Molecular markers of breast cancer metastasis
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Summary

Breast cancer is the most frequent malignancy among women in Western countries. The major cause of death from breast cancer is not the primary tumor, but the development of metastases in vital organs. Approximately 40% of the patients with breast cancer will develop metastases and ultimately die of their disease. Consequently, the prevention and control of lethal metastatic spread is the most important goal of breast cancer treatment. In the clinic, prognostic indicators are used to identify those patients who have a high risk of metastasis development, and who therefore should receive adjuvant therapy (chemotherapy or hormonal treatment) after local treatment of the tumor in the breast. However, none of the established prognostic markers, including tumor size, lymph node status, and histological grade, can accurately predict for an individual patient whether a breast tumor is most likely to metastasize (reviewed in Chapter 1). Instead, these markers only provide a general estimation of metastasis risk.

To identify new prognosis markers and therapeutic targets, it is essential to understand the extraordinarily complex metastatic process. To colonize a distant organ, a tumor cell must complete a sequential series of steps before it becomes a clinically detectable lesion. Some of these steps include invasion through the extracellular matrix, intravasation, survival in the blood stream, extravasation into a distant organ, development of a vascular network, and progressive growth at the distant site. A widely held hypothesis is that cancer metastasis arises from rare cells in the primary tumor that acquire additional genetic changes during the later stages of tumor development which enable these cells to complete the whole metastatic cascade (reviewed in Chapter 1).

This thesis describes two ways of gaining insight into the biology of the metastatic process: first, the detection and prognostic role of disseminated breast cancer cells, the ultimate cause of distant metastases, and second, the gene expression profiles of primary breast carcinomas and matching metastases.

In contrast to prognostic markers detected in the primary tumor, the identification of circulating tumor cells in blood offers dynamic information regarding the clinical evolution of the metastatic process.

Chapter 2 describes the development of an mRNA-based assay system to detect tumor cell genes by applying quantitative real-time PCR and a panel of four marker genes. Two of the genes employed, p1B and PS2, were derived from our SAGE analysis designed to identify differentially expressed genes in breast cancer, whereas CK19 and EGP2 had already been successfully used by others. Using a quadratic discriminant analysis including all four marker genes, we determined an increased marker gene expression in the peripheral blood of 30/103 patients with advanced breast cancer (29%), while elevated expression levels were absent in all 96 samples of healthy females.

In Chapter 3 we show that patients with advanced breast cancer have a significantly worse progression-free and overall survival if mRNA of tumor cells can be detected in their peripheral blood. The assay we have developed therefore has a clear biological correlation and may reflect the presence of circulating breast cancer cells. Whether the detection of circulating
tumor cells in early breast cancer patients is of relevance for the future clinical management of the disease, however, remains to be established.

Due to the convincing data obtained with our mRNA-based approach in peripheral blood, we developed a similar test to detect breast cancer cells in lymph nodes (Chapter 4). In breast cancer to date, the axillary lymph node status at primary diagnosis remains the most valuable individual prognostic marker for disease course and recurrence. A less invasive method for the assessment of the lymph node status than the standard treatment involving the complete dissection of 10-30 axillary lymph nodes, is the sentinel lymph node biopsy. In this approach, the first lymph node that receives drainage from the primary tumor is mapped. If the sentinel node does not contain tumor cells, no further axillary clearance is carried out. Using a sensitive real-time PCR approach including four marker genes (CK19, p1B, EGP2 and SBEM), 7/70 sentinel lymph nodes tested (10%) that were free of metastases as determined by the standard histological evaluation showed an increased marker gene expression, suggesting the presence of disseminated breast cancer cells. Four of the seven sentinel nodes marked positive by real-time PCR indeed proved to contain tumor cells after careful review of the paraffin-embedded material. These results show that the application of our technique may be of clinical importance.

To improve our understanding of the molecular mechanisms of the metastatic process, we determined the gene-expression profiles of primary breast carcinomas and matching metastases. In Chapter 5 we show that gene expression profiles of primary breast tumors are maintained in their distant metastases, of which some are detected years later. Furthermore, no general subset of genes discriminating between the primary and metastatic breast tumors tested could be identified. This, however, might be due to the small sample size and the different sites of metastasis studied. The differentially expressed genes detected in the individual pairs of primary tumors and metastases mainly originate from the organs analyzed harboring metastatic spread.

Our findings challenge the prevalent model of metastasis, and imply that the genetic changes in primary tumors that favor metastasis occur much earlier in tumor progression than previously appreciated. Moreover, our study argues against the concept that the translocation of a tumor cell to a distant site in the body includes major changes in the gene expression of a tumor. In fact, the changes in gene expression of a metastatic colony when compared with the primary tumor are much more subtle than expected (Chapter 6).

Two major studies have described the use of microarray technology to assess the molecular classification of human breast cancer and have defined new subgroups based on gene expression that are relevant to patient management. Breast tumors analyzed by hierarchical clustering of the expression patterns of 'intrinsic' genes have been shown to subdivide into at least four molecular subtypes, which are associated with significantly distinct patient outcomes (Perou et al., Nature 406, 2000; Sorlie et al., PNAS 98, 2001). Using a supervised method, a 70-gene expression profile has been identified which accurately predicts the later appearance or absence of clinical metastasis within 5 years in young breast cancer patients (van 't Veer et al., Nature 415, 2002). In Chapter 7 we show that distant metastases display not only the overall gene expression profile but also the same molecular breast cancer subtypes and 70-gene prognostic
signature when compared to their primary breast carcinomas. These findings suggest that the metastatic nature of breast carcinomas is an inherent feature of breast cancer.

In breast cancer, the axillary lymph nodes are often the first sites to harbor metastases. We show that primary breast carcinomas and lymph node metastases do not differ at the transcriptional level by a common subset of genes. However, subtle differences in the expression of genes involved in extracellular-matrix remodeling, cell-matrix interaction and growth factor signaling are detected in individual pairs of matching primary and metastatic tumors (Chapter 8). Our data suggest that primary breast carcinomas are unique and complex organs that may use individual sets of genes to accomplish lymph node metastasis. Furthermore, in a dataset of 295 breast cancer patients, including 151 lymph node-negative and 144 lymph node-positive patients (van de Vijver et al., N Engl J Med 347, 2002), no expression signature predicting the lymph node status could be identified. These data imply that lymph node metastasis may occur independently of hematogenous metastasis, and therefore that the axillary lymph node status is not the most reliable predictor of disease course in breast cancer patients.

The experiments described in this thesis show that the presence of mRNA species that may originate from circulating tumor cells could be detected in blood and lymph nodes by a quantitative multimarker PCR assay. Furthermore, our results suggest that the presence of circulating tumor cells in peripheral blood are of prognostic benefit.

The research in this thesis has also contributed to a further understanding of the metastatic process, by showing that the overall gene expression profile, the molecular breast cancer subtype and prognostic expression profile of a primary breast tumor are maintained throughout the metastatic process. Currently, there is considerable interest in the use of microarray profiling to predict therapy response, which will eventually provide a further step toward the achievement of 'personalized medicine' for the treatment of breast cancer. The data presented in this thesis imply that treatment decisions based on the expression profile of a primary tumor might be a rational approach towards preventing the outgrowth of micrometastases.