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Matrilysin as a target for chemotherapy for colon cancer: use of antisense oligonucleotides as antimetastatic agents

Abstract Matrilysin (MMP-7) is the smallest member of the matrix metalloproteinase (MMP) family. It is frequently expressed in various types of cancer including colon, stomach, prostate, and brain cancers. Previous studies have suggested that matrilysin plays important roles in the progression and metastasis of colon cancer. Recently, we have examined the effects of a matrilysin-specific antisense phosphorothioate oligodeoxyribonucleotide on in vitro invasion and liver metastasis in nude mice of two human colon carcinoma cell lines (CaR-1 and WiDr). In culture, the antisense oligonucleotide effectively inhibited both the secretion of matrilysin by CaR-1 cells and their in vitro invasion through a reconstituted basement membrane. In a nude mouse model, the antisense oligonucleotide potently suppressed the experimental liver metastasis of WiDr cells from the spleen. These results suggest that matrilysin has an important role in the liver metastasis of human colon cancer and that matrilysin antisense oligonucleotides have therapeutic potential for the prevention of metastasis.

Key words Matrilysin · Antisense oligonucleotide
MMP · Colon cancer · Metastasis

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Introduction

It is widely accepted that matrix metalloproteinases (MMPs) play an essential role in tumor cell invasion through basement membranes and connective tissues [24]. These enzymes are also involved in other biological processes such as arthritis, wound healing, embryogenesis, and angiogenesis. Of the more than 17 members of the MMP family described to date, gelatinase A (MMP-2), gelatinase B (MMP-9), membrane-type MMPs (MT-MMPs), stromelysin (MMP-3), interstitial collagenase (MMP-1), and matrilysin (MMP-7) are frequently expressed in tumor tissues and are especially important for the invasive growth of tumor cells [24]. Therefore these MMPs are thought to be good targets for antimetastatic chemotherapy.

MMPs are synthesized as latent proenzymes and converted to active forms by limited digestion within their propeptide domains by serine proteinases and some activated MMPs. The activity of activated MMPs is almost irreversibly inhibited by their natural inhibitors, tissue inhibitors of metalloproteinases (TIMPs). TIMPs are known to inhibit tumor invasion in vivo [2, 22].

Recently, potent hydroxamic acid MMP inhibitors such as batimastat and marimastat have been developed by pharmaceutical companies [4, 26, 27]. These inhibitors inhibit tumor growth and in some cases tumor invasion in vivo, and some are now under clinical trial. However, these inhibitors have broad specificity for many MMPs and also inhibit metalloproteinases other than MMPs.

As a different type of antimetastatic agent, we have recently examined the antiinvasive and antimetastatic effects of an antisense oligodeoxyribonucleotide to matrilysin.

Matrilysin and colon cancer

Matrilysin is the smallest member of the MMP gene family and lacks the C-terminal hemopexin-like domain found in all other members of this family [28]. Its cDNA was originally cloned from a human carcinoma cDNA library as a putative metalloproteinase (pump-1) [14], and the protein product was first identified in the culture medium of a human rectal carcinoma cell line and called matrin [12]. Matrilysin is identical to a small uterine metalloproteinase purified from the postpartum uterus of rats [31]. Matrilysin has high proteolytic activity against various extracellular matrix proteins such as fibronectin, laminin, type IV collagen, and proteoglycans [12, 31]. Most MMPs, including interstitial collagenase, gelatinases A and B, and stromelysin, are overexpressed by stromal cells rather than cancer cells in human cancer tissues [20]. However, matrilysin is produced by carcinoma cells but not by stromal cells [18, 19].

It has been reported that the matrilysin gene is expressed in human cancers of the colon [7, 11, 15, 18, 33], breast [3], prostate [19], stomach [11, 15], brain [16], pancreas, kidney, and lung [15]. An immunohistochemical study showed that matrilysin is most frequently expressed in adenocarcinomas of the colon and pancreas and transitional cell carcinoma of the kidney (Table 1) [15]. We also found that matrilysin is synthesized in vascular endothelial cells adjacent to matrilysin-producing tumor cells but not those in healthy tissues (Table 1). In addition, cultured human umbilical vein endothelial cells secrete matrilysin into culture medium [15]. These results suggest the possible involvement of this enzyme in tumor angiogenesis, as well as tumor invasion.

Table 1 Immunohistochemical analysis of matrilysin expression by tumor cells and vascular endothelial cells in various human cancer tissues (*Ad-ca* adenocarcinoma, *HCC* hepatocellular carcinoma, *NSCLC* non-small cell lung carcinoma, *RCC* renal cell carcinoma, *SCC* squamous cell carcinoma, *SCLC* small cell lung carcinoma, *TCC* transitional cell carcinoma)

Primary organ	Type	Positive/total cases	
		Tumor cells	Endothelial cells
Esophagus	SCC	1/5	1/5
Stomach	Ad-ca	4/10	3/10
Colon	Ad-ca	10/10	7/10
Liver	HCC	0/8	0/8
Pancreas	Ad-ca	5/5	5/5
Kidney	RCC	0/7	0/7
	TCC	4/5	2/5
Lung	SCLC	3/5	0/5
	NSCLC	1/5	1/5
Thyroid	Ad-ca	0/5	0/5
Lymph node	Lymphoma	0/5	0/5
Skin	Melanoma	1/5	0/5

A reverse transcriptase polymerase chain reaction analysis has shown that the expression of the matrilysin gene correlates well with the Dukes' stage of colon cancers, and matrilysin mRNA levels are higher in metastatic liver tumors than in primary tumors [7]. We also found that the matrilysin gene is expressed in colorectal adenomas showing severe dysplastic changes but not in those showing mild to moderate dysplastic changes. Matrilysin mRNA is detected in familial colorectal adenomas irrespective of adenoma size and degree of dysplasia [25].

Yamamoto et al. [32] reported that overexpression of exogenous matrilysin gene in matrilysin-negative colon cancer cells increased their in vitro invasive activity. However, in a similar study Witty et al. [30] reported that overexpression of matrilysin in colon cancer cells did not increase their in vitro invasion but increased their in vivo tumorigenicity. Furthermore, Wilson et al. [29] demonstrated that intestinal tumorigenesis is suppressed in matrilysin-deficient *Min*/+ mice, which are heterozygous for the *ApcMin* allele. All these studies suggest that matrilysin contributes to early progression of colon cancer and its invasion and metastasis. In addition, these findings suggest that matrilysin is an important target in therapy for colon cancer and the prevention of metastasis.

Most MMPs, including matrilysin, are secreted in a latent proform (proMMPs). These proMMPs are effectively activated by serine proteinases such as trypsin and plasmin. We have recently found that pancreatic trypsin is expressed in various healthy human tissues including the colon, stomach, lung, liver, skin, and brain [9]. Trypsin is overexpressed in carcinoma cells and the surrounding vascular endothelial cells of some types of cancer tissues [6, 8]. Coexpression of matrilysin and trypsin suggests that trypsin is a natural activator of promatrilysin.

Matrilysin antisense oligonucleotide

Colon cancer is one of the most common human cancers, and frequently develops liver metastasis, the major cause of death in colon cancer patients. Studies have shown that the introduction of matrilysin antisense cDNA into colon cancer cells suppresses their in vitro invasion [32] and tumorigenicity in nude mice [30]. Therefore we attempted to examine the effect of matrilysin-specific antisense oligonucleotide on invasion and metastasis of human colon cancer cells. A phosphorothioate oligodeoxyribonucleotide of 15 bases was designed using a computer program and synthesized [13]. This antisense oligonucleotide effectively suppressed matrilysin mRNA levels and matrilysin secretion in the cultured human colon carcinoma cell line CaR-1 [13], which was used for the initial identification

and characterization of human matrilysin [12]. It also inhibited the in vitro invasion of CaR-1 cells through the reconstituted basement membrane Matrigel.

To examine the effect of the antisense oligonucleotide on metastasis, we used another human colon cancer cell line, WiDr, because CaR-1 cells had low tumorigenicity in nude mice [5]. WiDr cells secrete matrilysin at high levels, but do not secrete gelatinase A, gelatinase B, stromelysin, or interstitial collagenase at detectable levels. The antisense oligonucleotide suppressed matrilysin secretion into the culture medium by 40–80% in different experiments.

In an in vivo experiment to determine the effect of the antisense oligonucleotide on liver metastasis, 5×10^6 WiDr cells in 0.05 ml of serum-containing culture medium were injected into the spleens of nude mice. Antisense and control oligonucleotides were injected intraperitoneally at a dose of 120 μg per mouse every day from 1 day before to 10 days after the intrasplenic injection of cancer cells; 0.25 ml of phosphate-buffered saline (PBS) was used as the vehicle for these injections and one group of mice received only vehicle. Visible tumor nodules in whole livers were counted 11, 28, and 42 days after implantation without histological sectioning. Administration of the antisense oligonucleotide reduced the number of metastatic liver tumor nodules throughout the study compared with PBS or control oligonucleotide (Fig. 1) [5], but did not affect tumor growth in the spleen. These results suggest that matrilysin has an important role in the liver metastasis of human colon cancer and that matrilysin antisense oligonucleotides are useful for preventing metastasis.

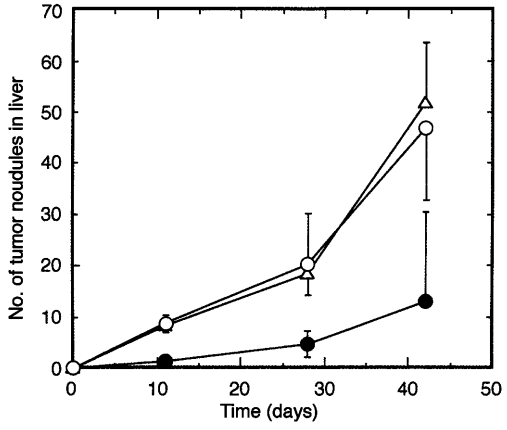

Fig. 1 Effect of matrilysin antisense oligonucleotide on experimental liver metastasis by WiDr human colon carcinoma cells in nude mice. Mice were injected intraperitoneally with matrilysin antisense oligonucleotide (●), control oligonucleotide (△), or PBS (○) every day from 1 day before to 10 days after implantation of WiDr cells. Tumors were counted at 11 days (n = 5 in each group), 28 days (n = 5 in each group), and 42 days (PBS, n = 8; control oligonucleotide, n = 9; antisense oligonucleotide, n = 13). Each point represents the mean ± SD

Discussion and perspectives

Hydroxamic acid MMP inhibitors are important candidates for new antineoplastic and antimetastatic drugs. In recent years, these MMP inhibitors have been widely used in basic research and clinical trials involving cancer patients. They significantly suppress tumor growth and experimental metastasis and angiogenesis, and increase survival time in tumor-bearing animals in some experimental models [4, 26, 27]. It is believed that some of these synthetic inhibitors will prove to be of therapeutic value in ongoing clinical trials in cancer patients. However, it also seems possible that chronic administration of these synthetic inhibitors will produce side effects due to their relatively broad specificity for MMPs. In this regard, the use of antisense oligonucleotides to MMPs might be another way to overcome tumor growth and metastasis.

The use of antisense oligonucleotides has therapeutic potential in a wide variety of diseases [17, 23]. The in vitro and in vivo antitumor effects of antisense oligonucleotides to various target genes, including protooncogenes and cellular genes encoding cytokines, cytokine receptors, and signal-transducing proteins, have been demonstrated [10]. A considerable number of antisense oligonucleotides have entered clinical trials. However, we know of no other group that has reported the use of MMP antisense oligonucleotides as antineoplastic or antimetastatic agents.

The data presented here demonstrate that the matrilysin antisense oligonucleotide effectively inhibits experimental liver metastasis of colon cancer cells in nude mice. This is the first report showing that an antisense oligonucleotide against MMP has antimetastatic activity in vivo. However, the matrilysin antisense oligonucleotide did not inhibit the growth of WiDr colon cancer cells in the spleen and liver of nude mice.

In this animal model, colon cancer cells were injected into the spleens of nude mice. Therefore this experimental system does not include the steps of release of tumor cells from a primary tumor and intravasation that occur in natural metastasis. Thus it is likely that the antisense oligonucleotide blocks the activity of matrilysin in extravasation. It should also be noted that the antimetastatic effect of the antisense oligonucleotide was greater than expected based on its inhibitory effect on matrilysin synthesis in cultured WiDr cells.

It has been reported that phosphorothioate oligonucleotides injected into mice either intraperitoneally or intravenously accumulate mainly in the liver and kidney [1, 21, 23]. Accumulation of oligonucleotide in the liver might explain the efficient inhibition of liver metastasis of colon cancer cells by the matrilysin antisense oligonucleotide.

Although many synthetic MMP inhibitors show antimetastatic effects in experimental metastasis models, they often fail to have significant antimetastatic effects in spontaneous metastasis models [4]. To evaluate the

therapeutic potential of matrilysin antisense oligonucleotides, it is important to test their activity in spontaneous metastasis models and with many different cancer cell lines. Nevertheless, our initial results suggest that matrilysin antisense oligonucleotides have promising therapeutic potential in human cancer.

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