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

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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+, HER3R and HER3 mutants (F94L, G284R, D297Y, D313H, K329T, T355I, L792V and E1261A) were plated, treated and stained. *p<0.05 versus WT. (B) MCF-7 cells with HER3WT and HER3T355I overexpression were seeded and treated x vehicle (DMSO), lapatinib (1µM), fulvestrant (1µM), SCH772984 (1µM) or indicated combinations and immunoblotted using indicated antibodies. (Right panel) EFH-1470 and MCF-7 cells with HER3WT and HER3T355I overexpression were seeded in six-well plates in triplicate and treated x 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) EFH-1470 and MCF-7 cells with HER3WT and HER3T355I overexpression were seeded on a basement membranes of matrigel x 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.

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 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.

Structure of HER3 induced by HER2 inhibition.
(A) 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: 1X9B) (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 toxocaine-rescued position at 355 (T355) in the HER3 ECD reveals that the bulbar hydrophobic residue produces a steric clash with the polar pocket.

Conclusions
➢ T355 mutation is oncogenic in both ER+ MCF-7 A T47D cell lines and results in phosphorylated EGFR, ErbB3 and ErbB4 and activation of downstream MAPK signaling.
➢ Genetic ablation of HER3 reduces ErbB levels. Studies are underway to determine how this occurs.
➢ T355 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
Funding provided by Susan G. Komen Career Catalyst Research Grant CCR14298180.