#1043 Expression of EGFR ligands betacellulin and tenascin C are associated with molecular response following gefitinib treatment in women with primary breast cancer

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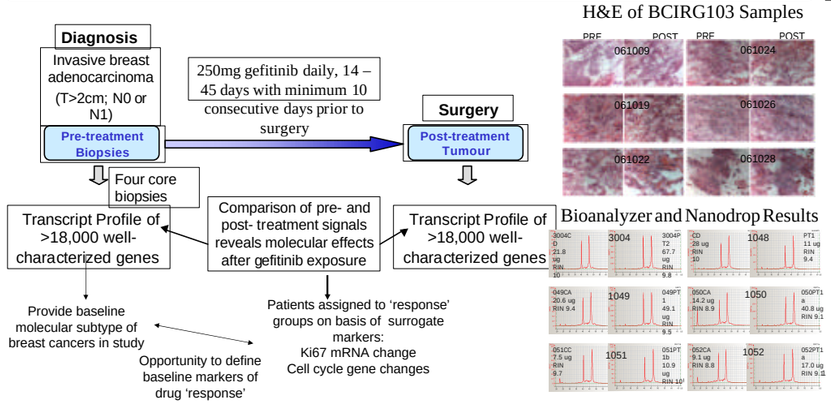
Abstract

Introduction: The epidermal growth factor receptor (EGFR) has been implicated in the pathogenesis of breast cancer. However, EGFR inhibitors have had limited clinical success in the treatment of breast cancer. BCIRG 103 was a pre-surgical study designed to evaluate the molecular changes that occur in primary breast cancer tissue after short-term exposure to the EGFR tyrosine kinase inhibitor gefitinib (IRESSA™, AstraZeneca). Here we use an analysis of variance (ANOVA) to identify genes associated with a molecular response to gefitinib in this study.

Methods: 59 women were enrolled and 43 were evaluable for molecular changes. Frozen tissue was obtained at baseline and after exposure to gefitinib. Agilent microarrays were performed on the baseline sample to determine the molecular subtype of breast cancer (ie luminal vs non luminal) and to identify genes associated with "molecular response" to gefitinib. As reported earlier (SABCC 2005, ASCO 2006), molecular response was assigned based on changes in Ki67 mRNA and cell cycle gene set mRNA when comparing the baseline sample to the post-exposure specimen. We assigned 43 patients evaluable for molecular changes to one of three groups: (1) molecular growth inhibition, 11 patients (2) molecular growth proliferation, 9 patients (3) no significant change in Ki67 or cell cycle genes, 23 patients.

BCIRG 103



H&E of BCIRG103 Samples

Bioanalyzer and Nanodrop Results

Data Analysis (I)

I. Assigning Molecular Response

- Experiments where Ki67 mRNA showed a significant change in expression ($p < 0.01$) were clustered across all sequences where there was a 1.75 fold change in at least two experiments.
- A set of 80 tightly correlated genes related to cell cycle, DNA repair, cell proliferation, and cell death were identified and used in the assignment of treatment experiment as inhibited, proliferating, or unchanged.
- Biological processes in the Ki67 gene set were determined using GoMiner[1] a tool for interpreting genomic data.

Assignment of Patients to Response Groups

'Response' Group	Ki67 mRNA	Predominant Change in Cell Cycle Cluster (mRNA)	No. Patients
Inhibition	⬇	⬇	10
	⬆ (minor change)	⬇	1
			11
Proliferation	⬆	⬆	8
	⟷	⬆	1
			9

GoMiner Ki67 Gene Set Analysis

Go ID	Total genes on Agilent Chip in Category	# Genes Changed in Category	p-value	Term
0008150	8814	53	0.0151	Biologic Process
0007049	550	30	0	Cell Cycle
0006259	431	18	0	DNA Metabolism
0006260	118	11	0	DNA Replication
0008283	425	5	0.0781	Cell Proliferation

Note: Cell Cycle category dominates the list of changed genes

Note: Each gene maybe found in more than one category

II. Principal Component Analysis (PCA)

- 40 baseline experiments comparing pre-treatment sample to a mixed reference were analyzed using Rosetta Resolver 5.1 PCA tool
- PCA data reduction was performed on 40 experiments across genes with a 2.0 fold change in at least 3 experiments (4502 genes).

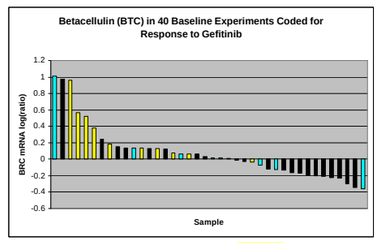
- Experiments were classified as luminal or non-luminal based on the expression of a set of well known markers. This revealed:

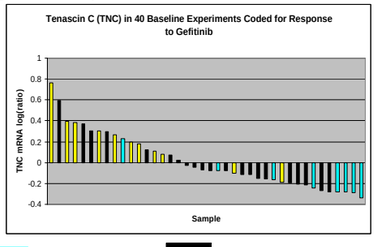
1) Classification as luminal/ non-luminal dominates gene expression
2) All HER2 amplified tumors are luminal in this data set
3) Molecular response to gefitinib does not correlate with a defined subtype

Data Analysis (II)

III. Analysis of Variance (ANOVA)

- Since the number of non-luminal experiments was small (7), biomarker analysis was confined to the luminal subset (33)
- ANOVA of this subset to compare 3 groups (9 inhibited, 6 proliferating, 18 no change)
- Selected genes biologically relevant to EGFR pathway for analysis[2] (338 available)
- Rosetta Resolver 5.1 ANOVA tool used for analysis
- Both betacellulin and tenascin C are ligands for EGFR[3,4] and have higher expression in patients with molecular inhibition following gefitinib exposure





Molecular response: Inhibited Proliferating No change

Conclusions

1. A cell cycle gene set successfully discriminated 3 **molecular response groups** following gefitinib exposure (inhibition, proliferation, and no significant change)
2. PCA analysis showed a strong association of overall gene expression profile with molecular subtype (luminal/ non-luminal) but not with response.
3. ANOVA identified elevated expression of betacellulin and tenascin C as being associated with molecular inhibition in luminals
4. BTC and TNC are both EGFR ligands and further studies validating their potential as biomarkers of clinical response to gefitinib and other EGFR targeted agents in breast cancer are warranted

References

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