Paper Outline

Multi-pathway modeling of response to AKT inhibitors in breast cancer

Modeling AKT inhibitor response in breast cancer using multi-pathway signatures

Predicting drug response to AKT inhibitors at the multi-pathway level in breast cancer

**Molecular profiling of breast cancer therapies using multi-pathway HER2/AKT/BAD signatures**

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Journal: Cancer Research?

Introduction

* Breast cancer statistics
* History of personalized medicine in breast cancer and why it’s inefficient
  + - Chemoresistance
    - HER2/Herceptin resistance story
* Emerging targeted AKT, mTOR therapies and the need for biomarkers
* Why the pathway-based approach is better
* AKT/BAD/IGF1R/HER/ERK in breast cancer
* Objective: To identify the deregulation of common pathways (AKT, BAD, HER2) in breast cancer and to leverage the deregulation status in modeling drug response predictions to provide individualized treatments.

Methods

1) Overexpression of pathway elements using adenovirus in HMEC cells

- Virus we used, infection time, MOI, all the specifics

*-* Western blot figures for each signatures (supplements)

- Just the phosphorylated proteins and the B-action

2) Data processing and normalization

Sequencing methods, processing (Rsubread), normalization, batch adjusting

- Signatures

- ICBP

- CCLE

- TCGA

3) Generation and validation of genomic signatures that represent pathway activity

- ASSIGN parameters, test data tests, correlation parameters, boot strap method

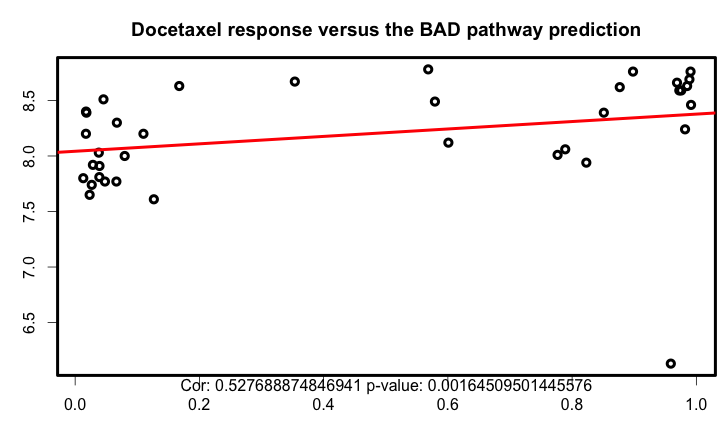
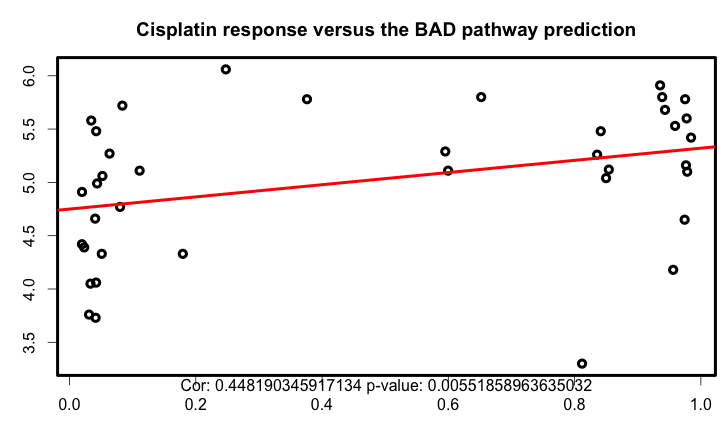
4) Building drug response prediction models in cell lines

5) Validating finding in cell lines and patient cells

-CCLE, GDSC, TCGA and patient cells if possible +Reagents + protocols

Results

1. BAD pathway activity as a biomarker for chemosensitivity:
   1. High BAD activity correlated well with chemosensitivity in cell lines for many classes of chemotherapies including taxane derivatives, platinum analogs and topoisomerase inhibitors.



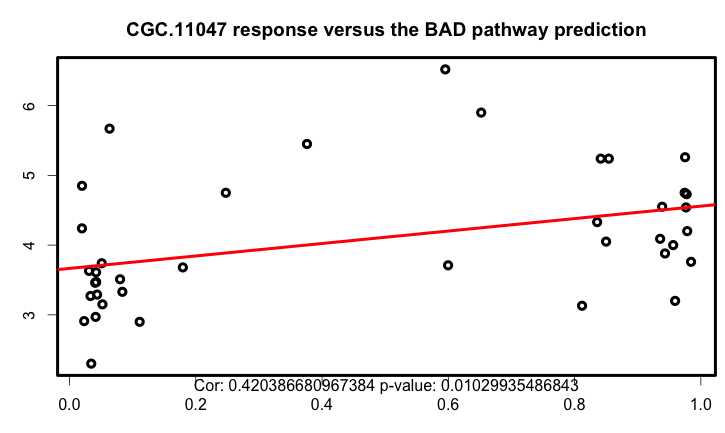


Figure: BAD pathway activation status is positively associated with chemo drugs.

* 1. Within subtypes, triple negative (Basal and Claudin-low) breast cancer has higher BAD activity making this subtype more sensitive to chemotherapies
     1. In ICBP, CCLE breast cancer cell lines

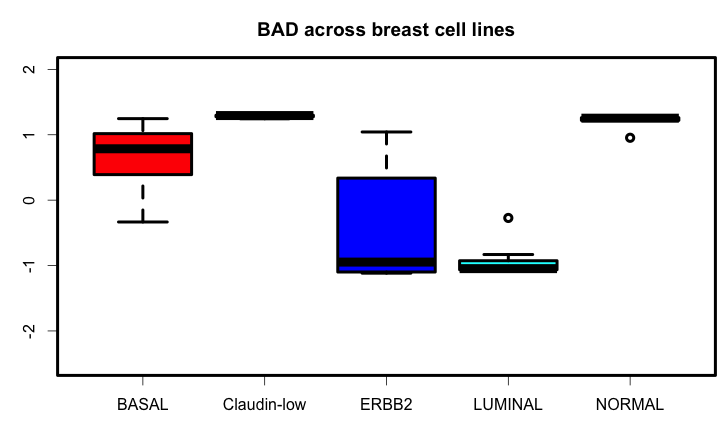


Figure: BAD pathway activation status is higher in triple negative breast cancer cell lines than receptor positive cell lines.

* + 1. In TCGA BRCA, basal-like subtype samples have the highest BAD activity and patients with this subtype of breast cancer are more likely to benefit from chemotherapy.

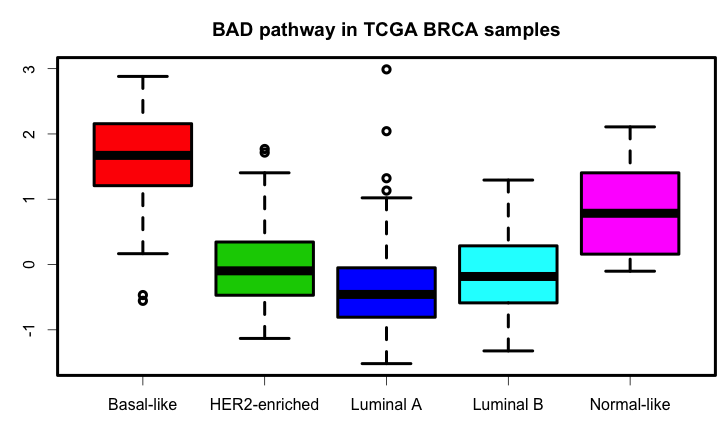


Figure: BAD pathway activation status is higher in triple negative TCGA BRCA samples than receptor positive ones.

1. In addition to chemosensitivity, BAD pathway provides insights about HER2, AKT, PI3K and mTOR targeted therapy sensitivity. BAD pathway activity is negatively correlated with HER2, AKT, PI3K and mTOR activity and can be used to identify resistance to therapies targeting these nodes.
   1. In ICBP breast cancer cell lines

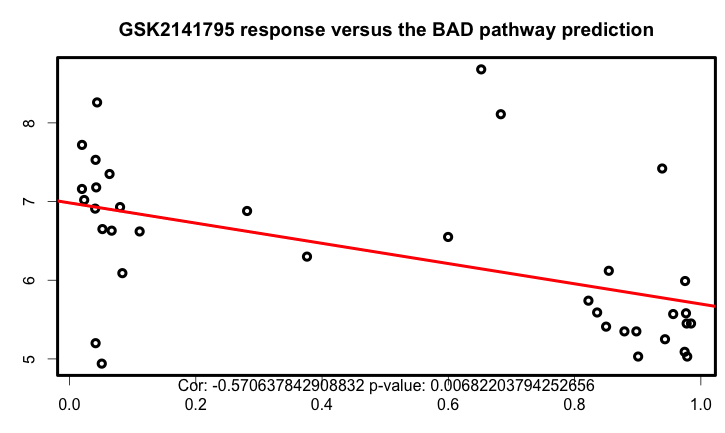
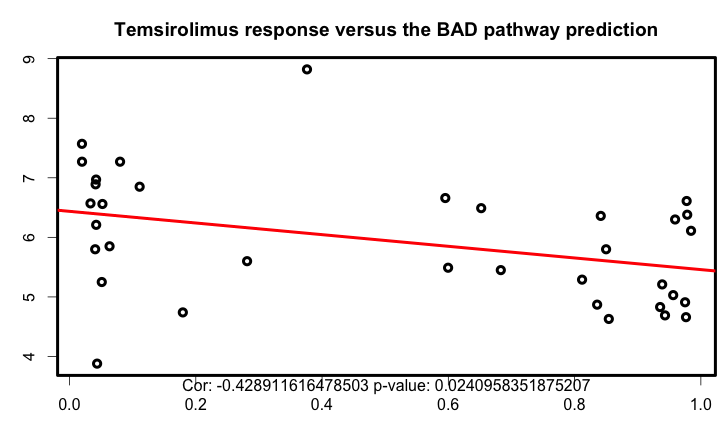


Figure: BAD pathway activation status is negatively associated to AKT, HER2 and mTOR targeted drugs.

* 1. Validate these findings in GDSC dataset. GDSC has Cisplatin and Docetaxel and a lot of other chemos and [Temsirolimus](http://www.cancerrxgene.org/translation/Drug/1016). CCLE has Paclitaxel, Topotecan, Irinotecan for validation.

1. Modeling AKT targeting drug response: AKT activity is high in Luminal and ERBB2 amplified cell lines. Therefore, AKT targeting therapies likely to work better in these two subtypes.

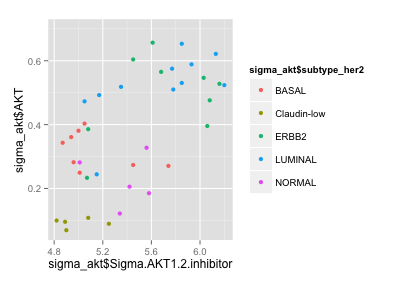


Figure : Due to high AKT activation status in many ERBB2 amplified and luminal cell lines, AKT targeting drugs are likely to work.

1. Modeling HER2 targeting drug response
   1. Compare HER2 amplification status and HER2 high pathway activity
   2. Comparing drug response based on the HER2 amplification versus HER2 high pathway activity in breast cancer cell lines. Pathway predictions are more specific to drug response for lapatinib than the HER2 amplification status. (Caveat is Lapatinib is HER2+EGFR inhibitor. It would be nice to study trastuzumab)

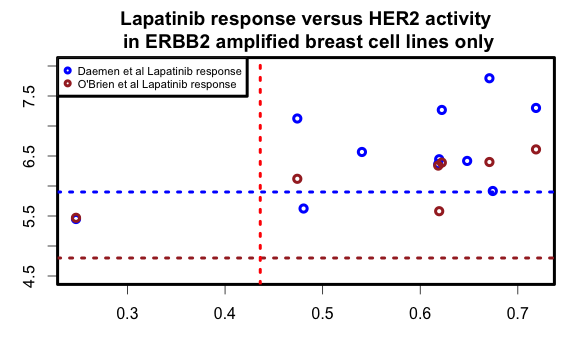


Figure: The blue dashed line divides the cell lines based on the drug response assay done by O’Brien et al and all blue dots are representing ERBB2 amplified cell lines. Cell lines above this line are “sensitive” to Lapatinib and below are “resistance”. The redline shows HER2 pathway prediction above which cell lines are likely to be sensitive to HER2 targeted therapy and below which cell lines are not likely to responsive to HER2 targeted therapy. The brown dots are showing ERBB2 amplified cell lines’ drug response and brown dashed line is showing above which cell lines are Lapatinib sensitive (data from Heiser at al). HER2 pathway predictions are more specific for HER2 targeted therapy response in these ERBB2 amplified cell lines.

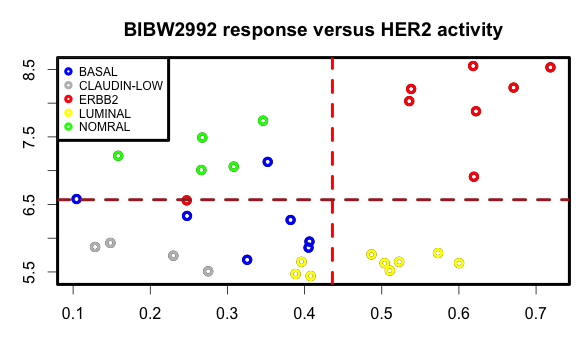


Figure: The points are showing ICBP breast cell lines’ BIBW2992 response and brown dashed line (mean of the drug response) is showing above which cell lines are BIBW2992 sensitive (data from Daemen at al). Cell lines above this line are “sensitive” to BIBW2992and below are “resistance”. The redline shows HER2 pathway prediction above which cell lines are likely to be sensitive to HER2 targeted therapy and below which cell lines are not likely to responsive to HER2 targeted therapy. HER2 pathway predictions are more specific for HER2 targeted therapy response in these ERBB2 amplified cell lines.

1. Building final model based on BAD, AKT and HER2 pathway predictions and subtypes.
   1. Illustrating that multi-pathway is better than single pathway. Boxplot the bootstrap iterations of single vs multi for HER, BAD, and AKT. Single pathway can not consider the pathway crosstalk and may lead to wrong pathway activation status. Demonstrate how results can vary based on pathway included for multipathway predictions.
   2. Chemodrugs

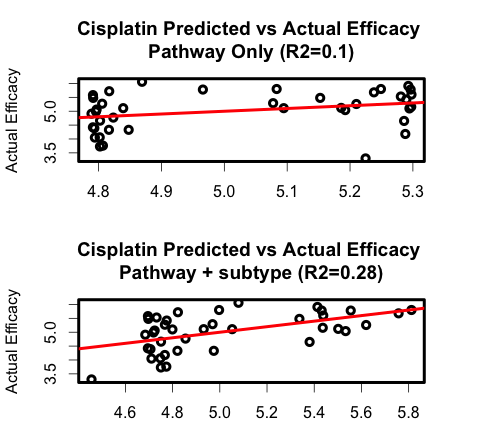
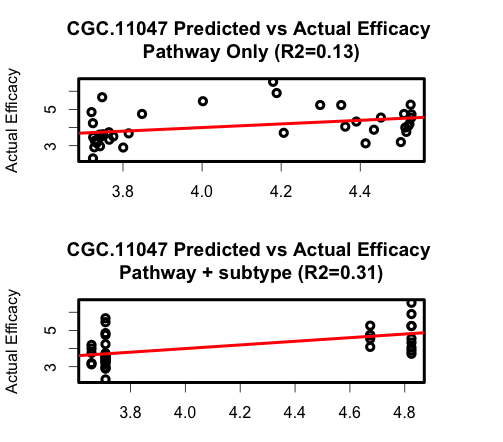


Figure : Bad pathway predictions together with subtype are informative of drug response in ICBP cell lines.

* 1. AKT and mTOR targeting drug: Sigma AKT, GSK2141795, rapamycin, temsirolimus, everlolimus. Compare performance of models of these drugs with Heiser at al AUC based models.

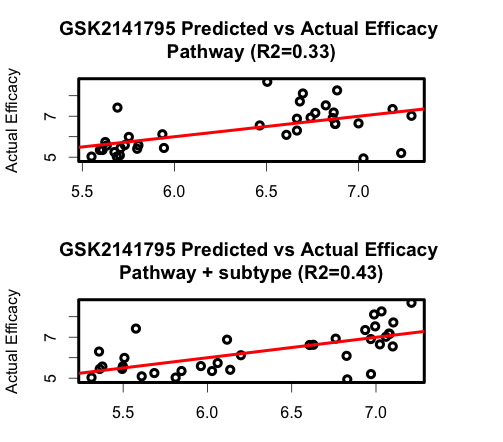
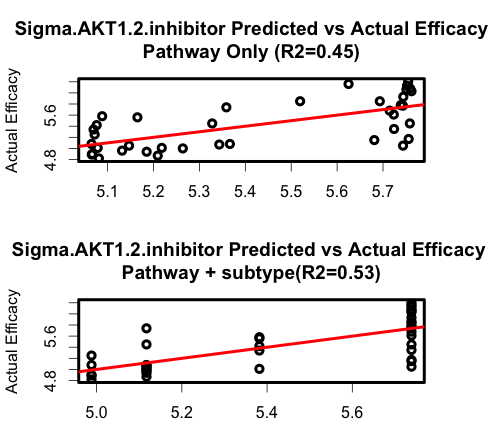


Figure : Targeted therapy response variability can be explained primarily by targeted pathway activation status. Sigma AKT ½ inhibitor and GSK2141795 both target AKT and their response are explained by AKT pathway activation status.

* 1. HER2 targeting drug: Lapatinib, BIBW2992

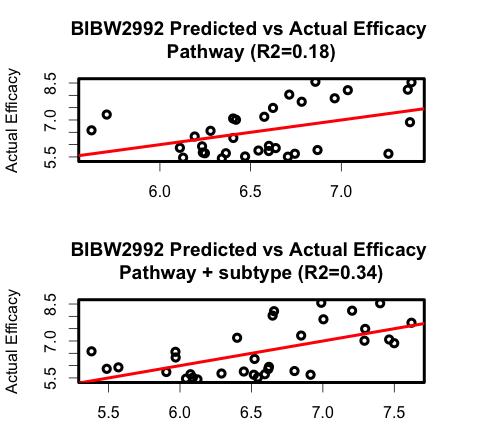
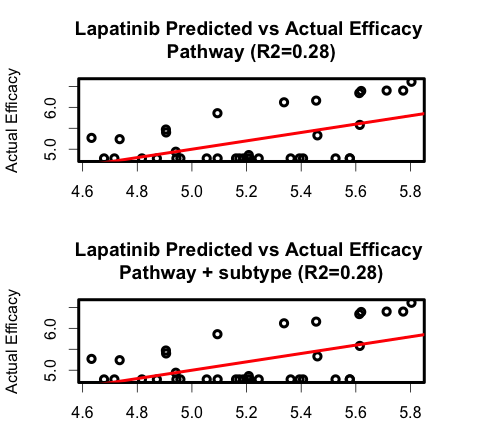


Figure : Targeted therapy response variability can be explained primarily by targeted pathway activation status. Lapatinib and BIBW2992 both target EGFR and HER2. For Lapatinib, subtype status did not contribute over HER2 pathway activation status.

* 1. Add snps, indel and proteomics data to the models. Show contribution from pathway prediction and subtypes

1. Validation of the models in independent cell lines and patient cells
   1. Maybe data from the groups that Sam mentioned
2. *Other ideas*

*- Something interesting about IGF1R pathway? IGF1R uperegulation during neoadjuvant therapy predicts poor prognostic marker in breast cancer (Heskamp et al , PLOS one, 2015). ASSIGN is dropping the most significant prior gene if adaptive signature feature is being used. IGF1R validation pending.*

*- Something interesting about ERK pathway? High ERK indicate chemoresistance after neoadjuvant chemotherapy (Balko et al, Nature Medicine 2012). ASSIGN is changing direction if adaptive signature feature is being used. ERK validation pending.*