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Award Number: W81XWH-06-1-0583

TITLE: The role of ERBP in breast cancer progression

PRINCIPAL INVESTIGATOR: Yijun Zhu, M.D.

CONTRACTING ORGANIZATION: Northwestern University
Evanston, IL 60208

REPORT DATE: September 2008

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 01-09-2008		2. REPORT TYPE final		3. DATES COVERED (From - To) 1 Sep 2006-31 Aug 2008	
4. TITLE AND SUBTITLE The role of ERBP in breast cancer progression				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-06-1-0583	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Yijun Zhu, M.D. Email: y-zhu2@northwestern.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Northwestern University Evanston, IL 60208				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Material Command Fort Detrick, Maryland 21702-501				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Metastasis likely occurs when primary tumor cells obtain additional genetic or epigenetic alteration. ERBP (estrogen receptor binding protein) is an estrogen receptor binding protein which potentiates the transcriptional activity of estrogen receptor. Unlike most coactivators which interact with AF2 domain of estrogen receptor, ERBP interacts with the DNA binding domain of estrogen receptor. The altered expression of ERBP could promote the metastasis through enhancing the expression of genes which are regulated by estrogen and are involved in the breast cancer metastasis. By overexpressing ERBP in breast cancer cells, we found that ERBP overexpression enhanced the migration and invasion capability of tumor cells. ERBP overexpression also promoted the tumor formation in nude mice. We identified 8 estrogen inducible genes which were up-regulated by ERBP overexpression. Finally, we found that expression of ERBP is increased in about 30% of breast cancers.					
15. SUBJECT TERMS Breast carcinoma; metastasis; estrogen receptor.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT unlimited	18. NUMBER OF PAGES 11	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION

Metastasis, a process during which primary tumor disseminates into distal sites, likely occurs when primary tumor cells obtain additional genetic or epigenetic alteration. ERBP (estrogen receptor binding protein) is an estrogen receptor binding protein which potentiates the transcriptional activity of estrogen receptor. Unlike most coactivators which interact with AF2 domain of estrogen receptor, ERBP interacts with the DNA binding domain of estrogen receptor. Recently, we found that the expression of ERBP was dramatically increased when ER positive breast cancer MCF-7 cells acquire the capability of metastasizing. The proposed studies tested the hypothesis that the acquisition of ERBP overexpression promotes the metastasis through enhancing the expression of genes which are regulated by estrogen and are involved in the breast cancer metastasis.

BODY

Task 1. To determine if overexpression of ERBP promotes tumor metastasis.

The MCF-7 cells were transfected with pcDNA3.1-ERBP or control pcDNA3.1 vector. The cells were selected with G418. Individual clones were picked up, expanded, and examined for ERBP expression. Two clones overexpressing ERBP and two control clones were selected for further analysis. We found ERBP overexpression enhanced the migration (Fig. 1A) and invasion capability (Fig. 1B) of MCF-7 tumor cells but had no significant effect on cell proliferation (Fig. 1C). ERBP overexpression also promoted the tumor formation in nude mice (Fig. 2), indicating that ERBP is involved in tumor progression. But no metastasis into lung was detected for both tumor cells overexpressing ERBP and control cells, indicating that overexpression of ERBP alone is not enough to confer the metastatic capability to MCF7 and additional factors remain to be identified.

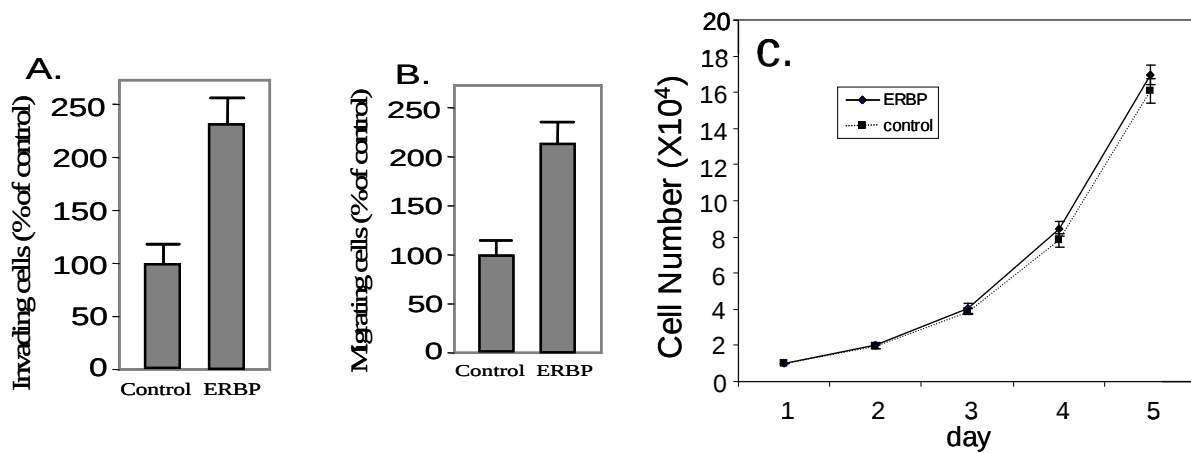
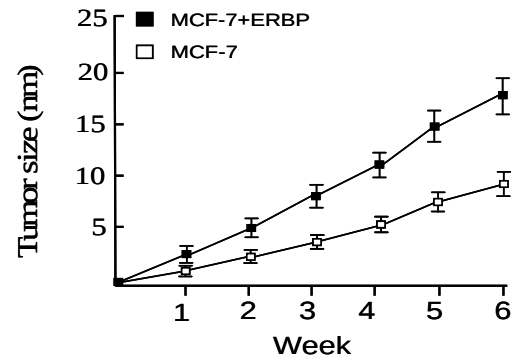


Fig. 1. A). Overexpression of ERBP increased the migration of MCF-7 cells through polycarbonate Transwell filters. B). Overexpression of ERBP enhanced the invasion of MCF-7 cells to the bottom layers of the Matrigel-coated membranes. Data represent the average of three independent experiments. C). Overexpression of ERBP had no effect on cell proliferation. Viable cells were counted by trypan blue staining at different times after initial seeding of 1×10^4 cells.

Fig. 2. The curve of tumor growth in nude mice. Two clones of MCF-7 cells or two clones of MCF-7 cells overexpressing ERBP (1×10^6 cells from each clone) were injected into nude mice, respectively. The experiment was performed in triplicate and the average tumor sizes were calculated.



Task 2. To identify the estrogen responsive genes regulated by ERBP.

A microarray analysis was performed with total RNA prepared from MCF-7 and MCF-7 overexpressing ERBP, both of which were treated with estrogen for 24h. We identified 8 estrogen inducible genes (Table 1) which were up-regulated by ERBP overexpression. WISP-2, GAS6 and amphiregulin are secreted proteins, which might be involved in the invasive growth promoted by ERBP.

Table 1. List of estrogen responsive genes up-regulated by ERBP

GeneBank No.	Gene Name	Fold
NM_003881	WISP-2	2.0
NM_003225	TFF1	2.1
NM_001657	Amphiregulin	2.2
AF245389	Greb1	2.2
NM_003489	NRIP1	2.3
NM_000926	Progesterone Receptor	2.5
NM_005080	XBP-1	2.8
NM_000820	GAS6	3.1

Task 3. To determine if ERBP expression is increased in metastatic breast cancers in comparison with primary breast cancers.

Total RNA was prepared from primary tumors and their metastatic tumors. We found that expression of ERBP is increased in about 30% of breast cancers (Fig. 3), indicating that ERBP plays a role in breast tumorigenesis. We did not see ERBP expression was increased in metastatic tumors, indicating that the expression of ERBP is not further increased in metastatic tumors.

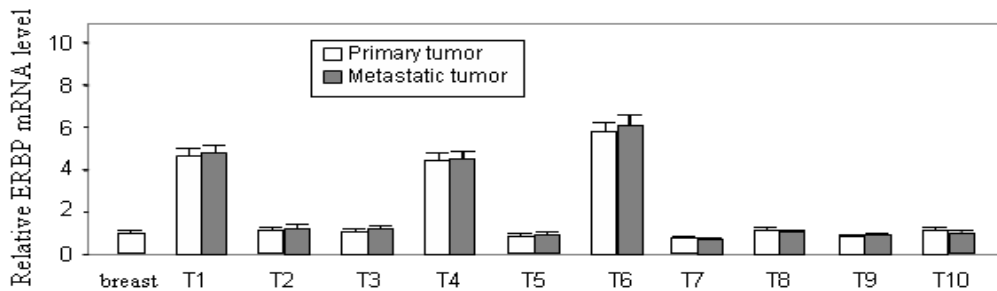


Fig. 3. ERBP expression was increased in about 30% of breast cancers. Tumor T1, T4 and T6 showed increased expression of ERBP with similar levels of expression in primary and metastatic tumors.

KEY RESEARCH ACCOMPLISHMENTS

- * We found ERBP overexpression enhanced the migration and invasion capability of tumor cells but had no effect on cell proliferation. ERBP overexpression also promoted the tumor formation in nude mice. ERBP overexpression alone did not confer metastatic property to MCF-7 cells.
- * We identified 8 estrogen inducible genes which were up-regulated by ERBP overexpression. These genes could play roles in the invasive growth promoted by ERBP
- * We found that expression of ERBP is increased in about 30% of breast cancers. But ERBP expression was not further increased in the metastatic tumors

REPORTABLE OUTCOMES

None

CONCLUSIONS

ERBP overexpression enhanced the migration and invasion capability of tumor cells and the tumor formation in nude mice but had no effect on cell proliferation. Expression of ERBP is increased in about 30% of breast cancers. ERBP expression was not further increased in the metastatic tumors

REFERENCES

None

APPENDICES

None.