AD_____

Award Number: DAMD17-01-1-0370

TITLE: Mechanism of ErbB1 and ErbB2 Hetero-oligomerization

PRINCIPAL INVESTIGATOR: Jong W. Yu
Mark A. Lemmon, Ph.D.

CONTRACTING ORGANIZATION: University of Pennsylvania
Philadelphia, Pennsylvania 19104-3246

REPORT DATE: July 2002

TYPE OF REPORT: Annual Summary

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20030130 187

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Annual Summary (1 Jul 01 - 30 Jun 02)

4. TITLE AND SUBTITLE
Mechanism of ErbB1 and ErbB2 Hetero-oligomerization

5. FUNDING NUMBERS
DAMD17-01-1-0370

6. AUTHOR(S)
Jong W. Yu
Mark A. Lemmon, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Pennsylvania
Philadelphia, Pennsylvania 19104-3246

E-Mail: jongyu@mail.med.upenn.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

We are developing an *in vivo* system using erbB/IL2 receptor chimerae in a B-cell line to investigate the interactions and mechanism of oligomerization between the epidermal growth factor (EGF) receptor family members erbB1 and erbB2. Since erbB2 overexpression has been strongly associated with breast cancer and has been shown to be a valuable target for breast cancer therapies, we are interested in dissecting its mechanism of activation. Heteromeric interaction between the intracellular domains of the IL2 receptor beta and gamma chain will serve as a reporter for direct interaction between the extracellular domains of erbB1 and erbB2 by mediating T or B-cell proliferation in the absence of IL2. To date, I have made B-cell lines stably expressing various erbB/IL2 receptor chimerae and I have been able to show erbB1 homo-oligomerization and hetero-oligomerization with erbB2 in an EGF (and IL2 independent) manner. With this assay, I plan to assess the ability of specific erbB receptor ligands to induce erbB1 and erbB2 hetero-oligomers and determine whether erbB1 and erbB2 interact as a hetero-tetramer rather than a hetero-dimer. By understanding how erbB1 and erbB2 interact, we hope to provide insight into the mechanism by which erbB2 mediates transformation and tumorigenicity in cells.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, erbB1, erbB2	9
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

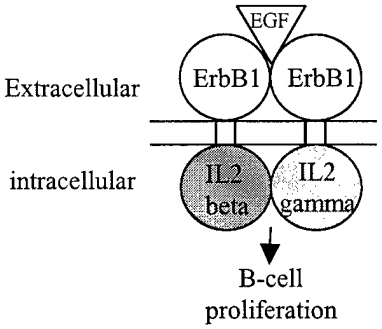
The epidermal growth factor (EGF) receptor family members are tyrosine kinase receptors which transduce extracellular signals by ligand mediated receptor homo- and/or hetero-oligomerization and subsequent transphosphorylation of the opposing oligomer member. This results in the recruitment of specific proteins leading to the activation of various signaling pathways. ErbB2, which possesses no known ligand, is thought to activate signaling pathways only through hetero-oligomerization with the other receptor members. ErbB2 has been shown to play a role in breast cancer, as amplification and overexpression of this gene are observed in a significant fraction of human breast cancers. Furthermore, high levels of erbB2 expression correlate with an aggressive tumor phenotype and poor patient prognosis. With the inception of Herceptin, a humanized antibody directed against erbB2, it has become clear that erbB2 may be an important target for breast cancer treatment. While it is unclear how erbB2 causes transformation and tumor formation, current evidence suggests that ligand dependent hetero-oligomerization may play a primary role. In our studies, we hope to dissect the mechanism of erbB2 hetero-oligomerization (and subsequent activation) with erbB1. We plan to achieve this by developing an *in vivo* reporter system using erbB/IL2 receptor chimerae, where ligand mediated oligomerization of the extracellular domains of the erbB receptors will in turn drive oligomerization of the IL2 receptor intracellular beta and gamma chains of these receptor chimerae. This intracellular beta-gamma chain association will generate a proliferative signal in B or T cells (which are erbB receptor null) in an IL2 independent manner. Using this approach, we hope to dissect and understand how erbB2 is normally activated and suggest novel therapies directed against the activation of erbB2 in breast cancer treatment.

Body

Aim1: Investigate EGF dependent erbB1 homo-oligomerization and erbB1-erbB2 hetero-oligomerization *in vivo* and identify structural determinants of hetero-oligomerization.

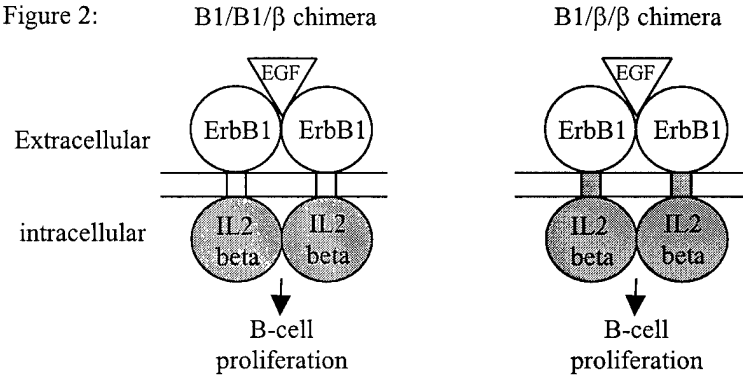
For this aim, I have created the various erbB1/IL2 and erb**B2**/IL2 receptor chimerae expression plasmids (Aim 1a) and created B-cell lines stably expressing these receptor chimerae (Aim 1b). While creation of stable cell lines expressing only one receptor chimerae has been feasible, creation of stable cell lines expressing two receptor chimerae has been difficult to achieve. In the case for determining epidermal growth factor (EGF) dependent erbB1 homo-oligmerization (as a positive control for this system), I have not been able to generate a cell line expressing both erbB1/IL2 receptor chimerae as shown in figure 1. In this figure, the heteromeric interaction of the intracellular beta and gamma chain of the IL2 receptor mediates B or T-cell proliferation in the absence of IL2.

Figure 1:



However I have made a Baf3 (mouse pro-B cell) cell line stably expressing only a single erbB1/IL2R beta chimera, and it has been shown that in this cell line only, a homomeric interaction of the intracellular beta chain of the IL2 receptor can also mediate a proliferative signal (figure 2).

Figure 2:



Upon testing testing these cell lines in a proliferation assay, I am able to see EGF dependent erbB1 homo-oligomerization (Aim1c); this is evident by an EGF dependent (and IL2 independent) proliferation of only Baf3 cells expressing these chimerae (figure 3). However the proliferative signal was not as robust as reported with similarly designed receptor chimerae (as compared with a saturating concentration of IL2). In this proliferation assay, I basically wash the cells, starve them for a brief period, treat with ligand of interest for approximately 16 hours, and finally treat with tritium labeled thymidine for the final three hours. The cells are subsequently harvested on a membrane and the incorporated tritium labeled thymidine is counted. Expression of receptor chimerae of each stable cell line was determined by flow cytometry, using a labeled antibody directed against the extracellular domain of erbB1. As seen in figure

4, expression of these chimerae appears to be sufficient. The line traces are Baf3 cells alone treated with antibody, and filled traces are Baf3 cells expressing receptor chimerae treated with antibody.

Figure 3:

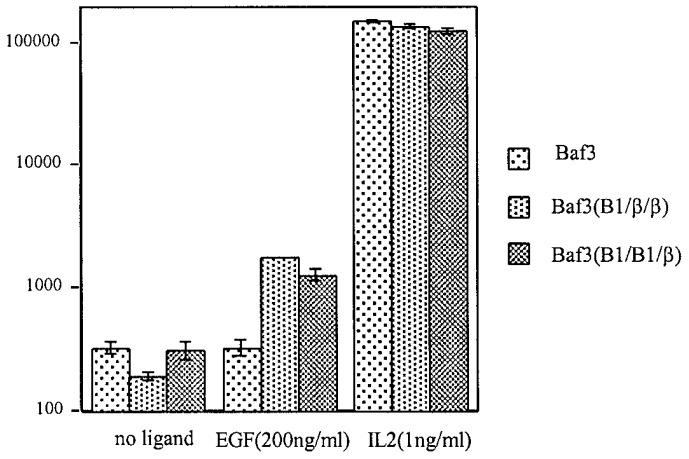
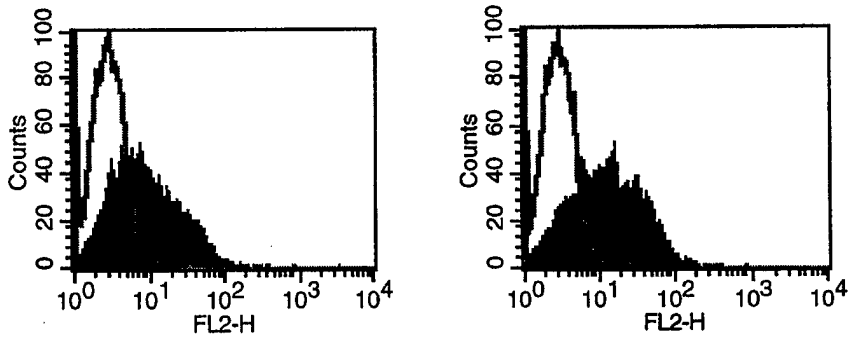


Figure 4:

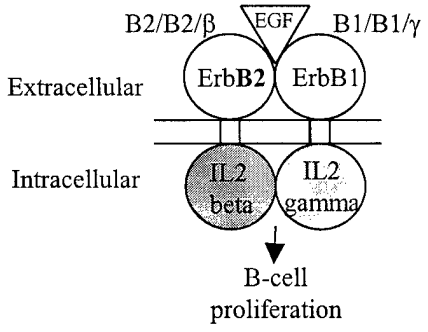


B1/B1/β chimera expressionin Baf3 cells

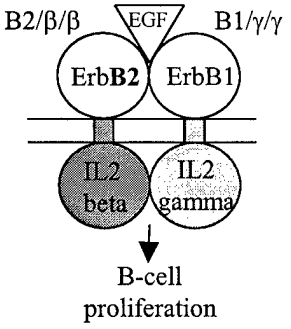
B1/β/β chimera expression in Baf3 cells

With these results, albeit not as robust as expected, I proceeded to test erbB1 and erbB2 hetero-oligomerization (Aim1d). I again used the Baf3 cell line to make stable cell lines; however, to avoid the proliferative effects of EGF dependent erbB1 homo-oligomerization (as seen above), I generated a stable cell line expressing an erbB1/IL2R gamma chimerae and an erb**B2**/IL2R beta chimerae. As seen in figure 5, EGF treatment of cells expressing both erbB1 and erbB2 receptor chimerae should result in hetero-oligomerization of the extracellular domains of erbB1 and erbB2. This in turn will result in the intracellular association of the IL2R beta and gamma chains mediating B-cell proliferation. In Baf3 cells expressing only a erbB1/IL2R gamma chimera, EGF will enable homo-oligomerization of the erbB1 chimerae, but the intracellular IL2R gamma-gamma association will not yield a proliferative response. In Baf3 cells expressing only an erb**B2**/IL2R beta chimera, EGF will not bind to or enable homo-oligomerization or the erbB2 chimera. Thus a proliferative signal should not be seen in this case as well.

Figure 5: A)

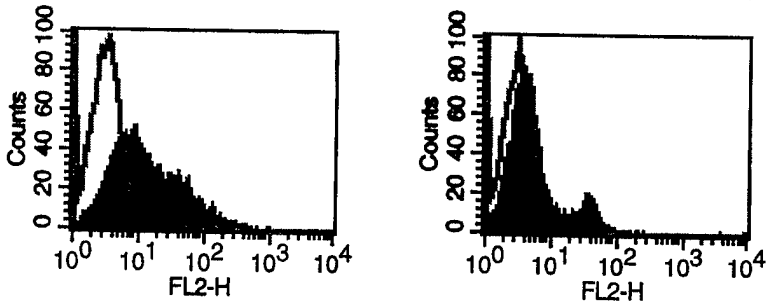


B)



I generated stable cell lines expressing both erb**B2** and erbB1 receptor chimerae by stably transfecting in the erbB2 chimera, selecting for expressing cells by fluorescence activated cell sorting (via an erbB2 antibody directed against the extracellular domain), and subsequently stably transfecting in the erbB1 chimera (Aim 1b). As mentioned earlier, I have been having problems generating stable cell lines expressing two receptor chimerae. In this case, I am able to generate cell lines expressing only one receptor chimerae somewhat easily, however upon introduction of the second chimera, I am only able to observe expression of this receptor only in a small percentage of the cells (after selection of expressing cells by fluorscence activated cell sorting). This is illustrated in figure 6, where expression of an erbB1 and erbB2 chimera are analyzed in the same cell line (double stable) using flow cytometry. Once again, the line traces are Baf3 cells alone treated with antibody and filled traces are Baf3 cells expressing receptor chimerae treated with the relevant antibody. Similar expression profiles are observed in cells expressing both the B2/β/β and B1/γ/γ receptor chimerae.

Figure 6:



B2/B2/β chimera expression

B1/B1/γ chimera expression

Despite the expression problem of the second stably transfected receptor chimera, I performed proliferation assays to determine if I can see EGF dependent hetero-oligomerization of erbB1 and erbB2 in this reporter system (Aim1d). In the case of figure 5A, EGF treatment of cells expressing these chimerae yielded a proliferative response (see figure 7A). Cells expressing the erbB2/IL2R beta chimera alone yielded no response when treated with EGF (figure 7A), as expected for reasons mentioned above. Cells expressing the erbB1/IL2R gamma chimera alone have been generated but have not been tested yet in this assay; however we predict that upon EGF treatment, no proliferative effect would be seen as mentioned above. This indicates that EGF dependent hetero-oligomerization can be observed between the erbB2/B2/β and erbB1/B1/γ chimerae. Similar results are observed in the case for figure 5B (see figure 7B), although a more robust proliferative signal is observed in this case. Given these results, it is evident that this *in vivo* system will be useful in dissecting the mechanism of hetero-oligomerization between erbB1 and erbB2. Furthermore, the results in figure 7B (also see figure 5B) suggest that the extracellular domains of erbB1 and erbB2 are sufficient for EGF dependent hetero-oligomerization *in vivo* (Aim 1d). It should be recognized that the results addressing structural requirements for erbB1-erbB2 hetero-oligomerization are in the context of receptor overexpression and ligand saturation. It is not clear whether other domains are required in the context of physiological receptor expression levels and physiological levels of circulating ligand. These results basically answer most of the questions addressed in aim 1, but more effort is required in generating stable cell lines which express both receptor chimerae sufficiently. This will certainly improve the proliferative signal observed for erbB receptor hetero-oligomerization and will enable me to address aim 1e, aim 2, and aim 3 in a more efficient manner.

Figure 7A:

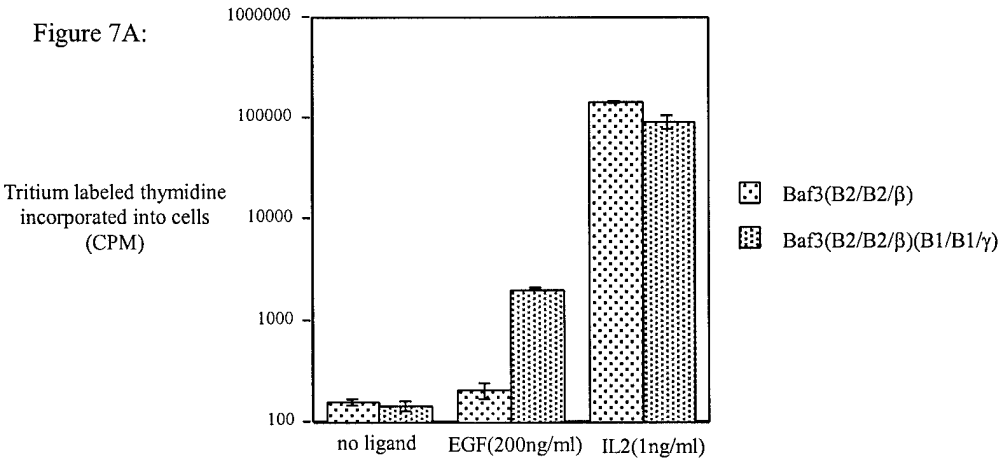
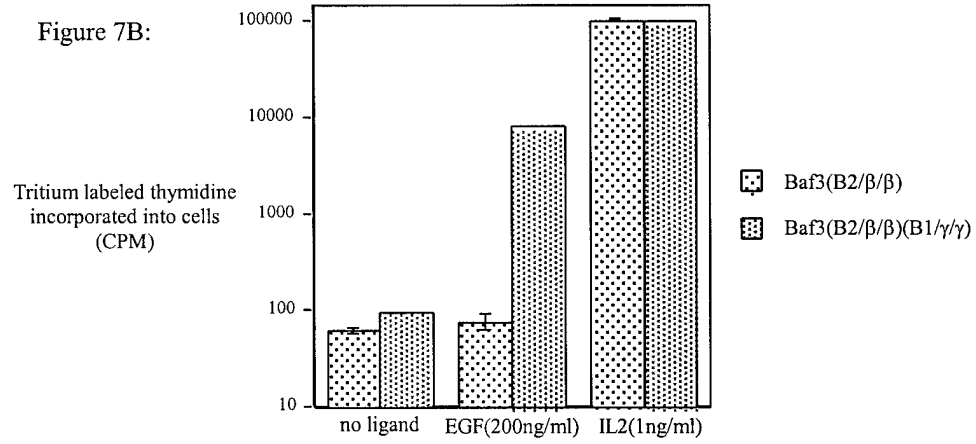


Figure 7B:



Key research accomplishments

- Created receptor chimerae constructs of various deletions of erbB1 and erbB2 (Aim 1a)
- Created stable cell lines expressing the various receptor chimerae (Aim 1b)
  -although more effort is required in this area to efficiently generate stable cell lines expressing sufficient levels of two or more receptor chimerae
- Observed EGF dependent erbB1 homo-oligomerization using this *in vivo* reporter system (Aim1c)
- Observed EGF dependent erbB1-erbB2 hetero-oligomerization using this *in vivo* reporter system (Aim1d)
- Identified structural determinants required for EGF dependent erbB1-erbB2 hetero-oligomerization (Aim 1d)

Reportable outcomes

None

Conclusions

The results presented in this report indicate that the *in vivo* reporter system proposed will be useful in dissecting the mechanism of hetero-oligomerization between erbB1 and erbB2. To date, I have been able to observe EGF dependent erbB1 homo-oligomerization as a control for this system. Furthermore, I have been able to observe EGF dependent erbB1-erbB2 hetero-oligomerization using this assay and I have been able to address the structural requirements for this hetero-oligomerization. While progress has been made in aim 1, further work is required to improve the assay. Particular effort, I believe, is required in establishing cell lines which express more than one receptor chimera at a sufficient level. Once we can establish this, we will be able to address the remaining aims of this proposal in an efficient and convincing manner. The results obtained from the remaining aims should provide insight into the regulation of activation of erbB2. We hope that by understanding this normal mode of regulation (or hetero-oligomerization), we will provide further insight into blocking the deleterious effects of erbB2 overexpression in breast cancer.

References

none

Appendices

none