Dual Stromelysin-3 Function during Natural Mouse Mammary Tumor Virus-ras Tumor Progression

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ABSTRACT

In human carcinomas, stromelysin-3/matrix metalloproteinase 11 (ST3, MMP11), the expression by nonmalignant fibroblastic cells located in the immediate vicinity of cancer cells is a bad prognostic factor. Using mouse models of primary tumors, it has been demonstrated that ST3 is a key player during local invasion, favoring cancer cell survival in connective tissue through an antiapoptotic function. To investigate the ST3 impact on additional phases of cancer cell invasion, we developed mammary gland cancer prone MTV-ras transgenic mice in wild-type (ras+/-;ST3+/+), or ST3-deficient (ras+/-;ST3/-/-) genotype and studied their whole natural cancer history. The tumor-free survival and delay between the first ras oncogenic hit and primary tumor appearance increased in ras+/-;ST3/-/- mice (P < 0.000001 and <0.000007, respectively). A systematic search for occult primary tumors and metastases revealed, in addition to a lower total number and size of primary tumors (P < 0.02), an unexpected higher number of metastases (P < 0.01) in ras+/-;ST3/-/- mice. Moreover, for a similar number and size of primary invasive tumors, ras+/-;ST3/-/- mice developed more metastases, indicating that the cancer cells evolving in ST3-deficient stroma have an increased potential to hematogenous dissemination. We conclude that the ST3 micro-environment is a consistently active partner of invading cancer cells but that its function differs throughout cancer progression, being tumor enhancer or repressor in processes leading to local or distal invasion. Such a dual effect for an MMP might shed light, at least partially, for the absence of survival benefit for patients included in anti-MMP clinical trials.

INTRODUCTION

The tumor microenvironment plays a crucial role on the issue of transformed epithelial cells and their aptitude to give rise to malignant tumors. To disseminate, invading cancer cells have to first survive and proliferate in a connective compartment that usually is not permissive for epithelial cells. In fact, in normal conditions, connective tissues are involved in the homeostasis of the epithelium through apoptotic signal of anoikis type (1–3). Thus, host connective tissue consisting of molecular, mechanical, and cellular (fibroblastic, inflammatory, and endothelial cells) components can be regarded as an integral part of the invasive tumors contributing to tumor progression (4). Manipulating the host–tumor interactions has therefore the potential of re-orienting malignant processes by re-establishing normal control mechanisms. To date, the molecules implicated in these processes are largely unknown, and molecular mechanisms remain elusive.

Breast cancer cells are known to dramatically remodel the surrounding connective tissue leading to the development of an important stroma through a phenomenon named desmoplasia. Several members of the family of the classical metal-dependent enzyme MMPs that includes the collagenases, gelatinases, stromelysins, matrilysin, metalloelastase, and the membrane-type metalloproteinases were reported to be implicated in this process. In addition, MMPs exhibit a bright range of biological functions; notably, they favor cell proliferation, death and migration, and tissue angiogenesis, all processes involved during tumor progression (5–8). The MMP-11, also named ST3, is a singular MMP whose substrate remains unknown (9). ST3 has been shown to be ectopically expressed in almost all invasive human breast carcinomas and in a large part of their associated metastases. This expression is restricted to a subset of nonmalignant fibroblasts located in the vicinity of cancer cells (10), suggesting the existence of a bi-directional cross-talk between malignant epithelial cells and adjacent normal fibroblastic cells. Both cell/cell contact and soluble molecules have been suggested to be responsible for ST3 expression in invasive carcinomas (11, 12). Finally, high ST3 levels are associated with poor patient clinical outcome of breast cancer patients. Similarly, ST3 has been shown to be a factor of bad prognosis for carcinomas of various other organs, including colon, head and neck, and prostate (10). Therefore, ST3 that is related to tumor progression might represent an appropriate target for specific inhibitors in future therapeutic approaches of carcinomas.

The critical role of stromal ST3 in primary tumor progression has been demonstrated experimentally using several tumor models established in wild-type (ST3+/+) or ST3-deficient (ST3−/−) mice (13). Intragastric 7,12-dimethylbenzanthracene procarcinogen gavage leads to higher number and size of induced carcinomas of the mammary gland when administered to ST3+/+ than to ST3−/− mice (14). Moreover, ST3-deficient mouse embryonic fibroblasts have lost their capacity to improve tumor take of cancer cells in nude mice after s.c. injection. Thus, ST3 is a paracrine factor that promotes cancer cell implantation in the connective host tissues, a process that occurs at the time of local invasive steps (14). This is consistent with the spatiotemporal pattern of ST3 expression by fibroblasts at the interface between invading tumor cells and connective tissue in human carcinomas (10). Finally, ST3 favors the development of tumors as early as 6 days after syngeneic cancer cell injection into s.c. connective tissue. It has been shown that, in this process, ST3 inhibits cancer cell death through apoptosis and necrosis (15, 16). Thus, ST3 helps, in a paracrine manner, the cancer cells to circumvent the anoikis signals naturally emitted by the stromal cells in response to the presence of misplaced epithelial cells inside the connective compartment (1–3). This antiapoptotic function confers to ST3 an additive originality among the MMPs because they are rather reported to be pro-apoptotic molecules (15, 16).

All studies performed thus far concerning the ST3 function in carcinomas have been focused on primary tumor development. The impact of ST3 on the additional steps of tumor progression resulting...
in dissemination, invasion at distance, and metastasis development remains unknown. To address this question, we have designed a tumor model that recapitulates the natural cancer history because it includes the phases of tumor initiation (epithelial cell transformation), promotion (cancer cell survival and proliferation), and progression (local invasion, hematogenous spreading, at distance invasion and implantation). This model was achieved by crossing MMTV-ras transgenic mice (17) and ST3-deficient mice (14). After intercrosses, we obtained mice expressing the ras transgene in a context of stably established wild-type (ras+/+;ST3+/+) or deficient (ras+/−;ST3−/−) mice ST3 genotypes. The ras family genes are the oncogenes most frequently detected in human (18), mouse (19), and rat (20, 21) tumors. The activation of ras-dependent pathway has been shown to be necessary and sufficient to the initial step of the hepatocyte growth factor/scatter factor-induced invasive growth. Additive pathways are then required to achieve the complete execution of the program leading to metastases (22). MMTV-ras mice contain a transgene in which expression of an activated v-Ha-ras oncogene is under the control of the MMTV transcriptional regulatory elements. As a result, ras is expressed at high levels in the mammary glands of mice which carry the transgene, predisposing them to tumors arising from this tissue. Appearance of mammary gland tumors in MMTV-ras mice is dependent on the number of cycles of pregnancy/lactation because MMTV promoter is mainly active during these periods (17).

In the present study, we have investigated the ST3 contribution to the whole process of tumor progression by studying the development of invasive primary tumors and metastases subsequently to ras-induced transformation of mammary gland epithelial cells in a host environment wild type or devoid of ST3. We show that ST3 increases the potency of a cancer cells to give rise to a primary tumor. However, we also show that ST3 reduces the potency of an invasive primary tumor to metastasize.

MATERIALS AND METHODS

Generation of the ras+/+;ST3+/+ and ras+/−;ST3−/− Mice. MMTV-ras mice in an inbred FVB genetic background were obtained from Charles River, Inc. These transgenic mice express an activated Ha-ras oncogene via two mutations, Gly12Arg and Ala59Thr (17). ST3-deficient mice in 129/SvJ genetic background were developed in our laboratory (14). In the targeting construct, exons 2–7 were deleted and replaced by the neomycin resistance gene. Offsprings of the ras+/+;ST3+/+ and ras−/−;ST3−/− genotypes were generated as littermates from common matings so that all animals in the study were of a FVB/129/SvJ mixed genetic background. A previous analysis has revealed that the tumorigenesis of these MMTV-ras transgenic mice was not greatly affected depending of the genetic background (23). Similarly, ST3 favors tumor development whatever the genetic background of the mice (13, 23). Similarly, ST3 favors tumor development whatever the genetic background of the mice (13–16). Offsprings were screened by Southern blot analysis for their ras and ST3 status. Briefly, a small piece of tail was cut from each animal at the time of weaning and then used to isolate and analyze the genomic DNA by standard procedure as described previously (14). Genomic DNAs were digested with SpeI restriction enzyme, and Southern analysis was performed by standard methods. An 800-bp p32-labeled SpeI/EcoRI fragment of the plasmid pBS 3–SB-ST3 was used to identify the 2.4-kb mutant and 8.2-kb normal ST3 alleles. A 5-kb p32-labeled BamHI fragment of the plasmid p poly III-ras was used to identify the 4.5-kb ras transgene.

Ras+/+;ST3−/− mice were indistinguishable from ras+/−;ST3+/+ mice in appearance and behavior. The mammary gland structure was similar in both sets of mice. Because ras oncogene expression was dependent of pregnancy, the mice were maintained in the presence of male breeders continuously from the time they reached sexual maturity (8 weeks) to allow the maximum number of pregnancies and lactation. Only multiparous females were included in the study. All mice were fertile, giving rise to an average of six pups per litter.

Screening for Mammary Gland Tumor Appearance. The study included 46 ras+/+;ST3+/+ and 37 ras+/−;ST3−/− mice. Animals were checked twice weekly for the presence of primary carcinomas of mammary gland by palpation. For each mouse, several parameters were taken into account, namely, the age of tumor onset, the date of the first pregnancy, and the number of pregnancies.

Systematic Search for Mammary Gland Invasive Tumors and Liver and Lung Metastases. The study included 16 ras+/+;ST3+/+ and 16 ras+/−;ST3−/− mice. Once a primary tumor was detected, its growth was monitored, and the animal was sacrificed when the tumor had reached 1 cm in diameter. All mice were autopsied, and all of the mammary glands, the liver, and the lungs were systematically removed for further histological analysis to check for the presence of occult primary tumors and for metastases.

Histological Tumor Evaluation. Tissues were fixed in phosphate-buffered formalin (4%) and embedded in paraffin. Histological examination was performed on H&E-stained sections under light microscopy. The number of tumors and their sizes were evaluated under light microscopy. For each sample, three sections taken at the largest part of the surgical pieces were evaluated.

Statistical Analyses. Data are presented as mean ± SD. All Ps were calculated using Student’s t test. Kaplan-Meier disease-free survival curves were analyzed by the Log-rank test (24). Ps ≤ 0.05 were considered significant.

RESULTS

ST3 Deficiency Increases Tumor-free Survival of MMTV-ras Mice. We generated two genotypic groups of animals which carried MMTV-ras-transgene but differed in their ST3 status, leading to ras+/+;ST3+/+, and ras+/−;ST3−/− mice (Fig. 1). All animals tested (46 ras+/+;ST3+/+ and 37 ras+/−;ST3−/−) developed mammary gland tumors (Fig. 2 and Table 1). The youngest ras+/+;ST3+/+ animal that developed carcinomas was 20 weeks old, and the oldest one was 45 weeks old, whereas the range for tumor appearance in the ras+/+;ST3−/− mice was from 15 to 85 weeks old. Tumor-free curves showed a highly significant (P < 0.00001) difference in the delay of tumor appearance depending of ST3 status. At 25 weeks of age, although ~50% of ras+/+;ST3+/+ mice presented tumors, <10% developed a tumor in the ras+/+;ST3−/− set of mice. The same percentage of tumors in ras+/+;ST3−/− was only reached at 35 weeks. Moreover, at 35 weeks only 5% of mice were devoid of tumors in the ras+/−;ST3+/+ set (Fig. 2).

ST3 Deficiency Increases the Delay between the First ras Oncogenic Hit and Clinically Detectable Tumor Onset. The characteristics of mice at the time of detection of the first primary tumor are presented in Table 1. The mean age for tumor onset for the ras+/+;ST3+/+ group was 25.52 weeks; whereas for ras+/−;ST3−/−, it was 37.27 weeks (P < 0.00017). At the time of tumor detection, ras+/+;ST3−/− mice exhibited one more preg-

Fig. 1. Genotyping of ras+/++;ST3+/+ and ras+/−;ST3−/− transgenic mice. Southern blot analysis of genomic DNA extracted from the two sets of mice. WT, wild type; KO, knocked-out. Lanes 1 and 2, ras+/+;ST3−/− mice; Lanes 3, 4, and 6, ras+/−;ST3+/− mice; Lanes 5 and 7, ras+/+;ST3+/+ mice.
A similar observation was already done using other models of tumorigenesis (13–16). For each mouse, the 10 whole mammary glands which consist of 2 inguinal, 2 abdominal, 2 first thoracic, 2 thoracic, and 2 cervical (25), were removed and studied for the presence of macroscopic and microscopic invasive primary tumors. A total of 229 and 135 invasive primary tumors was found in ras+/+;ST3+/+ and ras+/+;ST3/−/−, respectively (Table 2). Thus, the same animal and even the same gland can develop several tumor foci. The mean tumor number per mouse was ~40% lower for ST3-deficient mice than for ras+/+;ST3+/+ mice (8.69 +/- 6.37 versus 14.31 +/- 8.42, P < 0.02). In both sets of mice, the tumor number and size varied from mouse to mouse showing that individual factors are involved in tumor development. In addition, there were variations among the glands of the same mouse. Tumor distribution was similar in the two sets of mice with a preference for the thoracic localization (63.8% in ras+/+;ST3+/+ and 74% in ras+/+;ST3/−/−). The mean total tumor volume estimated under light microscope was ~2-fold higher in ST3 wild-type mice (64.5 +/- 53.32 mm3 and 35.93 +/- 24.43 mm3 for ras+/+;ST3+/+ and ras+/+;ST3/−/−, respectively; P < 0.03).

Whatever their ST3 Status, Primary Tumors Showed Various Histological Patterns. Only invasive tumors have been taken into account. Histological analysis performed on the 364 primary tumors did not reveal obvious differences depending on the ST3 status of the mice (Fig. 3, A–H). In the two sets of mice, we observed tumors of various sizes. They all corresponded to moderately to poorly differentiated invasive duct carcinomas. The majority of these tumors were growing as solid areas that contained virtually no stroma (Fig. 3, A and B). A few cases in both groups showed tumors characterized by dense desmoplastic reaction (Fig. 3, C and D). Some of the largest tumors were infiltrated by hemorrhage, resulting in a microcystic growth pattern (Fig. 3, E and F). Finally, few tumors with an overall solid pattern showed areas of squamous metaplasia with keratinization and cyst formation (Fig. 3, G and H). Moreover, tumors of different sizes and cyst with different histological phenotypes can coexist in the same mouse and even in the same gland.

**ST3 Deficiency Increases Metastasis Incidence.** Lung and liver of the 16 ras+/+;ST3+/+ and 16 ras+/+;ST3/−/− mice used for systematic primary tumor study were removed and screened for the presence of metastases under light microscope. Although liver was always devoid of metastases, 31% (5 of 16) ras+/+;ST3+/+ mice and 69% (11 of 16) ras+/+;ST3/−/− mice developed lung metastases (Table 2). Moreover, the total number of metastases was higher in ST3-deficient mice. A total of 10 and 34 metastases was detected in ras+/+;ST3+/+ and ras+/+;ST3/−/− mice, respectively. The mean number per mouse was significantly increased (0.63 +/- 1.08 versus 2.12 +/- 2.27, P < 0.01). Whatever the ST3 status, all metastases were very small, all being comprised between 0.2 and 1 mm3. Their histological analysis did not reveal any obvious differences depending of the ST3 status (Fig. 3, I and J).

**ST3 Deficiency Favors Cancer Cell Spreading.** In human cancer, a current clinical parameter used for prognosis is the size of the primary tumors. Thus, we plotted for each of the 16 ras+/+;ST3+/+ and 16 ras+/+;ST3/−/− mice the number of their primary tumors versus the number of the metastases that they developed (Fig. 4A).

### Table 2. Characteristics of primary tumors and metastases in ras+/+;ST3+/+ and ras+/+;ST3/−/− mice

<table>
<thead>
<tr>
<th>Parous mice</th>
<th>nb</th>
<th>Incidence</th>
<th>Age (weeks)</th>
<th>Total nb</th>
<th>nb/mouse</th>
<th>Total size/mouse</th>
<th>Incidence</th>
<th>Total nb</th>
<th>nb/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>ras+/+; ST3+/+</td>
<td>16</td>
<td>16/16</td>
<td>29.12 +/- 5.11</td>
<td>229</td>
<td>14.31 +/- 8.42</td>
<td>64.50 +/- 53.32</td>
<td>5/16</td>
<td>10</td>
<td>0.63 +/- 1.08</td>
</tr>
<tr>
<td>ras+/+; ST3/−/−</td>
<td>16</td>
<td>16/16</td>
<td>41.75 +/- 8.63</td>
<td>135</td>
<td>8.69 +/- 6.37</td>
<td>35.93 +/- 24.43</td>
<td>11/16</td>
<td>34</td>
<td>2.12 +/- 2.27</td>
</tr>
</tbody>
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*P* **p**-values from comparison between groups.

**Table 1. Mouse characteristics at the time of the onset of the first detectable primary tumor**

Mean values +/- SD and Ps are indicated. nd, not done.

Primary tumours | ras+/+; ST3+/+ | ras+/+; ST3/−/− | *p*  
--- | --- | --- | ---  
Incidence | 46/46 | 37/37 | nd  
Age of onset (weeks) | 25.52 +/- 7.68 | 37.27 +/- 12.99 | 0.00017  
Pregnancy (nb) | 4.28 +/- 1.28 | 4.97 +/- 1.92 | 0.05  
Delay after first pregnancy (weeks) | 16.87 +/- 5.26 | 26.00 +/- 10.06 | 0.000007

### Table 2. Characteristics of primary tumors and metastases in ras+/+;ST3+/+ and ras+/+;ST3/−/− mice

Animals were sacrificed and autopsied when they had at least one tumor of 1 cm in diameter. Mean values +/- SD and Ps are indicated. nd, not done.

- **A. Mouse characteristics**
  - Parous mice
  - nb
  - Incidence
  - Age (weeks)
  - Total nb
  - nb/mouse
  - Total size/mouse
- **B. Primary tumours**
  - Total nb
  - nb/mouse
  - Total size/mouse
- **C. Metastases**
  - Incidence
  - Total nb
  - nb/mouse

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Moreover, because several primary tumors were observed for each mouse, we also compared the total tumor size (sum of the volumes of all detected tumors) with the number of metastases (Fig. 4B). When we compared the two sets of tumors, consistent with the higher metastasis incidence reported above in ST3-deficient mice, we observed that, at equal primary tumor number or volume, the number of metastases was clearly higher in ras+/+;ST3−/− mice. Thus, although only 1 out of 5 ras+/+;ST3+/+ mice (20%) that developed metastases had <15 primary tumors, 10 out of 11 ras+/+;ST3−/− mice (91%) did it. Similarly, 80% ras+/+;ST3+/+ metastatic mice had a total tumor volume >50 mm³, whereas this is only true for 45% ras+/+;ST3−/− metastatic mice.

**DISCUSSION**

ST3 belongs to the tumor microenvironmental factors that, although not expressed by the cancer cells themselves, are involved during tumorigenesis. Using a mouse model recapitulating the natural cancer history in a context of stably defined wild-type or ST3-deficient genotype, we have studied the ST3 impact on the whole tumor progression processes, including local and distal invasive steps.

**MMTV-ras, a Tumor Model Relevant to Study ST3 Function during Tumor Progression.** Metastasis is an aleatory process, and animal models allowing to evaluate the ability of transformed cells to form primary tumors and spontaneously invade, enter the circulation, and successfully metastasize are uncommon (26, 27). In MMTV-ras transgenic mice, tumors of epithelial origin were turned on by activated Ha-ras oncogene expression driven by the MMTV promoter at each pregnancy/lactation cycle. Thus, using this model, we have access to an important parameter usually not known in human oncology that is the date of the occurrence of the first oncogenic event because it corresponds to the first pregnancy. All ras+/+;ST3+/+ and ras+/+;ST3−/− mice developed mammary gland cancers presenting characteristics reminiscent of those observed in human breast cancers (28): (a) it is generally admitted that several years lie between genetic alteration and clinical tumor detection. Accordingly, in the 2-year mouse life span scale, there is a relatively long time between the first ras expression and primary tumor appearance (12–77 weeks); and (b) breast cancers are highly heterogeneous. A primary tumor develops from a unique transformed cell and subsequently results from a clonal cell expansion. In our model, each ras targeted cell has the potency to develop a clonal tumor. However, although the ras oncogenic hit is of same nature and occurs at the same time for a given animal, we observed variations in histology and size of invasive primary tumors, even sometimes in a given mammary gland. This exemplifies the fact that ras is not sufficient to transform epithelial cells, but that additional genetic events whose nature and time of occurrence might vary from cell to cell are required. It has been reported that between 6 and 10 clonal successions may be necessary to generate highly malignant human cancer cells (29); and (c) in human disease, cancer cells from primary tumors have the capacity to disseminate and implant in distant organs. Similarly, MMTV-ras mice developed lung metastases. Together, these results show that MMTV-ras tumor history satisfactorily mimics the natural human cancer progression, whatever the mouse ST3 genotype.
ST3, an Enhancer Factor for Local Cancer Cell Invasion. The delay observed between the first oncogenic hit and primary tumor appearance dramatically increased in ras+/+:ST3+/- mice, indicating that cells committed to transform via activated ras expression have more difficulties to locally invade, survive, and/or implant in ST3-deprived host environment. Accordingly, the incidence of invasive primary tumors was ~40% lower in ST3-deficient mice than in wild-type mice, although they presented one more pregnancy/lactation cycle and, subsequently, one more period of ras expression. Because ST3 is never expressed by epithelial cells and that its normal fibroblastic expression does not occur in mammary gland during gestation and lactation (13), we can exclude that ST3 might increase the transcriptional activity of the MMTV promoter. Subsequently, the ras transgene should be expressed with similar efficiency in epithelial cells of ras+/+:ST3+/+ and ras+/+:ST3+/- mice. For the same reasons, the oncogenic potency of activated ras should be similar in epithelial cells whatever their ST3 genotype. Thus, this indicates that, in ST3-deficient mice, 40% of ras-activated cells did not give rise to the development of an invasive primary tumor, because they are either eliminated or kept in a dormant state. In fact, at both local and distal steps of invasion, cancer cells might either die, enter dormancy, or form tumors (30). Similar ST3 effect on primary tumor development was already observed in other tumor models using chemical oncogenic stimulus (14) or s.c. cancer cell injections (13, 15). Furthermore, it has been demonstrated that, at local step of invasion, ST3 circumvents apoptotic signals emanating from the connective tissue and directed against invading cancer cells (15, 16). Thus, consistent with already reported results, local invasive steps of MMTV-ras tumor progression are enhanced by ST3.

ST3, a Repressor Factor for Distant Organ Invasion. Mice having ST3-deficient genotype developed more lung metastases than those of the wild-type set. Thus, we are before the paradox to have, in absence of ST3, a lower number and volume of primary invasive tumors but a higher amount of metastases, the signature of a more aggressive cancer. In fact, although local tumors can easily be cured, the dissemination of malignant cells is deleterious for the patients. Thus, we should admit that, even if relatively small and clinically undetectable, ras+/+:ST3+/- primary tumors may already have the ability to dispatch metastatic cells to distant sites in the body. Moreover, this dissemination could occur very early during the natural cancer history. These data call into question the universality of the clinical paradigm positively correlating metastatic incidence to the primary tumor size. Accordingly, this conceptual question has been recently addressed based on various reflections done from human cancer data (31). Altogether, these results showed that ST3 plays an active repressor role in biological events leading to invasion of distant organs by cancer cells.

ST3, an MMP with Dual Function during Tumor Progression. In human carcinomas, high levels of ST3 expression in primary tumors of various organs are correlated with poor patient outcome (10). Similarly, some other MMPs are invariably up-regulated in the stromal compartment of invasive epithelial cancers and associated with poor prognosis (5–8, 32). It was therefore concluded that MMPs constitute valuable targets for therapy, and numerous companies have developed synthetic inhibitors. However, with few exceptions, clinical trials using MMP inhibitors have not led to significant therapeutic benefit (33) or even have led to significantly poorer patient survival (8, 34). In addition to the already proposed reasons for these disappointing results, including notably the broad spectrum of the used MMP inhibitors and timing of their administration, our data highlight that this could be attributable to MMP paradoxal effect at the primary and secondary tumor sites. In fact, if we analyzed the tumors developed in MMTV-ras mice using the routine clinical prognosis parameters, similarly, we can conclude that ST3 is associated to worse mouse outcome as shown by comparison of the ras+/+:ST3+/+ and ras+/+:ST3−/− tumor-free survival curves. Thus, the combination of the enhancer and repressor paradoxal functions of ST3 during tumor progression results in an apparent negative effect. In the present study, the repressor effect of ST3 on metastases was de-masked because we have the possibility, in addition to know the time of the first oncogenic hit, to visualize clinically occult lung metastases. Consistently with this hypothesis, in vivo mouse experiments have shown that, in some conditions, MMP inhibitors might promote metastases (35). Altogether, our data show that ST3 actively participates in tumorigenesis and that its function dramatically varies depending on the steps of tumor progression. This points out the need to establish the spatial and temporal significance of individual MMP during tumor progression to design more rational compounds.

How Is ST3 Able to Repress Metastasis Development? Our data imply that in addition to its antiapoptotic function during local invasive steps of tumor progression (15, 16), ST3 should have another molecular function that leads to decreased metastatic rate. It can be hypothesized that cancer cells that successfully invade local connective tissues devoid of ST3 have acquired for their own antiapoptotic function provided in wild-type mice by fibroblastic ST3 and therefore became apoptosis resistant and subsequently more aggressive. In this context, it has been reported that MMP-2 (36) and MMP-9 (37)-deficient mice developed less primary tumors but more aggressive. Another possibility might be that ST3 acts on processes associated with angiogenesis and hematogenous spreading of cancer cells. We have observed previously the precocity of the presence of more vessels in very small s.c. tumors developed in deficient mice (15). Interestingly, it has been reported that primary tumors have the capacity to maintain metastases under dormant status through the production of angiogenesis natural inhibitors, such as angiostatin and endostatin (38, 39). Moreover, several MMPs are able to cleave plasminogen and generate angiostatin (33, 40–42). Finally, the repression of metastasis development might result from the lung specificity of extracellular matrix and its associated factors (4) or of the emitted chemokines (43). In fact, we can presume that the nature of the tumor/host interface would be different in local and distant organs. In this context, although ST3 substrate(s) remain unknown, it has been shown that its antiapoptotic function at the primary tumor site results from ST3 enzymatic activity and is dependent on molecule(s) associated to the extracellular matrix (44).

Together, our results support the hypothesis that tumor microenvironmental factors, and among them ST3, are crucial throughout the whole natural tumor progression (45, 46). Moreover, they point out that, during this process, an identical MMP may play paradoxal functions and have a positive or negative impact on the local or distal cancer cell invasion.

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