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13. ABSTRACT *(Maximum 200*

This grant investigates the role of EMMPRIN, seprase and DPPIV in breast cancer invasion and metastasis. The aims are to evaluate these conceivable invasion-related molecules as prognostic markers for node-negative breast cancer. We identified, cloned and expressed a full-length cDNA that encoded for a human tumor derived collagenase-stimulating factor, called EMMPRIN. Vectors containing EMMPRIN sense and antisense inserts have been constructed and expression studies showed that EMMPRIN is expressed at invadopodia of invasive breast carcinoma cells where it interacts with membrane proteases and integrins. Using breast cancer lysates as immunogens and selecting hybridoma cell lines by breast cancer antigen ELISA, we have established hybridoma cell lines that produce mAbs potentially useful as tissue or serum prognostic markers. Using a new assay for antigen identification of their cognate mAbs based upon the transient expression of COS7 mammalian cells, we have identified the antigens of 29 mAbs generated from our laboratory. In paraffin sections of human breast carcinoma and malignant melanoma, anti-seprase antibodies stained foci of breast carcinoma, melanoma, and activated stromal fibroblasts and endothelial cells, suggesting that seprase may be a cell activation protease.

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Molecular diagnosis for breast malignancy

(5). INTRODUCTION

Nature of the problem: Serine integral membrane proteases (seprase and DPPIV) and EMMPRIN have been shown to localize predominantly to the invading front (invadopodia) of the breast cancer cells and to shed membrane vesicles. These molecules are promising indicators of the metastatic potential of breast cancer cells (malignancy antigens). Ideally, a measurable product of invasive cancer cells either localized at the invading front of the breast cancer tissues or shed in patient's blood would permit objective assessment of micrometastases formation. The purpose of this application is to perform a clinical evaluation of **invasion-related cell surface antigens, including seprase, DPPIV, MT1-MMP, EMMPRIN, and fibronectin,** as prognostic markers for node-negative breast cancer.

Background: Metastasizing cancer cells can invade the extracellular matrix using plasma membrane protrusions, termed invadopodia, that contact and dissolve the matrix. Various membrane-bound proteases and their associated proteins such as integrins localized on the invadopodial membranes are responsible for the extracellular matrix degradation and cell invasion. Work from our laboratory shows that secreted proteases including gelatinase A and integral membrane proteases (seprase, dipeptidyl peptidase IV [DPPIV], and membrane type-matrix metalloprotease [MT-MMP]) are associated with cell surface invadopodia and shed membrane vesicles. For example, invasive human breast carcinoma cell line MDA-MB-436 retains the invasive phenotype *in vitro*, constitutively expresses invadopodia-associated proteases, degrades and enters into a fibronectin-rich collagenous matrix. We suggest that invadopodia-associated proteases are ideal targets for the diagnosis and treatment of cancer as their presence in association with primary tumors may signal increased metastatic potential. An approach toward the development of new prognostic markers for breast malignancy involves production of monoclonal antibodies directed against membrane proteases in a mixture of invadopodial glycoproteins. The hybridoma cell lines that produced monoclonal antibodies against invadopodia-associated proteases were selected by antibody localization at invadopodia and antibody immune reactivity against breast cancer antigens. Membrane protease accessibility at the cell surface invadopodia and shed membrane vesicles can therefore be used to detect surface proteases on micrometastases or to detect components shed by micrometastases in serum.

(1) Cell surface proteases of breast cancer cells. Evidence shows that integral membrane proteases, both metallo- (MT-MMP) and serine- (seprase and DPPIV) types, are associated with membrane protrusions of carcinoma cells and fibroblasts where matrix degradation occurs. We thus suggest that these proteases form structurally and functionally linked complexes on invadopodia that play an important role in the cell surface proteolysis during the invasion of cancer cells into the extracellular matrix. We have cloned a cDNA that encodes the 97-kDa subunit of seprase {Goldstein, Ghersi, et al. 1997 ID: 7995}. Its deduced amino acid sequence predicts a type II integral membrane protein with a cytoplasmic tail of 6 amino acids, followed by a transmembrane domain of 20 amino acids and an extracellular domain of 734 amino acids. The carboxyl terminus contains a putative catalytic region (~200 amino acids) which is homologous (68% identity) to that of the nonclassical serine protease DPPIV. RT-PCR analysis showed that

seprase and DPPIV mRNA were present in the invasive breast carcinoma cell lines MDA-MB-436 and HS578t, as well as in fibroblasts and activated endothelial cells. Furthermore, we demonstrated the importance of localization of the MT-MMP to invadopodia in invasion and showed the involvement of the transmembrane/cytoplasmic domain in invadopodial localization {Nakahara, Howard, et al. 1997 ID: 8027}.

(2) Breast cancer EMMPRIN. We have cloned and sequenced a full length cDNA encoding a 20-58 kDa glycoprotein, which is located on the outer surface of human tumor cells, and which interacts with fibroblasts to stimulate expression of several matrix metalloproteinases in the fibroblasts. We show that a human tumor derived collagenase-stimulating factor, now called extracellular matrix metalloproteinase inducer (EMMPRIN), is associated in membrane protrusions of carcinoma cells where matrix degradation occurs. Northern analysis of mRNA from six breast carcinoma cell lines showed that they all express high levels of EMMPRIN message. We suggest that EMMPRIN on carcinoma invadopodia may activate stromal fibroblasts and angiogenic endothelia, which express SIMP members (seprase & DPPIV) and MT1-MMP. Thus, EMMPRIN may play an important role in the activation of fibroblast/endothelial invasion during breast cancer progression.

(3) Serum fibronectin of breast cancer patients. Because of our progress in obtaining mAbs useful for breast cancer antigen ELISA, we have moved forward to perform pilot serum studies. The use of circulating tumor markers to develop serum tests for screening breast cancer patients have been suggested {Schwartz, Schwarting, et al. 1993 ID: 4874}{Mansour, Ravdin, et al. 1994 ID: 5624}{Chen, Lee, et al. 1994 ID: 6847}. For example, it was found that increases in the serum concentrations of carcinoembryonic antigen (CEA) associated with progression of cancers of the gastrointestinal tract, lung, and breast (Beard and Haskell, 1986). Previous studies also considered fibronectin in serum as a potential breast cancer marker (Ylatupa et al., 1995).

Goals: We have generated several panels of monoclonal antibodies that recognize seprase, DPPIV and EMMPRIN as determined by antigen identification by their respective monoclonal antibodies using transient transfection with cognate expression plasmids in COS-7 mammalian cells. Using these antibodies we found that EMMPRIN and protease molecules were expressed in human breast carcinoma and melanoma cells, when these cells were identified to be invasive using fibronectin- and collagen degradation assays. The cells made contact with the matrix as well as stromal fibroblasts and endothelial cells, localized protease molecules to invadopodia, activated soluble and matrix-bound gelatinase A, and degraded and invaded the matrix. We established monoclonal antibody-based ELISA and Western blot analyses for quantitative detection of seprase, DPPIV, fibronectin and EMMPRIN in breast cancer and melanoma cells, in tumor tissues, in sera of experimental metastasis mice as well as in sera of node-negative breast cancer patients. Furthermore, the invadopodial protease complexes may potentially be exploited for therapeutic advances by the development of specific antibodies and inhibitors that block their activities, and by the use of monoclonal antibodies to target cytotoxic molecules to micrometastases.

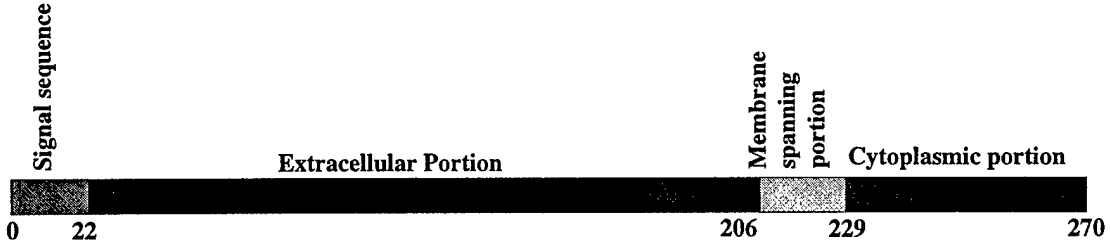
(6). BODY

Task 1. Characterization of breast cancer EMMPRIN.

1a. Screening of MDA-MB-231 cDNA library with anti-EMMPRIN mAbs, and positive clones will be sequenced. Year 1

In this study, we have used antibody screening of λgt11 cDNA library of MDA-MB-231 cells to isolate a complementary DNA encoding a protein previously named "p10 maligrin". An open reading frame of three overlapping clones was identified as a human tumor derived collagenase-stimulating factor, now called extracellular matrix metalloproteinase inducer (EMMPRIN) {Biswas, Zhang, et al. 1995 ID: 6777}. Computer database searches indicate that EMMPRIN is a member of the immunoglobulin superfamily and that the deduced amino acid sequence of EMMPRIN is identical to that recently reported for human basigin and M6 antigen, molecules of previously undetermined biological function. Thus, EMMPRIN has following properties:

- The deduced amino acid sequence from EMMPRIN cDNA predicts a type I integral membrane protein. It is a 58-kDa glycoprotein and its non-glycosylated form is a 27-29-kDa protein.
- EMMPRIN, identical to human basigin and M6 antigen, is a member of the immunoglobulin superfamily (e.g. NCAM, ICAM and VCAM) which has been shown to interact with Integrins.
- It is present in human lung and breast carcinoma cells and it functions by stimulating fibroblasts to produce MMP-1, MMP-2, and MMP-3.



1b. Expression of EMMPRIN: transfection of EMMPRIN expression vector into low expressing cells, and analysis of transfectants for collagenase-stimulating activity or binding to integrins, C4BPα and membrane proteases (invadopodial activity). Years 2 & 3.

We have expressed EMMPRIN in COS7 monkey kidney fibroblastic cells (transient expression). As most transient transfection models, we have obtained 20% expression efficiency. However, COS7 was engineered to exhibit extremely high protein expression, i.e., 100- to 1,000-fold increase in protein expression. In transfected COS7 cells, EMMPRIN is expressed as soluble and cellular forms with M(r) ranging from 20- to 58-kDa, which interacts with untransfected cells to stimulate production of several matrix metalloproteinases by COS7 cells in the medium. We determined the effect of EMMPRIN overexpression in production of gelatinase A (also called MMP-2 and 72-kDa type IV collagenase) in the total cell population. Using gelatin zymography to assess

gelatinase A in cell conditioned media, we found that overexpression of EMMPRIN in 20% COS7 cells stimulated the production of gelatinase A two folds in the total COS7 cell-population, a result confirming that of Biswas and colleagues {Kataoka, De Castro, et al. 1993 ID: 6768}. Linda Howard, C.C. Lee, and Jerry Santos have studied EMMPRIN expression in six breast carcinoma cell lines, one normal epithelial line and two fibroblastic lines using Northern blotting analysis as well as RT-PCR of mRNA profile. They found by Northern blotting that while EMMPRIN message was high in breast carcinoma cells, but it was low in normal epithelial cells and fibroblasts (Figure 1A below).

In MDA-MB-231 cells, mAb B26 recognizes the native 58-kDa EMMPRIN by

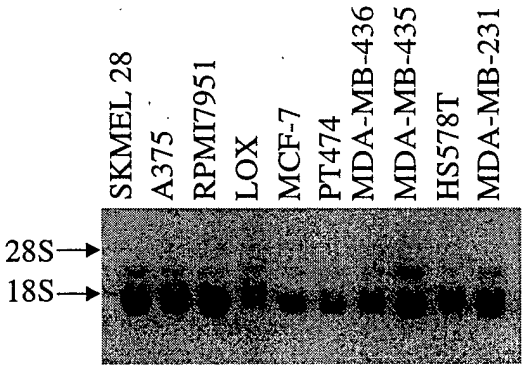
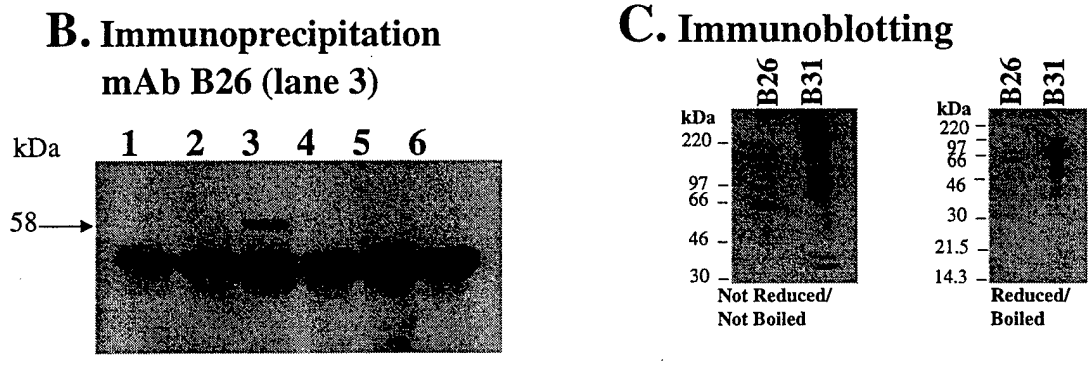


Fig. 1



both immunoprecipitation and immunoblotting analyses (Figure 1B,1C above). Antibody B26 was used to isolate a complementary DNA encoding EMMPRIN from a λgt11 cDNA library derived from MDA-MB-231 cells. EMMPRIN protein expression was correspondent with mRNA expression in three breast carcinoma cell lines, MDA-MB-231, MDA-MB-436 and MCF-7, which showed different degrees of cell invasiveness as assayed by *in vitro* fibronectin/gelatin degradation/invasion assay {Chen, Yeh, et al. 1994 ID: 6844}. Expression levels of EMMPRIN in these cells correspond with cell invasiveness: from high to low invasiveness, MDA-MB-231 > MDA-MB-436 > MCF-7.

To examine the role that EMMPRIN may play in the interaction with fibroblasts, immunocytochemical localization of EMMPRIN was performed as described {Chen, Lee,

et al. 1994 ID: 6847}. Using mAb B26, EMMPRIN co-localizes with invadopodial antigens (IA) and a p90 membrane glycoprotein (p90) in invasive breast carcinoma cells (HS578T), demonstrating that EMMPRIN is an invadopodial antigen (Figure 2A,2B below). In addition, EMMPRIN localized on invadopodia of invasive MDA-MB-231 cells but diffusely on surfaces of non-invasive MCF-7 cells. These results shed light on the function of EMMPRIN in tumor invasion. EMMPRIN may exist its cellular function by association with specific molecules localizing at invadopodia, possibly integrins.

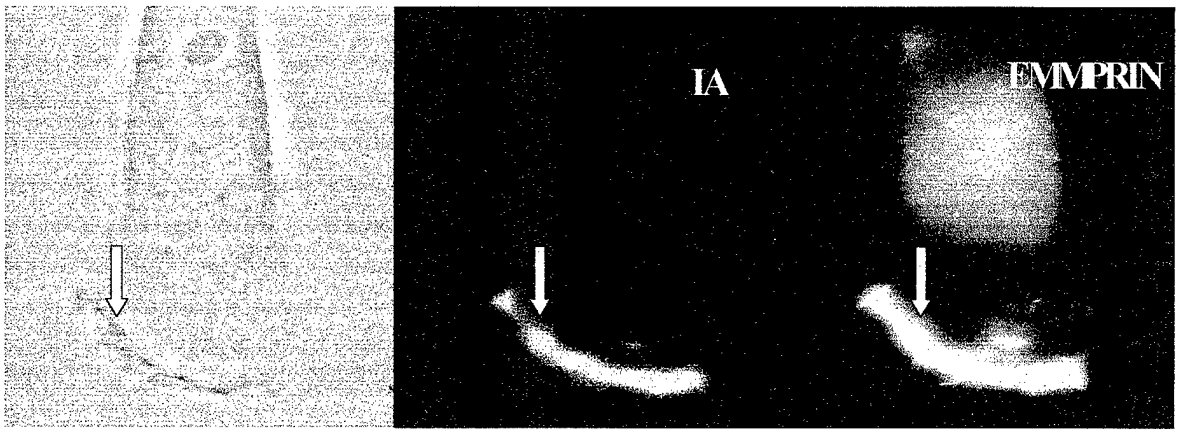


Fig. 2A

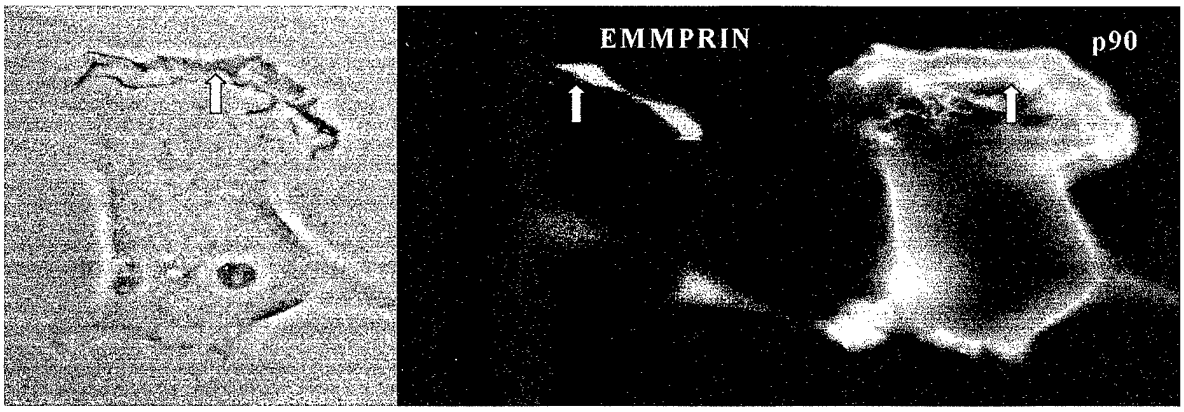


Fig. 2B

1c. Suppressing EMMPRIN expression: transfection of antisense orientation of mammalian vector into breast cells and confirmation of antisense RNA expression, and analysis of invadopodial activity.
<u>Years 3 & 4</u>.

We have constructed vectors containing EMMPRIN sense and antisense inserts and transfected them into MCF7 cells (low expresser, non-invasive). Prominent transfectants will be established and they require several months of cell cloning and characterization of clones. Our experience with seprase cloning {Goldstein, Ghersi, et al. 1997 ID: 7995} suggests that we may be able to obtain positive clones in near future. In addition, we have used vectors containing EMMPRIN sense and antisense inserts to transfect MDA-MB-231 cells (high expresser, invasive). Prominent transfectants will be established and the clones will be characterized in the last year of this grant period.

1d. Mapping of functional domains of EMMPRIN: mutation (deletion) of cDNA, and expression of mutant EMMPRIN in breast cells and analysis of effect of mutation on invadopodial activity. Years 3 & 4

EMMPRIN may associate with integrin for its localization at invadopodia {Sincock, Mayrhofer, et al. 1997 ID: 7743}. We plan to identify potential integrins in association with EMMPRIN and putative EMMPRIN domain responsible for this association.

Task 2. Production of monoclonal antibodies directed against tumor cell surface antigens, which are useful for clinical studies.

2a. Screening of breast cancer cells, MDA-MB-231, in paraffin sections with existing mAbs, and selection of diagnostically useful mAbs against malignancy antigens. Years 1 & 2.

The goal of this originally proposed Task was to select available antibodies that are useful in diagnostic tests of a large set of breast cancer Paraffin-embedded samples. We obtained only three anti-seprase mAbs, D8, D28 and D43, that could stain breast cancer Paraffin-embedded samples. We have established a new assay for antigen identification of their cognate mAbs based upon the transient expression of COS7 mammalian cells. Using this assay, we have confirmed the antigens of several mAbs generated from our laboratory: mAb B26 directed against EMMPRIN, 6 mAbs against seprase, and 22 mAbs against DPPIV.

2b. Production of new monoclonal antibodies directed against breast cancer antigens, and positive hybridoma clones will be established. Year 3.

To produce new monoclonal antibodies directed against breast cancer antigens, we have developed a new strategy of mAb production: the use of breast cancer lysates as immunogens and selection of hybridoma cell lines by breast cancer antigen ELISA. We have initiated new experiments by direct immunization of rats with carcinoma lysates derived from node-**positive** breast cancer patients. We used malignant breast cancer tissues, ~15 gm in weight, as starting material and, after homogenization in PBS, membrane glycoproteins were subjected to purification by WGA-agarose chromatography. We have immunized 6 rats with WGA binding proteins and established 4 hybridoma cell lines that were selected based upon breast cancer antigen ELISA. Using the recently developed strategy of mAb production: the use of breast cancer lysates as immunogens and selection of hybridoma cell lines by breast cancer antigen ELISA, we have established three hybridoma cell lines, M4, S22 and E98, that produce mAbs potentially useful as tissue or serum prognostic markers.

Task 3. Tissue prognostic markers for node-negative breast cancer.

3a. Staining paraffin sections of lung-micrometastases in experimental invasion and metastasis mice with available mAbs, and analysis of the results. Year 2.

Three mAbs D8, D28 and D43 that recognized seprase could stain paraffin-

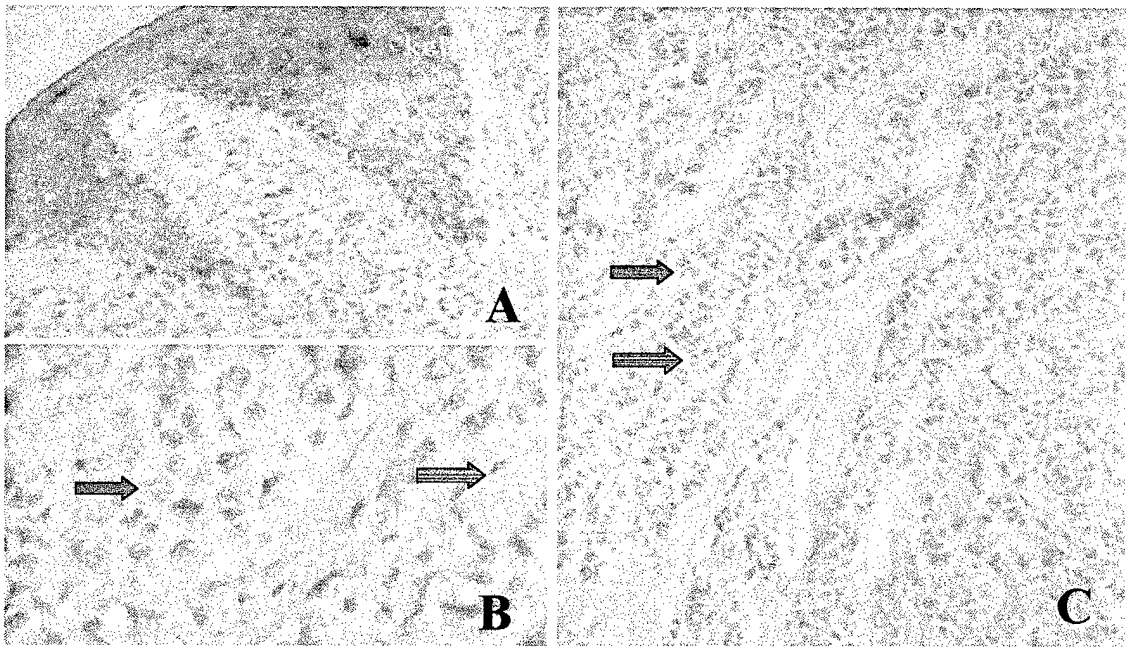


Fig. 3

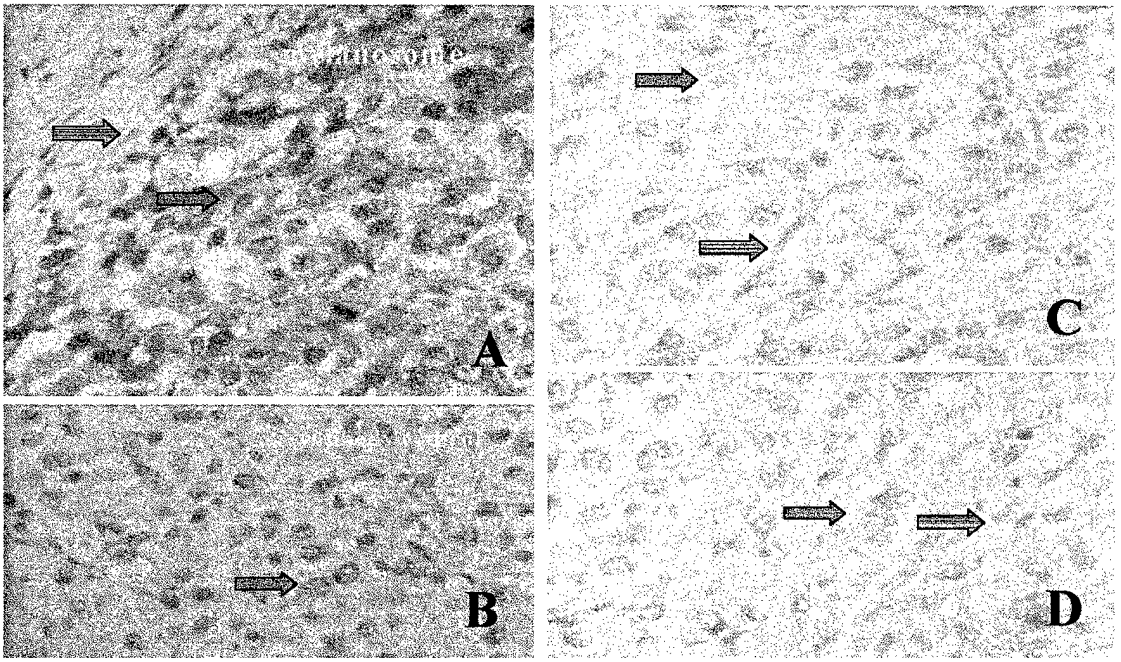


Fig. 4

embedded sections. We have used these mAbs to stain paraffin-embedded materials, including lung-micrometastases in experimental metastasis mice, small human melanoma, and human breast carcinoma (Task 3a, 3b, and 3c, respectively). We found that mAb D8, D28 and D43 against seprase stained only LOX human melanoma cells in primary tumors as well as lung metastases but they did not label any host (mouse) stromal cells in the experimental metastasis model. The result is very interesting as metastasizing cells seem to maintain seprase expression and anti-human seprase mAbs do not recognize mouse antigens.

3b. Staining paraffin sections of small human melanoma (13) containing invading front (<1 cm) with anti-seprase mAbs, and analysis of the results. Year 3.

In collaboration with Dr. A.-K. Ng of Maine Blood Research Foundation, we have examined the seprase protein expression *in situ* in small human melanoma. Anti-seprase mAbs D8, D28 and D43 stained paraffin-embedded sections of human melanoma tissues. Antibody D8 gave best staining results: it stains both tumor cells and fibroblasts in 5/8 human skin melanoma samples when keratin mAb AE1/3 staining was used as a positive staining control (an example was shown in Figure 3 above). Other 5 samples were found to be negative due to poor preservation. Similarly, when mAb HMB45 staining for melanosomes was used as positive staining of samples, mAbs D28 and D43 stained both tumor cells and fibroblasts in 5/8 human skin melanoma samples (Figure 4 above). These results were published in abstract form and we are prepared a manuscript in combination with the work on biochemical identification of melanoma seprase and DPPIV (see list of manuscripts below).

3c. Staining paraffin sections of human breast carcinoma (20) with anti-seprase mAbs or available mAbs, and analysis of the results. Year 3.

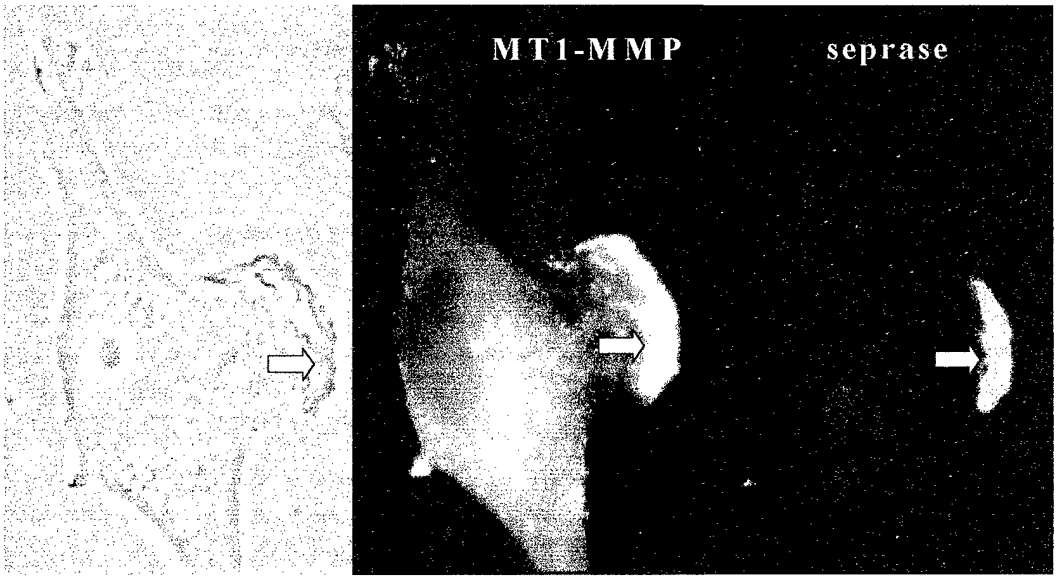


Fig. 5

In collaboration with Dr. A.-K. Ng and Dr. Tetsu Yamane, we examined the seprase protein expression *in situ* in small human breast carcinoma (20 samples). In cultured cells, seprase and MT1-MMP co-localize with invadopodial antigens (IA) in invasive breast carcinoma cells (HS578T), demonstrating that both seprase and MT1-MMP form a part of invadopodial antigens (Figure 5).

Antibody staining of paraffin-sections of breast cancer tissues showed that seprase

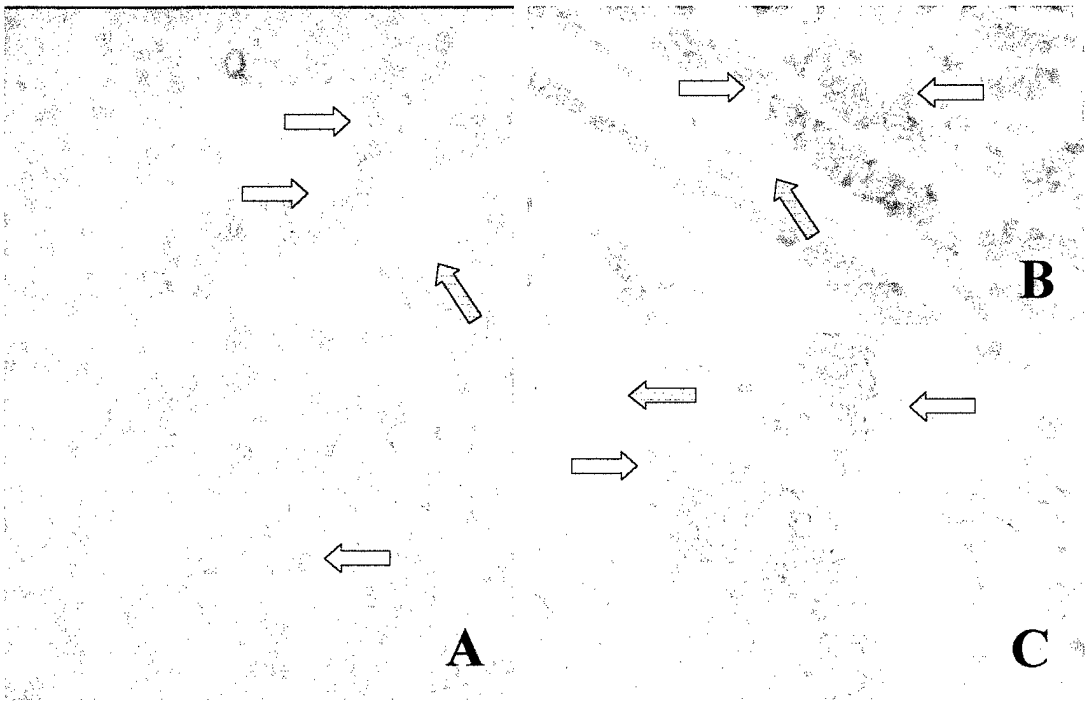


Fig. 6

was expressed in malignant breast ductal carcinoma cells (Figure 6 orange arrows) and adjacent fibroblasts (Figure 6 green arrows) but not in distant normal tissue cells.

Immunofluorescent staining of frozen sections of breast carcinoma samples showed that seprase was expressed in malignant breast ductal carcinoma (Figure 7 panel B, red cell clusters) and adjacent angiogenic microvessel endothelia (Figure 7 panel A arrows, indicating factor VIII staining for angiogenic endothelia).

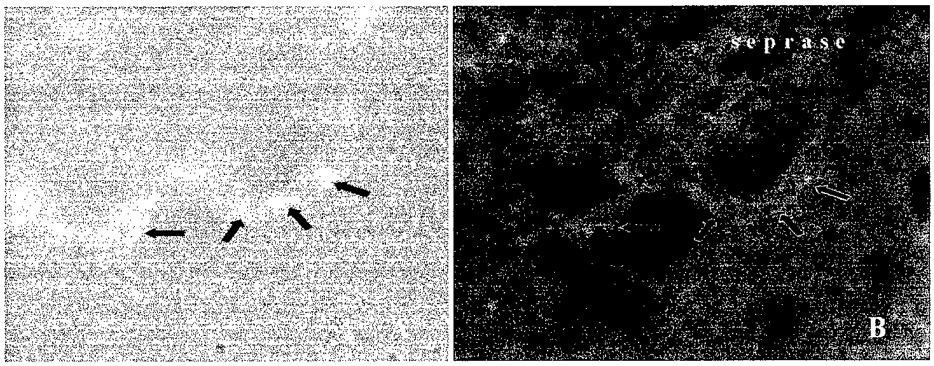


Fig. 7

Consistently, molecular cloning of melanoma seprase {Goldstein, Ghersi, et al. 1997 ID: 7995} shows that melanoma seprase is identical with fibroblast activation protein α and it is expressed in breast carcinoma cells as well as activated fibroblasts and endothelial cells {Scanlan, Raj, et al. 1994 ID: 6906}. Thus, the data suggest that seprase is also a cell activation protease{Rettig, Garin-Chesa, et al. 1988 ID: 5791}{Garin-Chesa, Old, et al. 1990 ID: 5794}.

3d. ELISA Screening of breast cancer tissue lysates from node negative- & positive- breast cancer patients (100), as compared to normal with available mAbs, and statistical analysis of the results. Years 3 & 4.

Because of the limit of number of mAbs available for paraffin-embedded materials, we had added a new breast cancer antigen ELISA to address the role of seprase, DPPIV, EMMPRIN, and fibronectin in node-negative breast cancer (see Task 4b below). Now, we have obtained three mAbs M4, S22 and E96 that recognize breast cancer antigens and these mAbs are potentially useful tools for diagnosis of breast cancer. Specifically, main goal in the updated Task 3 is to perform breast cancer antigen ELISA using breast cancer tissue lysates from node negative- & positive- breast cancer patients and statistical analysis of the results.

3e. Studies on EMMPRIN, seprase and DPPIV message RNA of 20 representative tumor tissues of node negative breast cancer patients with RT-PCR approach, and analysis of the results. Year 4.

We have established RT-PCR analyses to determine the levels and alterations of mRNA. Reverse Transcriptase - PCR (RT-PCR), a procedure that we have already tested for seprase mRNA in several *in vitro* cell lines {Goldstein, Ghersi, et al. 1997 ID: 7995}(see *List of Grant Manuscripts*). The method demonstrates the presence of mRNA corresponding to seprase in highly invasive melanoma cell line LOX, moderately invasive melanoma line RPMI7951, moderately invasive breast carcinoma line MDA-MB-436, and embryonic lung fibroblast line WI-38, but not in non-invasive melanoma line SKMEL28. Strikingly, it showed that a truncated seprase mRNA appears in all lines: RMPI7951, LOX, MDA-MB-436 and 231, and HUVEC. We believe that this PCR method will give us a pleasant surprise that could resolve the altered protease mRNA(s) in malignant carcinomas.

Task 4. Serum prognostic markers for node-negative breast cancer.

4a. Establishment of breast cancer antigen ELISA using breast cancer cells in culture (6 cell lines) and in experimental (mouse) melanoma metastasis model. Years 1& 2.

As described previously in 1996 Progress Report, we established mAb-based ELISA for the quantitative detection of DPPIV and seprase in cultured breast cancer and melanoma cells as well as in sera of experimental metastasis mice. Serum ELISA tests have been conducted using serum samples from 100 mice injected s.c. with LOX cells. Serum samples were taken at the time of initial cell inoculation (day 1) and at the day (day 36) where lung metastases were detected. We performed three times of this experiment (each with 33 mice) and conformed metastases that were originally described by Fodstad and colleagues {Fodstad, Kjonniksen, et al. 1988 ID: 190}{Fodstad, Aamdal, et al. 1988 ID: 192}{Fodstad & Kjonniksen 1994 ID: 6001}. We collected sera from these mice (0.2-ml each bleeding each mouse) and tested for the presence of human melanoma seprase using "sandwich" ELISA. Unfortunately, "sandwich" ELISA for human melanoma seprase did not detect significant shed seprase in mouse sera. As

discussed in Tasks 2b & 3 above, seprase alone may not be effective as a prognostic markers of breast cancer and we have established several mAbs and new antigen ELISA for detecting breast cancer antigens. This new development will be applied to Task 4b, i.e. evaluation of these breast cancer antigens as serum prognostic markers in node-negative breast cancer patients. We concluded that the animal work should be ended Year 2.

4b. Studies on serum screening of breast cancer patients using same samples (200) at the time of initial surgery, the latest recurrence-free follow-up time, and the first recurrence follow-up time that are available in the Serum Bank of the Lombardi Cancer Center, and statistical analysis of the results. Years 3 & 4.

Fibronectin: We have recently developed a panel of mAbs directed against cellular fibronectin, isolated from human term placenta using WGA chromatography. We used this major serum glycoprotein as a target to develop serum ELISA screening of node-negative patients, and examined the idea of "breast cancer serum markers". Cellular fibronectin (with mAb D27) is localized on basement membranes underlying embryonic endothelia and it is found in high levels in human sera - estimated 0.1 to 6 μg per ml of serum - using quantitative ELISA. A pilot cellular fibronectin serum test was established to determine whether serum antigen levels change during breast cancer progression and near the time of recurrence as compared to the serum antigen level of health women. Initial serum ELISA tests were conducted using paired serum samples from 10 normal individuals and from 20 patients taken at the time of initial therapy and at the first follow-up visit where recurrence or nonoccurrence were detected. As shown in Figure 8 below, we performed variance and paired t-tests to determine the relationship of recurrence and serum cellular fibronectin levels. We did find a significant difference between these of healthy women and patients, however, we did not find any significant difference in the serum cellular fibronectin of same pair of patients from the time of the initial sample to the time of recurrence ($p=.85$). However, there was a significant difference in cellular fibronectin between recurrent and nonrecurrent subjects regardless of the time of measurement ($p=.002$). The association between cellular fibronectin and recurrence did not vary according to the time at which the sample was taken. As cellular fibronectin in serum has been considered as a potential breast cancer marker {Ylatupa, Haglund, et al. 1995 ID: 7174}, our pilot studies (and reviewer's comments) suggest that we need to continue this investigation in order to pinpoint the role of cellular fibronectin in occurrence or recurrence of breast cancer.

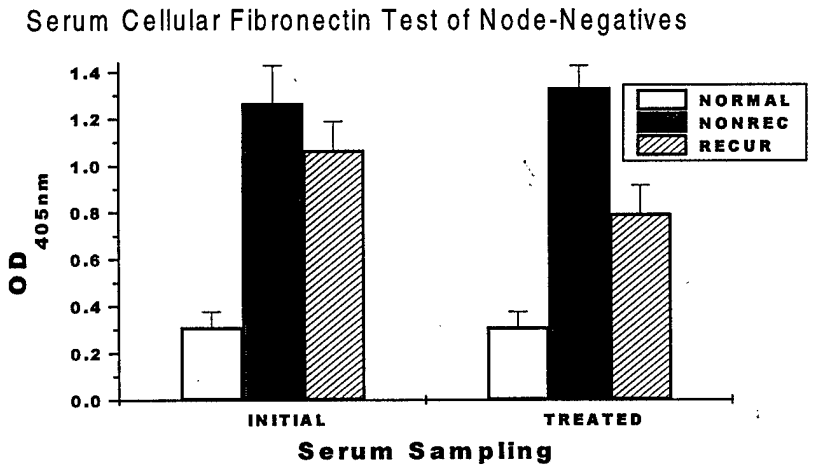


Figure 8. Result of a pilot serum test showing serum cellular fibronectin levels of paired serum samples (NONREC and RECUR) from 10 patients taken at the time of initial therapy (INITIAL) and at the first follow-up visit where recurrence was detected (TREATED), as compared to serum cellular fibronectin levels of 10 health women (NORMAL). We did not find any significant difference in the serum cellular fibronectin of same pair of patients from the time of the initial sample to the time of recurrence (p=.85). However, significant difference occurs between these of healthy women and patients.

ELISA tests for other breast cancer antigens: As discussed above, we have obtained three mAbs M4, S22 and E96 that recognize breast cancer antigens. We have found that EMMPRIN, seprase, DPPIV and breast cancer antigens are potential serum antigens. Future studies in Task 4b will use available mAbs to evaluate if they are serum prognostic markers for node-negative breast cancer. Recent studies indicate that tumor surface antigens can act as possible circulating tumor markers. For example, serum DPPIV **activity** has been suggested to be useful in the diagnosis of benign versus malignant tumors {Maes, Scharpe, et al. 1994 ID: 6887}{Kubota, Iizuka, et al. 1994 ID: 6897}. Furthermore, we will use a combinations of these molecules as potential serological markers as previously suggested (Schwartz et al., 1993; Mansour et al., 1994; Chen et al., 1994a).

(7). CONCLUSIONS

Task 1:

- Transient expression of EMMPRIN in COS7 monkey kidney fibroblastic cells stimulates production of gelatinase A (also called MMP-2 and 72-kDa type IV collagenase) in the fibroblastic cell population. This result suggests that EMMPRIN

could serve as a collagenase-stimulating factor, leading to increased degradation of interstitial or basement membrane matrix components and thus to enhance both stromal and tumor cell invasion.

- Northern and Western blot analyses demonstrate that while EMMPRIN message and protein were high in breast carcinoma cells, but they were low in normal epithelial cells and fibroblasts.
- EMMPRIN is expressed at invadopodia of invasive breast carcinoma cells where it interacts with membrane proteases and integrins.

Task 2.

- To produce new monoclonal antibodies directed against breast cancer antigens, we have developed a new strategy of mAb production: the use of breast cancer lysates as immunogens and selection of hybridoma cell lines by breast cancer antigen ELISA. At this stage, we have established three hybridoma cell lines, M4, S22 and E98, that produce mAbs potentially useful as tissue or serum prognostic markers.

Task 3.

- In paraffin sections of human breast carcinoma and malignant melanoma, anti-seprase antibodies stained foci of breast carcinoma and melanoma, as well as activated stromal fibroblasts and endothelial cells, suggesting that seprase may be a cell activation protease.
- A breast cancer lysate ELISA was established to address potential prognostic roles of seprase, DPPIV, EMMPRIN, and fibronectin in node-negative breast cancer.

Task 4.

- A breast cancer serum ELISA was established to address potential prognostic roles of seprase, DPPIV, EMMPRIN, and fibronectin in sera of node-negative breast cancer patients. Pilot studies using anti-cellular fibronectin mAbs and three mAbs M4, S22 and E96 showed promise of this assay in a large scale study as described in Task 3 above.

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