Research Highlights

Highlights from the latest articles in biomarkers

Master regulators may provide more consistent prediction of breast cancer prognosis than other genes

Evaluation of: Lim WK, Lyashenko E, Califano A: Master regulators used as breast cancer metastasis classifier. *Pac. Symp. Biocomput.* 504–515 (2009).

Genome-wide expression profiling has been used with some success to assess risk of disease progression. Unfortunately, genes identified in different data sets to predict disease progression often have little overlap, which limits their usefulness, since as a panel or signature they are not prognostic in other subjects with breast cancer. The authors argue that the reason for this lack of stability across different sample sets is that the genes identified are most often ‘passengers’ rather than ‘drivers’ of a disease phenotype, such as poor outcome. They rationalize that the most differentially expressed genes are generally far downstream, and not the effectors of a phenotype, noting that oncogenes and tumor suppressors are generally not the most differentially expressed genes.

The authors have developed an algorithm to identify targets of transcription factors (TFs) that they have validated in B cells, T cells and glial cells. In the current manuscript, they demonstrate that the algorithm can identify TFs that may induce or suppress specific gene signatures associated with poor prognosis in breast cancer, and that this ‘master regulator’ algorithm is more stable in classifying different breast cancer samples than two recently published gene signatures reported to predict disease progression in women with breast cancer.

The authors demonstrate that the two published gene signatures only had 9% overlap, whereas the master regulator TFs had 48% overlap between the two breast cancer sample sets, suggesting that the master regulator signature is more reliable across a variety of breast cancer samples in predicting breast cancer disease progression. Interestingly, performance of the master regulator classifier decreased as more genes were used in the classification, suggesting that only the most highly significant predictors should be included in the classification set.

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Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Two-step amplification to optimize information obtained from minute amounts of formalin-fixed tissue

Evaluation of: Dietrich D, Lesche R, Tetzner R *et al.*: Analysis of DNA methylation of multiple genes in microdissected cells from formalin-fixed and paraffin-embedded tissues. *J. Histochem. Cytochem.* (2009) (Epub ahead of print).

The amount of tissue available to scientists for analysis from individual patients continues to decrease. This is explained both by the less invasive techniques used to collect the sample and by better disease detection leading to the identification of earlier-stage disease. This decrease in sample amount comes at a time when the

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ability of scientists to perform multiple-gene assessment is rapidly increasing. The current report proposes a procedure for the simultaneous quantification of DNA methylation of multiple genes using minute amounts of formalin-fixed breast cancer tissue collected using laser-capture microdissection. The procedure consists of bisulfate treatment that has been optimized for samples containing only a few cells and multiplex amplification of all genes of interest, followed by locus-specific amplification and quantitative bisulfate sequencing, which incorporates a normalization domain into the PCR product.

In the report, ten epigenetic biomarkers were analyzed in tissue samples from two breast cancer patients obtained at multiple sites of invasive and intraductal cancer and benign tissue. The authors were able to successfully amplify and quantify methylation status for all ten genes. The results were consistent with a previously published real-time PCR-based quantitative DNA methylation assay, and the new assay allows for quantitative methylation analysis of multiple loci from laser-capture microdissected formalin-fixed tissue samples. This technique should prove useful in the assessment of methylation status of multiple genes in a variety of disease processes where only limited sample is available.

The expression of angiogenesis and chemotaxis genes may help explain why African–American breast cancer patients have a worse prognosis

Evaluation of: Martin DN, Boersma BJ, Yi M *et al.*: Differences in the tumor microenvironment between African–American and European–American breast cancer patients. *PLOS One* 4(2), E4531 (2009).

Even after controlling for socio–economic factors and access to healthcare, African–American breast cancer patients have worse survival rates than European–Americans. African–Americans have a higher percentage of estrogen receptor-negative, basal-like subtype tumors. However, even when considering only tumors that are not basal-like, African–American breast cancer cases have worse outcomes than non-African–American cases. This suggests that there are differences in the biology of African–American compared with European–American tumors.

The authors tested the hypothesis that tumor biology could explain differences in survival by performing laser-capture microdissection and genome-wide mRNA expression using Affymetrix U133A GeneChips of tumor epithelium and adjacent stroma in 18 African–American and 17 European–American patients. The goal of the study was to identify biological networks rather than individual genes. The most significant differences were found in cell cycle control and chemotaxis gene networks in tumor epithelium and angiogenesis gene networks in tumor stroma. The authors looked for the optimal gene panels in tumor epithelia and stroma to differentiate African–American and European–American specimens. A two-gene panel consisting of *PSPHL* and *CRYBB2* were best at differentiating African–American and European breast cancer epithelia.

A five-gene pattern, which included *PSPHL*, *CXCL10*, *CXCL11*, *ISG20* and *GMD5*, correctly differentiated the vast majority of African–American from European–American tumor stroma. Immunohistochemical analysis of microvessel density and macrophage infiltration in 248 breast cancer patients confirmed the presence of increased angiogenesis in African–American tumors.

This study adds to a growing body of literature that supports the hypothesis that there are biological differences between African–American and European–American tumors, which likely explain the poorer survival in African–Americans. The authors suggest chemotherapeutic interventions (sirolimus, resveratrol and chlorpromazine), which may target genes that are upregulated in African–American cancers and thereby more successfully treat African–American patients.