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Review

Matrix metalloproteinase stromelysin-3 in development and pathogenesis

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Summary. The extracellular matrix (ECM) serves as a medium for cell-cell interactions and can directly signal cells through cell surface ECM receptors, such as integrins. In addition, many growth factors and signaling molecules are stored in the ECM. Thus, ECM remodeling and/or degradation plays a critical role in cell fate and behavior during many developmental and pathological processes. ECM remodeling/degradation is, to a large extent, mediated by matrix metalloproteinases (MMPs), a family of extracellular or membrane-bound, Zn^{2+}-dependent proteases that are capable of digesting various proteinaceous components of the ECM. Of particular interest among them is the MMP11 or stromelysin-3, which was first isolated as a breast cancer associated protease. Here, we review some evidence for the involvement of this MMP in development and diseases with a special emphasis on amphibian metamorphosis, a postembryonic, thyroid hormone-dependent process that transforms essentially every organ/tissue of the animal.

Key words: Matrix metalloproteinase, Stromelysin-3, Metamorphosis, *Xenopus laevis*, Tumor invasion, Metastasis

Introduction

The vast majorities of the cells that constitute a tissue or organ are in constant contacts with each other and/or with the extracellular matrix (ECM) (Hay, 1991). The cell-cell and cell-ECM interactions not only influence the local cellular environment but impact upon the three-dimensional structure of the tissue or organ. The ECM is a complex structure composed of many proteins and other macromolecules (Hay, 1991; Timpl and Brown, 1996). The ECM serves as a structural support media for the cells it surrounds. The ECM can interact directly with nearby cells through their cell surface receptors, especially the integrins (Damsky and Werb, 1992; Schmidt et al., 1993; Brown and Yamada, 1995). Furthermore, it stores many signaling molecules such as growth factors, and ECM remodeling can alter the availability of these molecules (Vukicevic et al., 1992; Werb et al., 1996). In addition, as most cells are surrounded by ECM, cell-cell interactions clearly depend on the nature of the ECM. The interaction between the cell and ECM is thus a dynamic, multi-facet one which influences cell shape, behavior, and ultimately the fate of the cell (Hay, 1991; Ruoslahti and Reed, 1994). Thus, alterations in the ECM is expected to play critical roles in organogenesis and organ function.

ECM remodeling and degradation is believed to be largely through the action of matrix metalloproteinases (MMPs). MMPs are extracellular or membrane-bound enzymes that are capable of digestion specific components of the extracellular matrix. Here, we will first provide a brief summary on the properties of MMPs, in particular MMP11 or stromelysin-3 (ST3), and then review some of the studies that support the involvement of ST3 in developmental and pathological processes.

Matrix metalloproteinases

MMPs are a large family of extracellular or membrane-bound proteolytic enzymes (Alexander and Werb, 1991; Birkedal-Hansen et al., 1993; Barrett et al., 1998; Nagase, 1998; Parks and Mecham, 1998; Pei, 1999; McCawley and Matrisian, 2001). They can be divided into 5 subfamilies, collagenases, gelatinases, stromelysins (ST1, ST2, and ST3), membrane-type MMPs, and others. MMPs consist of multiple domains, including the pre- and pro-peptides, a catalytic domain, a hinge region, and in most MMPs, the hemopexin domain (Fig. 1). In addition, gelatinase A (MMP2) and B

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(MMP9) also contain the fibronectin (FN)-like domain, and membrane type (MT)-MMPs has a transmembrane domain (Fig. 1).

MMPs are synthesized as preenzymes and the pre-peptide is cleaved upon secretion into the ECM as proenzymes with exceptions of ST3 and MT-MMPs (Fig. 1). The proenzymes are enzymatically inactive due to the formation of the fourth coordination bond with the catalytic Zn^{2+} ion by the conserved cysteine residue in the propeptide (van Wart and Birkedal-Hansen, 1990). The proenzymes can be activated in the ECM or on cell surface through proteolytic removal of the propeptide (Nagase et al., 1992; Birkedal-Hansen et al., 1993; Kleiner and Stetler-Stevenson, 1993; Barrett et al., 1998; Nagase, 1998; Murphy et al., 1999). Due to the presence of the conserved furin-recognition motif RXKR (Fig. 1), ST3 and MT-MMPs are activated intracellularly through a furin-dependent process (Pei and Weiss, 1995; Sato and Seiki, 1996; McCawley and Matrisian, 2001).

The mature or activated MMPs have different but often overlapping substrate specificities (Barrett et al., 1998; Uria and Werb, 1998; McCawley and Matrisian, 2001; Overall, 2002). Collectively, they are capable of cleaving all protein components of the extracellular matrix. The collagenases are primarily responsible for degradation of native collagen fibers. In addition, they can degrade entactin, tenascin, aggrecan, and gelatin (denatured collagen). The gelatinases are capable of cleaving gelatin, fibronectin, laminin, aggrecan, elastin, and vitronectin, as well as some native collagen, especially type IV collagen. ST1 and ST2 have a broader substrate spectrum, capable of digesting collagen, gelatin, laminin, fibronectin, elastin, tenascin, aggrecan, vitronectin, proteoglycans, fibrin/fibrinogen. ST3, however, has only weak activity against ECM proteins such as collagen IV, fibronectin, laminin, aggrecan. MT-MMPs cleave proteoglycans, fibronectin, laminin, vitronectin, gelatin, and collagen. The substrates of the other MMPs vary from considerably from each other with matrilysin having a broad spectrum of ECM substrates, similar to ST1 and ST2.

In addition to ECM substrates, increasing studies have found that MMPs are capable of degrading non-ECM extracellular or membrane-bound proteins (Barrett et al., 1998; Uria and Werb, 1998; McCawley and Matrisian, 2001; Overall, 2002). For example, many mature MMPs can cleave other pro-MMPs to activate them. Other non-ECM substrates include pro-growth factors, whose cleavage by MMPs results in active growth factors, proteinase inhibitors, and cell surface proteins such as integrins and E-cadherin, etc. Thus, MMPs may influence cell behavior and tissue development though both ECM remodeling and other mechanisms.

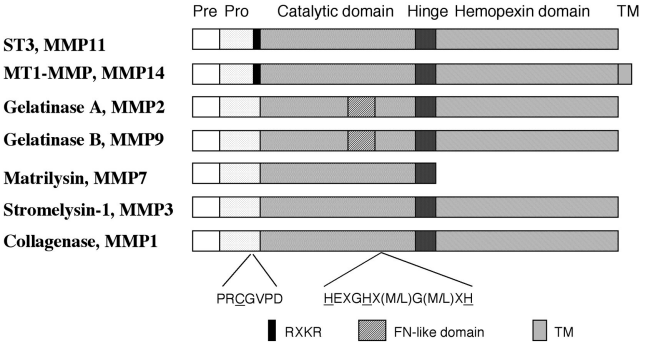

Fig. 1. Structure of MMPs. MMPs generally contain four domains. These are the pre- and pro-peptides, and catalytic and hemopexin domains from N- to C-terminus, respectively. Some exceptions exist. These include matrilysin, which does not have a hemopexin domain, and membrane type MMPs (MT-MMPs), which contain a transmembrane/cytoplasmic (TM) domain at the carboxyl end, and gelatinases, which contain a fibronectin (FN)-like domain. A conserved peptide is present in the propeptide of all MMPs where a C residue (underlined) is involved in the coordination with the catalytic Zn^{2+} ion in the inactive proenzyme. In the catalytic domain, a conserved region contains three H residues (underlined) that coordinate with the catalytic Zn^{2+} ion. Finally, MT-MMPs and ST3 contain the conserved RXKR sequence for furin-dependent intracellular activation of the enzymes.

Stromelysin-3

ST3 (MMP11) was initially isolated as a breast cancer associated gene (Basset et al., 1990). Molecular and biochemical analyses have revealed that ST3 has several interesting and unique properties compared to most other MMPs. First, it is activated intracellularly due to the presence of the furin recognition motif (RXKR) (Fig. 1) in the propeptide and thus secreted in its mature, enzymatically active form (Pei and Weiss, 1995). Second, unlike most other MMPs, ST3 has only a weak activity toward ECM proteins but a much stronger activity against non-ECM proteins α1-proteinase inhibitor and IGFBP-1 (Murphy et al., 1993; Pei et al.,

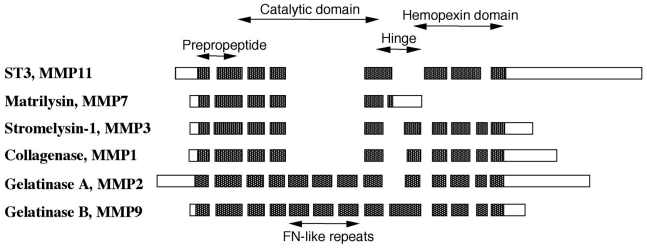

Fig. 2. Structure of MMP genes. MMPs have similar intron/exon organizations with the exception of ST3. ST3 (Anglard et al., 1995; Li et al., 1998) has only 8 exons instead of 10 exons as in the interstitial collagenase (Fini et al., 1987; Collier et al., 1998) and stromelysin-1 (Breathnach et al., 1987) genes, or 13 exons as in gelatinases (Huhtala et al., 1990; Collier et al., 1991; Huhtala et al., 1991), which contain the fibronectin-like repeats, or only 6 exons as in matrilysin (Gaire et al., 1994), which lacks the carboxyl hemopexin domain. The individual exons are shown as bars. The dotted bars indicate the coding region and the open bars represent the 5'- and 3'-UTRs. The individual domains of the coding region are indicated on the top or bottom.

1994; Manes et al., 1997). In addition, ST3 gene has a unique organization in the hemopexin domain that is conserved from human (Anglard et al., 1995), mouse (Ludwig et al., 2000), to frog (Li et al., 1998) (Fig. 2). The hinge region and the hemopexin domain are encoded by 4 exons in ST3 instead of 6 in other MMPs or 2 in matrilysin (MMP7) due to the lack of the hemopexin domain in MMP7 (Fig. 2). Finally, it has been reported that alternative splicing and promoter usage of the human ST3 gene generates a transcript encoding an intracellular active MMP (Lu et al., 2002). These findings suggest that ST3 has unique functions compared to other MMPs.

Expression of stromelysin-3 in developmental and pathological processes

MMPs have been studied extensively largely due to their association with tumor invasion and metastasis (Tryggvason et al., 1987; Stetler-Stevenson et al., 1993; MacDaougall and Matrisian, 1995; Lochter and Bissell, 1999). While they are not expressed at high levels in most adult organs under normal physiological conditions, the expression of the mRNAs and proteins for various MMPs shows strong correlations with tissue remodeling and organogenesis in development in various animal species ranging from human to amphibians (Matrisian and Hogan, 1990; Sang, 1998; Uria and Werb, 1998; Salamonsen and Woolley, 1999; Vu and Werb, 2000; Sarras et al., 2002).

Since the initial cloning of ST3 from a human breast cancer (Basset et al., 1990), extensive studies have been carried out on the expression profiles of ST3 under various pathological conditions. ST3 has little expression in normal adult organs but is expressed in a number of pathological processes, including wound healing, atheroscelrotic lesions, and rheumatoid arthritis (Wolf et al., 1992; Nawrocki et al., 1994; Basset et al., 1997; Schonbeck et al., 1999). Most studies have been on human carcinomas. ST3 is expressed in most human carcinomas, including breast, non-small cell lung, basal cell, and colorectal carcinomas, etc. (Urbanski et al., 1992; Wolf et al., 1992; Basset et al., 1993; Hahnel et al., 1993; Muller et al., 1993; Polette et al., 1993; Wolf et al., 1993; Rouyer et al., 1994; Kossakowska et al., 1996; Basset et al., 1997). Furthermore, high levels of ST3 associate with tumor progression and poor prognosis (Muller et al., 1993; Anderson et al., 1995; Chenard et al., 1996; Ahmad et al., 1998; Tetu et al., 1998; Lochter and Bissell, 1999). Such correlations may not be surprising since a key step in tumor invasion and metastasis is the migration of tumor cells into various host tissues/organs. Tumor cells may activate MMPs to remodel the ECM in order to migrate across the ECM barrier that is present in various organs/tissues. Interestingly, like most other MMPs, ST3 is not expressed in tumor cells themselves. Instead, tumor cells induce the expression of ST3 in the surrounding stromal cells (Basset et al., 1990, 1997; Mari et al., 1998) (and references above). How ST3 may participate in tumor invasion and metastasis remains unclear. It may do so by remodeling the ECM. On the other hand, as stated above, ST3 has only weak activity toward ECM proteins compared to other MMPs *in vitro*, although this may not be the case *in vivo*. Given the ability of ST3 to cleave non-ECM proteins, it may also influence tumor cell growth and development through other pathways.

While ST3 has little expression in adult organs under normal physiological condition, it is highly expressed in a number of developmental processes. High levels of its mRNA are present during embryo implantation, in many developing/remodeling organs such as the limb, tail, and snout, etc. (Basset et al., 1990; Lefebvre et al., 1995; Alexander et al., 1996; Chin and Werb, 1997). In mammals, at least in the developing limb and in mammary gland during post-lactation involution, ST3 expression correlates with apoptosis (programmed cell death) (Basset et al., 1990; Lefebvre et al., 1992; Uria and Werb, 1998), suggesting a role for this MMP in facilitating cell death during normal physiological processes.

We have been studying the function of MMPs during amphibian metamorphosis in *Xenopus laev*is. Amphibian metamorphosis is a postembryonic developmental process initiated and controlled by thyroid hormone (TH) (Dodd and Dodd, 1976; Shi, 1999; Shi et al., 2001). TH exerts its effects on target tissues via binding to thyroid hormone receptors, which are transcription

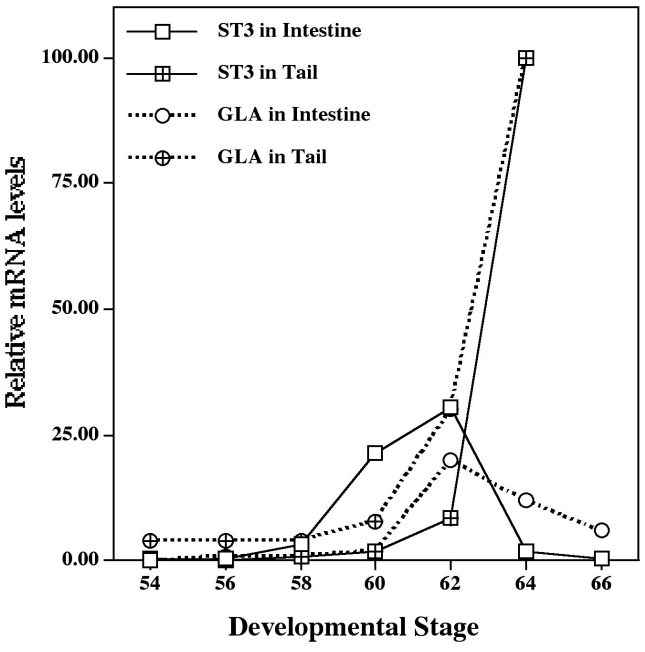

Fig. 3. Organ-dependent temporal regulation of gelatinase A (GLA) and ST3 during *Xenopus laevis* metamorphosis. The mRNA levels for GLA and ST3 are based on (Patterton et al., 1995) and are plotted on different scales with the expression level in tail at stage 64 set to 100.

factors belonging to the nuclear receptor super-family. Thyroid hormone receptor modulates gene expression by binding to specific DNA sequences in target genes (Tsai and O'Malley, 1994; Mangelsdorf et al., 1995). Thus, it is believed that TH induces a gene regulation cascade in individual tissues/organs to effect their transformations. Among the genes which are regulated by TH during *Xenopus laevis* metamorphosis are several MMP genes, including ST3, gelatinase A (GLA), gelatinase B (GLB), collagenase-3 and collagenase-4 (Patterton et al., 1995; Stolow et al., 1996; Shi and Ishizuya-Oka, 2001; Jung et al., 2002).

Through Northern blot and in situ hybridization analyses, we and others have shown that *Xenopus* collagenase-3, collagenase-4, GLA, GLB, and ST3 are expressed in tissues where apoptosis occurs during metamorphosis (Patterton et al., 1995; Ishizuya-Oka et al., 1996, 2000; Berry et al., 1998a,b; Damjanovski et al., 1999; Shi and Ishizuya-Oka, 2001; Jung et al., 2002). Interestingly, unlike other MMPs, the expression of ST3 mRNA and protein is tightly correlated spatially and temporally with apoptosis during intestinal remodeling and tail resorption (Patterton et al., 1995; Ishizuya-Oka et al., 1996; Berry et al., 1998a,b; Damjanovski et al., 1999; Ishizuya-Oka et al., 2000; Jung et al., 2002). For example, in the remodeling intestine, ST3 mRNA is upregulated by stage 58, reaching peak levels by stage 60-62 (Fig. 3). Thus, its expression precedes cell death in the intestine, which begins at stage 60 during natural metamorphosis (Ishizuya-Oka et al., 1996). On the other hand, collagenase-3 and collagenase-4 have little expression during intestinal metamorphosis while GLA is upregulated only when or after cell death has taken place (cell death is completed by stage 62) (Fig. 3) (Patterton et al., 1995; Ishizuya-Oka et al., 1996). Spatially, ST3 is expressed in the fibroblastic cells underlying the apoptotic tadpole epithelial cells in the intestine (Fig. 4) (Patterton et al., 1995; Damjanovski et al., 1999). In addition, ST3 expression is also correlated with the remodeling of the ECM (the basal lamina or basement membrane) that separates the epithelium and the connective tissue (Ishizuya-Oka et al., 1996; Ishizuya-Oka et al., 2000). Similarly, high levels of ST3 mRNA are present in the connective tissue underlying the apoptotic skin epidermis and surrounding the dying muscle cells in the resorbing tail (Damjanovski et al., 1999). Presumably, as in the intestine, the basement membrane underlying these dying cells also remodels during metamorphosis.

The basal lamina is composed of laminin, entactin,

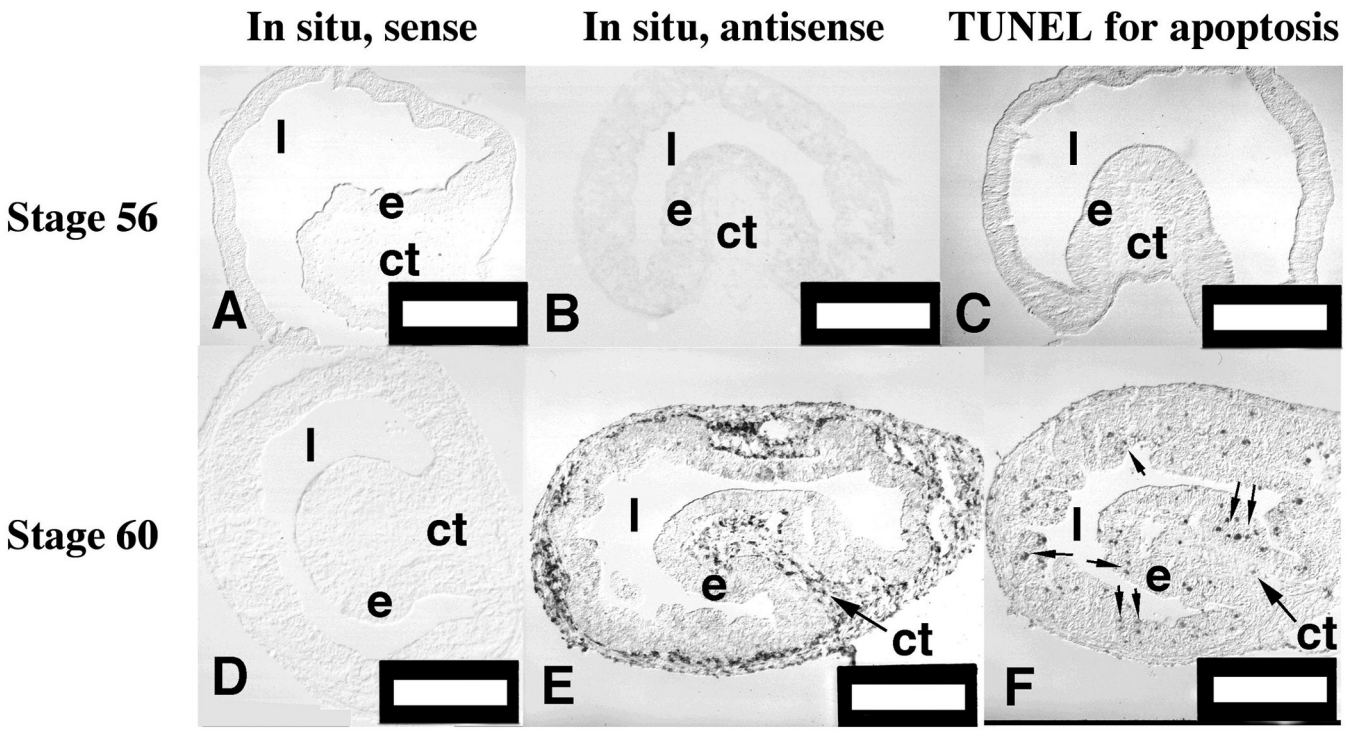

Fig. 4. Association of ST3 expression with larval epithelial cell death during intestinal remodeling. Stages 56 **(A, B, C)** and stage 60 **(D, E, F)** intestines were analyzed by in situ hybridization for ST3 expression **(A, B, D, E)** and by TUNEL assay for apoptotic cells **(C, F)**. There was no detectable ST3 expression at stage 56 **(B)**. ST3 expression was strong in all regions of the connective tissue at stage 60 but not in the epithelium or muscles **(E)**. Likewise, at stage 56, there was no apoptotic signals **(C)**, while there was many apoptotic signals within the epithelium at stage 60 (arrows). Note that the control hybridization with the sense RNA probe did not detect any signal, as expected. ct: connective tissue; e: epithelium; l, lumen: White bars: 250 μm. See (Damjanovski et al., 1999) for more details.

collagens, and proteoglycans, etc. (Hay, 1991; Timpl and Brown, 1996). In premetamorphic *Xenopus laevis* tadpoles, the intestinal basal lamina is thin. It becomes much thicker and multiply folded during metamorphosis along with massive epithelial apoptosis (Ishizuya-Oka and Shimozawa, 1987; Murata and Merker, 1991). Toward the end of metamorphosis, with the progress of intestinal morphogenesis as the adult epithelial cells differentiate, the basal lamina becomes thin and flat again (Shi and Ishizuya-Oka, 1996). Thus, it is remodeled by not totally degraded, consistent with the selective activation of different MMPs and concurrent synthesis of new ECM proteins during metamorphosis. Since ST3 has only week activity toward known ECM proteins *in vitro*, it is likely that ST3 may indirectly participate in the modification of the basal lamina, although one cannot rule out the possibility that ST3 may have strong activity toward certain ECM proteins under *in vivo* condition.

Functional studies on the involvement of stromelysin-3 in developmental and pathological processes

Extensive biochemical studies on various MMPs have revealed their biochemical and molecular properties and led to the identification of many *in vitro* ECM and non-ECM substrates (see above). On the other hand, it has been much more difficult to study the biological functions of MMPs. While potential roles of MMPs in tumor invasion have been supported by many animal studies with natural and synthetic MMP inhibitors (Nelson et al., 2000; Sternlicht and Bergers, 2000), human clinical trials with synthetic MMP inhibitors in advanced cancers have repeatedly yielded disappointing results (Coussens et al., 2002). This might be due to the fact the ECM remodeling/degradation is not the determining factor in tumor invasion and metastasis. Since often metastasis occurs when

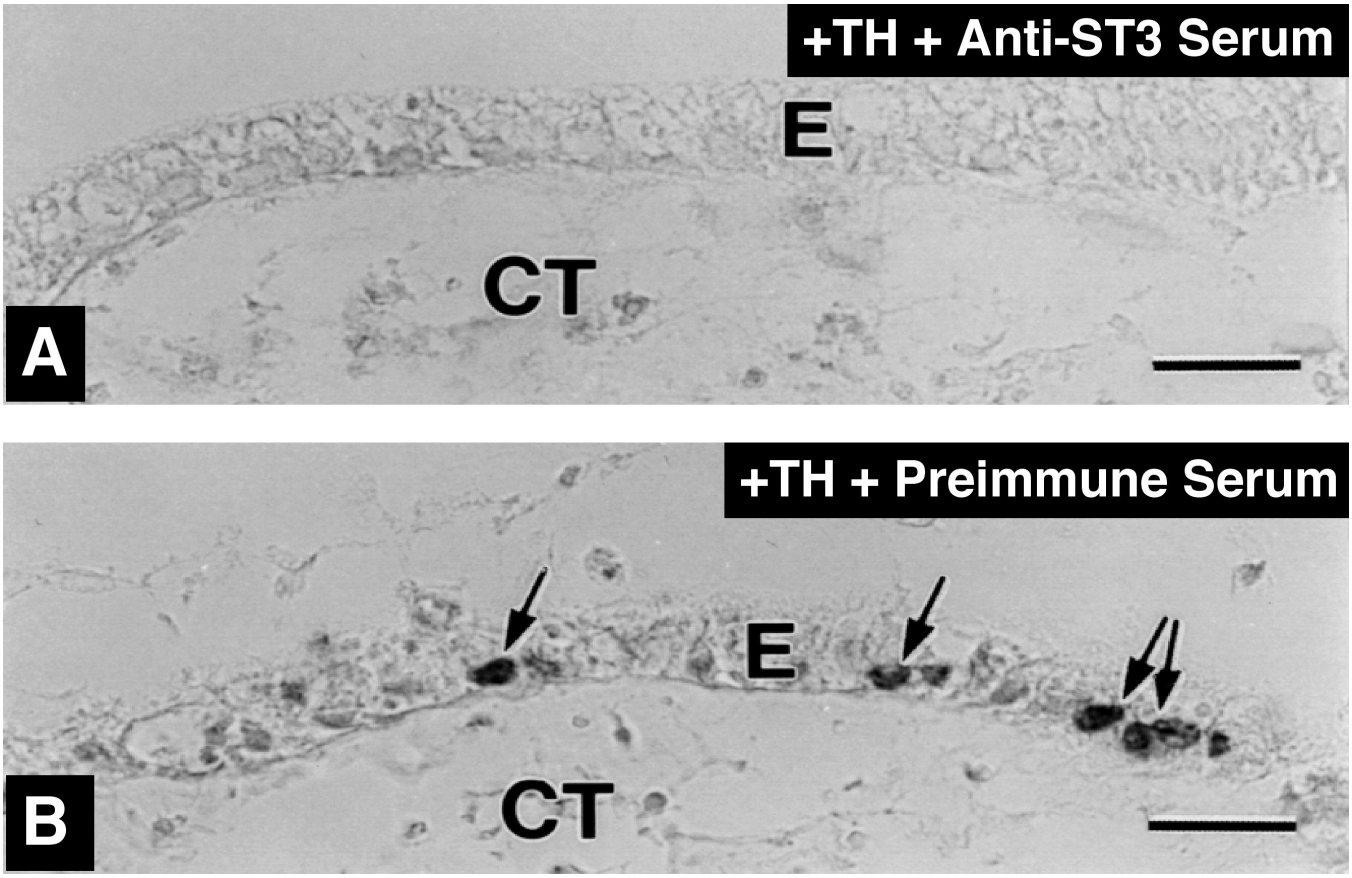

Fig. 5. Blocking ST3 function inhibits TH-induced epithelial apoptosis. Intestinal fragments from premetamorphic tadpoles were cultured *in vitro* for 3 days in the presence of TH and 1% anti-ST3 serum **(A)** or 1% preimmune serum **(B)** and apoptosis (arrows) was detected with the TUNEL method. Note the presence of apoptotic nuclei (arrows) in the absence **(B)** but not presence **(A)** of the anti-ST3 antibody. E: epithelium; CT: connective tissue. Bars: 20 μm. See (Ishizuya-Oka et al., 2000) for more details.

secondary genetic changes take place in the tumor cells. Tumor cells, in the absence of sufficient MMP activity, may find other means, e.g., employing other proteases, to get through the ECM barrier in order to migrate into other tissues/organs. One might expect that MMP inhibition will not block but merely delay tumor progression, which might be difficult to measure clinically. On the other hand, given the ability of MMPs to cleave non-ECM proteins, such as growth factor precursors, they may also influence tumor cell growth and development. Indeed, a number of studies suggest that MMPs play important roles in the regulation of tumor initiation and growth (Fingleton et al., 1999; Lochter and Bissell, 1999). For example, ST3 knockout mice have reduced tumor incidence and smaller tumors after carcinogen treatment (Masson et al., 1998). On the other hand, ST3-expressing MCF7 cells produce more tumors when injected into mice than MCF7 cells expressing the antisense ST3 mRNA (Noel et al., 1997, 2000). Mechanistically, ST3 expression in tumor cells appear to suppress tumor cell apoptosis *in vivo* and functions in a process that requires its proteolytic activity (Noel et al., 2000; Wu et al., 2001).

The role of ST3 in mammalian development remains unclear despite its interesting spatial and temporal developmental expression profiles. ST3 knockout mice are apparently normal and fertile with no observable behavior defects. This is likely due to redundancy in MMPs activity in development. Indeed, similar lack of or week phenotypes were reported for mice lacking other MMPs (Shapiro, 1998; Vu et al., 1998) with the exception of MT1-MMP (Holmbeck et al., 1999; Zhou et al., 2000). On the other hand, studies with MMP inhibitors demonstrated a requirement for MMP activities in development (Alexander et al., 1996; Chin and Werb, 1997).

In the frog model system, we have shown that transgenic overexpression of *Xenopus* collagenase-4, ST3 or a mammalian MT-MMP leads to embryonic defects and lethality in *Xenopus laevis* (Damjanovski et al., 2001). In contrast, a catalytically inactive ST3 mutant has no effects on animal development. These findings are consistent with the tight regulation of MMP expression during *Xenopus* embryogenesis (Damjanovski et al., 2000).

As stated above, ST3 expression is tightly correlated with TH-induced cell death during metamorphosis in different organs. To investigate the role of ST3 in this process, we have taken advantages of the ability to induce metamorphosis in organ cultures *in vitro*. Since ST3 is an extracellular proteinase, we reasoned that we may be able to block the function of endogenous ST3 by simply adding inhibitors to the organ culture medium. For this purpose, we generated a polyclonal antibody against the catalytic domain of *Xenopus* ST3, which is capable of inhibiting the ability of ST3 to cleave the *in vitro* substrate α1-protease inhibitor. When this antibody is added to the culture medium of intestinal fragments, it inhibits the TH-induced epithelial apoptosis as well as the remodeling of the basal lamina separating the epithelium and connective tissue (Fig. 5) (Ishizuya-Oka et al., 2000). Thus, ST3 may directly or indirectly modify the ECM to influence TH-induced cell death during metamorphosis.

Conclusions

The MMP stromelysin-3 shares many similarities with other MMPs but also has several unique properties. It has little expression in adult organs under normal physiological conditions but is upregulated in a number of pathological processes especially tumor invasion. More recent studies suggest that it may also be involved in tumor initiation. Developmentally, ST3 is expressed in many processes involving cell migration and tissue morphogenesis, especially where cell death takes place. At least in the case of frog metamorphosis, strong evidence exists to support a role of ST3 in regulating cell fate during development. How ST3 participate in pathogenesis and development remains unclear, largely due to the lack of information on ST3 substrates *in vivo*. As is the case for other MMPs, despite the identification of *in vitro* substrates for ST3, there have been little *in vivo* studies to support their cleavage by ST3 during pathogenesis or development. This would remain as a major challenge in the near future.

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