

Fibroblast remodeling of adsorbed collagen type IV is altered in contact with cancer cells

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Summary. A series of co-culture experiments between fibroblasts and H-460 human lung carcinoma cells were performed to learn more about the fate of adsorbed type IV collagen (Coll IV). Fibroblasts were able to spatially rearrange Coll IV in a specific linear pattern, similar but not identical to the fibronectin (FN) fibrils. Coll IV partly co-aligns with fibroblast actin cytoskeleton and transiently co-localize with FN, as well as with $\beta 1$ and $\alpha 2$ integrin clusters, suggesting a cell-dependent process. We further found that this Coll IV reorganization is suppressed in contact with H460 cells. Zymography revealed strongly elevated MMP-2 activity in supernatants of co-cultures, but no activity when fibroblasts or cancer cells were cultured alone. Thus, we provide evidence that reorganization of substrate associated Coll IV is a useful morphological approach for *in vitro* studies on matrix remodeling activity during tumorigenesis.

Key words: Adsorbed collagen IV reorganization, Fibroblasts and cancer cells co-culture, MMP-2

Introduction

It is now widely accepted that the dynamic cells behavior in tissues is mainly regulated by stimuli from the extracellular matrix (ECM). In addition to its conventional role in providing a scaffold for building tissues, the ECM acts as a directional highway for cellular movement (Silletti et al., 1998; Tiger et al., 2001) and provides instructional information for regulating terminal cell differentiation (Watt, 2002), cell growth (Danen and Yamada, 2001), as well as tumor

progression and metastasis (Felding-Habermann, 2003; Bhowmick et al., 2004). Tissue cells deposit in the ECM various fibrillar and non-fibrillar macromolecules, which form a composite structure consisting of matrix proteins, such as collagen, fibronectin (FN) and laminin, embedded in the pool of proteoglycans (Ayad et al., 1994). Once synthesized, the adhesive proteins are spatially organized (mainly by the mesenchymal cells) at different levels of complexity, ranging from the three-dimensional fibrillar network of the rough connective tissues (Humphries et al., 2004; Larsen et al., 2006) to the sophisticated structure of basement membrane (Kuhn, 1994; Kalluri, 2003). The cells interact with the ECM via integrins (Humphries et al., 2006), a family of transmembrane glycoproteins consisting of non-covalently associated α and β -subunits (Luo and Springer, 2006) which are responsible for the modulation of various cell functions through specific cell signaling (Mitev and Miteva, 1999; Stupack and Cheresch, 2002; Clark et al., 2005). It is well documented that cancer cells lose their ability to produce and organize ECM (Silletti et al., 1998; Yi and Ruoslahti, 2001; Felding-Habermann, 2003; Larsen et al., 2006) tending to destroy and invade the basement membranes (Deriyugina et al., 1998; DeClerck et al., 2004). The major enzymes that degrade ECM are the matrix metalloproteinases (MMP) (Stamenkovic, 2003), tissue plasminogen activator (tPA) (Myohanen and Vaheri, 2004) and urokinase (Wei et al., 2007), but it seems that MMP-2 plays an important role in tumorigenesis (Strongin et al., 1995; Steffensen et al., 2002; Stamenkovic, 2003; DeClerck et al., 2004), as it is capable of degrading type IV collagen (Coll IV) - a main microfilamentous component of the basement membranes (Kuhn, 1994; Kalluri, 2003). In general, the fate of the collagen, and particularly the Coll IV matrix depends on the balance between its synthesis and degradation (Amano et al., 2001; Sottile and Hocking,

2002), which are strictly controlled by stimuli from the surrounding cells (Lynch and Matrisian, 2002; Larsen et al., 2006), which alter with tumor progression and metastasis (Saad et al., 2002; Stewart et al., 2004; Wong and Hynes, 2006). In addition, cancer cells are able to induce specific matrix remodeling responses in other cell types, for example, in endothelial cells to start neovascularization (Kalluri, 2003; Wong and Hynes, 2006), but also in stromal fibroblasts to provoke local matrix degradation (Sato et al., 1999, 2004; Stewart et al., 2004). A line of investigations show that tumor cells alter fibroblast collagen matrix, inducing both activation of MMP-2 (Boyd and Balkwill, 1999; Zigrino et al., 2000; Saad et al., 2002) and down regulation of collagen synthesis (Fenhalls et al., 1999). It is not clear however if they disturb its organization.

Studying the balance between matrix assembly and degradation during tissue remodeling is important for understanding mechanisms that regulate a great number of normal and pathological processes, such as development and wound healing, as well as ischemic injury, atherosclerosis, angiogenesis, invasion and metastasis (Deryugina et al., 1998; Tiger, et al., 2001; Lynch and Matrisian, 2002; Kalluri, 2003; Stamenkovic, 2003; DeClerk et al., 2004). An elevated expression of matrix metalloproteinases (MMPs) has been correlated with the growth and metastasis of preexisting tumors (Golubkov and Strongin, 2007). In the line of tumor-stromal cell interactions, the aim of the present study was to provide new morphological insights into the concept that tumor cells are able to constrain stromal cells to remodel Coll IV matrix. For that purpose, we co-cultured fibroblasts and H460 lung carcinoma cells on substrata pre-adsorbed with Coll IV, with the idea of following the fate of this non-fibrillar protein by immunofluorescence. Surprisingly, we found that fibroblasts alone were able to remove and rearrange Coll IV in a specific linear pattern, which transiently co-localize with fibronectin fibrils, and both $\beta 1$ and $\alpha 2$ integrins. Considering the possible physiological relevance of this phenomenon, we further extended this study to learn more about the expected balance between matrix organization and matrix degradation activity of

fibroblasts, and to see if it is altered in contact with cancer cells. Details of this investigation are presented herein.

Material and methods

Cell cultures

Human dermal fibroblast cell line CCD-25SK was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA; #1474). Normal human lung fibroblasts (NHLF) were purchased from Clonetics (via Cell Systems, St. Katharinen, Germany; #CC-2512). The human lung carcinoma cell line H460 was purchased from NCI (Frederick Cancer Research Facility, Frederick, MD, USA; #503473). CCD-25SK cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, RMB; #638307) supplemented with 10% fetal bovine serum (FBS, Life - Technologies, Paisley, Scotland; #16140) and 2 mM L-Glutamine (RMB; #210277), whereas cancer cells were cultured in RPMI-1640 (RMB, #209945) with 10% FBS and 2 mM L-Glutamine (RMB; #210277).

Co-culture experiments

To study the fate of adsorbed Coll IV, glass coverslips (22x22 mm, Assistent, Germany) were placed in 6-well tissue culture plates (Costar #CLS3506) and coated with 50 $\mu\text{g/ml}$ Coll IV (Sigma, #C0543) for 30 min at room temperature. Collagen was diluted in 0.1% acetic acid. After three times washing with PBS, 2.5×10^4 fibroblasts were added to each well in 2 ml serum-containing RPMI medium and left to spread for 2 h in a humidified CO_2 incubator. Then 1.2×10^5 H460 cells were added (usually 5:1 ratio if not specially indicated) in 1 ml medium and cultured for an additional 4 h (short-term experiments), or up to 24 hours (long-term experiments), then fixed and processed for immunofluorescence for Coll IV (see Table 1).

The samples to be processed for gelatin zymography (see below) were treated in different way, following the routine protocol. Briefly, the co-cultivation here was

Table.1. Protocol for Immunofluorescent stainings (see Materials and Methods).

Antigen	Primary antibody	Dilution	Conditions	Pretreatment	Secondary antibody	Visualization
Collagen IV	Mouse monoclonal antibody against human Coll IV from Chemicon	1:500	"	no	Goat anti-mouse Cy3 labeled antibody from Jackson Immunoresearch	
Fibronectin	Rabbit polyclonal anti FN antibody from Sigma	1:100	"	no	Goat anti-rabbit Cy2 labeled antibody from Jackson Immunoresearch	Laser Scanning Confocal Microscope type LSM 53 (Zeiss)
Actin	FITC labeled Body-P phalloidin from Molecular Probes	1:30	20°C, 20 min	yes	no	
$\alpha 2$ integrin	Mouse monoclonal antibody against $\alpha 2$ integrin from Chemicon	1:100	20°C, 30 min	yes	Goat anti-mouse Cy3 labeled antibody from Jackson Immunoresearch	
$\beta 1$ integrin	Mouse monoclonal antibody against $\beta 1$ integrin from Sigma	1:100	"	yes	"	

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carried out in serum-free DMEM/F12 (Life Technologies, Paisley, Scotland; #21331-020), supplemented with 2 mM L-Glutamine and Penicillin/Streptomycin (RMB #210404). Fibroblasts were seeded at 2.5×10^4 cells/cm² in 24-well cell culture plates (Costar, USA; #3524) for 2 h, and then HM or LM H460 cells were added in different ratios (as indicated) and co-cultured for an additional 48 h. The conditioned media were collected, centrifuged at 2000 rpm in an Eppendorf centrifuge and processed for gelatin zymography as below.

Gelatin Zymography

Gelatinolytic activity in the supernatants was detected after 4-fold concentration on a 10 kDa filter Amicon (Microcon Centrifugal Filter Devices YM-10; #42407), then 15 μ l aliquot of each supernatant was mixed with 15 μ l sample buffer (0.04 M Tris HCl pH 6.8, 4% SDS, 33% glycerin, 0.04% bromphenol blue) and heated to 100°C for 10 min. The samples were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using an 8% gel containing 0.1% gelatin. Running buffer was TG-SDS (RMB; #1666894). The gels were re-natured for 2x15 min in 2.5% Triton X-100 to remove SDS and incubated in activation buffer (50 mM Tris-HCl, 5 mM CaCl₂, 200 mM NaCl and 0.02% Brij 35) overnight. After staining with Coomassie brilliant blue R-250 (0.5% in 30% Methanol/10% acetic acid) and destaining, gelatinolytic activity was detected as unstained bands on a blue background.

Immunofluorescence

All samples were fixed with 3% paraformaldehyde (5 min) and saturated with 1% albumin in PBS. Some of them, as indicated, were permeabilized before saturation with 0.5% Triton X-100 in PBS (5 min). All antibodies used were commercial and their data and the applied dilutions are shown in Table 1. To visualize Coll IV reorganization the samples were stained and non-permeabilized with monoclonal anti-collagen IV antibody. To study the co-localization of Coll IV with FN (SIGMA, #F2006), 50 mg/ml soluble FN was added to the medium 30 min before fixation and then a double staining immunofluorescence protocol was applied. The cells were first stained for Coll IV, followed by anti-FN antibody (see Table 1). To study co-localization of collagen IV with actin, α 2 and β 1 integrin, the fixed and permeabilized cells were first stained for Coll IV and then the samples were incubated either with FITC labeled Body-P phalloidin to visualize actin, or with monoclonal antibodies against β 1- or α 2-integrins as described in Table 1. For negative control experiments the primary or the secondary antibodies were omitted.

Results

Fibroblast reorganization of Type IV Collagen

Within 4 h of incubation in the presence of serum in the medium, CCD-25SK fibroblasts were able to mechanically remove and reorganize adsorbed Coll IV (Fig. 1A). Typical dark streaks of Coll IV removal

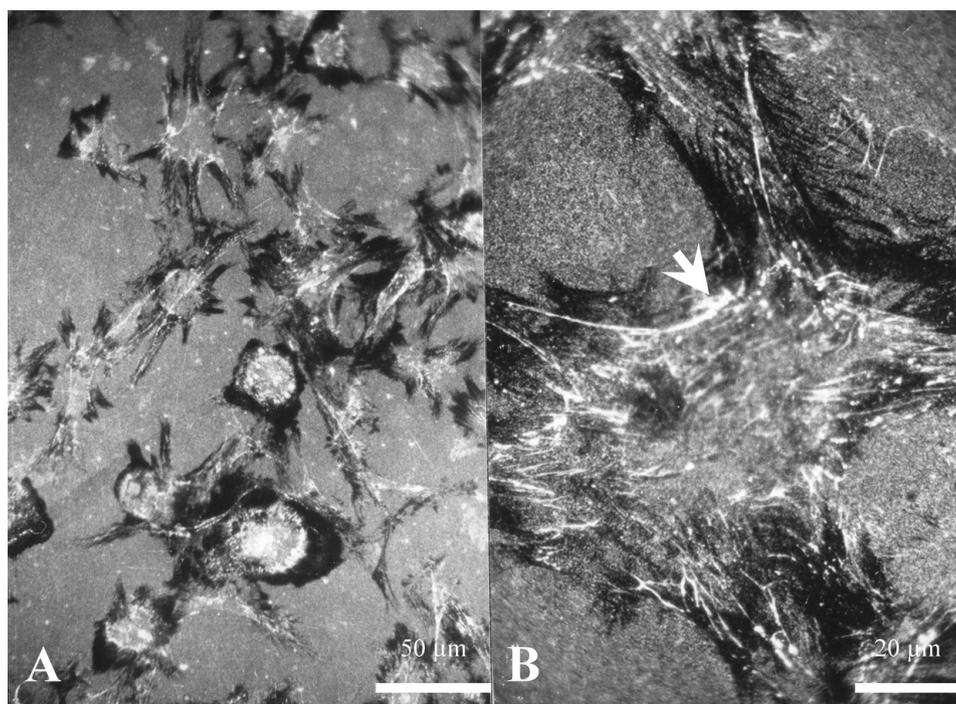


Fig. 1. Reorganization of type IV collagen by human fibroblasts. CCD-25SK fibroblasts were seeded for 4 h or 24 h on type IV collagen coated glass coverslips in the presence of 10% serum in the medium, then fixed and stained for collagen type IV. The samples were viewed and photographed using an inverted fluorescence microscope, type Axiovert 25 (Zeiss) at low 20x (**A**) and high 100x magnification (**B**); the white arrow on 1B indicates fibril-like formation of reorganized adsorbed collagen type IV.

appeared at cell borders. At higher magnification it can be seen that a significant amount of Coll IV is organized in a specific linear pattern (1B) (white arrow). After 24 h the fluorescent background of adsorbed collagen tended to disappear, apart from the organized Coll IV, which remains on the substratum (not shown here, see on Fig. 6C).

In order to exclude (1) any cross-reactivity with FN fibrils that could appear during the time of incubation, and (2) to follow the fate of Coll IV beneath the cells, in a separate experiment fibroblasts were seeded on FN coated substrata for 4 h (coated at 20 $\mu\text{g/ml}$, 30 min at 37°C) and then stained for Coll IV using the same as above protocol. In addition, half of the samples were permeabilized (with 0.5 % Triton X-100 for 5 min) and the rest were not. The results are shown in Fig. 2, where the upper row shows two representative pictures of cells cultured on Coll IV that were permeabilized (A), or not (B), before staining. The lower row represents another set of samples, where the cells were cultured on FN instead Coll IV substrata and stained again for Coll IV, permeabilized (C) or not (D), respectively. No significant differences between the permeabilized and non-permeabilized samples in respect to Coll IV reorganization, and no evidence for cross-reactivity with

FN, were found.

Cell surface distribution of $\alpha 2$ and $\beta 1$ integrin subunits

As shown in Fig. 3, there exist partial co-localization of linearly reorganized Coll IV with both $\alpha 2$ (Fig. 3A) and $\beta 1$ -integrin (Fig. 3B) subunits (seen in orange on merged red and green channels), strongly suggesting an involvement of collagen receptors in this process. Assuming that the $\beta 1$ -subunit, together with $\alpha 5$, comprises also the main FN receptor, we designed an additional experiment where soluble FN was added to the culture system. As shown in Fig. 4A, distinct zones of co-localization between Coll IV and FN fibrils were also observed, particularly on the cell borders, suggesting that Coll IV reorganization is dependent on the FN translocation during fibrillogenesis. The co-localization pattern, however, was only partial, indicating mostly FN-independent translocation of Coll IV. Finally, as seen in Fig. 4B, some of the reorganized Coll IV co-aligned with actin filaments.

Collagen IV in contact with H-460 cancer cells

H460 carcinoma cells also tended to rearrange Coll

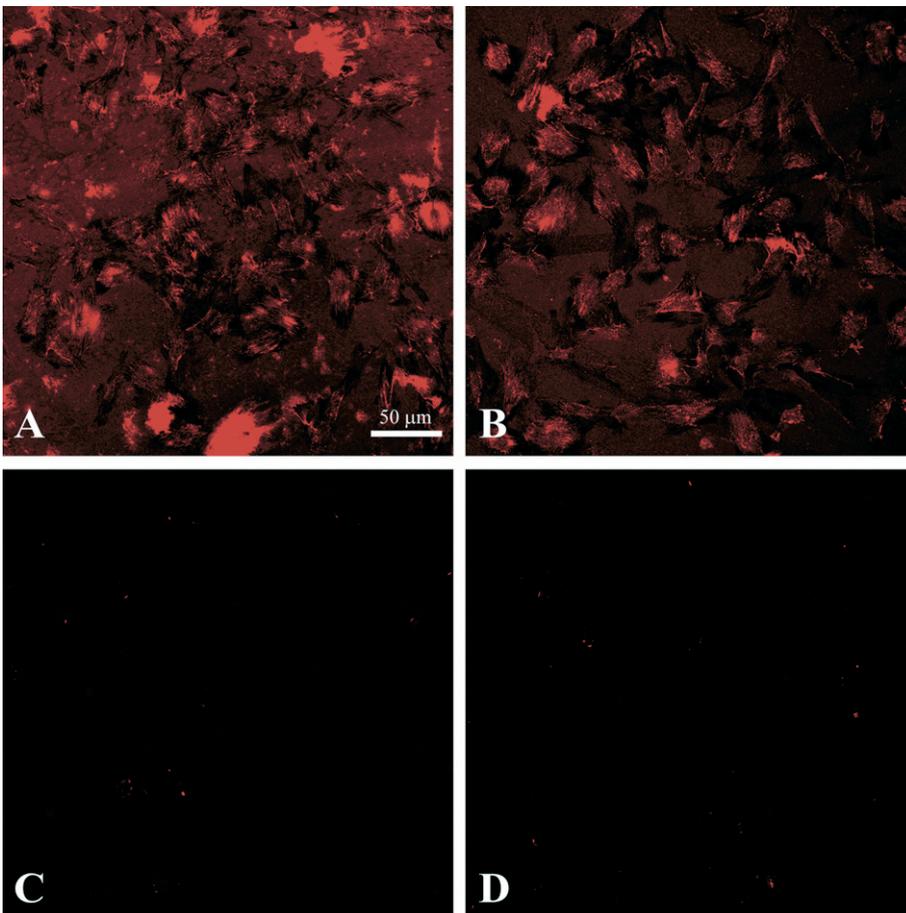


Fig. 2. Control immunofluorescence data showing the absence of cross-reactivity with FN and no effect of permeabilization. Fibroblasts were seeded on Coll IV (A, B - control samples) or FN coated substrata (B and C) for 4 h and then all of them stained for Coll IV. In addition, half of the samples were permeabilized with 0.5 % Triton X-100 (A, C) and the rest (B, D) not. No evidence for cross reactivity and no significant differences between permeabilized and non-permeabilized samples in respect to Coll IV reorganization were found.

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IV, but in a different pattern than fibroblasts. As shown in Figure 5, modest zones of Coll IV removal (Fig. 5A,B) and its “corona-like” arrangement at the periphery

of otherwise circumferentially spread H460 cells were typically observed. Rearranged Coll IV was clustered predominantly at cell borders and in very few short linear structures (Fig. 5B) (see white arrow head).

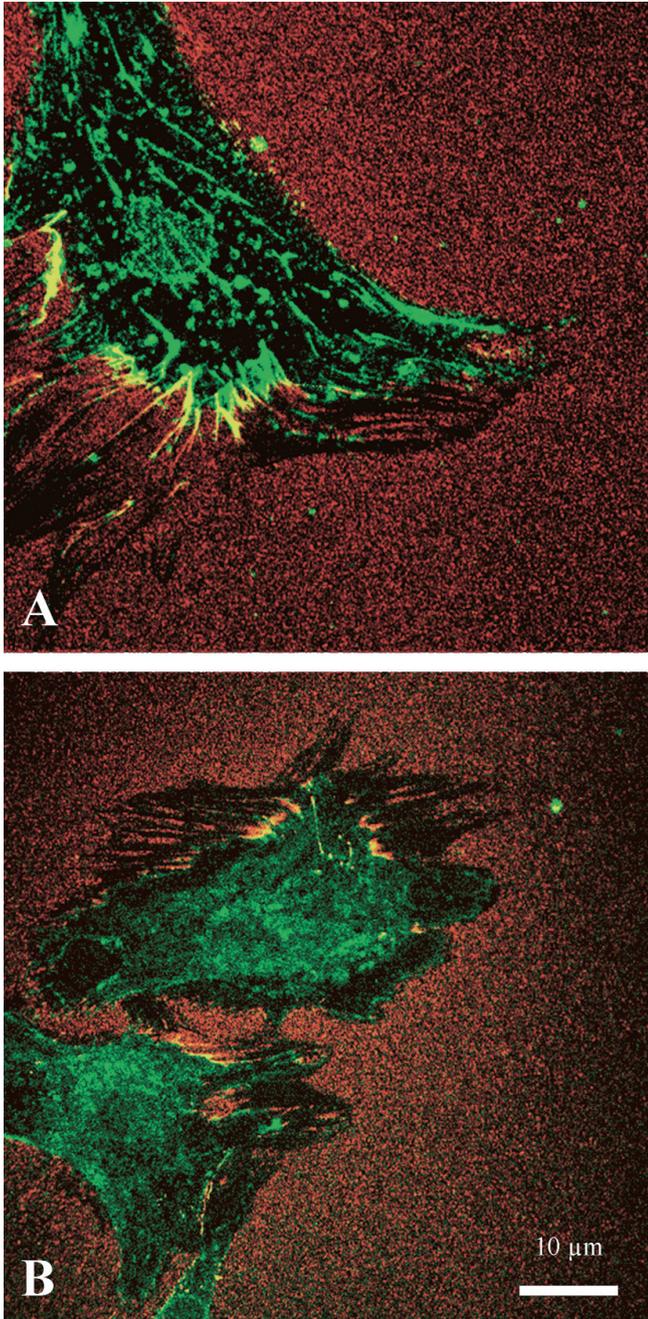


Fig. 3. Co-localization between linearly organized type IV and $\alpha 1$ or $\beta 2$ integrin subunits. CCD-25SK fibroblasts were seeded for 4 h on type IV collagen as above, then fixed and double stained for collagen type IV (**A and B**, red) and $\alpha 2$ (**A**, green) or $\beta 1$ (**B**, green) integrin. The samples were viewed at high magnification (x 100) and digitalized on LSM-530 (Zeiss) using simultaneously the green and the red channels. The co-localization results in orange staining.

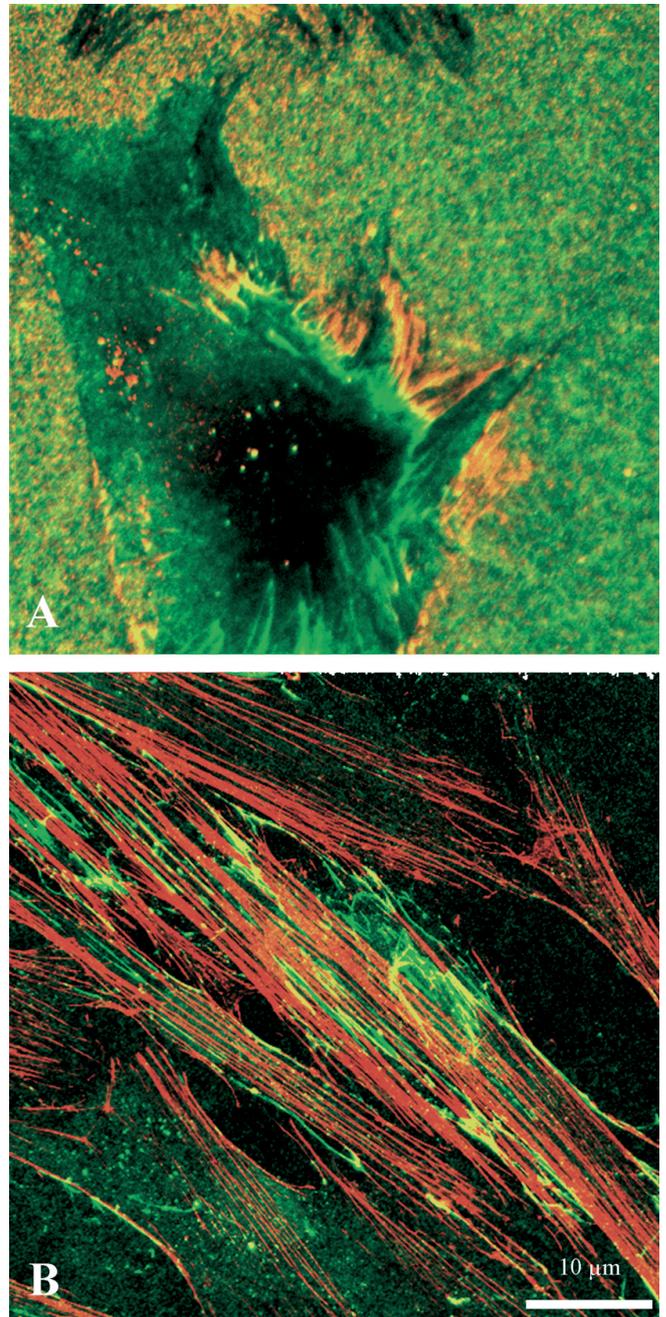


Fig. 4. Co-localization between linearly organized type IV collagen and fibronectin or actin. CCD-25SK fibroblasts were seeded for 4 h on type IV collagen as above, then fixed and double stained for collagen type IV (**A and B**, green) and fibronectin (**A**, red) or actin (**B** red). The co-localization results in orange staining. The samples were viewed and digitalized on LSM-530 (Zeiss).

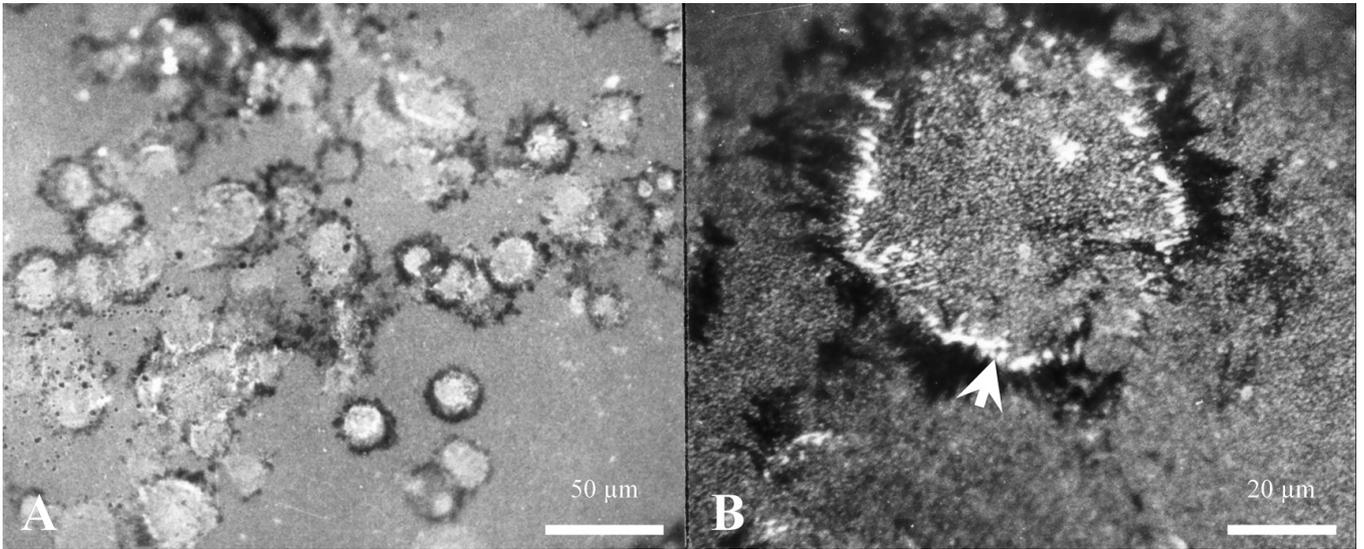
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Fig. 5. Reorganization of type IV collagen by H460 cells. The cells were seeded for 4 h in serum containing medium on type IV collagen coated glass coverslips, then fixed and stained for collagen type IV. The samples were viewed and photographed using an inverted fluorescence microscope, type Axiovert 25 (Zeiss) at low 20x (**A**) and high 100x magnification (**B**). The white arrow head on 5B, indicates “corona”-like reorganization of adsorbed collagen type IV.

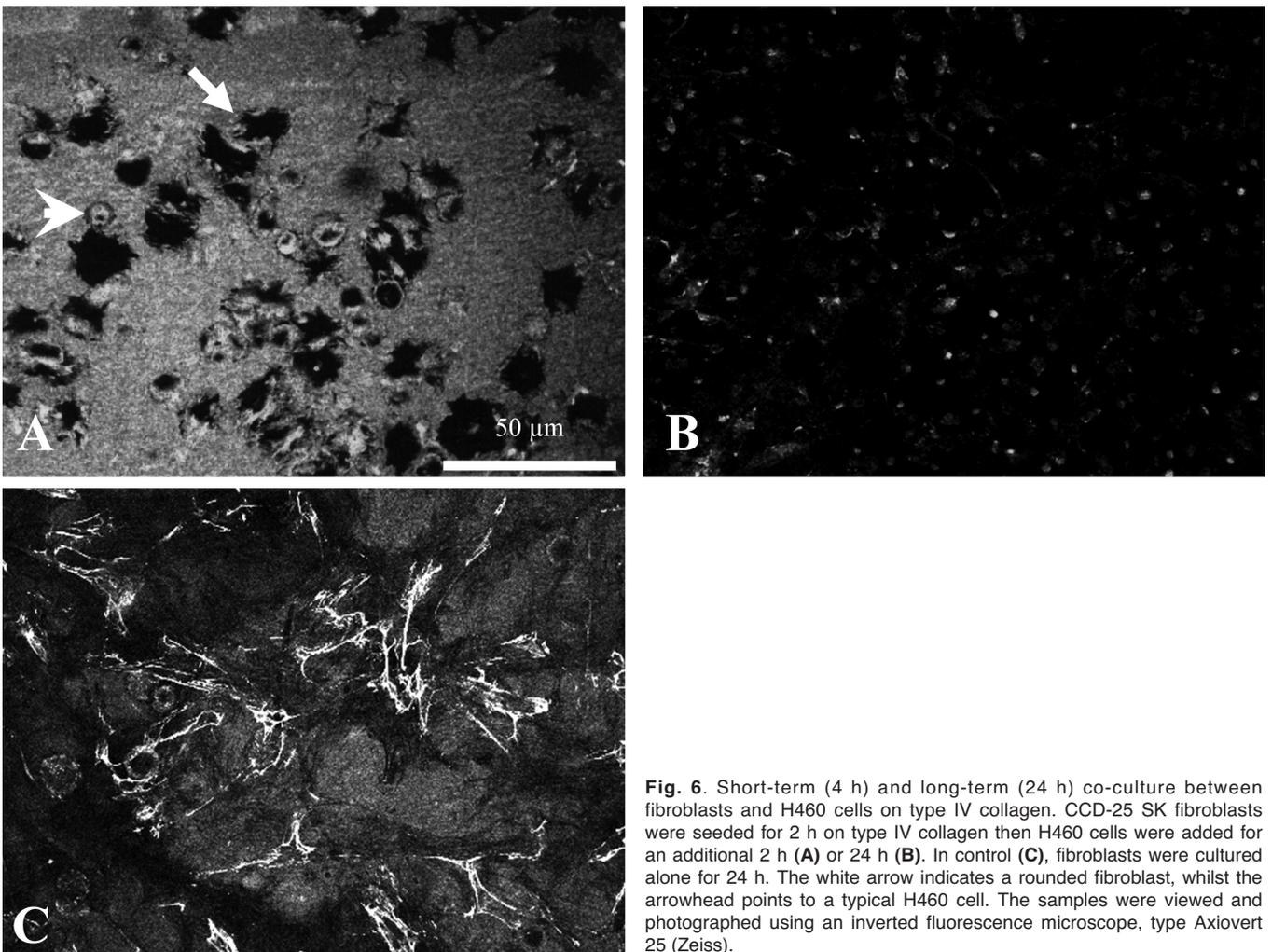


Fig. 6. Short-term (4 h) and long-term (24 h) co-culture between fibroblasts and H460 cells on type IV collagen. CCD-25 SK fibroblasts were seeded for 2 h on type IV collagen then H460 cells were added for an additional 2 h (**A**) or 24 h (**B**). In control (**C**), fibroblasts were cultured alone for 24 h. The white arrow indicates a rounded fibroblast, whilst the arrowhead points to a typical H460 cell. The samples were viewed and photographed using an inverted fluorescence microscope, type Axiovert 25 (Zeiss).

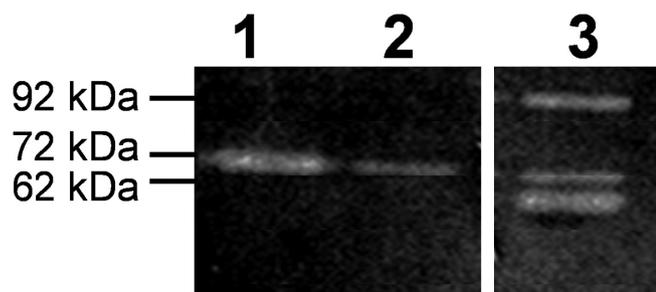


Fig. 7. Activation of MMP-2 in co-culture experiments between H460 and CCD 25SK fibroblasts. Zymography of the supernatants from co-culture of CCD-25 dermal fibroblasts with H460 cells for 72 h Lane 1: Fibroblasts (CCD-25SK) alone. Lane 2: H460 alone. Lane 3: NCI-H460/CCD-25SK 5:1

Co-culture experiments of H460 carcinoma cells with fibroblasts on coll IV

A typical result, when 3:1 cancer cells to fibroblasts were co-cultured for 4 hours (Fig. 6A) and for 24 hours (Fig. 6B) shown in Figure 6. As it was easy to distinguish cancer cells (Fig. 6A) from fibroblasts (Fig. 1A) by their round shape and approximately twice smaller size, no specific markers were used. Within four hours the cancer cells did not change their remodeling activity (see Fig. 6A), since the same pattern of local remodeling of adsorbed Coll IV was observed at cell periphery (Fig. 6A arrowhead). Moreover, they tended to suppress the fibroblast Coll IV reorganization activity and induced an obvious change in their overall morphology. From a typically extended cell shape in controls (Fig. 1A) fibroblasts started to round up and erase their borders within the surrounding fluorescent background (see white arrow in Fig. 6A), presumably because of the degradation of adsorbed collagen matrix beneath. After 24 h, both adsorbed and organized Coll IV was mostly destroyed, although a part of the organized collagen still remained on the substrate (Fig. 6B). For the same time the control fibroblasts retained their collagen reorganization activity (Fig. 6C), as well as their typical extended cell shape (not shown).

Expression and activation of MMP-2

When we co-cultured fibroblasts with H460 carcinoma cells, clear evidence for their ability to activate pro-MMP-2 was obtained after 48 h of contact. As seen in Fig. 7 (lane 3), H460 co-cultured with CCD-25SK fibroblasts was able to cleave the inactive pro-MMP-2 form (72 kDa) to create the active 62 kDa MMP-2, an effect confirmed also when primary human lung fibroblasts (NHLF) and H460 carcinoma cells co-culture were made (Fig. 8). Thus, we found that both types of fibroblasts secrete an inactive MMP-2 into the culture medium (Figs. 7, 8, lanes 1). The H460 cells

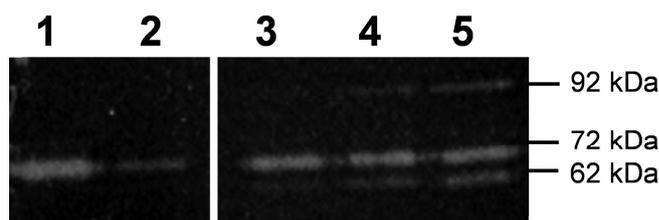


Fig. 8. Zymography of the supernatants from co-culture of human lung fibroblasts (NHLF) with H460 cells for 72 h in different ratios Lane 1: Fibroblasts (NHLF) alone. Lane 2: H 460 alone. Lane 3: H 460/NHLF 1:1. Lane 4: H 460/NHLF 2:1. Lane 5: H 460/NHLF 3:1. Note: fibroblasts secrete inactive MMP-2 into the culture medium; H460 cell line produce low amounts of inactive MMP-2 and activate MMP-2 when co-cultured with the fibroblasts.

alone also produced inactive enzyme (Figs. 7, 8, lanes 2). MMP-2 activation was almost independent of the amount of cancer cells added to CCD-25SK (not shown), which suggests that fibroblasts are responsible for this effect. NHLF fibroblasts however, seemed to be more dependent on cell number, since the increase of H460 carcinoma cell ratios from 1:1 to 2:1 and 3:1 (Fig. 8, lane 3, 4 and 5, respectively) intensified the activation.

Taken together, these data suggest that the fibroblasts secrete inactive (72 kDa) MMP-2 into the culture medium. The H460 cells also produce some inactive MMP-2 and are able to activate MMP-2, but only in direct contact with fibroblasts.

Discussion

Two types of activity of stromal cells could be distinguished in this study, the matrix organizing activity and the matrix degradation activity, both of which are altered in tumor progression. A novel observation is that fibroblasts are able to reorganize adsorbed Coll IV in a specific linear (fibril-like) pattern, which is similar, but not identical to the substratum associated FN fibrils (Grinnell, 1986; Altankov et al., 1996). In addition, we provide clear evidence that no cross-reactivity with the FN fibrils occurs. One important issue is whether this reorganization in vitro is physiologically relevant, as fibroblasts in 2D cultures differ dramatically from these residing in their normal 3D environment (Beningo et al., 2004). Coll IV is a non-fibrillar protein found in basement membranes, where, together with laminin, provides the major structural support for epithelial cells (Kuhn, 1994). It is widely accepted that Coll IV self-assembles into a meshwork by antiparallel interactions and extensive disulfide bounding of four molecules to form 7S domain (Kalluri, 2003). Furthermore, lateral interaction between C-terminal globular domains and condensation create an irregular two-dimensional meshwork that is the constituent of basement membranes (Vanacore et al., 2005). The observed linear

reorganization of adsorbed Coll IV by fibroblasts in our experiments seems to be dependent on the function of at least one collagen specific integrin receptor heterodimer, $\alpha 2\beta 1$, since it is co-localized with both $\alpha 2$ and $\beta 1$ subunits. Similar observations have been made for Coll IV, which is also linearly organized during early basement membrane assembly in an *in vitro* 3D skin-culture (Fleischmajer et al., 1998), suggesting again a cell-dependent process. While the molecular mechanisms which endow the spatial distribution and organization of Coll IV in basement membranes are still debatable, our data suggest that a driving force for Coll IV reorganization might be its association with FN fibrils, as was already demonstrated for fibrinogen (Tzoneva et al., 2002) and for some other fibrillar collagens (Velling et al., 2002). On the other hand, the double staining experiments showed only a partial co-localization between FN and Coll IV, confirming an independent translocation of collagen IV onto the cell surface. It also confirms the absence of any antibody cross-reactivity. Hence, our results support the view that deposition of collagen is regulated by the cells, both directly through collagen-binding integrins, such as $\alpha 2\beta 1$, and indirectly through $\beta 1$ integrin dependent polymerization of FN (Sottile and Hocking, 2002). In contrast to the earlier belief that collagen I and collagen III polymerization occurs via self-assembly, Velling et al. (2002) showed that a preformed FN matrix is essential for collagen network formation and that collagen-binding integrins strongly enhance this process. Furthermore, Sottile and Hocking (2002) found that FN polymerization into the extracellular matrix is required for the deposition of type I collagen, and this is indirectly supported by the observed coextensive linear assembly of Coll IV along the actin cytoskeleton, an organization typical for FN matrix fibrils (Wu et al., 1995; Christopher et al., 1997; Clark et al., 2005), as well as for the substratum reorganized FN (Grinnell, 1986; Altankov et al., 1996). FN is synthesized by many adherent cells, which then assemble it into a fibrillar network (Wierzbicka-Patynowski and Schwarzbauer, 2003). During assembly, FN undergoes conformational changes that expose fibronectin-binding sites and promote intermolecular interactions needed for fibril formation (Mao and Schwarzbauer, 2005). It should also be considered that the FN molecule has at least two binding sites for collagen (Danen et al., 2001; Mao and Schwarzbauer, 2005; Larsen et al., 2006), and corresponding binding sites for FN have been identified in the collagen molecule (Sottile and Hocking 2002; Velling et al., 2002; Zoppi et al., 2004). Earlier work of Dzamba et al. (1993) found that FN binding site in type I collagen regulates FN fibril formation in fibroblasts. On the other hand, Chernousov et al., (1998) reported that Schwann cells use directly a collagen IV-dependent mechanism for FN fibril assembly. Consistently, our results suggest that fibroblasts reorganization of adsorbed particularly Coll IV is likely to be dependent on the collagen specific integrins, but FN matrix may

serve as a template for its initial binding and orientation on the substratum.

We further demonstrated in this study that fibroblasts may change their matrix-dependent behavior in contact with cancer cells. The latter tend to alter their matrix forming activity and constrain them to destroy the existing collagen IV matrix. Indeed, after 24 h almost no adsorbed collagen IV was left when fibroblasts were co-cultured with H460 cancer cells. The remaining small fibrils and clusters probably means that the substratum organized collagen IV is more resistant to degradation. This observation is supported by zymography, which showed an elevated level of active MMP-2 in co-cultures of fibroblasts with H460 cells. The cells, however, obviously need direct contact with each other, since no active MMP-2 was found in the supernatants of either fibroblasts or H460 cancer cells when cultured alone, as well as when fibroblast-conditioned medium was added to the tumor cells (not shown here). MMP-2 is released in an inactive (72 kDa) pro-form, and two key molecules regulate its activation: the membrane bound MT1-MMP (MMP-14) and tissue inhibitor of metalloproteinase-2 (TIMP-2) (Kolenbrock et al., 1997; Lee et al., 1997; Jiang et al., 2002; Bernardo and Fridman, 2003). Our observation is consistent with the results of Sato et al. (1999), who showed an enhancement of MT1-MMP production and sequential activation of pro-MMP-2 in A431 cells when co-cultured with human dermal fibroblasts. Moreover, the same authors demonstrated later that cell-cell contact between cervical carcinoma cells and peripheral stromal fibroblasts augments the production and activation of MMPs, and therefore the subsequent imbalance between MMPs and TIMPs may result in the progression of cervical carcinoma cells *in vivo* (Sato et al., 2004).

Taken together, our results point to the existing dualistic activity of stromal fibroblasts towards the surrounding collagen matrix: one, directed on the continuous organization of this matrix, and the other, on its specific degradation, processes that are normally well tuned in the tissues. Both of these fibroblast activities however, seem to be misbalanced in contact with cancer cells.

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