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Annual Progress Report

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INTRODUCTION

The objectives of the present proposal are to identify and clone the protein recognized by the anti-meprin antibody; to study its functional characteristics relevant to invasion; and to examine its prevalence in breast cancer patients. We hypothesized that breast cancer cells are capable of breaking down the extracellular matrix barrier and this plays an important role in breast cancer invasion and metastasis. This study is based on our previous observation that breast cancer tissue expresses a meprin-like protein, which can be identified by immunostaining of breast cancer tissue using anti-meprin antibody.

During the first year, our research was focused on the expression of meprin alpha in bacteria and raising antibody to the recombinant protein in order to have a tool for the identification of the meprin-like protein in tissues and extracted proteins. We also performed immunohistology studies of human breast tumor and kidney tumor tissues, and produced data on experimental tumor growth with and without inhibition of meprin.

BODY

During the second year, we attempted to clone the protein recognized by the anti-meprin antibody, which is expressed in breast cancer tissue. We used human breast cancer cDNA expression library in lambdaSCREEN (Novagen). Plating this library allows IPTG-induced expression of cDNA inserts as polypeptides with the following

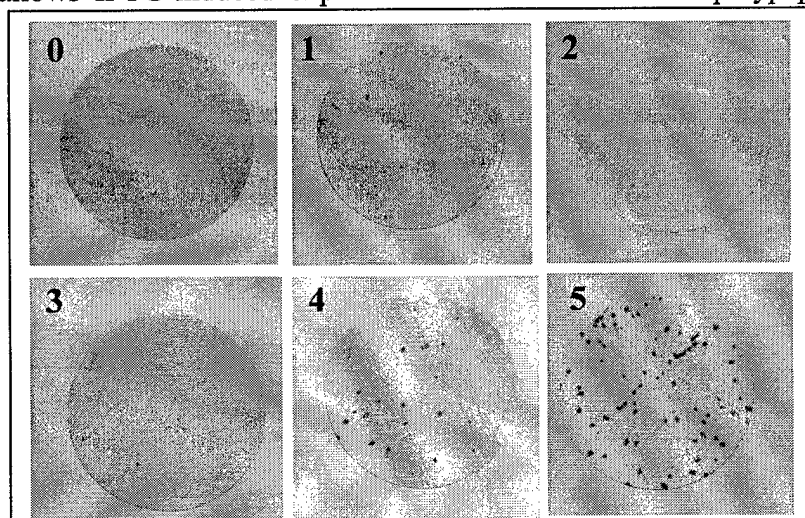
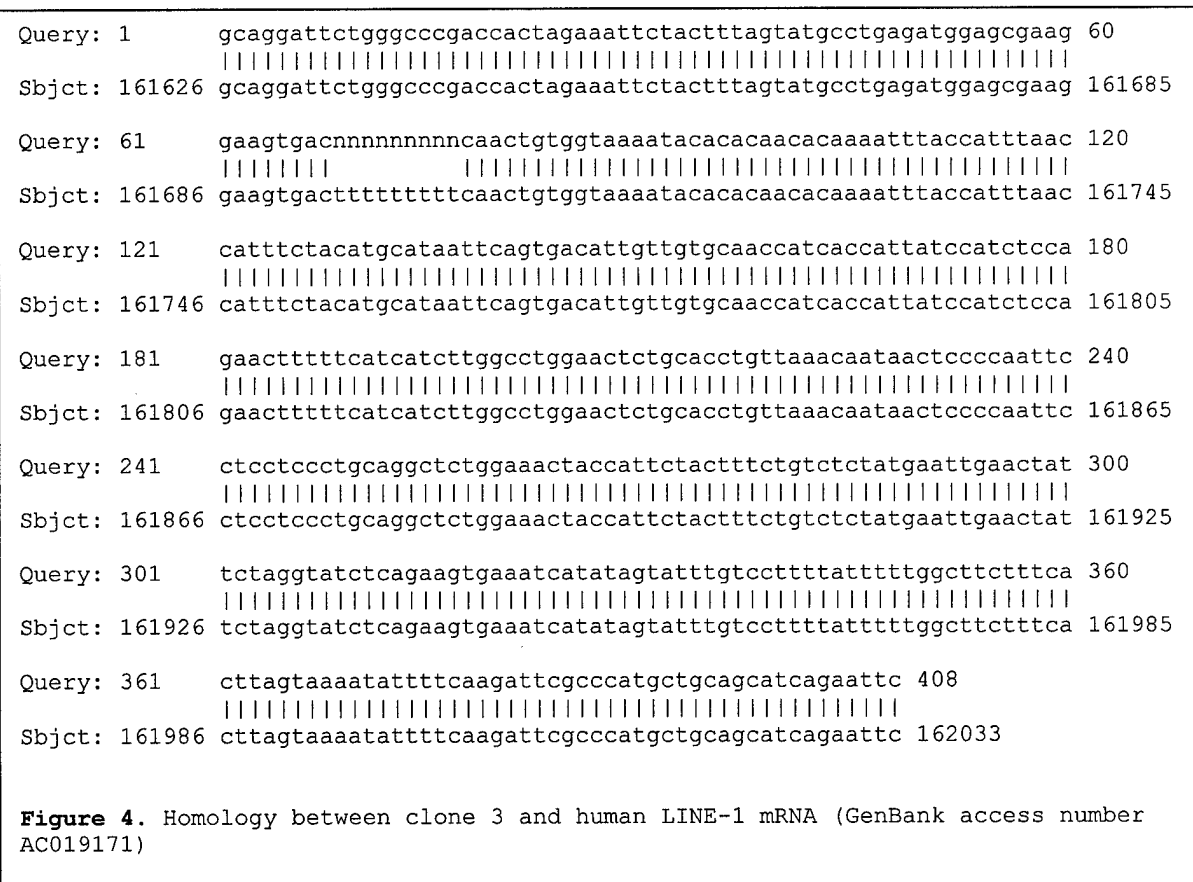
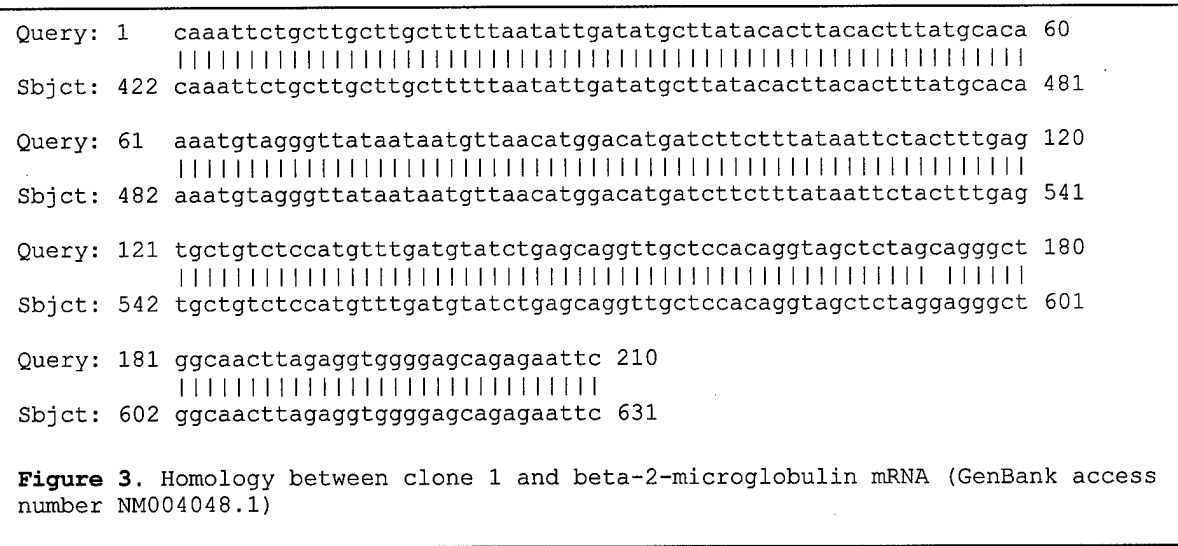


Figure 1. Screening of lambdaSCREEN (Novagen) human breast cancer cDNA expression library with anti-meprin. Last round shows purified clone 1.

detection using antibody. After 5 rounds of cloning (Figure 1), we have selected five clones reacted with anti-meprin antibody, and purified four of them to homogeneity. Some nucleotide homology was observed between the clones (Figure 2), however, none of them was meprin. Two clones were 97% homologous to beta-2-microglobulin (B2M), and two other were

99% homologous to LINE-1 retrotransposon. Although B2M expression was found by Klein et al (1) to correlate with breast tumors, it is a frequent finding during library



screening. More interestingly, LINE-1 retrotransposones encode a 40-kDa protein (p40) with unknown function (2). Several studies showed that this protein is expressed in invasive breast carcinomas, but not in non-malignant carcinomas or normal breast tissue (2, 3).

To express, purify and further characterize the cloned proteins, they have been sub-cloned in pJYN expression vector (Figure 5).

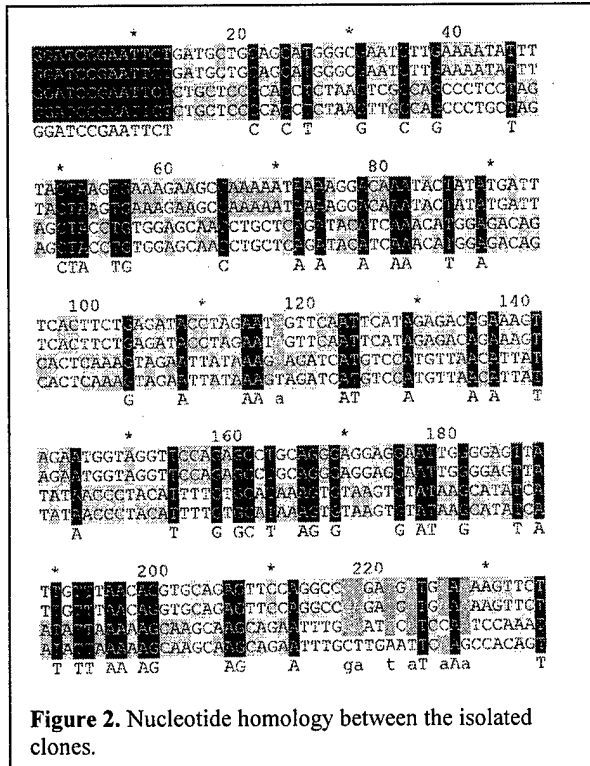


Figure 2. Nucleotide homology between the isolated clones.

homologous to LINE-1 retrotransposon sequence coding p40 protein with unknown function, which was shown to be expressed in invasive breast tumors; (5) sequences are sub-cloned in pJYN vector for *in vitro* expression.

REPORTABLE OUTCOMES

Wang JY, Singh AB, Walker PD, Basnakian AG, Shah SV (2002) Meprin-like metalloproteinase: a possible participant in breast tumor development. To be presented at: Era of Hope 2002 Department of Defense Cancer Research Program Meeting, September 25-28, Orlando, FL

CONCLUSIONS

Our data may indicate that p40 is a meprin-like protein which is important for breast cancer invasion and metastasis. These data are in good correlation with our previously obtained results suggesting that breast tumor development is dependent on the meprin or meprin-like proteinase activity in the tissue.

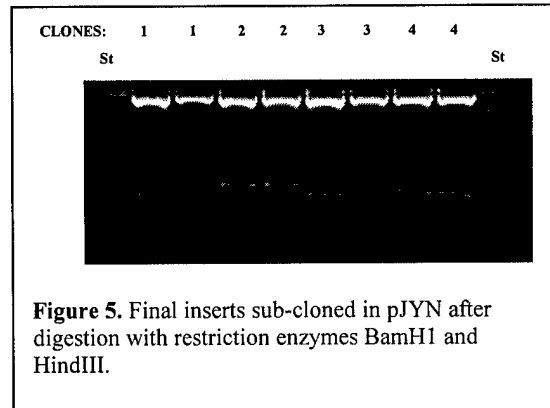


Figure 5. Final inserts sub-cloned in pJYN after digestion with restriction enzymes BamHI and HindIII.

KEY RESEARCH ACCOMPLISHMENTS:

- (1) library screened with anti-meprin for meprin-like protein expression;
- (2) four positive clones have been identified;
- (3) two of them are homologous to beta-2-microglobulin, and two other are

REFERENCES

- (1) Klein T, Levin I, Niska A, Koren R, Gal R, Schachter J, Kfir B, Narinski R, Warchaizer S, Klein B. Correlation between tumour and serum beta 2m expression in patients with breast cancer. *Eur J Immunogenet* 1996 Dec;23(6):417-23
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APPENDICES

N/A