

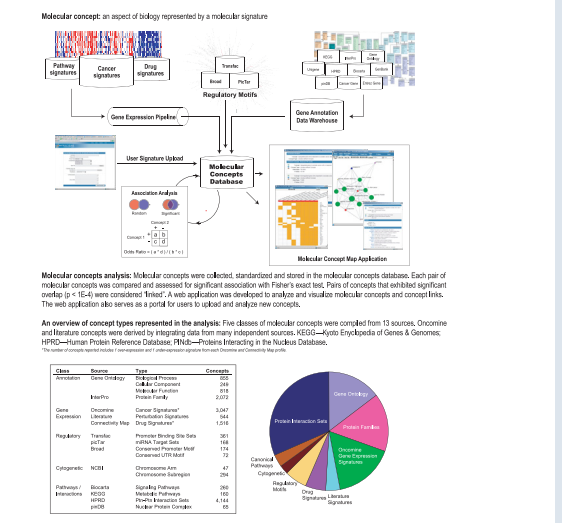
Gene Expression Modules Associated with Cetuximab Response in Metastatic Colorectal Cancer Predict Additional Patient Populations Likely to Respond

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Abstract

KRAS mutation is a negative predictor of response to anti-EGFR therapy in metastatic colorectal cancer (CRC). While KRAS mutation is almost always linked to a lack of response, patients with wild type KRAS exhibit a variable response, providing an opportunity for further stratification. Here, we analyzed global gene expression profiling data from a cetuximab trial to identify biology-driven co-expression modules positively and negatively associated with response. We identified two modules positively correlated with response and seven modules negatively associated with response. Using OncoPrint, a cancer genomics database and analysis platform, we ascribed functional annotations to the modules and linked them to additional tumor populations, suggesting additional indications that may receive benefit from cetuximab therapy. Consistent with the Ras mutation data, we identified a Ras activation module associated with lack of response to cetuximab, demonstrating that a gene expression module could serve as a surrogate for Ras mutation status. Most notably, we identified a carcinoma-like proliferation module, including the EGFR ligands epiregulin and amphiregulin, strongly associated with cetuximab response. Interestingly, this module was associated with subsets of a number of epithelial malignancies relative to normal tissue, including cancers of the colon, lung, bladder and esophagus. Unlike metastatic CRC, in which the module demonstrated variable expression, primary CRC showed near universal over-expression of the module, indicating that primary tumors may respond more frequently to cetuximab than metastatic tumors and that the more primary tumor-like or epithelial-like a metastatic CRC is, the more likely it is to respond. Several modules in addition to Ras activation correlated with a lack of response to cetuximab including those associated with metastasis / invasion, leukocytes, stromal response, cellular defense and interferon response. A decision tree analysis resulted in a predictive model that predicts KRAS mutant patients to progressive disease (25 / 27 patients), KRAS wild type patients with low expression of the carcinoma-like module to also have progressive disease (16 / 19) and KRAS wild type patients with high expression of the carcinoma-like module to have disease control with cetuximab (17 / 24). When applied to a panel of lung cancer cell lines, the model correctly predicted sensitivity to gefitinib, another EGFR inhibitor, in all cell lines but one. In summary, a co-expression module-based approach provided a framework to both study the functional context of drug response associations and link the drug response modules to additional tumor populations that may receive clinical benefit from a targeted therapy.

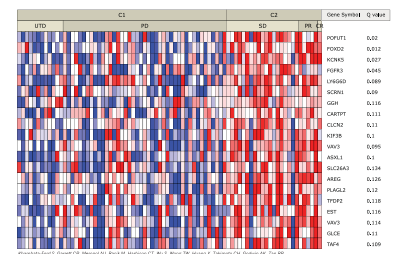
Methods



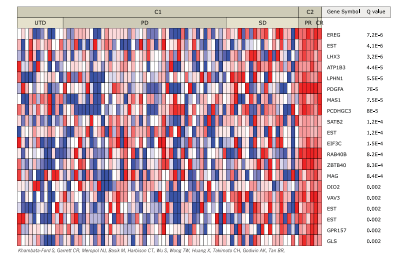
Results

Figure 1. Differential Expression Analysis of Metastatic Colorectal Cancer Stratified by Cetuximab Response.

(A) Disease control vs. progressive disease analysis. Tumor gene expression data from patients with disease control, including stable disease (n = 19), partial response (n = 5) and complete response (n = 1), were compared to data from patients with progressive disease (n = 43) and patients in which response was unable to be determined (n = 12).



(B) Response vs. no response analysis. Gene expression data from patients with partial response and complete response were compared to data from patients with stable disease, progressive disease and patient in which response was unable to be determined. The top twenty most significant genes are depicted as ranked by Student's T-test. Q-values indicate gene-specific false discovery rates. Red and blue indicate relative over- and under-expression, respectively.



Abbreviations
UTD: Unable to Determine
PD: Progressive Disease
SD: Stable Disease
PR: Partial Response
CR: Complete Response

Figure 2. Gene Expression Clusters in Metastatic Colorectal Cancer Organized by Cetuximab Response.

Unsupervised hierarchical clustering of the 10,000 most variable features identified 12 gene clusters correlated at R>0.5 with at least 10 features. Twenty representative features are depicted per cluster. Functional annotations were ascribed to each cluster using OncoPrint Concepts Analysis, which performs association analysis across 20,000+ gene sets spanning biological annotations, pathway signatures, normal tissue signatures and cancer signatures. Clusters were tested for association with response and progression signatures. Clusters associated with response or disease control have labels colored blue. Clusters associated with progression have labels colored red. The number of features in each cluster is provided in parentheses.

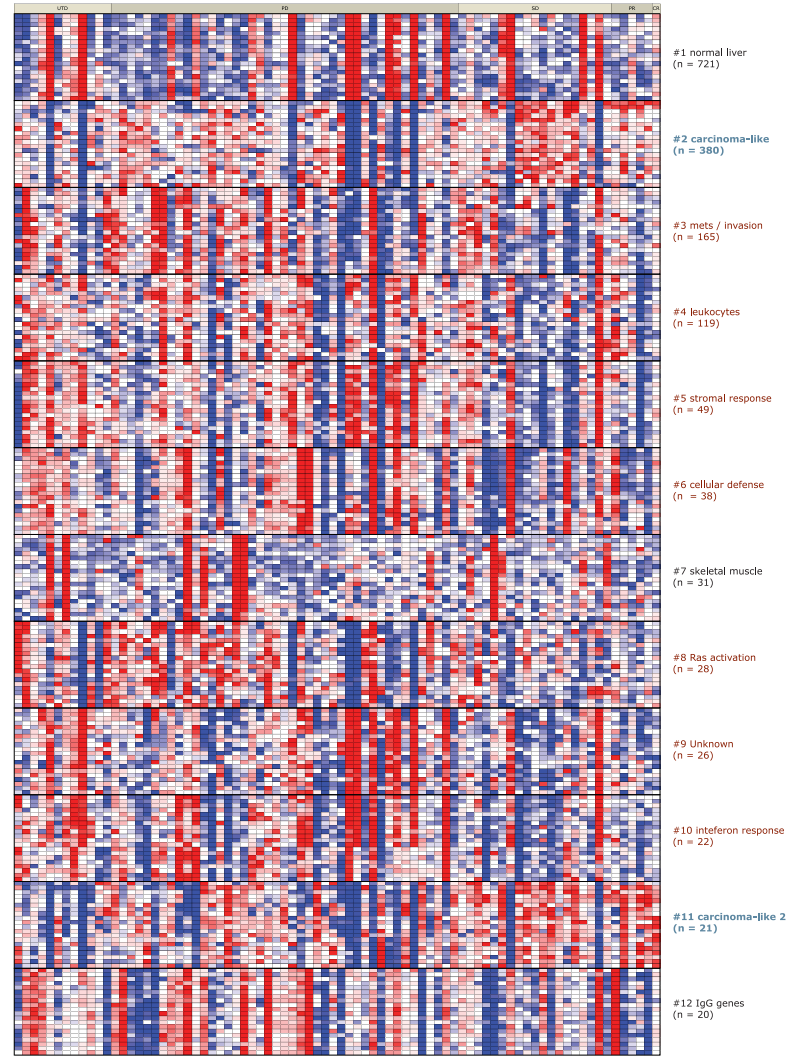


Figure 3. Carcinoma-like Cluster Associations.

OncoPrint Concepts Analysis was performed on the carcinoma-like cluster gene set to identify significant biological and clinical associations. Selected associations are presented. Each heat map includes twenty genes from the carcinoma-like cluster rank-ordered by the associated analysis.

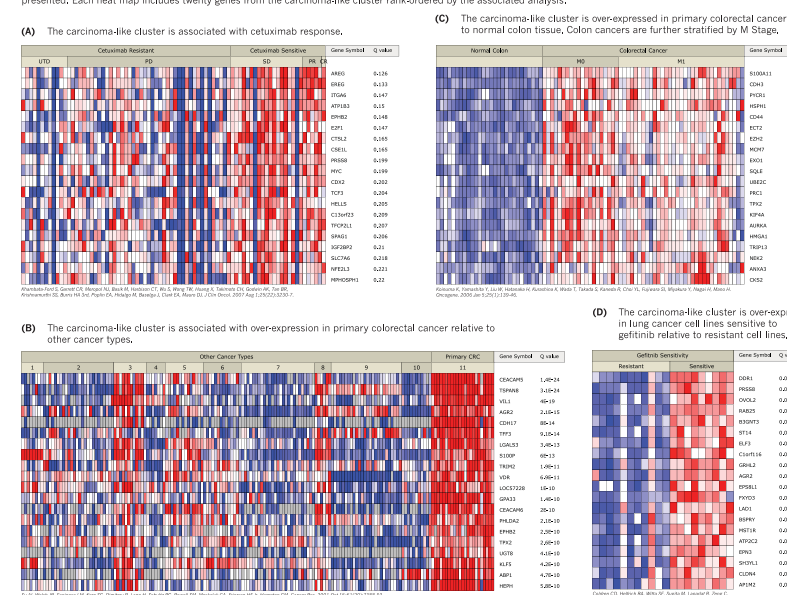


Figure 5. Decision Tree for Cetuximab Response.

The Weka ID3 decision tree algorithm was used to define an optimal predictor of cetuximab response. Mean scores from each of the 12 clusters in addition to K-Ras mutation status were used as input variables. Samples were classified as progressive disease or disease control as the outcome variable. The algorithm identified a two-node classifier. The number of correct predictions out of total predictions is provided in parentheses.

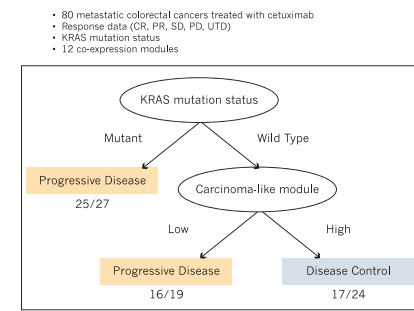
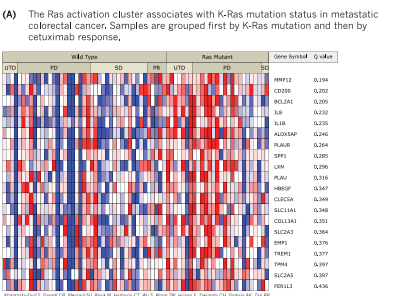
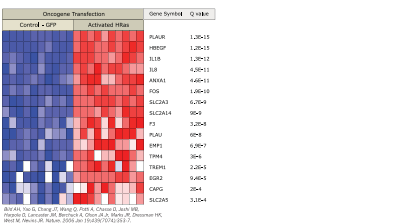


Figure 4. Ras Activation Cluster.

OncoPrint Concepts Analysis was performed on the carcinoma-like cluster gene set to identify significant biological and clinical associations. Selected associations are presented.



(B) The Ras activation cluster associates with over-expression in human mammary epithelial cells after transfection with activated H-Ras.



Conclusions

- Unsupervised hierarchical clustering identifies co-expression modules that associate with cetuximab response in metastatic colorectal cancer.
- The carcinoma-like cluster is positively associated with response and is generally over-expressed in primary colorectal cancer.
- The carcinoma-like cluster also associates with gefitinib response in lung cancer cell lines.
- The Ras activation cluster associates with K-Ras mutation in tumors and H-Ras transfection in cell lines.
- An optimal predictor of cetuximab response includes both K-Ras mutation status and expression of the carcinoma-like cluster.