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Hypothesis driven gene expression profiling in breast cancer

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Chapter 7

Summary

Summary

Understanding the biology that leads to the development and progression of a tumor; and most notably the mechanisms that result in metastatic disease that turns cancer into an incurable disease state, is a crucial component of cancer research. Unraveling the biological phenomena in tumor genesis and development can not only dissect patients into prognostic groups, but a mechanism driven approach allows for the identification of potential targets for therapy.

Wound healing has been linked to cancer based on similarities in biological processes including proliferation, angiogenesis, cell motility and matrix remodeling.

Epidemiologically, chronic wound and inflammatory states are well-known risk factors for cancer development: examples are the connection between cirrhosis and liver cancer, gastric ulcers and gastric carcinoma, and burn wounds and subsequent squamous cell carcinoma.

Chapter 1

The first chapter of this thesis provides a brief outline of the research and clinical questions underlying this thesis.

Chapter 2

The second chapter contains an overview of the current status of research in the field of microarray analysis in breast cancer and radiation oncology. Different methods used in the analysis of microarray data are described, including unsupervised and supervised analysis and hypothesis driven gene expression analysis. It subcategorizes clinical areas of interest in breast cancer research and discusses the current status of gene expression profiling in response prediction to (neo-adjuvant) chemotherapy, prognosis prediction, local recurrence prediction and prediction of distant metastases. Furthermore it reviews literature on prediction of normal tissue toxicity and response to treatment for radiation treatment. Lastly, it discusses the current status of implementation of gene expression profiling in the clinic and in clinical trials.

Chapter 3

In chapter three we describe validation of the hypothesis driven gene expression profile the Wound-response Signature (WS) and show its possible clinical application. Based on the hypothesis that features of the molecular program of normal wound healing might play an important role in cancer metastasis, we use a previously identified in vitro model for wound healing, and use this “Wound-response Signature” to reveal links between wound healing and cancer progression in breast tumors. In a consecutive series of 295 early breast cancer patients, we show that both overall survival and distant metastasis-free survival are markedly diminished in patients whose tumors expressed this Wound-response Signature compared to tumors that did not express this signature. In this patient group, 126 patients expressed the activated WS, whereas 169 patients showed a quiescent WS. Patients with an activated WS had an overall survival at 10 years of 50% versus, 84% ($p < 10^{-10}$; logrank) for patients with a quiescent WS. For distant

metastasis free probability at 10 years this was 51% versus 75% ($p < 10^{-6}$; logrank). A gene expression centroid (average expression profile) of the Wound-response Signature provides a basis for prospectively assigning a prognostic score that can be scaled to suit different clinical purposes. The Wound-response Signature improves risk stratification independently of known clinical and pathologic risk factors and previously established prognostic signatures based on unsupervised hierarchical clustering (“molecular subtypes”) or supervised predictors of metastasis (“70-gene prognosis signature”).

Chapter 4

In chapter four we have used gene expression profiling by microarray analysis to identify gene expression profiles that can help to predict local recurrence after breast conserving therapy in individual patients. We have used gene expression data from a previously published series of 295 breast carcinomas from patients treated in the Netherlands Cancer Institute (NKI 295 data set), but limited the analysis to patients that had undergone breast conserving therapy (lumpectomy and whole breast radiation). The 161 patients that satisfied these criteria, were divided into a training and validation series (81 and 80 patients each respectively, including 9 and 8 patients who experienced a local recurrences during follow up (median 7.7 years)). By using previously established gene expression profiles with proven value in predicting metastasis-free and overall survival (wound-response signature, 70-gene prognosis profile and hypoxia-induced profile) and training towards an optimal prediction of local recurrences in a training series, we establish a classifier for local recurrence after breast-conserving therapy. Validation of the different gene lists shows that the wound-response signature is able to separate patients with a high (29%) or low (5%) risk of a local recurrence at 10 years (sensitivity 87.5%, specificity 75%). In multivariable analysis the classifier is an independent predictor for local recurrence. Our findings indicate that gene expression profiling can identify subgroups of patients at increased risk of developing a local recurrence after breast-conserving therapy.

Chapter 4

Chapter five takes the use of hypothesis driven gene expression profiling to the next level when we correlate previously identified signatures to biological pathways represented in gene modules. These gene modules represent specific functional pathways associated with specific cancer subtypes and thereby possible susceptibility of these tumors to targeted therapies. By using the Wound-response signature as an example we show that expression of the activated WS in breast tumors is strongly associated with induction of both a mitochondria gene module and a proteasome gene module. Induction of the proteasome module is correlated with poor survival. We then tested whether or not targeting the mitochondrial defect, by inhibiting glycolysis, or inducing proteasome inhibition would selectively kill cells that express the WS. We used breast epithelial cells (MCF10A) that normally don't express the WS as control and induced the WS in these cells by overexpression of MYC and CSN5. We found that 3-bromopyruvic acid, which inhibits glycolysis, selectively killed breast cells expressing the mitochondria gene expression module and wound signature. In addition, inhibition of

proteasome activity by bortezomib, a drug approved for human use in multiple myeloma, abrogated wound signature expression and selectively killed breast cells expressing the wound signature. Furthermore, we show that in breast cancer cell lines, that all express the activated WS, the degree of activation correlates with sensitivity to proteasome inhibition; e.g. cells that show the highest activation of the WS are most sensitive to proteasome inhibition. Lastly we show that the correlation between the WS and induction of the proteasome module is present in six independent breast cancer data sets, from early stage to locally advanced disease. Thus, gene module maps may enable rapid translation of complex genomic signatures in human disease to targeted therapeutic strategies.

Chapter 6

In chapter six we wished to extend the use of hypothesis driven gene expression analysis through the combinations of various gene signatures to improve the prediction of outcome in breast cancer. We use the previously published NKI-295 data set with updated clinical follow-up. Tumors were assigned to three prognostic groups using the previously described Wound-response (WS) and hypoxia-response signatures (HS), and the outcome in each of these subgroups was evaluated. These three groups are (1) quiescent WS/non-hypoxic HS (n=121); (2) activated WS/non-hypoxic HS or quiescent WS/hypoxic tumors (n=129) and (3) activated WS/hypoxic HS (n=45). The distant metastasis free probability at 15 years is 76%, 53% and 36% for group 1, 2 and 3, respectively (log rank $p < 10^{-8}$; HR 2.3 (95%CI 1.8–3.1)) and the overall survival is 79%, 59% and 27% (log rank $p < 10^{-12}$; HR 2.8 (95%CI 2.1–3.8)) In multivariate analysis, this signature is not only independent of clinical and pathological risk factors; it is also the strongest predictor of outcome. Compared to a previously identified 70-gene prognosis profile, obtained with supervised classification, the combination of signatures performs roughly equally well and might have additional value in the ER-negative subgroup. In the subgroup of lymph node positive patients, the combination signature outperforms the 70-gene signature in multivariate analysis. We subsequently analyzed clinico-pathological variables, the combination signature and the 70-gene prognosis profile. The 70-genes prognosis profile lost its significance in predicting overall survival in the lymph node positive patients in the presence of the combination signature; age and the combination signature were the only variables that remained significant (HR 0.43 for age above 40 and HR 1.84 for the combination signature). Interestingly, the HR for the combination signature represents the difference between the good (group 1) and intermediate groups ((group 2), and also the difference between the intermediate (group 2) and the poor group (group 3), meaning that the HR for good versus poor is approximately 3.68. In addition, in multivariate analysis, the WS/HS combination is a stronger predictor of outcome compared to the recently reported invasiveness gene signature combined with the WS. Only the WS/HS signature remains a significant predictor in the model (HR 1.75 vs. 1.41) and p-value 0.002 vs. 0.093).