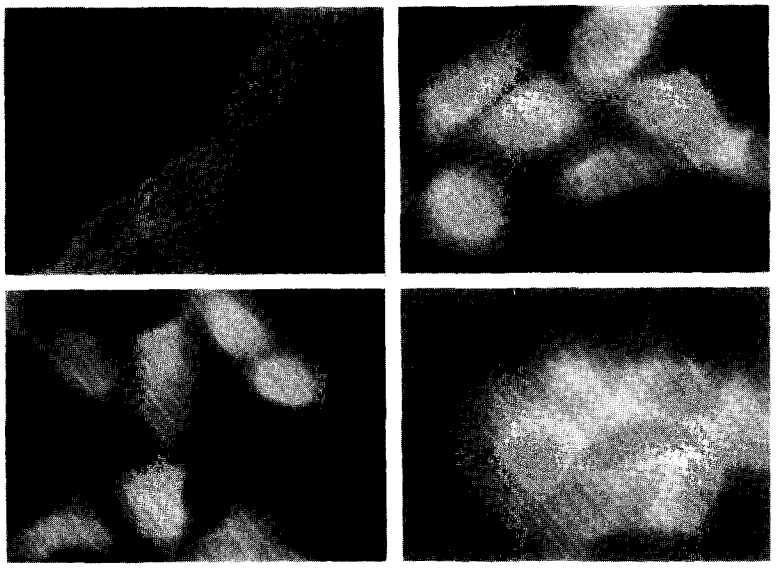

Fig. 8. Neoplastic cells, but not non-neoplastic cells, on fibronectin/tenascin substrate show immunofluorescence for collagenase. (A) NIH3T3 fibroblasts show no immunofluorescence for collagenase, (B) human tongue carcinoma cells SCCKN, (C) *K-ras*-2 transformed Cle-H3 fibroblasts and (D) human salivary gland adenocarcinoma cells SGT-1 show immunofluorescence when grown on fibronectin/tenascin substrate.

nullified the effect of TN in NIH3T3 and Cle-H3 cells. These findings may imply that TN interferes with the action of FN in SCCKN and SGT-1 but not in NIH3T3 and Cle-H3 on LN. Therefore, the binding potential of a cell to ECM proteins may not necessarily distinguish the invasive or metastatic cells from the normal cells, which may differ in different cell types. However, differences in binding potential may certainly lead to signalling pathways rather than a simple attachment to the substrate, which may, in turn, affect the cellular morphology and invasive behaviour of a given cell type. As supported by our findings, it may be that binding to TN, LN and FN was rather transient, with maximum adhesion being observed by 30–60 min.

As interactions of cell surface receptors that bind with ECM molecules may mediate signal transduction, resulting in activation of a variety of cell functions [25, 41, 42], we were able to demonstrate the expression of collagenase, one of the potent component in degrading the basement membrane and extracellular matrix proteins by invading tumour cells when grown on matrix coated with FN and TN in combination. Due to the transient adhesion of cells on FN/TN substrate and subsequently as cells were easily washed away from the adhered surface, we were unable to carry out quantitative altertions in the production of collagenase. In the present study, FN, TN, LN alone or TN and LN combined matrix could not modulate the production of collagenase which may participate in the degrading functions of ECM components.

Furthermore, we were able to demonstrate that cellular binding to FN, LN and TN are incompletely inhibited by RGDS peptide. Tenascin has been found to mediate cellular attachment through a RGD-dependent receptor [32]. RGDS-independent cell adhesion sites have been identified [43, 44]. However, the cellular receptors for TN are yet to be fully characterised. Therefore, TN may have binding sites other than RGDS-dependent sites. We have observed that the secretion of TN by tumour cells is under growth factor control and is capable of transmitting a signal such as secretion of collagenase. The nature of the signal and the effects on the responding cells are, however, unknown. The results of the present study raise the possibility that TN may be secreted by tumour cells and could function in an autocrine signal transduction fashion by transmitting an as yet undetermined signal that might play a role in tumour growth, differentiation, invasion and metastasis. These data further suggest that alteration of the composition of the ECM by addition of proteins such as TN, in combination with other ECM proteins, may regulate cellular behaviour. The secretion of TN by tumour cells themselves and an interaction with FN may induce ECM-degrading enzymes such as collagenase in an *in vitro* model. If the same is true *in vivo*, although a number of factors and interactions may implicate the ultimate outcome, TN may have a potential role in tumour growth, adhesion, migration, tissue remodelling, invasion and metastasis.

1. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 1991, **64**, 327–336.
2. Nicolson GL. Molecular mechanisms of cancer metastasis: tumor and host properties and the role of oncogenes and suppressor genes. *Curr Opinion Oncol* 1991, **3**, 75–92.
3. Lester BR, McCarthy JB. Tumor cell adhesion to extracellular matrix and signal transduction mechanisms implicated in tumor cell motility, invasion and metastasis. *Cancer Metas Rev* 1992, **11**, 31–44.
4. Alexander CM, Werb Z. Extracellular matrix degradation. In Hay E D, ed. *Cell Biology of Extracellular Matrix*, 2nd edn. New York, Plenum Press, 1991, 255–302.
5. Tucker RP, McKay SE. The expression of tenascin by neural crest cells and glia. *Development* 1991, **112**, 1031–1039.
6. Liotta LA. Tumor invasion and metastasis—role of extracellular matrix: Rhoads Memorial Award Lecture. *Cancer Res* 1986, **46**, 1–7.
7. Chiquet-Ehrismann R, Matsuoka Y, Hofer U, Spring J, Bernasconi C, Chiquet M. Tenascin variants: differential binding to fibronectin and distinct distribution in cell cultures and tissues. *Cell Reg* 1991, **2**, 927–938.

8. Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T. Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. *Cell* 1986, **47**, 131–139.
9. Ekblom P. Aufderheide E. Stimulation of tenascin expression in mesenchyme by epithelial–mesenchymal interactions. *Int J Dev Biol* 1989, **33**, 71–79.
10. Erickson HP, Bourdon MA. Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. *Annu Rev Cell Biol* 1989, **5**, 71–92.
11. Chiquet M, Wehrle-Haller B, Koch M. Tenascin (cytotactin): an extracellular matrix protein involved in morphogenesis of the nervous system. *Semin Neurosci* 1991, **3**, 341–350.
12. Halfter W, Chiquet-Ehrismann R, Tucker RP. The effect of tenascin and embryonic basal lamina on the behavior and morphology of neural crest cells *in vitro*. *Dev Biol* 1989, **132**, 14–25.
13. Wehrle B, Chiquet M. Tenascin is accumulated along peripheral nerve and allow neurite outgrowth *in vitro*. *Development* 1990, **100**, 401–415.
14. Mackie EJ, Halfter EW, Liverani D. Induction of tenascin in healing wounds. *J Cell Biol* 1988, **107**, 2757–2767.
15. Murakami R, Yamaoka I, Sakakura T. Appearance of tenascin in healing skin of the mouse: possible involvement in seaming of wounded tissues. *Int J Dev Biol* 1989, **33**, 439–444.
16. Anbazhagan R, Sakakura T, Gusterson BA. The distribution of immunoreactive tenascin in the epithelial–mesenchymal junctional areas of benign and malignant epithelia. *Virchows Arch B Cell Pathol* 1990, **59**, 59–63.
17. Chiquet-Ehrismann R. What distinguishes tenascin from fibronectin? *FASEB J* 1990, 4, 2598–2604.
18. Sage EH, Bornstein P. Extracellular proteins that modulate cell-matrix interactions. SPARC, tenascin, and thrombospondin. *J Biol Chem* 1991, **266**, 14831–14834.
19. Whitby DJ, Longaker MT, Harrison GK, Adzick NS, Ferguson MW. Rapid epithelization in fetal wounds is associated with the early deposition of tenascin. *J Cell Sci* 1991, **99**, 583–586.
20. Shrestha P, Sakamoto F, Takagi H, Yamada T, Mori M. Enhanced tenascin immunoreactivity in leukoplakia and squamous cell carcinoma of the oral cavity: an immunohistochemical study. *Oral Oncol, Eur J Cancer* 1994, **30B**, 132–137.
21. Shrestha P, Sumitomo S, Ogata K, *et al.* Immunoreactive tenascin in tumors of salivary glands: evidence for enhanced expression in tumor stroma and production by tumor cells. *Oral Oncol, Eur J Cancer* 1994, **30B**, 393–399.
22. Aggeler JM, Frisch SM, Werb Z. Collagenase is a major gene product of induced rabbit synovial fibroblasts. *J Cell Biol* 1984, **98**, 1656–1661.
23. Tremble P, Chiquet-Ehrismann R, Werb Z. The extracellular matrix ligands fibronectin and tenascin collaborate in regulating collagenase gene expression in fibroblasts. *Mol Biol Cell* 1994, **5**, 439–453.
24. Unemori EN, Werb Z. Reorganization of polymerized actin: a possible trigger for induction of procollagenase in fibroblasts cultured in and on collagen gels. *J Cell Biol* 1986, **103**, 1021–1031.
25. Werb Z, Tremble P, Behrendtsen O, Crowley E, Damsky CH. Signal transduction through the fibronectin receptor introduces collagenase and stromelysin gene expression. *J Cell Biol* 1989, **109**, 877–889.
26. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970, **227**, 680–685.
27. Washizu K, Kimura S, Hiraiwa H, *et al.* Development and application of an enzyme immunoassay for tenascin. *Clin Chim Acta* 1993, **218**, 15–22.
28. Chiquet-Ehrismann R, Kalla P, Pearson CA, Beck K, Chiquet M. Tenascin interferes with fibronectin action. *Cell* 1988, **53**, 383–390.
29. Adams JC, Watt FM. Regulation of development and differentiation by the extracellular matrix. *Development* 1993, **117**, 1183–1198.
30. End P, Panayatou G, Entwistle A, Waterfield MD, Chiquet M. Tenascin: a modulator of cell growth. *Eur J Biochem* 1992, **209**, 1041–1051.
31. Aukhil I, Slemp CC, Lightner VA, Nishimura K, Briscoe G, Erickson HP. Purification of hexabrachion (tenascin) from cell culture conditioned medium, and separation from a cell adhesion factor. *Matrix* 1990, **10**, 98–111.
32. Bourdon MA, Rouslahti E. Tenascin mediates cell attachment through an RGD-dependent receptor. *J Cell Biol* 1989, **108**, 1149–1155.
33. Grumet M, Hoffman S, Crossin KL, Edelman GM. Cytotactin, an extracellular matrix protein of neural and non-neural tissues that mediate glia-neuron interaction. *Proc Natl Acad Sci USA* 1985, **82**, 8057–8079.
34. Kruse J, Keilhauer G, Faissner A, Timpl R, Schachner M. The J1 glycoprotein—a novel nervous system cell adhesion molecule of the L2/HNK-1 family. *Nature* 1985, **316**, 146–148.
35. Lightner V, Erickson HP. Binding of hexabrachion (tenascin) to the extracellular matrix and substratum and its effect on cell adhesion. *J Cell Sci* 1990, **95**, 263–277.
36. Lotz MM, Burdsal CA, Erickson HP, McClay DR. Cell adhesion to fibronectin and tenascin: quantitative measurements of initial binding and subsequent strengthening response. *J Cell Biol* 1989, **109**, 1795–1805.
37. Spring J, Beck K, Chiquet-Ehrismann R. Two contrary functions of tenascin: dissection of the active sites by recombinant tenascin fragments. *Cell* 1989, **59**, 325–334.
38. Chakrabarty D, Jan Y, Levine A, McClenic B, Varani J. Fibronectin/laminin and their receptors in aberrant growth control in FR3T3 cell transformed by ha-ras oncogene and epidermal growth factor gene. *Int J Cancer* 1989, **44**, 325–331.
39. Plantfaber LC, Hynes RO. Changes in integrin receptors on oncogenically transformed cells. *Cell* 1989, **56**, 281–290.
40. Prieto AL, Anderson-Fiscone C, Crossin KL. Characterization of multiple adhesive and counteradhesive domains in the extracellular matrix protein cytotactin. *J Cell Biol* 1992, **119**, 663–678.
41. Damsky CH, Werb Z. Signal transduction by integrin receptors for extracellular matrix: cooperative processing of extracellular information. *Curr Opin Cell Biol* 1992, **4**, 772–781.
42. Hynes RO. Integrins, versatility, modulation and signaling in cell adhesion. *Cell* 1992, **69**, 11–25.
43. Vaughan L, Zisch AH, Weber P, *et al.* Cellular receptors for tenascin. In *Extracellular Matrix in the Kidney. Contrib Nephrol* Karger, Basel, 1994, **107**, 80–84.
44. Zisch AH, D'Alessandri L, Ranscht B, Falchetto R, Winterhalter KH, Vaughan L. Neuronal cell adhesion molecule contactin/F11 binds to tenascin via its immunoglobulin-like domains. *J Cell Biol* 1992, **119**, 203–213.

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