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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 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

Figure 3. Homology between clone 1 and beta-2-microglobulin mRNA (GenBank access number NM004048.1)

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\[\text{Sequence}\]
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Figure 4. Homology between clone 3 and human LINE-1 mRNA (GenBank access number AC019171)

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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 5.** Final inserts sub-cloned in pJYN after digestion with restriction enzymes BamH1 and HindIII.

**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**

**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.
REFERENCES

APPENDICES
N/A