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Selective expression of lysyl oxidase (LOX) in the stromal reactions of broncho-pulmonary carcinomas

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Summary. Lysyl oxidase (LOX) is the extracellular enzyme that initiates the main pathway of collagen and elastin cross-linking. LOX has also been correlated with the *ras* recision gene, a putative tumour suppressor isolated from revertants of *ras*-transformed fibroblasts. The present study investigates the potential correlation of LOX-dependent matrix protein cross-linking in the stromal reaction of lung carcinomas, with reference to the architecture of the main stromal reactions accompanying the neoplastic breast tissues. A strong LOX expression was associated with the hypertrophic scar-like stromal reaction found at the front of tumour progression in squamous carcinomas, adenocarcinomas, large cell carcinomas, or at sites of initial extense in bronchiolo-alveolar carcinomas. In contrast, little or no LOX expression was found within the stromal reaction of invasive carcinomas, small cell carcinomas, and neuro-endocrine carcinomas. The significance of LOX expression and of the stromal reaction are discussed, in light of data that associate LOX expression with tumours displaying a rather good prognosis.

Key words: Lysyl-oxidase, Bronchopulmonary carcinoma, Stromal reaction, Collagen, Myofibroblasts

Introduction

The extracellular matrix (ECM) represents the particular environment for tumour cell expansion and plays a crucial role in cell differentiation and tumour morphogenesis (Martinez-Hernandez, 1988). The significance of the stromal reaction in cancer has been interpreted either as a limitation mechanism against tumour progression by the formation of a physical barrier with limitation of angiogenesis and increase of metalloprotease inhibitors, or as a promoting mechanism for tumour growth with the loosening of the matrix by proteases and angiogenesis (Lagacé et al., 1985). We have recently reported that the early development of a cross-linked matrix rich in lysyl oxidase (LOX) around ductal breast carcinomas may represent a possible host defense mechanism in breast tumours, whereas the synchronous stromal reaction of invasive tumours lacking LOX may favor tumour dispersion (Peyrol et al. 1997).

LOX is an extracellular amine oxidase which initiates collagen and elastin cross-linking (Smith-Mungo and Kagan, 1998). LOX belongs to a new family of four members, together with the newly discovered LOXL1, LOXL2, and LOXL3 (Kenyon et al., 1993; Saito et al., 1997; Decitre et al., 1998; Jang et al., 1999; Jourdan Le Saux et al., 1999). Though the involvement of LOX in collagen cross-linking has been well documented, with the three other enzymes, only LOXL1 protein expression has been studied and associated with extracellular matrix remodelling (Decitre et al., 1998). The LOX gene has also been correlated with the *ras recision gene* (*rrg*), a putative tumour suppressor isolated from non-tumourigenic revertants of ras-transformed fibroblasts (Kenyon et al., 1991). It has been suggested that this tumour suppressor activity is due to the formation of extracellular cross-linked collagen modulating the cell phenotype (Contente et al., 1990). This hypothesis is consistent with the early development of a LOX-rich cross-linked matrix surrounding ductal breast carcinomas (Peyrol et al., 1997). However, the suppressor activity of LOX may also be accounted for by an intracellular or intranuclear localization (Wakasaki and Ooshima, 1990; Li et al., 1997), as the microinjection of recombinant LOX blocks the p21-Ha-*ras* induced oncogenic phenotype of *Xenopus laevis* oocytes (Di Donato et al., 1997). The steady state level of LOX transcription is high in fibrocompetent cells (myofibroblasts and smooth muscle cells) and low in malignantly transformed cells, in c-H-*ras*- or v-Ki-*ras*-transformed cells, or in metastatis-derived mouse prostate cancer cell lines (Contente et al., 1990; Smith-Mungo and Kagan, 1998; Reynaud et al.,

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1999). LOX expression is not detectable in tumour and stromal cells from invading ductal breast carcinomas or in primary and metastatic prostate cancers (Peyrol et al., 1997; Ren et al., 1998) but is high in myofibroblasts and myoepithelial cells of non invading (*in situ*) ductal breast cancers (Peyrol et al., 1997). The coordinated up-regulation of LOX and type I collagen appears to be a constant feature of fibrogenic processes (Sommer et al., 1993; Desmoulière et al., 1997), but has not been found in invading tumours (Peyrol et al., 1997; Ren et al., 1998, Trivedy et al., 1999). LOX was colocalized with type I collagen within pulmonary Wegener's granulomas, circumscribing the inflammatory mural infiltrate and forming a fibrosing front between the inflammation and the dense peripheral fibrosis (Gindre et al., 1995).

The study of LOX expression, as a marker of an early stromal reaction and of an invasive phenotype of tumour cells, has been extended here to broncho-pulmonary carcinomas (BC), known as highly polymorphic tumours of variable origins. At the present time, the clinicopathological evaluation and the histological classification of the different BC phenotypes do not take into account the stromal host response which constitutes the tumour biotope, the structural framework necessary for tumour cell differentiation and growth, and the adaptable medium for the intercellular signalling network of cytokines and growth factors (Mountain, 2000). This study was undertaken to determine whether specific stromal reactions could be correlated to specific polymorphic BC, with reference to the architecture of the main stromal reactions accompanying the neoplastic breast tissues (Peyrol et al., 1997) and to ECM remodelling associated to lung fibrosis (Takiya et al., 1983; Peyrol et al., 1990): the LOX-rich scar-like stromal reaction of *in situ* ductal breast carcinomas; the loose stroma associated with invasive ductal breast carcinoma, or the peculiar organization of the connective tissue within scirrhous areas of invasive carcinomas. The dense scar-like peritumoural stroma of *in situ* breast tumours was constituted by an abundant deposit of type I or type III collagen fibres forming closely apposed bundles, with active elastogenesis and elastin-associated microfibril synthesis. LOX was strongly expressed by myofibroblasts and myoepithelial cells, resulting in highly cross-linked fibrils. This scar-like fibrous stroma circumscribed *in situ* tumours and was found at the front of invasive tumours. In contrast to the *in situ* lesions, LOX was found to be all but absent from the stromal reaction of invasive ductal breast tumours, even in situations of abundant collagen synthesis within scirrhous regions. This uncoupling of LOX and collagen synthesis resulted in the formation of a loose angiogenic stroma, with a framework of type I and III collagen fibres, and rich in elaunin fibres or elastin-associated microfibrils. With regard to these patterns, the analytical approach to document the variations in stromal architecture of BC as a function of varied ECM protein expression included the localization and extension of the stroma, the mode of organization of the ECM at the ultrastructural level, the determination of the master ECM proteins (including LOX), and the cell type phenotype.

Materials and methods

Specimens

Thirteen surgical lung samples were selected from a collection of 1000 biopsies of the surgical pathology department of the Hospital of Caen (France). They were chosen because they were representative of each class of BC according to the WHO classification of tumours: 3 squamous carcinomas: well, moderately and poorly differentiated; among the 7 adenocarcinomas, 1 acinar, 1 solid with mucous secretion, 1 papillary; 2 non sclerosing bronchiolo-alveolar carcinoma (NSBAC) (1 mucinous, 1 non mucinous); 2 metastases with BAC pattern (primitive sites: colon and stomach); 2 neuroendocrine carcinomas: (1 small cell type and 1 large cell type); and 1 large cell carcinoma.

Among the adenocarcinoma subtype, the BAC was distinguished by the absence of other primary adenocarcinoma, the growth along alveolar septa with preservation of the general framework, the well-differentiated histology without large solid or cribriform cores, the absence of papillary structure with fibrovascular cores, and the absence of fibrosis.

The tumoural part of the surgical specimens was partly fixed in 3% buffered formalin and embedded in paraffin for histological evaluation and immuno-peroxidase staining, or frozen in liquid nitrogen for immunofluorescent detections.

Immunohistochemistry

Immunoperoxidase staining using a streptavidin-biotin complex and diaminobenzidine as chromogen was performed on 5 μm sections of lung tissue fixed with 3% formalin and embedded in paraffin. Immunoflurorescent staining was performed on 6 μm cryostat sections of fresh frozen tissue. The following antibodies were used: polyclonal antibodies anti-bovine type III procollagen, anti-human types I, III, IV and V collagen, anti-human fibronectin, anti-murine laminin, and anti-human elastin were from Pasteur-Lyon Institute, France; monoclonal antibodies anti-α-smooth muscle actin (M851 Dako), anti-desmin (M760 Dako), anti- bovine fibrillin (M11C3 Interchim). Polyclonal antibody against the anti-murine LOX was obtained as described (Sommer et al., 1993). Antibodies against collagen, elastin, fibronectin, laminin, α-smooth muscle actin or desmin, were used on frozen sections, while antibodies against α-smooth muscle actin and LOX were used on formalin-fixed tissues.

Electron microscopy

Small fragments of tumour tissues were sequentially fixed with cacodylate-buffered glutaraldehyde and

osmium tetroxide, dehydrated and embedded in epoxy resin. Semi-thin sectioning and methylene-blue-azur II staining allowed selection of tumour areas. Consecutive ultrathin sections were stained with methanolic uranyl acetate and lead citrate, and observed with a JEOL 1200 EX transmission electron microscope.

Results

This study was carried out on sample sections from BC representative of the different classes defined by the WHO. In spite of the large heterogeneity of the observed samples, the immunohistochemical detection of LOX associated to the evaluation of the stromal reaction permitted identification of different situations displaying similarities with the stromal reactions of ductal breast cancers, i.e., a typical fibrous stromal reaction associated with adenocarcinomas, an angiogenic stromal reaction rich in microfibrils associated with small cell carcinomas, and a pseudoseptal stromal reaction specifically associated with BAC. Each type of stromal reaction clearly displayed a differential expression pattern for LOX.

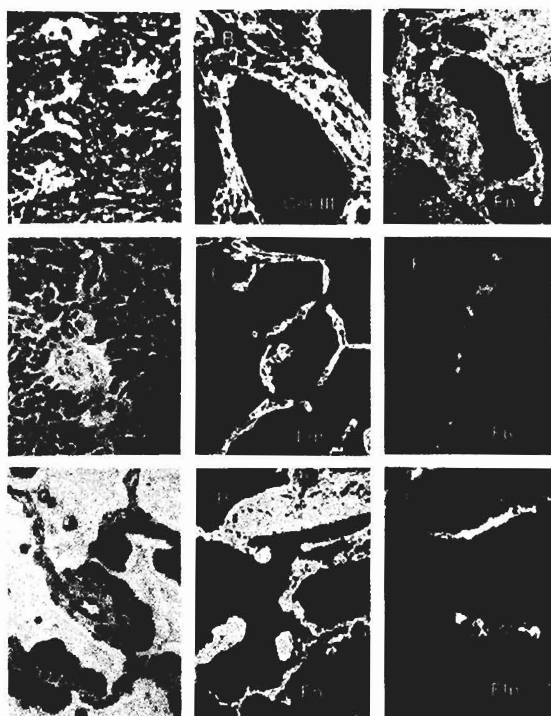

Fig. 1. Histological aspects and immunofluorescent labelling of stromal matrix proteins in adenocarcinoma (a-c), neuroendocrine carcinoma (d-f), bronchioloalveolar carcinoma (g-i). **a.** HPS staining shows the extense of the fibrous stromal reaction supporting tumour cell proliferation. **b.** Immunofluorescence of type III collagen shows its abundance and its major contribution to the stromal fibrillar framework. **c.** Immunofluorescence of fibronectin shows its abundant and diffuse distribution among the stromal framework. **d.** HPS staining highlights the scarce development of the highly vascularized stroma reaction among the wide tumour cell masses. **e.** Immunofluorescence of laminin underlines the extension and the close distribution of peritumoural and perivascular basal laminae. **f.** Immunofluorescence of fibrillin allows the detection of oxytalan fibres crossing the thin intertumoural stroma. **g.** HPS staining clearly discriminates the alveolar septum thickening beneath the tumour cell focus. **h.** Immunofluorescence of fibronectin clearly indicates its contribution to the septal thickening accompanying tumour cell spreading. **i.** Immunofluorescence of elastin shows the early development of elastic fibres along the axis of the invaded alveolar septum. x 250

The fibrous stromal reaction

This pattern was typically represented by non-small cell adenocarcinomas. Tumour cells grew as clusters or trabeculae together with desmoplasia that was infiltrated by a variable amount of polymorphic inflammatory cells (Fig. 1A). The stroma looked like a hypertrophic scar presenting all stages of maturation: a loose edematous pattern or mixed fibro-cellular pattern with numerous myofibroblasts or sclerotic acellular network. This desmoplastic stroma was predominantly associated with squamous, tubulopapillary or solid adenocarcinomas and large cell carcinomas.

The major matrix components were fibrillar collagens (type I, not shown, and type III, Fig. 1B) closely intermingled with glycoprotein components (fibronectin, Fig. 1C) and elastic fibres scattered inside (see Fig. 3A). LOX expression was strongly labelled within the stromal reaction of these carcinomas, independently of their anaplasia and differentiation. LOX labelling clearly highlighted the fibrillar framework of the concomitant stromal reaction (Fig. 2A); it also discriminated the tumour stroma interface in areas of late densely organized desmoplasia (Fig. 2B); and it decreased with the extense of inflammatory cell infiltration (Fig. 2C).

At the ultrastructural level, groups of densely packed collagen fibre bundles were disposed in parallel with the stromal axis (Fig. 3A). The close environnement of tumour cells showed basal lamina degradation (Fig. 3B) while among the clusters of inflammatory cells, altered collagen and elastic fibres were frequently encountered. Numerous myofibroblasts, positive for α-smooth muscle actin (data not shown) and characterized by a myoïd cytoskeleton, sustained this polymorphic extracellular matrix (Fig. 3B).

The microfibrillar angiogenic stromal reaction

A highly vascularized non-inflammatory stroma was encountered in small cell carcinoma - (NEC) - independently of its differentiation (Fig. 1D). Tumour cells diffusely grew either as massive and extensive fields of small poorly differentiated cells or as organoid trabeculae of more differentiated tumour cells.

The stromal reaction was restricted to thin hyaline non-inflammatory connective tissue sheets not associated with desmoplasia. The matrix fibrillar collagens and fibronectin were poorly represented (data not shown) and the main stromal components were laminin and fibrillin accompanying the synthesis of

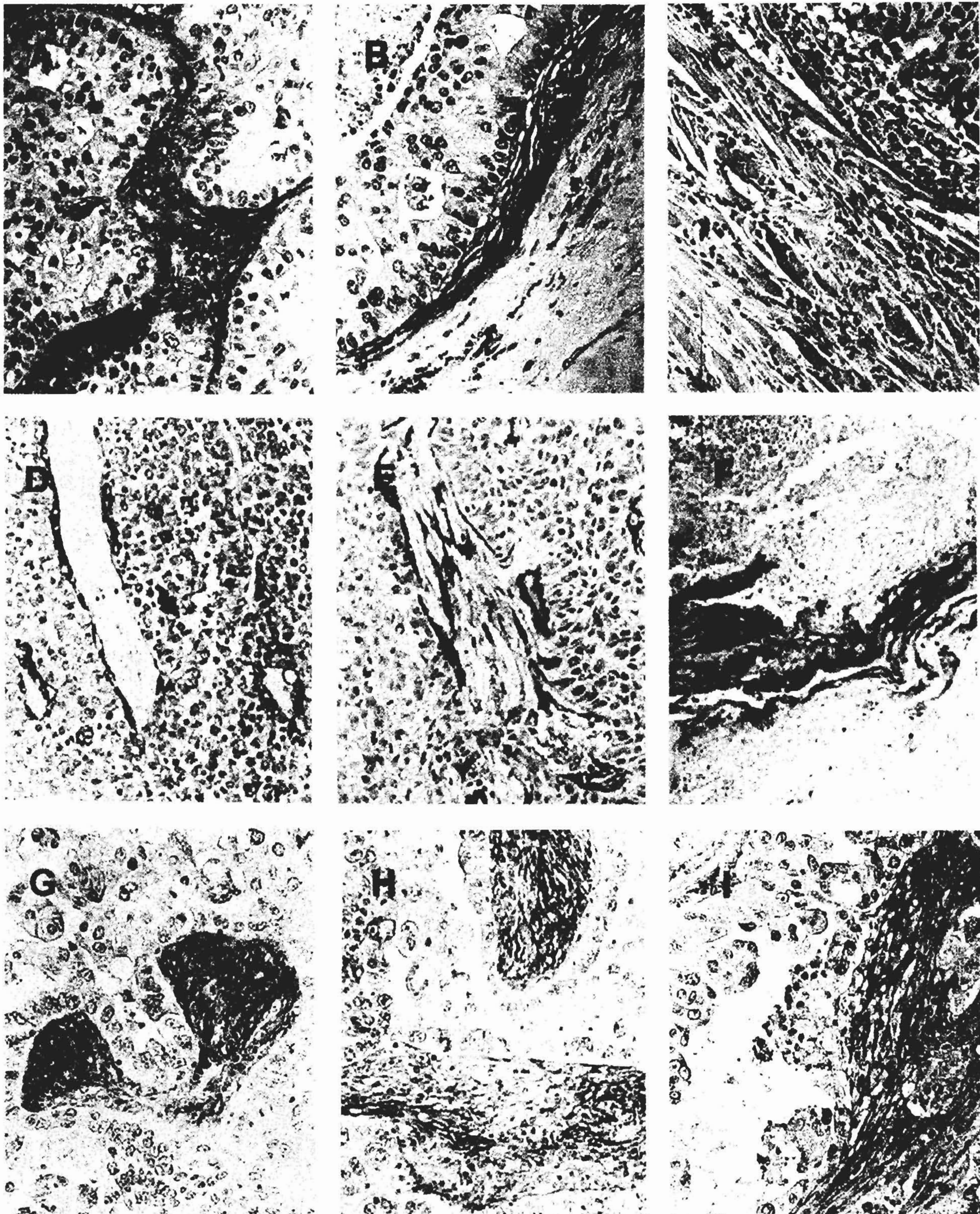

Fig. 2. Immunoperoxidase labelling of LOX in adenocarcinoma (a-c), neuroendocrine carcinoma (d-f), bronchioloalveolar carcinoma (g-h). **a.** LOX labelling clearly highlights the fibrillar framework of concomitant stroma reaction. x 250 **b.** LOX labelling is detected at the tumour stromal interface in areas of late densely organized desmoplasia. x 250. **c.** LOX labelling is fainter in stromal reactions including an abundant inflammatory cell component. x 250. **d.** LOX labelling delicately delineates the scarse matrix interposed between tumour cell masses and neovessel walls. x 250. **e.** LOX labelling is more important in the stroma reaction of moderately differentiated NEC when desmoplasia and inflammatory infiltration accompany angiogenesis. x 250. **f.** LOX labelling was strong and dense in the thickened alveolar septa adjacent to the progression front of the tumour; it progressively decreased until disappearance in the non invaded lung parenchyma. x 100. **g and h.** LOX labelling emphasizes the fibrillar framework of stromal buds supporting tumour cell spreading. x 250. **h and i.** The extense of LOX labelling depends on tumour cell spreading extension (buds or trabeculae). x 250

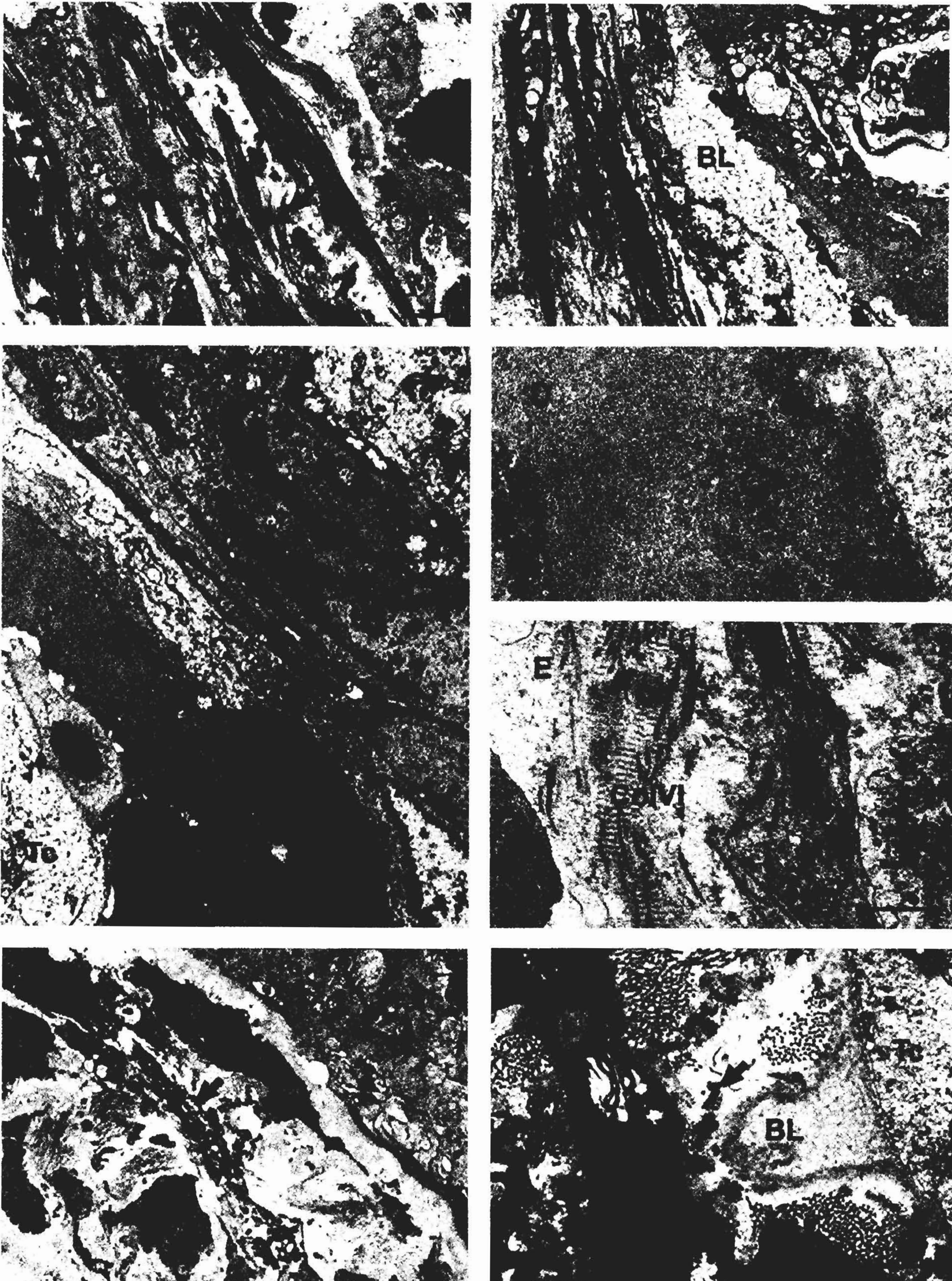

Fig. 3. Transmission electron microscopy of adenocarcinoma (a-b), neuroendocrine small cell carcinoma (c-e), and bronchiolo-alveolar carcinoma (f-g). **a.** Groups of densely-packed collagen fibre bundles are disposed in parallel with elaunin and elastic fibres along the stromal axis. **b.** Myofibroblasts sustains this polymorphic extracellular matrix; altered basal laminae surround tumour cells. **c.** Neovessels are the main component of the thin stroma disposed between tumour cell masses. **d.** High magnification of the material interposed between tumour cells and endothelial cells shows accumulation of elastin-associated microfibrils. **e.** Among the fibrillar components, type VI collagen is abundant. **f.** Myoid cells, elastic fibres and collagen fibre bundles constitute the stromal reaction to tumour cell spreading. **g.** Elastin-associated microfibrils of oxytalan and elaunin fibres contribute to link stromal myoid cells and collagen fibre bundles to peritumoural basal lamina which appears dystrophic. BL: basal laminae; ColVI: type VI collagen; Mf: myofibroblasts; Tc: tumour cells. Elastic-associated microfibrils (c and d) are indicated with *. Elastin (e), elaunin and oxytalan elastic fibrils (f and g) are indicated with arrows. Bar: 1 μm.

neovessels (Fig. 1E,F). The slight LOX labelling, co-localized with type I collagen (data not shown), delicately delineated the scarce matrix interposed between tumour cells and neovessel walls (Fig. 2D). On the other hand, LOX was enhanced in the discretely desmoplastic and inflammatory response of moderately differentiated neuroendcrine carcinoma (NEC) (Fig. 2E). Noticeably, LOX labelled a scar-like stroma found in the thickened alveolar septa adjacent to the progression front of the tumour and progressively decreased until disappearance in the non invaded lung parenchyma (Fig. 2F).

At the ultrastructural level, large trabeculae of elastin-associated microfibrils fused with perivascular basal lamina accompanied the neoangiogenesis process (Fig. 3C,D). The degradation pathways seemed to target preferentially peritumoural basal laminae and axial elastic fibres. Periodic assemblies of beaded filamentous type VI collagen were also found in abundance among a sparse and loose inter-tumoural collagenic network (Fig. 3D,E).

The pseudo-septal stroma

This stromal reaction profile preserved the lung architecture and concerned the non-sclerosing BAC, mucinous or not. The tumour cells grew by creeping along the alveolar septa or by aerogenous spreading and became substituted to the normal pneumocyte lining (Fig. 1G). In invaded NSBAC, the alveolar wall was thickened with enhanced fibronectin deposit (Fig. 1H) including numerous elastic fibres (Fig. 1I). The LOX staining clearly emphasized the thickened septa edged by tumour cells (Fig. 2G). The LOX-rich stromal reaction was either focused in fibrillar buds (Fig. 2G) or extended along the septa, accompanying widely spread tumour cell areas (Fig. 2H,I).

At the ultrastructural level, the major interstitial components were associated with the basal lamina and the elastic network represented by its three complementary structural forms, elastic fibres, elaunin and oxytalanic fibrils, with a predominance of the latter two (Fig. 3F). The fibrillar collagens were organized as a discrete matrix network (Fig. 3F,G). It was noticeable that most stromal myofibroblastic cells presented a myoid smooth muscle cell phenotype (Fig. 3G) with a desmin-rich (not shown) cytoskeleton.

Discussion

Identification of distinct stromal reaction patterns

This work highlighted at least three situations distinguished according to histological criteria for each stroma. The stromal reaction of the non-small cell BC exhibited features of hypertrophic and dense scar-like ECM, with a dense deposit of highly cross-linked collagen. The stromal reaction accompanying the small cell BC and NEC displayed a tiny ECM deposit. The stromal reaction of non-sclerosing BC was specified by a preservation of the interstitial framework and the extensive deposition of basement membrane. The lympho-epithelial type BC exhibited no ECM neosynthesis.

A strong LOX expression was associated with the typical hypertrophic scar-like stromal reaction characterized by the neosynthesis of fibrillar collagens and elastin by myofibroblasts, found at the front of tumour progression and disappearing later in dense, probably highly cross-linked scar-like stroma. As this stromal reaction was also characterized by the combination of both fibrogenic and degradative pathways, the resulting LOX expression seems to fit with the desmoplasia evolution. For example, in squamous carcinomas, adenocarcinomas and large cell carcinomas, the major expression takes place at the site of extracellular matrix production; while a mild LOX expression is associated with a balance between matrix neogenesis and degradation, and the disappearance of LOX expression occurs at the end of the matrix maturation process.

In contrast to this LOX-rich scar-like stromal reaction, the ECM associated with NEC or other small cell BC presented a low level of LOX. The enzyme was restricted to the maturation of discrete periodic collagen fibres and is not localized to sites of angiogenesis. It might be interesting to decipher whether this lack of LOX expression characterizes tumour-associated neoangiogenesis, in contrast to normal angiogenesis, which is dependent on LOX activity (Ingber and Folkman, 1988). In moderately differentiated NEC, the enhancement of stromal LOX expression increased and suggests the initial building step of a fibrous framework. This focal increase of LOX expression in NEC was localized in the thickened alveolar septa surrounding the tumour, reflecting the interstitial fibrosing front facing the tumour. LOX expression highlighted elastin and collagen maturation into a continuous and singularly differentiated cell-matrix network involving respectively tumour cell, peritumoural basement membrane, oxytalanic fibrils, elaunin fibres, collagen fibres and smooth muscle cell basement membrane, all together sustained by myoid stromal cells.

Significance of LOX activity

A putative defensive role for LOX expression and the subsequent collagen cross-linking in hypertrophic scar-like stromal reaction has been inferred from its expression pattern in ductal breast and prostate cancers (Peyrol et al., 1997; Ren et al., 1998). This effect might be caused by the decrease of a degradation rate of type I collagen (Vater et al., 1979), preventing ECM degradation by matrix metalloproteinases and subsequent tumour cell invasion. This hypothesis defines a defensive role for the neoformed connective tissue barrier, which might be considered as a limitation against invasiveness (Lagacé et al., 1985). Such a

situation is encountered in non small cells BC with a variability that reflects dynamics of the balance between tumour progression and host defense.

Little or no LOX expression was found within the stromal reaction of invasive ductal carcinomas (Peyrol et al., 1997), both in NEC and small cell BC. The corresponding scarce matrix includes constant features of neogenesis of neovessel components and a highly degradative pathway, with evidence of matrix degradation, representative of a permissive stroma around tumour cells. This situation of a decrease of LOX expression, an uncoupling with collagen synthesis, and an increase in stromal proteases (Furcht et al., 1994) should in principle favor tumour cell dispersion through non-cross-linked and easily degradable collagen matrices and tumour cell growth upon selective matrix substratum (Paweletz and Boxberger, 1994).

On the other hand, the expression pattern of LOX in the stroma reaction accompanying BAC argues for a completely different effect. In this case, LOX is expressed at the sites of tumour cell aerogenous spreading. Collagen and elastin cross-linking might create a singular matrix acting as a support for adhesion and migration of tumour cells, a feature which fits well with the absence of degradative pathways in BAC.

Different hypotheses have been advanced on the significance of the stromal reactions bordering cancer cell proliferation. Few experimental analyses have addressed this issue. In Lewis lung carcinoma, increased desmoplasia was associated with lower metastatic potential, while in the BL6 melanoma cell invasion model in mice, reduced collagen synthesis was correlated with increased invasion (Barsky and Gopalakrishna, 1987; Nakanishi et al., 1994). According to Schürch et al. (1982), the early and dense stromal reaction of ductal breast cancer could inhibit tumour progression by the formation of a physical barrier with limitation of angiogenesis and increase in metalloproteases inhibitors. Our recent observations of an abundant LOX-rich and LOXL1-rich deposit of collagen types I, III and IV and elastin circumscribing *in situ* ductal breast carcinomas was interpreted as a similar phenomenon, with the potential to restrict tumour cell growth (Peyrol et al., 1997; Decitre et al., 1998). Therefore, the LOX-rich scar-like stromal reaction of non-small cell BC may limit tumour progression.

Regulation of LOX expression

Tumour cells were never positive for LOX. This observation is consistent with previous findings describing the down-regulation of LOX gene in malignantly transformed cells, in oncogene-transformed fibroblasts (c-H-*ras*, v-Ki-*ras*, v-*fes*, v-*raf*, v-*abl*, or v-*sis*), in metastatis-derived mouse prostate cancer cell lines, in tumour cells from invading ductal breast carcinomas, or in primary and metastatic prostate cancers (Contente et al., 1990, Peyrol et al., 1997; Ren et al., 1998; Smith-Mungo and Kagan, 1998). It has been recently observed that the LOX promoter can be negatively regulated in *ras*-transformed fibroblasts by the IRF-response element and the LOcoll region, located at positions -1,280 to -1,257 of the LOX promoter and displaying 79% similarity with a COL1A1 promoter region (Tan et al., 1996; Reynaud et al., 1999). Interestingly, a high steady state expression level of LOX gene transcription has been recently reported in the highly invasive MDA-MB-231 human breast carcinoma cells (Kirschmann et al., 1999).

This study confirms the myofibroblast origin of LOX in reactive stroma. The involvement of myofibroblasts in the organization of reactive stroma in human epithelial tumours is well documented (Schmitt-Gräff and Gabbiani, 1992). Around non-invasive ductal *in situ* breast tumours, activation of the LOX and α_1(I) collagen genes takes place within myofibroblasts, and this leads to a retractile strengthening of the early peri-tumoural stroma. It is not yet clear whether myofibroblasts are involved or not in the limitation or propagation of tumours. While myofibroblasts expressing LOX might be clearly associated to tumour restriction in non-invasive ductal breast tumours (Peyrol et al., 1997), they have also been associated with the progression of experimental tumours (Schmitt-Gräff and Gabbiani, 1992). Many studies have emphasized the heterogeneity of myofibroblasts that was established with respect to their cytoskeleton (α-smooth muscle actin, desmin, vimentin; Sappino et al., 1990). However, though their role in fibrogenesis and ECM degradation is well known, the control of this switch between synthesis and degradation has still to be understood. It should be noted that a tight link between LOX gene activation and early myofibroblast differentiation has been clearly demonstrated in the murine bile duct ligation model where LOX expression occurs very early during ECM remodelling and precedes myofibroblast differentiation (Desmoulière et al., 1997). The signals for these processes are yet undefined though transforming growth factor ß1 is considered as the best candidate for controlling both the differentiation of myofibroblasts (Desmouliere et al., 1993) and the activation of LOX and type I collagen genes (Feres-Filho et al., 1995; Rossi et al., 1988).

The potential marker value of LOX and of the stromal reaction

The international basis of histological diagnosis is based on the histological classification according to the World Health Organization. In association with the tumour-node-metastasis (TNM) staging system for lung cancer (Mountain, 2000), it allows randomized therapeutic assays, epidemiological studies and prognostic evaluation. The present study may introduce the stromal reaction and LOX expression as additional parameters to the BC evaluation. The reactional LOX-rich and hypertrophic scar-like stromal reaction has been associated with non small cells BC, squamous cell

carcinoma, to all subtypes of adenocarcinomas except BAC, and to large cell carcinomas. These tumours either respond better to surgery, or, for unresectable ones, to radiotherapy with or without specific chemotherapy. It should be noted that the squamous cell BC, with an important scar-like and LOX-rich stromal reaction, have the best prognosis followed by adenocarcinomas and large cell carcinomas. In contrast, the absence of a true LOX-rich scar-like stromal reaction characterizes moderately and poorly differentiated NC and small cell BC, which are treated by sequential multi-agent chemotherapy and radiotherapy though the survival of these patients is generally poor (Ruckdeschel et al., 1994). The behaviour of BAC is dependent on the subtype (mucinous or not, sclerosing or not) and the multifocality. The stromal reaction of the non-sclerosing BAC, mucinous or not, is remarkable by the strict preservation of the interstitial framework, and by a characteristic thickening of septa labelled with LOX and edged by tumour cells. It should be noted that surgery succeeds for the non-sclerosing BAC with 75% five-year survival in the localized forms. The multifocal forms, with a low LOX expression, escape this good prognosis. Altogether, as in breast and prostate cancers, this study points to the putative correlation between the expression of a LOX-rich scar-like stable stroma and a favorable limitation of lung carcinomas, as found in breast tumours. Further studies should address this issue.

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