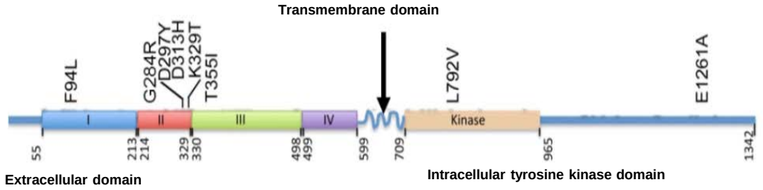
# Role of HER3 signaling pathways in ER+ and HER2+ breast cancers

Rosalin Mishra[1*], Samar Alanazi[1], Hima Patel[1], Long Yuan[1], Joan T. Garrett[1]

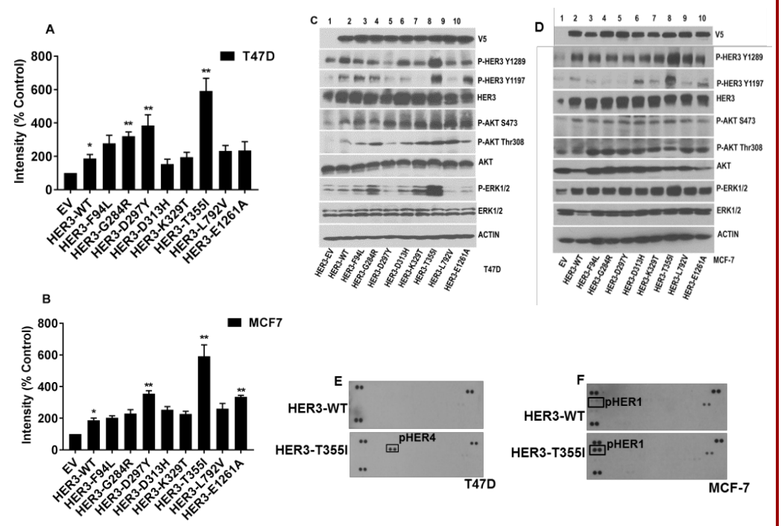
[1,*]James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH,

## Background

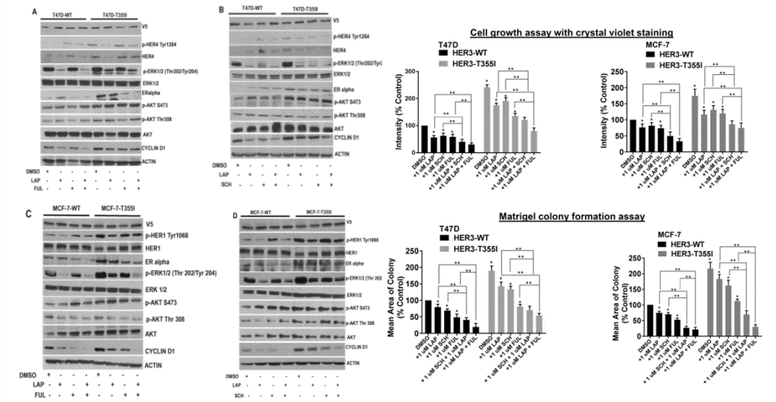
- There are 4 members of the ErbB family of receptor tyrosine kinases: HER1 (EGFR), HER2 (ErbB2), HER3 (HER3) and HER4 (ErbB4).
- HER3 is as essential as HER2 for maintaining cell viability in HER2-overexpressing breast cancer cells.
- HER3 is emerging as an important protein in luminal breast cancers, potentially promoting resistance to endocrine therapy.
- HER3 is mutated in 2% of primary breast cancers and mutated in 14% of metastatic ER+ breast cancers (n=22).



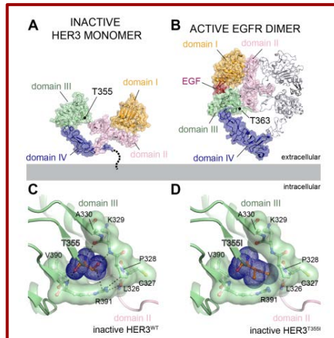

## Results



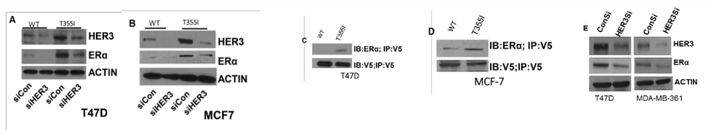

**Oncogenic potential of ER+ T47D and MCF-7 cells stably expressing HER3 mutations.** (A) T47D cells expressing HER3$^{EV}$, HER3$^{WT}$ and HER3 mutants (F94L, G284R, D297Y, D313H, K329T, T355I, L792V and E1261A) were plated, treated and stained. *p<0.05 versus EV and **p<0.05 versus WT. (B) MCF-7 cells with HER3 constructs were plated and stained under above similar conditions. *p<0.05 versus EV and **p<0.05 versus WT. (C-D) Signaling pathways in serum starved T47D and MCF-7 cells expressing HER3 EV, WT and mutants determined by western blot. Actin served as loading control. (E-F) RTK arrays used to determine tyrosine phosphorylation of various RTKs from lysates of T47D and MCF-7 cells expressing HER3$^{WT}$ and HER3$^{T355I}$ serum starved overnight.



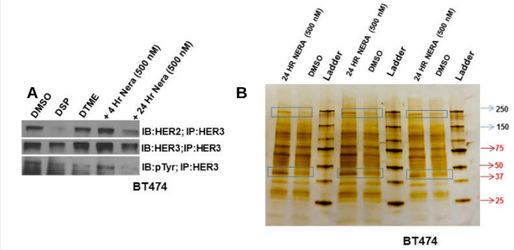

**Blocking HER1/HER4, ER and ERK1/2 signaling inhibits downstream signaling and the proliferation of ER+ cells with HER3$^{WT/T355I}$.** (A-D) ER+ T47D and MCF-7 cells with HER3$^{WT}$ and HER3$^{T355I}$ overexpression were seeded and treated ± vehicle (DMSO), lapatinib (1µM), fulvestrant (1µM), SCH772984 (1µM) or indicated combinations and immunoblotted using indicated antibodies. (Right panel, top) ER+ T47D and MCF-7 cells with HER3$^{WT}$ and HER3$^{T355I}$ overexpression were seeded in six-well plates in triplicate and treated ± vehicle (DMSO), lapatinib (1µM), fulvestrant (1µM), SCH772984 (1µM) or indicated combinations. Media and inhibitors were replenished every second day and stained with crystal violet (Right panel, bottom) ER+ T47D and MCF-7 cells with HER3$^{WT}$ and HER3$^{T355I}$ overexpression were seeded on a basement membrane of matrigel ± vehicle (DMSO), lapatinib (1µM), fulvestrant (1µM), SCH772984 (1µM) or indicated combinations. The average size of each cellular structure was quantified using ImageJ and expressed relative to respective control. *p<0.05 versus WT,**p<0.05 versus respective treatment groups as indicated.



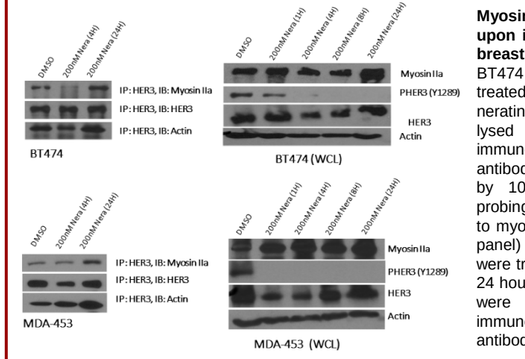

**Structural analysis of HER3$^{T355I}$.** (A-B) The locations of the conserved T355 residue mapped onto the structure of the inactive HER3 extracellular domain (ECD) monomer (PDB ID: 1M6B) (A) and the active EGFR ECD homodimer (PDB ID: 3NJP) (B) illustrating the conformational changes associated with ligand dependent receptor activation. In EGFR, domains I and II rotate around the domain II/III hinge as a single rigid body to form an extended, activated conformation poised to interact with dimerization partners. For clarity, one monomer of the EGFR ECD homodimer is shown using the same surface representation as for the inactive HER3 ECD monomer. The second monomer is shown as grey ribbons. (C) Cartoon representation of interactions between T355 and residues of the hinge region pocket in the inactive HER3 ECD. (D) Cartoon representation of the modelled isoleucine residue at position 355 (I355) in the HER3 ECD reveals that the bulkier hydrophobic residue produces a steric clash with the polar pocket.




**Abrogation of HER3 inhibits ERα expression.** (A & B) MCF-7 and T47D cells with HER3$^{WT}$ and HER3$^{T355I}$ expression were transfected with a siHER3 or siCon for 48 hr and analyzed using specific antibodies. Actin was used as loading control. (C & D) MCF-7and T47D cells with HER3$^{WT}$ and HER3$^{T355I}$ were immunoprecipitated using V5 antibody and analyzed using ERα antibody. **(E) Knocking down HER3 from Erα cells without HER3 overexpression suppresses HER3 expression.**




**Identification of HER3 binding partners upon inhibition of HER2 in HER2+ breast cancer.** (A) BT474 cells were immunoprecipitated using a HER3 antibody. An immunoprecipitation assay was performed and the products were analyzed by 10% SDS-PAGE followed by HER3, p-Tyr and HER2 immunoblots. DTME (dithiobismaleimidoethane) crosslinker, DSP (dithiobis(succinimidyl propionate)) crosslinker. (B) Silver staining of protein bands present upon inhibition of HER2 with neratinib and absent under DMSO control treatment from HER3 immunoprecipitates. Upper bands represent myosin IIa as determined by mass spectrometry. Lower band identified as actin by mass spectrometry. (C) BT474 cells were immunoprecipitated using a HER3 antibody. An immunoprecipitation assay was performed and the products were analyzed by 10% SDS-PAGE followed by probing immunoblots for antibodies to myosin IIa and HER3 .




**Myosin IIa levels are increased upon inhibition of HER2 in HER2+ breast cancer cells** (left panel) BT474 and MDA453 cells were treated with DMSO or 200 nM neratinib for 4 or 24 hours. Cells were lysed and subjected to immunoprecipitation with a HER3 antibody. Products were analyzed by 10% SDS-PAGE followed by probing immunoblots for antibodies to myosin IIa, HER3 and actin. (right panel) BT474 and MDA453 cells were treated with DMSO or 1, 4, 8 or 24 hours with 200 nM neratinib. Cells were lysed and subjected to immunoblot analysis with indicated antibodies.

## Conclusions

- T355I mutation is oncogenic in both ER+ MCF-7 & T47D cell lines and results in phosphorylated EGFR, ErbB3 and ErbB4 and activation of downstream MAPK signaling.
- Genetic ablation of HER3 reduces ERα levels. Studies are underway to determine how this occurs.
- T355I mutation might promote HER3 signaling by shifting the conformational equilibrium of the receptor to an extended conformation, even in the absence of a ligand.
- Inhibition of both EGFR/ErbB4 and MAPK signaling significantly suppresses the proliferation of ER+ cells expressing HER3 T355I mutant.
- Inhibition of HER2 results in increased levels of myosin IIa. Myosins constitute a superfamily of motor proteins that bind to actin and produce mechanical force through magnesium-dependent hydrolysis of ATP. Experiments are underway to determine the role of myosin IIa as an adaptive response to inhibition of HER2.

## Acknowledgements

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