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2019

## ErbB3 Signaling and its Effect on Spheroid Formation in Ovarian Cancer

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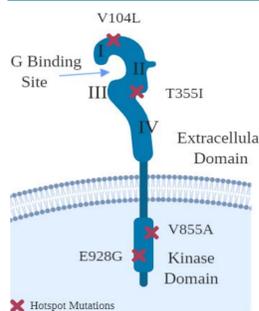
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## Introduction



**Figure 1: Diagram of ErbB3 Hotspot Mutations** that have been identified in many types of cancers.

The ErbB family of receptor tyrosine kinases are often mutated or overexpressed in cancer making them important targets for cancer therapy.

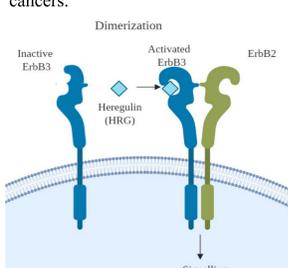
The ErbB family activates signaling pathways leading to proliferation, growth and drug resistance.

ErbB3, one member of the ErbB family, has limited kinase activity and is thought to favor heterodimerization with ErbB2.

Mutations in ErbB3 that fall within specific hotspots have been identified in a variety of cancers (Figure 1)

### Why Ovarian Cancer?

- 5th leading cause of cancer deaths in women in the U.S.
- Relapse rate is 75%.
- Multiple ErbB receptors as well as the interacting receptor, Met, are expressed in ovarian cancer making it a good model to study ErbB interactions
- Ovarian cancer is thought to disseminate through the peritoneal cavity as free-floating cancer spheroids.



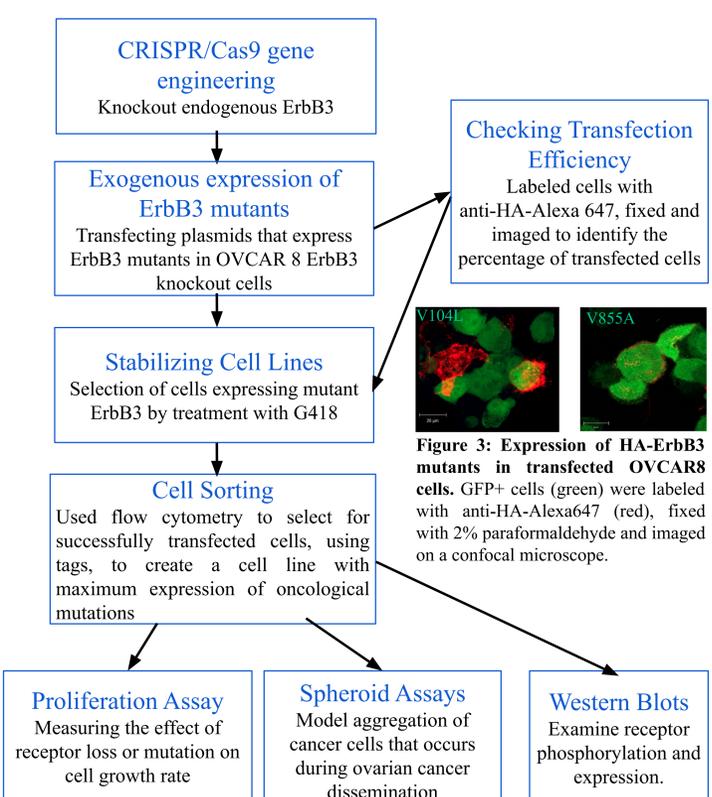
**Figure 2: ErbB3/ErbB2 Heterodimerization** ErbB3 can adopt an active conformation when bound to a ligand, heregulin. ErbB3 can form homo- and hetero-dimers which activates different pathways.

By understanding how ErbB3 is activated in ovarian cancer and how the activation affects cell adhesion, therapies can be developed to reduce ovarian cancer relapse.

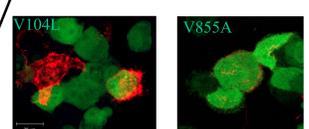
### Hypothesis

ErbB3 gain of function mutations may alter interactions with ErbB2 and affect spheroid formation and spreading.

## Methods

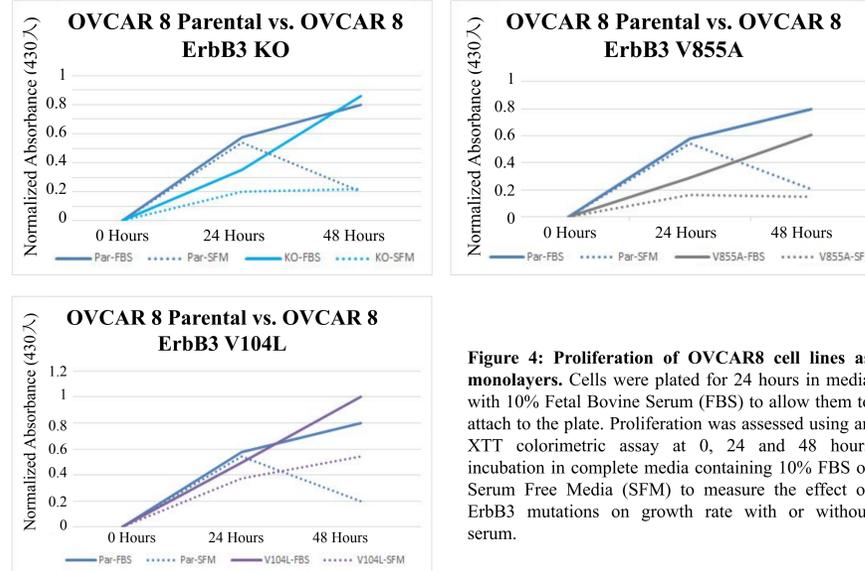


**Checking Transfection Efficiency**  
 Labeled cells with anti-HA-Alexa 647, fixed and imaged to identify the percentage of transfected cells



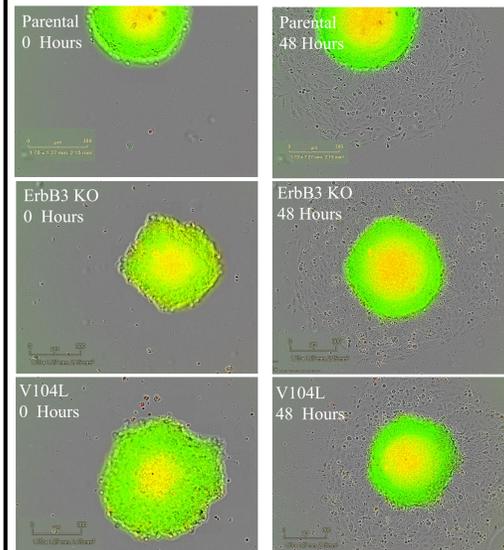
**Figure 3: Expression of HA-ErbB3 mutants in transfected OVCAR8 cells.** GFP+ cells (green) were labeled with anti-HA-Alexa647 (red), fixed with 2% paraformaldehyde and imaged on a confocal microscope.

## Proliferation Assay



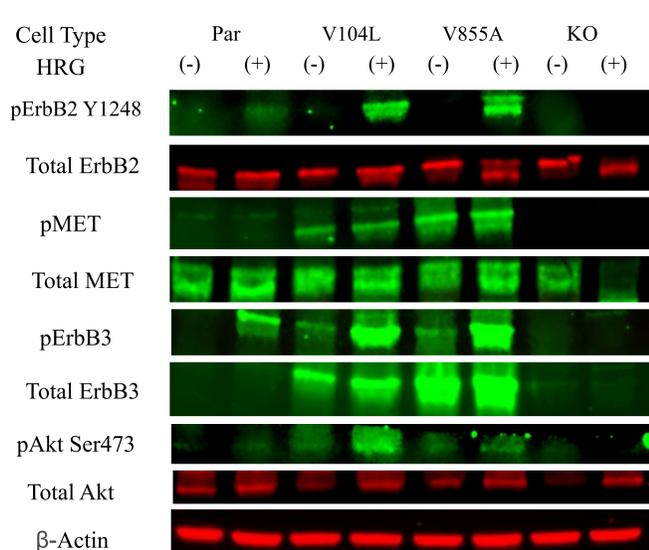
**Figure 4: Proliferation of OVCAR8 cell lines as monolayers.** Cells were plated for 24 hours in media with 10% Fetal Bovine Serum (FBS) to allow them to attach to the plate. Proliferation was assessed using an XTT colorimetric assay at 0, 24 and 48 hours incubation in complete media containing 10% FBS or Serum Free Media (SFM) to measure the effect of ErbB3 mutations on growth rate with or without serum.

## Spheroid Spreading on Fibronectin



**Figure 6: Spheroids on Fibronectin** Spheroids are placed on fibronectin to mimic how spheroids may interact and spread on epithelial membranes on tissues and organs in the body.

## Receptor Signaling in ErbB3 KO and ErbB3 Mutant OVCAR8 Cell Lines



**Figure 7: Protein Analysis of OVCAR 8 cell lines.** Cells were plated as a monolayer +/- stimulation with 12 nM HRG for 2 minutes. Fluorescent westerns show the expression level of ErbB2, MET, ErbB3 and Akt (downstream signaling protein) in OVCAR 8 Parental (Par), ErbB3 V104L, V855A and ErbB3 KO.

## Conclusion

OVCAR8 ErbB3 V104L exhibits serum-independent growth as a monolayer suggesting that this mutant may activate proliferation pathways in the absence of ligand. This is also supported by the phosphorylation of MET in the absence of ligand. Surprisingly, ErbB3 KO's rate of growth is faster than the parental in 10% FBS between 24 to 48 hours.

ErbB3 KO and V104L and V855A mutations result in less compact spheroids compared to parental and ErbB2 KO spheroids.

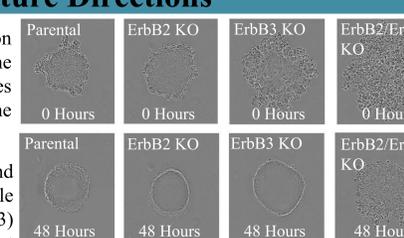
Certain oncogenic mutations in ErbB3 may improve ErbB3 interactions with MET.

Targeted therapies have focused on ErbB2/ErbB3 interactions as key oncogenic partners. However, the importance of ErbB3/MET interactions should not be ignored when designing future therapies.

## Future Directions

Perform co-immunoprecipitation (Co-IP) assays to determine whether mutant ErbB3 increases ErbB3/Met interactions in the absence of ligand.

Examine spheroid formation and receptor interactions of double knockout (ErbB2 and ErbB3) OVCAR 8 cells to see which effect on spheroid morphology is dominant.



**Figure 8: Loss of ErbB3 and ErbB2 in OVCAR8 cells further loosens spheroids even after 48 hours.**

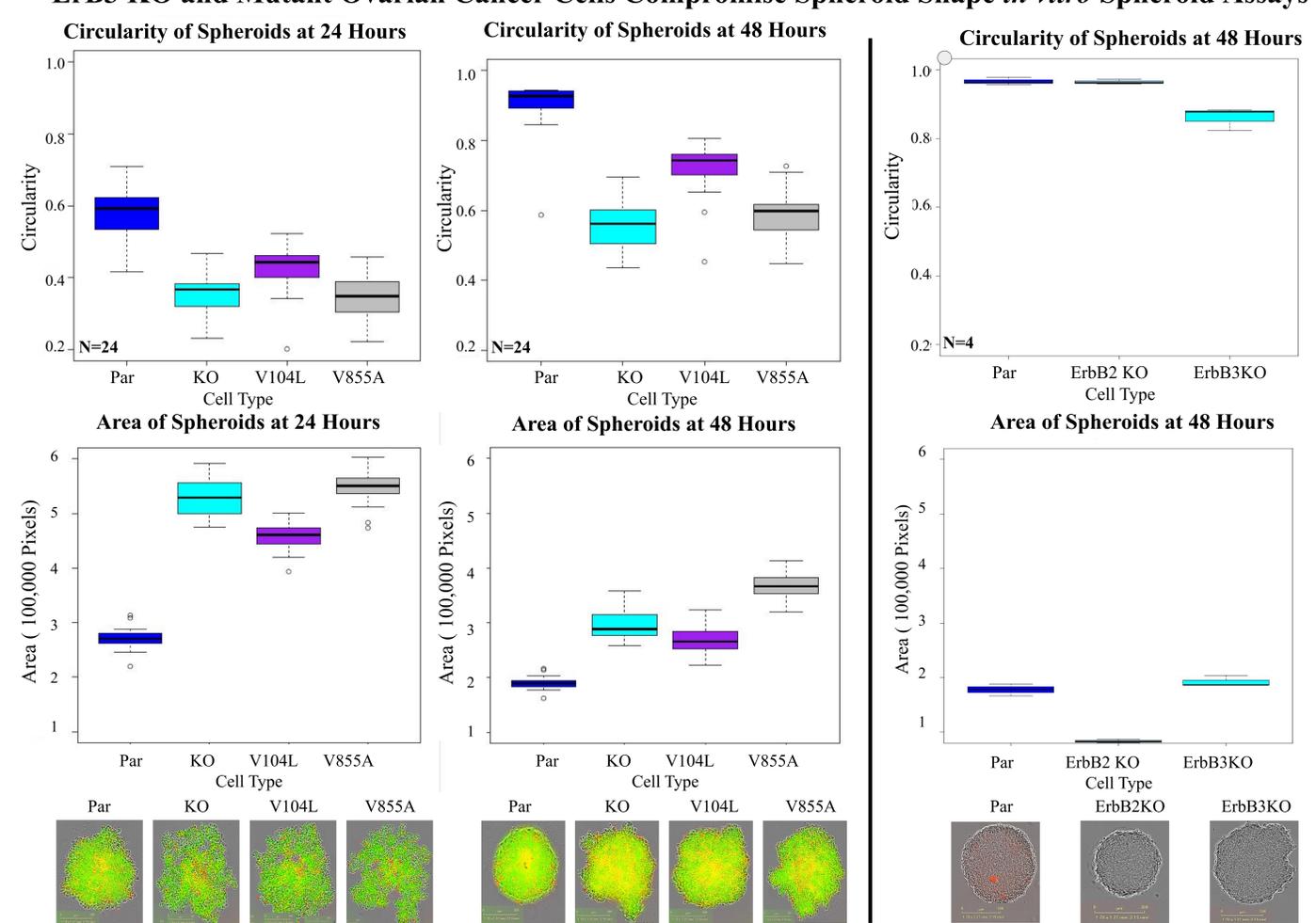
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## Acknowledgements

Thanks to Erica Pascetti who developed the initial protocol for spheroid spreading on fibronectin. Cedric Cleary and Eunice Choi developed the CRISPR/Cas9 knockout lines. Imaging for this work used the University of New Mexico Cancer Center Fluorescence Microscopy Shared Resource, funded by NCI 2P30 CA118100 (PI Willman, C.) "UNM Cancer Center Support Grant". Thanks to The Hathaway Lab and the UNM Department of Cell Biology for use of the Incucyte imaging system. Other support was provided by the UNM SpatioTemporal Modeling Center (NIGMS P50GM085273) and the Phi Beta Psi Charity Trust Fund.

## Erb3 KO and Mutant Ovarian Cancer Cells Compromise Spheroid Shape *in vitro* Spheroid Assays



**Figure 5: Both OVCAR 8 ErbB3 KO and Mutant ErbB3 V104L and V855A form looser spheroids than parental OVCAR 8, whereas ErbB2 KO make tighter, compact spheroids.** 10,000 cells were plated into cell-repellent plates and imaged every two hours to view spheroid formation. All cell lines express GFP (green). YoY3 dye (red) was used to visualize cell death. Using ImageJ, the area and circumference of spheroids was measured. Circularity, a ratio measurement ( $4\pi \times [\text{Area}] / [\text{Perimeter}]^2$ ) was calculated at 24 and 48 hours to compare spheroid compactness.

**The more compact the spheroid formation, the greater the invasive behavior of the cancer cell line.**