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Stromelysin-3: a paradigm for stroma-derived factors implicated in carcinoma progression

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1. Introduction

Most human malignant tumors are carcinomas which usually comprise two interdependent compartments, the cancer cell themselves and the stroma. The stroma, which differs from normal connective tissue at both morphological and biochemical levels [1,2], is an integral part of tumors and has been long proposed to be necessary for tumor growth [3–5]. The stroma provides the vascular supply that tumors require for expansion beyond a minimal size [6], and possibly also specific signals for tumor growth and metastasis [5,7]. The hypothesis that proteinases could represent such signals is supported by the recent observation that a number of extracellular proteinases implicated in carcinoma progression, including stromelysin-3 (ST3), are not produced by cancer cells, as this has been long believed, but by stromal cells [8–10].

The proteinases implicated in cancer progression include urokinase-plasminogen activator, cathepsin B, D and L, and various matrix metalloproteinases (MMPs) [11–13]. Taken together, these enzymes can degrade virtually all components of the extracellular matrix (ECM), suggesting that they may contribute to tumor progression by disrupting the ECM and thus allowing invasive cancer cells to migrate into adjacent tissues [14]. However, cancer cell invasion represents only a part of the malignant phenotype, and a number of recent observations suggest that extracellular proteinases, including some MMPs such as ST3, could also contribute to other aspects of the malignant phenotype [10,15,16].

2. The matrix metalloproteinase family

The MMP family has currently 17 members, which belong to various subgroups (Table 1). MMPs (also known as matrixins) were initially defined as enzymes that degrade at least one component of the ECM, contain a zinc ion and are inhibited by chelating agents, are secreted in a latent form and require activation for proteolytic activity, are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs), and share amino-acid similarities [17]. However, some of the recently identified members of the MMP family do not fulfil all of these criteria, suggesting that the MMPs should now be defined solely as a class of extracellular zinc-dependent proteinases belonging to the metzincin family [18], and being characterized by amino acid similarities and inhibition by TIMPs.

It is noteworthy that the present classification used for MMPs is not fully satisfactory. Thus, ST3 has not the broad spectrum of proteolytic activities shared by the other stromelysins, while the metalloelastase has a broad substrate range which extends beyond that of elastin alone. Conversely, both gelatinase A and B can cleave elastin, in addition to a number of ECM molecules which are also substrates for stromelysin-1 2 or matrilysin [15].

3. Stromelysin-3 structural and functional properties

The ST3 gene was identified by differential screening of a human breast cancer cDNA library among a group of genes expressed in invasive carcinomas at higher levels than in breast fibroadenomas [8]. Thereafter, the mouse ST3 cDNA was cloned from a placenta library [19], while that for rat was cloned from a healing skin wound library [20] and that for xenopus was found by a differential screening aimed at identifying genes expressed during tadpole metamorphosis [21]. ST3 exhibits structural characteristics of other members of the MMP family, including the presence of a putative zinc-binding segment with three liganding histidine residues (Fig. 1). ST3 was initially included into the stromelysin subgroup because it had the same four-domain structure as the previously described stromelysin-1 or -2 [22], but subsequent analyses have suggested that in fact it represented the first member of a new MMP subgroup [23–26].

First, comparison of the sequence of the ST3 catalytic domain with that of other members of the MMP

Table 1
The matrix metalloproteinase family

Enzyme	MMP number	Predominant cellular expression in carcinoma
Collagenases		
Collagenase-1 (interstitial)	MMP-1	Stromal cells
Collagenase-2 (neutrophil)	MMP-8	?
Collagenase-3	MMP-13	?
Collagenase-4	MMP-18[a]	?
Stromelysins		
Stromelysin-1	MMP-3	Stromal cells
Stromelysin-2	MMP-10	Cancer cells
Matrilysin	MMP-7	Cancer cells
Gelatinases		
Gelatinase A	MMP-2	Stromal cells
Gelatinase B	MMP-9	Stromal cells
Membrane type		
MT1-MMP	MMP-14	Stromal cells
MT2-MMP[b]	MMP-15	?
MT3-MMP[b]	MMP-16	?
MT4-MMP	MMP-17	?
Other types		
Stromelysin-3	MMP-11	Stromal cells
Metalloelastase	MMP-12	Stromal cells
—	MMP-19[a]	?
Enamelysin	MMP-20	?

Data from [10,13,15,16,51,88–94].

[a] Although the MMP described by Cossins et al. [89] was initially termed MMP-18, this MMP should now be referred to as MMP-19, as that described by Pendas et al. [90] and Sedlacek et al. [91]. MMP-18 corresponds to collagenase-4 [88].

[b] Although the MT-MMP described by Takino et al. [93] was initially termed MT2-MMP, this MMP should now be referred to as MT3-MMP, and that found by Will and Hinzmann [94] as MT2-MMP.

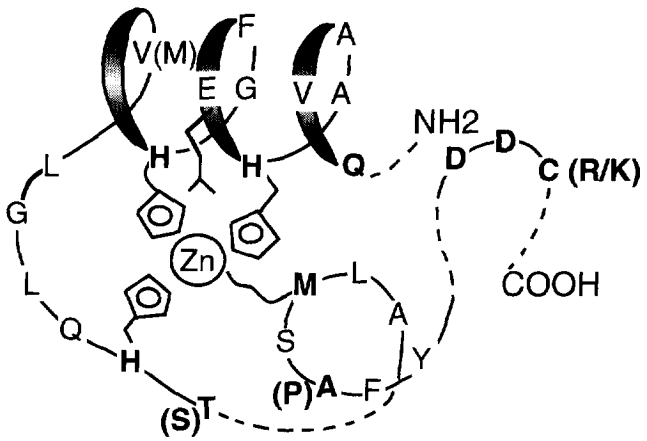

Fig. 1. Catalytic-zinc environment in human, mouse, rat and xenopus stromelysin-3. Human ST3 differs from all other matrixins, including mouse, rat and xenopus ST3, by the presence of an alanine (A) instead of a proline (P) C-terminally to the 'Met-turn' [18]. This proline/alanine substitution, which involves a residue belonging to the S_1' pocket [95], has been shown to play a role in the inability of C-terminally truncated forms of human ST3 to cleave ECM components [32]. Another difference between human ST3 and the other matrixins is the presence of a cysteine (C) residue C-terminally to the aspartic doublet (D-D) which is conserved in all matrixins and believed to play a role in stabilizing the catalytic site [96]. The three mammalian ST3, but not that of the xenopus, are characterized among other MMPs by the presence of a threonine (T) instead of a serine (S), C-terminally to the third histidine (H) liganding the catalytic zinc atom [97]. The xenopus protein also differs from human, mouse and rat ST3 by the presence of a methionine (M) instead of a valine (V) C-terminally to the second histidine residue. All other amino acids in the ST3 catalytic-zinc environment are identical in the four species, particularly the glutamine (Q) which is specific to ST3 among other MMPs and whose side chain defines the bottom of the S_1' pocket. Amino acids are represented by the one-letter code.

family indicates that ST3 is nearest to bacterial metalloproteinases, in an evolutionary sense, than the other stromelysins [23,25,26]. Second, ST3 has extra amino acids at the junction between the pro- and catalytic domains [8,19–21]. These additional amino acids are functionally important since they include a typical RXRXKR cleavage site for convertases of the furin-type [27]. The ST3 RNRQKR motif is 100% conserved

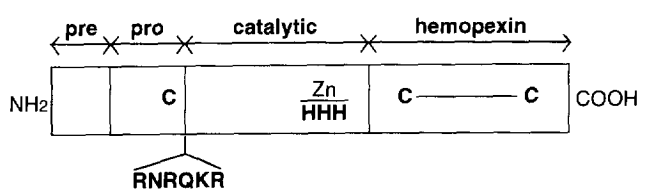

Fig. 2. Domain structure of stromelysin-3. Among the MMPs exhibiting four-protein domains [15], ST3 is characterized by the presence of a short stretch of amino acids located at the junction between the pro- and catalytic domains, which includes a typical RNRQKR cleavage site for convertases of the furin type and which is 100% conserved from xenopus to human. Amino acids are represented by the one-letter code.

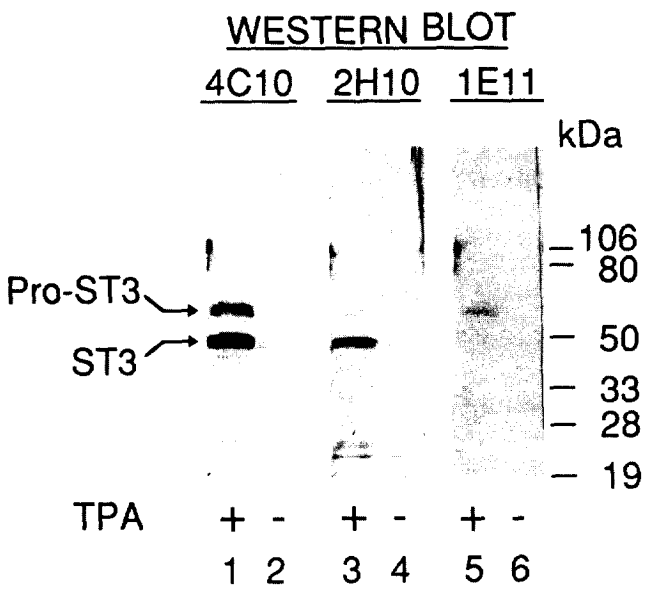

Fig. 3. Western blot analysis of human fibroblastic stromelysin-3. Conditioned media from HFL1 human diploid fibroblasts (ATCC CCL 153), stimulated or not with TPA (12-*O*-tetradecanoyl-13-phorbol-acetate, 10 ng/ml), were concentrated 100 × by ammonium sulfate precipitation and analyzed by immunoblotting with monoclonal antibody 5ST-4C10 against the ST3 catalytic domain (lanes 1 and 2) or with monoclonal antibody 1ST-2H10 against the 25 C-terminal amino acids of ST3 (lanes 3 and 4) or with monoclonal antibody 6ST-1E11 against the ST3 propeptide (lanes 5 and 6). The results indicate that the major ST3 form secreted by HFL1 fibroblasts corresponds to a protein with an apparent molecular weight of 49 kDa, which has lost the N-terminal prodomain and retained an intact C-terminal domain.

from Xenopus to human (Fig. 2) and has been shown to be functional in all three mammalian ST3 identified so far [20,28,29]. Thus, pro-ST3 is intracellularly processed by furin or furin-like enzymes and predominantly secreted as a potentially active molecule (mature form) (Fig. 3), in contrast to other MMPs which are secreted as proforms requiring activation in the extracellular space [15]. Finally, and most importantly, ST3 has unusual functional properties. Studies carried out with the mouse and human enzymes have shown that, in contrast to other MMPs, high molecular weight forms of recombinant ST3 do not exhibit enzymic activities detectable by casein or gelatin zymography (Fig. 4) [30–32]. Furthermore, the human ST3 mature form has been found to not cleave any of the major ECM components [31], although low molecular weight forms of the mouse enzyme truncated at their C-terminal part demonstrate caseinolytic activity (Fig. 4) and stromelysin-like activities towards a large number of ECM components [30,32]. While these findings indicate that the C-terminal part of mouse ST3 may have an inhibitory function which is released by cleavage within the C-terminal domain, such a C-terminal truncation is ineffective in the case of the human enzyme [32]. The sole substrate so far found to be cleaved by both

human and mouse ST3 is α1-proteinase inhibitor [31,32], suggesting that this inhibitor and other serine proteinase inhibitors of the serpin family may be the physiological ST3 substrates [31]. However we note that most, if not all MMPs, can cleave α1-proteinase inhibitor, and in some cases with an apparently much higher efficiency than ST3 [33,34].

Based on its capability to cleave α1-proteinase inhibitor and also to bind the TIMPs [30,32], it appears reasonable to believe that ST3 is a MMP, although it has unique functional properties and for which no specific substrate has been identified yet. Alternatively, ST3 may correspond to a protein having kept a MMP-like structure, including an apparently functional zinc-binding site, but that has evolved to acquire a new biological function. In this respect, we note that ST3 is a divergent member in the MMP family, which originated by gene duplication about 800 million years ago [35].

4. Stromelysin-3 gene expression

4.1. Human malignancies

The ST3 gene is expressed in most invasive primary carcinomas and in a number of their metastases, but more rarely in sarcomas and other non-epithelial malignancies (Table 2) [36–54]. Observations made in skin [38,52], head and neck [39], and breast [42,44] carcinomas have shown that the highest levels of ST3 gene expression were usually detected in tumors suspected to have the highest local invasiveness or highest malignant potential. ST3 gene expression was also observed in some non-invasive carcinomas and other precursor lesions, including breast in situ carcinomas, bladder transitional papillary carcinomas, uterine cervix dysplasias and in situ carcinomas, and colon adenomas [40,45,48]. In these cases, expression was most often detected in precursor lesions having the highest probability to evolve toward invasion, with ST3 levels usually lower than in the corresponding invasive carcinomas.

Table 2
Stromelysin-3 gene expression in human cancer

Histological classification	Number of tumors	
	Total	ST3 positive (%)
Primary invasive carcinomas		
Adenocarcinomas[a]	180	162 (90)
Squamous cell carcinomas	132	123 (93)
Metastases[b]	34	21 (62)
Non-epithelial malignancies	34	7 (21)
Precursor lesions[c]	91	35 (38)

Expression was analysed ar RNA or protein level. Data from [48].
[a] Renal adenocarcinomas were not included in the statistics.
[b] From breast and colon carcinomas.
[c] Breast in situ carcinomas, bladder transitional papillary varcinomas, uterine cervix dysplasias and in situ carcinomas.

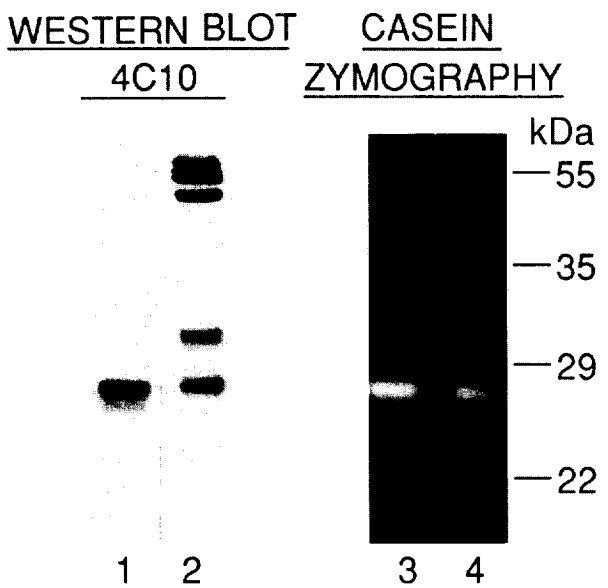

Fig. 4. Electrophoretic analysis of mouse recombinant stromelysin-3. Mouse recombinant ST3 expressed from a myeloma cell line was purified and analyzed by immunoblotting with monoclonal antibody 5ST-4C10 against the ST3 catalytic domain (lanes 1 and 2) and by casein zymography (lanes 3 and 4). The low molecular weight forms of mouse ST3, which have lost the predicted propeptide and the majority of the C-terminal domain, show caseinolytic activity in contrast to the high molecular weight forms [30]. Reprinted by permission of the American Society for Biochemistry and Molecular Biology.

Taken together, these observations suggest that levels of ST3 expression may be used to identify patients at greatest risk for cancer recurrence. Indeed, it has been shown that recurrent breast carcinoma was more frequent in the patients with high levels of ST3 RNA or protein in the tumor than in those with low ST3 levels (Fig. 5) [47,54]. Thus, while it appears reasonable to believe that ST3 expression may be used to identify tumors associated with poor prognosis, the question is to know whether ST3 can serve as an independent prognostic variable. In this respect, multivariable analyses of significant size are now necessary to rank in importance ST3 and the other proteolytic factors having prognostic power in human carcinomas [12].

In all the carcinomas so far examined both ST3 RNA and protein have been specifically detected in fibroblastic cells of tumor stroma observed in the immediate vicinity of cancer cells. We note that these fibroblastic cells, although they are not malignant per se, are often the predominant stromal cell type in human carcinomas and must be regarded as authentic tumoral cells [55]. The stroma constitutes a second component of solid tumors and should be in no case confused with the non-cancerous connective tissue surrounding the tumor. This is

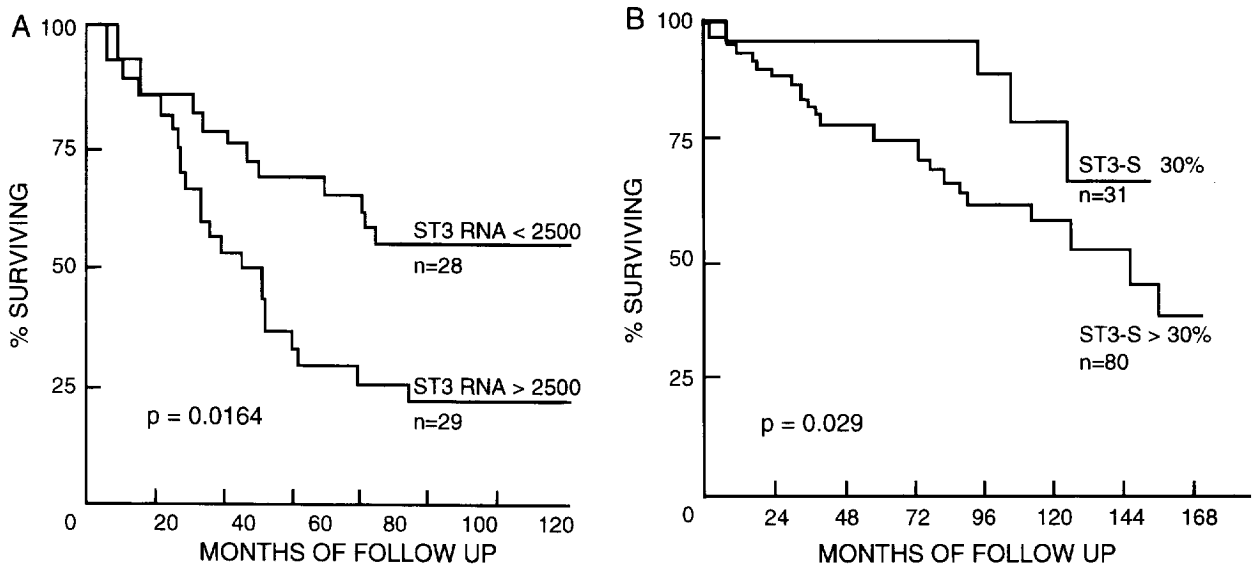

Fig. 5. Relationship between stromelysin-3 gene expression levels in tumor tissue and overall survival in patients with invasive human breast carcinoma. (A) RNA expression. Tumors were stratified for low (< 2500 units) or high (> 2500 units) ST3 transcript levels evaluated by quantitative in situ hybridization [47]. Reprinted by permission of Wiley-Liss, a subsidiary of John Wiley and Sons, Inc. (B). Protein expression. Tumors were stratified for low (ST3-S ≤ 30%) or high (ST3-S > 30%) ST3 protein levels evaluated by semiquantitative immunohistochemistry using monoclonal antibody 5ST-4A9 against the ST3 hemopexin domain [54]. Reprinted by permission of Wiley-Liss, a subsidiary of John Wiley and Sons, Inc.

particularly well illustrated by ST3 expression in human carcinomas, where the ST3-expressing fibroblastic cells are found intermixed with cancer cells inside the tumoral mass itself and not in the peritumoral tissue (Fig. 6). In agreement with these observations, cancers such as kidney adenocarcinomas and small cell lung carcinomas which progress without inducing a prominent fibroblastic stroma, are also those exhibiting the lowest levels of ST3 gene expression [48].

4.2. Mouse tumors

In striking contrast with the observations made in human carcinomas, nude mouse tumors generated by injection of human breast cancer cells, or those occurring in mouse strains developing mammary carcinomas with high frequency, were rarely found to be associated with ST3 gene expression (Table 3). This may be explained by the fact that these tumors grow much faster than their human counterparts and can achieve considerable tumor size in a few weeks without developing a prominent tumor stroma that is required to observe high levels of ST3 expression (compare Fig. 7(A) and 7(B)). However, some observations suggest that other factors specific to ST3 and necessary for its expression are lacking in mouse tumors. Thus the SPARC gene, which encodes an ECM-associated glycoprotein [56], is expressed at comparable high levels both in fibroblastic cells of human breast carcinomas and in stromal cells of mouse mammary tumors [57].

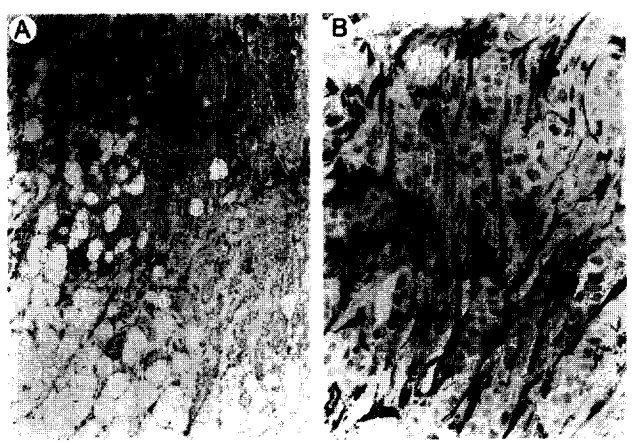

Fig. 6. Immunolocalization of stromelysin-3 in fibroblastic cells of invasive human breast carcinoma. Photomicrographs of paraffin-embedded tissue sections stained with hematoxylin after indirect immunoperoxidase staining of ST3 with monoclonal antibody 5ST-4A9 against the ST3 hemopexin-like domain, are shown. (A). Periphery of an invasive breast carcinoma. ST3-positive fibroblastic cells are observed in the tumoral tissue at a distance from the leading edge of the invasive front, where cancer cells directly interact with surrounding normal adipose tissue (original magnification: × 100). (B). Center of an invasive breast carcinoma. ST3-positive fibroblastic cells are intermixed with cancer cells inside the tumoral mass (original magnification: × 400).

Table 3
Stromelysin-3 gene expression in mouse tumors

Tumor type	Number of tumors	
	Total	ST3 positive
Subcutaneous[a]		
MCF7	7	0
MDA-MB-231	6	1
Mammary		
PS strain[b]	5	0
wap-*myc*[c]	2	0
wap-*ras*[c]	12	2

Expression was evaluated by Northern blot analysis with a mouse ST3 cDNA probe [19].
[a] Injection with MCF7 (ATCC HTB 22) or MDA-MB-231 (ATCC HTB 26) human breast cancer cells in mammary fat pad of nude mice.
[b] Ref. [98].
[c] Transgenic mice expressing *myc* or *ras* oncogenes under the control of the wap gene promoter [99].

4.3. Non-cancerous tissues

ST3 gene expression has been observed in the endometrium, [8,58] in the placenta [8,19], in the ovary [20], and in a number of conditions associated with tissue remodeling, including mammalian embryonic development [8,59], amphibian metamorphosis [21], mammary gland apoptosis [19], and cutaneous wound-healing [20,38,60]. As in the case of human carcinomas, ST3 gene expression in non-cancerous tissues was specifically observed in fibroblastic cells, with the exception of the placenta, where both ST3 RNA and protein were found in syncytiotrophoblastic cells [61], and the embryonic brain, where ST3 transcripts were specifically detected in neuroepithelial cells of the floor plate [59]. Although ST3 expression is associated with a large number of conditions involving intense tissue remodeling, specific modifications appear to be required for triggering this expression, since benign lesions associating both epithelial and fibroblast proliferations may or may not express the ST3 gene. Thus in the breast, focal expression of the ST3 gene could be observed in some lesions of sclerosing adenosis (Fig. 8), while expression is exceptionally detected in fibroadenomas [8,40,62]. In the skin, however, ST3 was expressed in dermatofibromas, which correspond to benign fibrous nodules, although it was not expressed in fibrosarcomas [52].

In summary, the ST3 gene is expressed in various tissue remodeling processes, both normal and pathological. In human carcinomas, ST3 is specifically expressed by fibroblastic cells. Although this was initially regarded as being characteristic of the ST3 gene [8], it is now clear that most extracellular proteinases implicated in human carcinoma progression are in fact predominantly expressed by stromal cells [9,10]. These findings, together with the observation that a number of molecules involved in the regulation of extracellular proteolysis were also predominantly expressed by stromal cells, has led to the concept that extracellular proteinases, and particularly MMPs, were not specifically involved in cancer cell invasion and metastasis, but could also contribute to other aspects of the malignant phenotype including the earlier stages of tumor

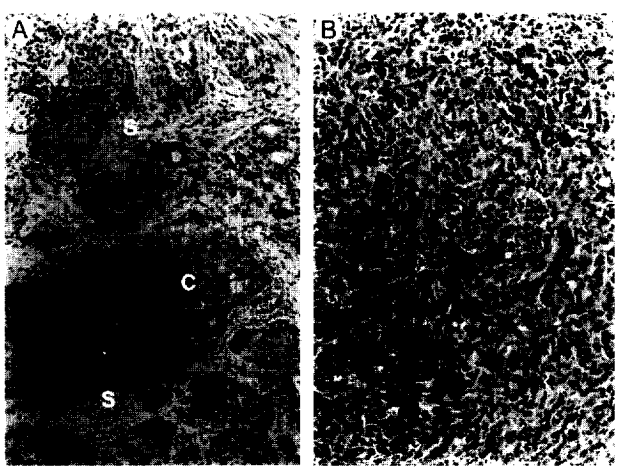

Fig. 7. Comparative histological analysis of human and mouse mammary gland carcinomas. Photomicrographs of paraffin-embedded tissue sections stained with hematoxylin, are shown (original magnification: x100). (A). An invasive human breast carcinoma, where cancer cells (C) are embedded in a prominent tumoral stroma (S), is shown. (B) A tumor raised in a nude mouse after injection of MCF7 human breast cancer cells (ATCC HTB 22) in mammary fat pad is shown. Note that the cancer cells have rapidly proliferated with almost complete absence of tumor stroma formation.

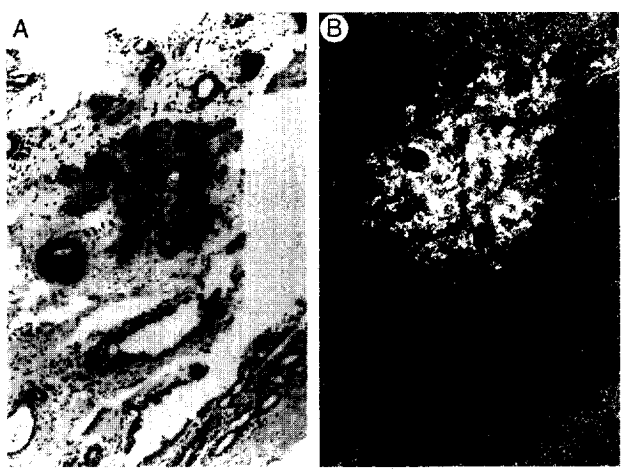

Fig. 8. In situ hydridization of stromelysin-3 RNA in human breast sclerosing adenosis. Bright-field (A) and dark-field (B) photomicrographs of a paraffin-embedded breast tissue section stained with hematoxylin after in situ hydridization with [^{35}S]-labeled ST3 antisense RNA and autoradiography, are shown. ST3 transcripts (white silver precipitate grains in panel B) are specifically detected in fibroblastic cells surrounding proliferative epithelial cells, in a tissue area showing typical breast sclerosing adenosis. (Original magnification: × 100).

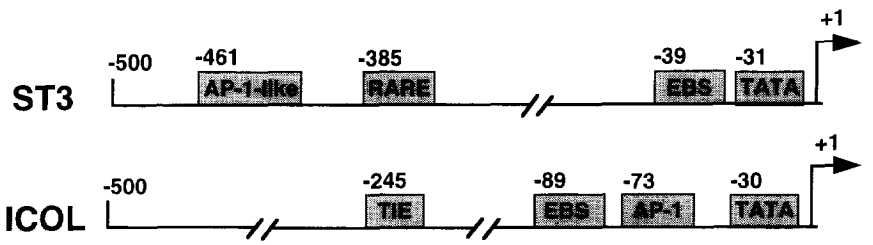

Fig. 9. Schematic representation of human stromelysin-3 and interstitial collagenase gene promoters. The ST3 promoter is characterized by the presence of a retinoic acid responsive element (RARE) and the absence of AP-1 binding site on its proximal part. The AP-1-like element found in the ST3 promoter at nucleotide -461 is not functional [66]. The interstitial collagenase (ICOL) promoter is a paradigm of MMP promoters containing an AP-1 binding site, that can be repressed by retinoic acid. TIE, transforming growth factor-β inhibitory element; EBS, Ets binding site.

progression [10,15,16]. As this is discussed below, such a possibility appears particularly relevant in the case of ST3.

5. Regulation of stromelysin-3 gene expression

The fibroblastic cells expressing the ST3 gene in human carcinomas being consistently found in the immediate vicinity of cancer cells, it appears reasonable to believe that during cancer progression ST3 expression could be triggered by some mediators produced by malignant cells. Such factors should be expressed early during cancer progression since, as discussed above, ST3 gene expression is observed in a number of pre-invasive lesions. This early stromal expression of the ST3 gene is reminiscent of the situation observed for cancerous angiogenesis, since Weidner et al. [63] have found that a subset of in situ breast carcinomas of the comedo type were angiogenic. It is currently believed that such an increase in the number of blood vessels is, at least in part, due to binding of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) expressed by cancer cells, to specific receptors at the endothelial cell surface [64]. Therefore, a comparable scenario may be proposed for ST3, whose fibroblastic expression is known to be induced by various growth factors, of which basic fibroblastic growth factor (bFGF) was found to be the most potent [8,65]. However, these findings were obtained ex vivo, and it is presently unknown whether such factors are also operating in vivo.

The in vivo situation is obviously much more complex. Aside from soluble growth factors and inflammatory cytokines, the expression of several MMPs can be induced by cellular contacts and/or ECM interaction with integrins [15]. Furthermore, in the case of ST3, a number of evidence indicates that its expression is also induced by retinoic acid. The ST3 promoter contains a retinoic acid responsive element (RARE) (Fig. 9), whose functionality has been demonstrated both in transient transfection experiments using COS-1 cells [66], and in human diploid fibroblasts for the endogenous gene [67]. This observation is puzzling since no such RARE was observed in other MMP promoters (Fig. 9) [68], which are known to be repressed and not activated by retinoic acid [69,70].

6. Stromelysin-3 contribution to tumor progression

The possible contribution of ST3 to cancer progression is supported by a number of clinical observations, such as those indicating that high ST3 expression levels are predictive of a poor clinical outcome in breast cancer (Fig. 5) [47,54]. Such a possibility is also supported by experimental findings showing that ST3 expression promotes tumor take in nude mice [71]. In these experiments, MCF7 human breast cancer cells were stably transfected with cDNAs encoding either human or mouse ST3, and subcutaneously injected into nude mice. While ST3 expression was not found to increase invasive nor proliferative properties of transfected cells [71], it was found to reduce the tumor-free

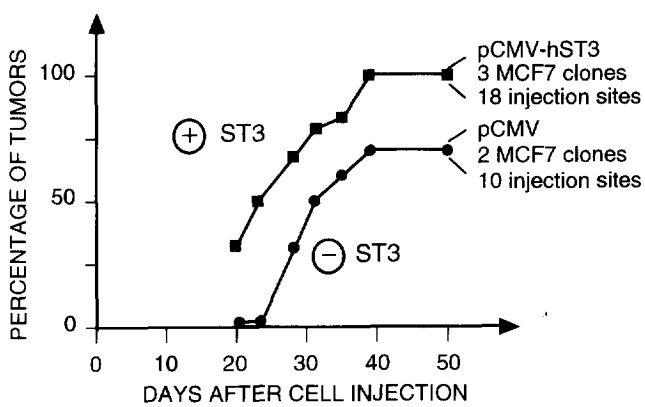

Fig. 10. Increased tumor take of MCF7 human breast cancer cells expressing stromelysin-3. MCF7 breast cancer cells (ATCC HTB 22), stably transfected with either the pCMV vector alone (pCMV) or the vector containing a full-length human ST3 cDNA (pCMV-hST3) were subcutaneously injected into nude mice. ST3-expressing MCF7 cells were associated with a reduced tumor-free period ($P < 10^{-4}$) and a higher percentage of tumors. Data from Ref. [71].

period (Fig. 10), indicating that ST3 is involved in tumor formation rather than in tumor growth in the nude mouse model.

These findings showing that ST3 can promote tumor formation without modifying the invasive potential of cancer cells seem consistent with the in vitro observations showing that the ST3 mature form cannot digest any of the major ECM components tested so far [30–32]. Surprisingly, however, comparable findings to those obtained for ST3 and MCF7 breast cancer cells using the nude mouse model have also been observed for matrilysin, a MMP which exhibits a broad spectrum of activities toward the ECM [15]. Indeed, Witty et al. [72] have found that human colon carcinoma cells stably transfected with a matrilysin cDNA did not increase their invasive capability, although they exhibited an increased tumorigenicity when injected into the cecum of nude mice. These observations suggest that MMPs such as matrilysin and ST3 could contribute to the survival and implantation of cancer cells outside of their compartment of origin. Such an action could be achieved by controlling the activation or mobilization of growth factors and/or cytokines, these acting on cancer cells either directly, or indirectly in promoting stromal cell migration and proliferation. Although the ST3 target has not yet been identified, we note that some MMPs, aside their activities on the ECM, were found to cleave growth factor controlling molecules, such as insulin-like growth factor-binding protein-3 (IGFBP-3) [73] or the ecto-domain of fibroblast growth factor receptor 1 (FGFR1) [74].

Taken together, these observations suggest that during cancer progression the role of MMPs in general and particularly that of ST3 is not limited to facilitating malignant cell invasion alone, but is also likely to participate in other aspects of the malignant phenotype, including cell proliferation and/or survival.

7. Implications for cancer treatment

Besides surgery and radiation, the treatment of human tumors relies heavily on the use of cytotoxic agents, targeted at the increased metabolic and/or proliferative activities of cancer cells. These agents have most often toxic side-effects on normal cells, which limit their use at curative dose for long periods of time. The possibility to develop less toxic agents exerting antitumor activities by targeting unique molecular pathways is therefore important [75,76], and there is evidence to suggest that proteinase inhibitors may represent such molecules [77]. Indeed, several synthetic MMP inhibitors have demonstrated anti-tumor activity in various experimental models of cancer [78–83], and while phase III clinical trials have recently been initiated, the results of phase II studies carried out using such inhibitors appear promising [84,85]. In this context, the observation that ST3 and other proteinases produced during the progression of human carcinomas originate from the tumor stroma and not from the cancer cells, opens interesting perspectives. Targeting stromal cells rather than cancer cells as is the case in conventional chemotherapy, may facilitate drug delivery since the tumor stroma is more easily accessible from the blood stream than cancer cells. Other possible benefits from targeting stromal cells (or stromal cell products) is the decreased likelihood of the development of drug resistance and the possibility to generate anti-tumor agents capable of acting synergistically with the conventional cytotoxic agents directed against cancer cells. In preventing the normal functioning of tumor stroma, tumors should be deprived of essential support services necessary for survival and growth [1].

The challenge now facing researchers is to design highly specific and potent MMP inhibitors [86]. It is presently uncertain whether all the MMPs that are expressed in human carcinomas actually contribute to tumor progression or represent a host reaction against cancer spreading. Furthermore, it is reasonable to believe that synthetic MMP inhibitors will be administered to cancer patients during long periods of time, and therefore inhibitors with specificity limited to only a few MMPs should be used in order to prevent undesirable side effects [87]. In this context, human ST3 is an attractive target because of its unusual structural and functional properties. ST3 being expressed early during tumor progression, its targeting could be envisaged both in individuals known to be at risk of developing invasive malignancies, or as adjuvant therapy following resection of a primary tumor.

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