# ErbB-Met Signaling Interactions in Lung Cancer

Jerrica Breindel, Betsy Cowell, Jonathan Haskins, and David F. Stern

*Department of Pathology, Yale University, New Haven CT, 06520*

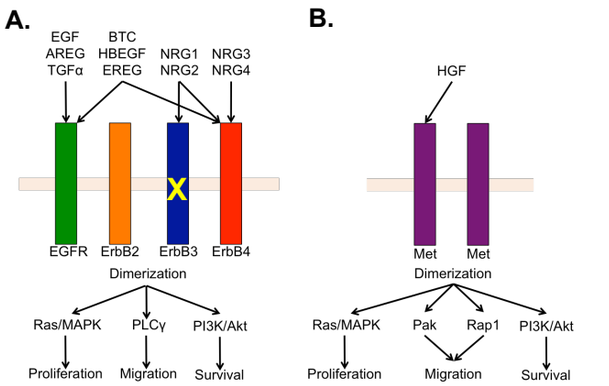
## ABSTRACT

Amplification of the receptor tyrosine kinase (RTK), Met, is a resistance mechanism that can overcome tyrosine kinase inhibition of the activated epidermal growth factor receptor (EGFR) in Non-Small Cell Lung Cancer (NSCLC). EGFR belongs to a family of RTKs known as the ErbB family, which consists of four members: EGFR, HER2, ErbB3, and ErbB4. The ErbB receptors can signal as homo- or hetero-dimers and activate many cell survival and growth signaling pathways. While studying Met as a resistance mechanism, it was discovered that EGFR and Met seem to signal to one another in untreated lung cancer cell lines, suggesting these receptors may have a relationship independent of drug resistance.[1-5] While studies have shown that Met can signal and physically interact with EGFR and ErbB3, it is unclear what the consequence of this signaling interaction is and if this is through one single ErbB receptor or through an ErbB heterodimer. It is also unclear if ErbB-Met signaling occurs through a direct interaction or if Met is activated downstream of ErbB signaling cascades.

To test the signaling interactions between these receptors, each ErbB receptor has been expressed alone or in combination with Met in 32D cells, a mouse hematopoietic cell line that lacks endogenous expression of any of these receptors or their ligands. These cells have revealed that while activation of EGFR by its ligand EGF is sufficient to stimulate Met phosphorylation, hetero-dimerization of EGFR with ErbB3 can enhance this cross activation. We have also determined that ErbB activation of Met occurs through Ras/ErK signaling and may be dependent on stabilization of Met protein.

To determine the consequences of ErbB-Met signaling interactions, both 32D and NSCLC cell lines were tested for Met-dependent phenotypes. While Met has no role in EGFR-dependent survival or proliferation, we found that Met is important for EGF-induced migration and invasion of NSCLC cell lines containing both wild type and activated EGFR. These results show that Met is important for the aggressive phenotypes of NSCLC and that ErbB-Met signaling may be an important drug target to prevent progression of cancers that harbor this signaling axis.

## BACKGROUND




**ErbB and Met Signaling. (A)** ErbB family members are activated by ligand binding which induces dimerization. Once dimerized, ErbB receptors can activate many downstream signaling pathways. ErbB3 has no kinase activity and, therefore, requires an active dimerization partner, **(B)** Met is similarly activated by ligand binding which induces dimerization and downstream signaling.

## MATERIALS & METHODS

**32D cells**

- Mouse lymphoblastic cell line
- Do not express ErbB receptors or ligands
- Do not express Met or HGF

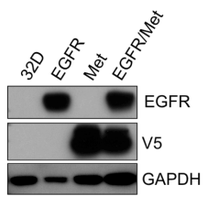



**Figure 1. 32D cells expressing EGFR and Met.** 32D cells were electroporated with constructs expressing EGFR, MetV5, or both.

## RESULTS

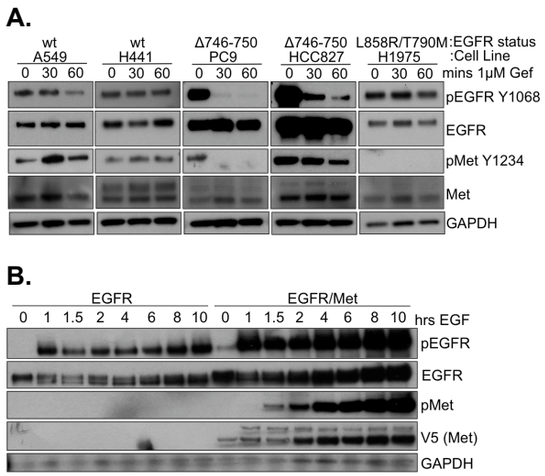



**Figure 2: EGFR activation is sufficient to activate Met phosphorylation. (A)** Non-Small Cell Lung Cancer cell lines with different EGFR mutational status were treated with the EGFR tyrosine kinase inhibitor, Gefitinib (Gef), for the indicated times. **(B)** 32D cells expressing EGFR or EGFR/Met were starved for 4 hours then treated with 10ng/ml EGF for the indicated times.

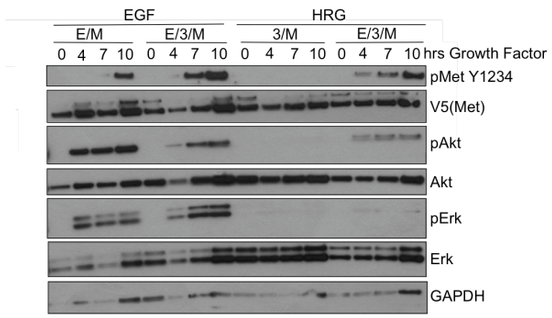



**Figure 3. ErbB3 signaling enhances EGF-induced Met phosphorylation and can activate Met through a dimerization partner.** 32D cells expressing EGFR/Met (E/M), EGFR/ErbB3/Met (E/3/M), or ErbB3/Met (3/M) were starved for 4 hours then stimulated with 10ng/ml EGF or 50ng/ml HRG for the indicated times.

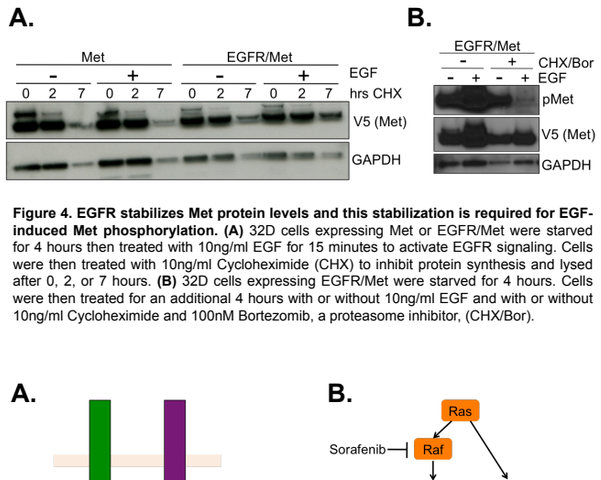



**Figure 4. EGFR stabilizes Met protein levels and this stabilization is required for EGF-induced Met phosphorylation. (A)** 32D cells expressing Met or EGFR/Met were starved for 4 hours then treated with 10ng/ml EGF for 15 minutes to activate EGFR signaling. Cells were then treated with 10ng/ml Cycloheximide (CHX) to inhibit protein synthesis and lysed after 0, 2, or 7 hours. **(B)** 32D cells expressing EGFR/Met were starved for 4 hours. Cells were then treated for an additional 4 hours with or without 10ng/ml EGF and with or without 10ng/ml Cycloheximide and 100nM Bortezomib, a proteasome inhibitor, (CHX/Bor).

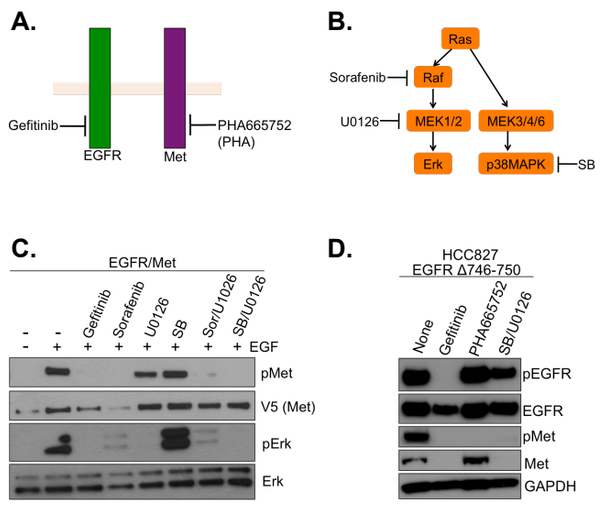



**Figure 5. EGFR activates Met phosphorylation and stabilization through the Ras-Erk signaling pathway. (A)** diagram of EGFR and Met inhibitors, **(B)** diagram of the Ras-Erk signaling pathway and inhibitors used, **(C)** 32D cells expressing EGFR/Met were starved for 4 hours then treated for another 4 hours with 1uM of the indicated drugs and 10ng/ml EGF, **(D)** HCC827 cells were treated with 1uM of the indicated drugs for 24 hours.

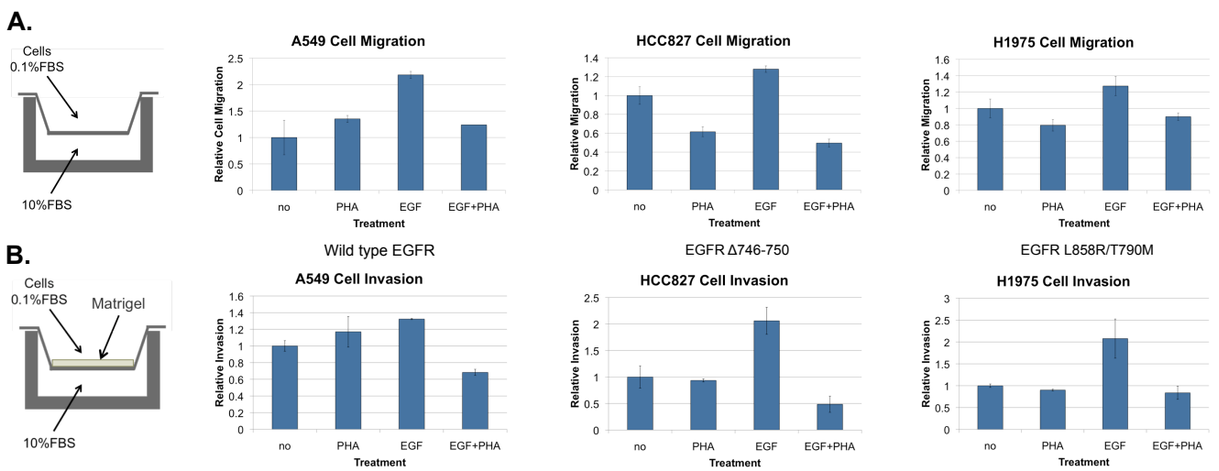
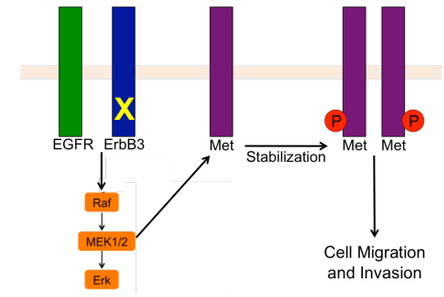



**Figure 6. Met is involved in EGF-induced cell migration and invasion in NSCLC cell lines with both wild type and activated EGFR. (A)** NSCLC cell lines were plated in the top of a transwell migration chamber in 0.1%FBS and allowed to migrate towards 10%FBS for 48 hours. Cells were given no treatment (no) or were treated with 1uM PHA665752 (PHA), 10ng/ml EGF (EGF), or both (EGF+PHA), **(B)** NSCLC cell lines were treated as in (A) but were plated in a migration chamber containing a layer of Matrigel.

## CONCLUSIONS

- EGFR activation leads to delayed Met phosphorylation (Figure 2)
- ErbB3 enhances EGF-induced Met phosphorylation (Figure 3)
- Met cannot act as a dimerization partner for ErbB3 (Figure 3)
- ErbB3 requires an active signaling partner to induce Met phosphorylation (Figure 3)
- EGFR signaling stabilizes Met protein and this stabilization is required for EGF-induced Met phosphorylation (Figure 4)
- EGFR induces Met stabilization and phosphorylation through the Ras-Erk signaling pathway in 32D cells and a NSCLC cell line (Figure 5)
- Met is important for EGF-induced cell migration and invasion in NSCLC cell lines with both wild type and mutant EGFR (Figure 6)

### Model of ErbB-Met Signaling




## SUMMARY

Until now, signaling interactions between Met and the ErbB family were thought to be important only as a drug resistance mechanism in NSCLC. Now, it is clear that this signaling interaction occurs in drug naïve cancer cell lines to promote aggressive phenotypes. We have found that activation of multiple different ErbB family members can lead to increased Met protein stability and phosphorylation. This signaling occurs indirectly through the Ras-Erk pathway and ultimately leads to Met-dependent increases in cell migration and invasion. These results confirm the potential benefit of using Met inhibitors in combination with other therapies to treat NSCLC. Currently, Met inhibitors are being investigated for use in patients with Met amplification as a resistance mechanism to EGFR tyrosine-kinase inhibitors. Our results, however, show that Met inhibitors may also be beneficial to patients with wild type EGFR or other drug resistance mechanisms, such as EGFR T790M mutations, since the ErbB-Met signaling axis is also active in these cell lines. In corroboration with these results, a recent clinical trial using Met and EGFR inhibitors found an unexpected benefit for patients with wild type EGFR.[6] Overall, we have found an ErbB-Met signaling axis that is promoting aggressive phenotypes in NSCLC cell lines and should be pursued as a drug therapy option for NSCLC patients with both wild type and mutant EGFR.

## REFERENCES

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