AWARD NUMBER DAMD17-97-1-7259

TITLE: c-erbB-2 Receptor Signalling and Breast Cancer Metastasis

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REPORT DATE: August 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
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Fort Detrick, Maryland 21702-5012

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Overexpression of the c-erbB2 gene has been shown to be correlated with poor prognosis and the number of lymph node metastases in breast cancer patients. Our recent work has demonstrated that c-erbB2 indeed enhanced the intrinsic metastatic potential of human breast cancer MDA-MB-435 cells. We hypothesize that the erbB2-encoded receptor tyrosine kinase (RTK) may enhance metastatic potential through the RTK-signaling molecules. The purpose of this study is to test whether the increased c-erbB2 tyrosine kinase activity and tyrosine autophosphorylation on the carboxyl-terminal tail may be required for the downstream signaling involved in breast cancer metastasis. We have basically completed the Task 1, 2, and 3 of the Objective 1 as stated in the Statement of Work of our proposal. We have transfected the wild-type and mutant erbB2 genes into MDA-MB-435 cells and established a panel of wild-type and mutant erbB2 gene transfectants, which can be used as the experimental system to further our studies to understand the mechanism of erbB2 gene enhanced human breast cancer metastasis. Currently, we are comparing the metastatic potential of wild-type with erbB2 mutant transfectants that express mutated erbB2 proteins in vitro and in vivo (Task 4 and 5).
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2. Appendices
A. Introduction
Breast cancer is one of the most common malignancies among women in the United States, and metastasis from this cancer is the major cause of death for these patients. Therefore, it is extremely important to uncover the basis of breast cancer metastasis. Overexpression of the c-erbB2 (also known as HER-2, neu) gene has been shown to be correlated with poor prognosis and the number of lymph node metastases in breast cancer patients. Our recent work has demonstrated that stable transfection of the human c-erbB2 gene into the low c-erbB2-expressing MDA-MB-435 human breast cancer cells (named 435.eB transfectants) indeed enhanced the intrinsic metastatic potential of these cells [Tan, 1997]. Because overexpression of the c-erbB2 gene has been found in ~ 30% of breast tumors, it is very important to examine the molecular mechanisms underlying the enhanced metastatic potential induced by c-erbB2 overexpression and then to design new strategies to treat this type of breast cancer metastasis. We hypothesize that the c-erbB2-encoded receptor tyrosine kinase (RTK) may enhance metastatic potential through the RTK-signaling molecules. The purpose of this proposed study is to test whether the increased c-erbB2 tyrosine kinase activity and tyrosine autophosphorylation on the carboxyl-terminal tail may be required for the downstream signaling involved in breast cancer metastasis. To address this question we will study: (1) The requirement of the tyrosine kinase domain and tyrosine autophosphorylation sites in the c-erbB2 receptor for mediating signals leading to metastasis. (2) The immediate downstream signals of c-erbB2 that may contribute to increased metastatic potential.

B. Specific Aims
The specific Aims 1 and 2 have not been modified.

C. Study Results and Significance
Aim 1. The requirement of the tyrosine kinase domain and tyrosine autophosphorylation sites in the c-erbB2 receptor for mediating signals leading to metastasis.
We basically completed the Task 1, 2, and 3 of the Objective 1 as stated in the Statement of Work of our proposal. To determine whether the tyrosine kinase activity and other structural motifs in the cytoplasmic domain of erbB2 receptor are required for enhancing metastatic potential, we have subcloned and transfected the kinase-deficient dominant-interfering mutant (K753M),
autophosphorylation-site mutant (Y1248F), and c-terminal-deletion mutant (C1025) and constitutively activated mutant (V659E) of the c-erbB2 receptor into human breast cancer MDA-MB-435 cells. We have established a panel of erbB2 gene transfected stable transfectants by using G418 selection. To identify those transfectants that actually produced the mutated erbB2 proteins, we performed immunoblot analysis by using antibodies against the extracellular domain of erbB2 protein. Immunoblot analysis results show that these mutant erbB2 gene transfected cell lines expressed very high mutated erbB2 proteins at different expression levels (Appendices Fig. 1). To determine the structural changes in erbB2 mutants will result in specific changes in tyrosine-phosphorylated proteins that correlate with their effects on metastatic potential in MDA-MB-435 breast cancer cells, the tyrosine phosphorylation pattern of erbB2 proteins have been examined in vivo. As described in Appendices Fig. 2, western blotting using anti-phosphotyrosine antibodies was performed on protein lysates from MDA-MB-435 transfectants that express wild-type or mutant erbB2 proteins. As we expected, very low level of tyrosine phosphorylation of erbB2 protein has been found in transfectants that expressing the kinase-defective K753M mutant. This result indicate that K753M mutant protein is kinase defective in MDA-MB-435 cells. We also detected a reduction in tyrosine phosphorylation of erbB2 proteins in MDA-MB-435 transfectants expressing either the Y1248F, C1025 mutant proteins compared to those expressing the wild-type erbB2 protein, which indicate lower intrinsic tyrosine kinase activities of Y1248F, C1025. Further more, we detected a higher tyrosine phosphorylation of erbB2 proteins in MDA-MB-435 transfectants expressing the constitutive activated V659E mutant proteins compared to those expressing the wild-type erbB2 protein, which indicate higher intrinsic tyrosine kinase activities of this mutant.

Currently, we are comparing wild-type with erbB2 mutant transfectants that express mutated erbB2 proteins for their metastatic potentials in vitro and in vivo (Task 4 and 5).

D. Plans

Aim 1. To evaluate the requirement of the tyrosine kinase domain and tyrosine autophosphorylation sites in the c-erbB2 receptor for mediating signals leading to metastasis.

Aim 1.1. Part 2 and Aim 1.2 Compare wild-type with mutant erbB2 transfectants for their metastatic potential and to evaluate whether deficiencies of tyrosine kinase activity in the c-erbB2 mutants that may affect metastatic potential in MDA-MB-435 transfectants.

Please refer to the original proposal for the detail.

Aim 2. To investigate the immediate downstream signals of c-erbB2 that may contribute to increased metastatic potential.
Please refer to the original proposal for the detail.

E. Conclusions
We have established a panel of wild-type and mutant erbB2 gene transfectants, which can be used as the experimental system to further our studies to understand the mechanism of erbB2 gene enhanced human breast cancer metastasis.

F. Reference

G. Human Subjects: No change.

H. Vertebrate Animals: No change.

I. Publications: No paper has been published so far.

J. Inventions and Patents: None.
**Fig. 1** Western Blotting Shows Wild-type and Mutant erbB2 Proteins Expression

**Fig. 2** Western Blotting shows ErbB2 mutant Transfectants erbB2 Protein Tyrosine Phosphorylation levels