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Minireview

Distant metastasis: not out of reach any more

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Abstract

Metastasis is the major cause of death in breast cancer patients. Gene-expression studies have shown that the likelihood of metastasis can be predicted from analysis of primary tumors. Two recent papers in *BMC Medicine* and *BMC Cancer* have established new operational expression signatures containing a limited number of genes involved in angiogenesis or cell proliferation.

Breast cancer is the second leading cause of cancer-associated mortality in women in Western countries. The main event leading to death in breast cancer patients is the development of metastases - secondary locations of cancer cells in sites distant from the primary tumor. Today, the number of patients with metastatic breast cancer has declined, thanks largely to improvements in the systemic adjuvant treatment of early-stage disease, designed to eradicate micrometastases. Nevertheless, approximately 6-10% of patients have metastatic disease at the time of diagnosis and 30% of initially non-metastatic patients will eventually develop metastatic relapse. The prognosis for these patients is poor, with an estimated 5-year survival of only 21%.

Despite a wealth of studies, metastasis is not well understood and is poorly controlled clinically. Recent data have suggested that the capacity to metastasize is due to factors both extrinsic and intrinsic to the tumor cells [1]. Intrinsic factors are associated with tumor-cell aggressiveness. Extrinsic factors are associated with the peritumoral stroma, immune response and neo-angiogenesis, and probably include other, more elusive factors linked to treatment response or host susceptibility. It is clear that therapeutic targeting of both is needed to prevent and to treat metastasis. This is clinically evident by the efficacy of bevacizumab, a monoclonal antibody against vascular endothelial growth factor (VEGF), in combination with chemotherapy in treating metastatic breast, lung and colon cancers.

The potential of a tumor to metastasize can be detected early and before the occurrence of metastasis by using gene-expression profiling [2]. This finding challenged the original idea that metastases arise from cells within the primary tumor that have acquired the ability to metastasize after a stepwise accumulation of alterations and release of host barriers. In breast cancer, at least five molecular subtypes (luminal A, luminal B, basal, ERBB2+ and normal-like) have been identified and display different propensities to metastasize, and prognostic multigene expression signatures have been established.

These signatures are valuable in two ways. First, they can be used in the clinic to guide treatment. Second, they provide clues to understanding the metastatic process. Two recent articles, published in *BMC Medicine* and in *BMC Cancer*, report prognostic gene-expression signatures associated,

respectively, with distant metastases and with the metastatic potential of breast cancer. These studies improve our knowledge of metastasis and propose means to detect it.

A 13-gene set associated with tumor response to hypoxia and metastasis

Hu *et al.* [3] used gene-expression analysis by DNA microarrays to compare a series of primary tumors and metastases. They established a clinical 'MetScore' that combines lymph-node status and metastasis status at time of diagnosis and ranges from 0 (negative for both node and distant metastasis) to 3 (distant metastasis present). They show that distant metastases are, at the whole-genome transcriptional level, more distinct from non-metastatic primary tumors and regional metastases than the latter are to each other. They determined a set of 1,195 genes whose expression was associated with a MetScore. When the gene set was used to classify samples by hierarchical clustering, a subset of non-distant metastatic primary tumors (MetScores 1 and 2) resembled distant metastases. As expected from previous prognostic studies, basal and ERBB2+ tumors correlated most highly with MetScore 3. Several gene clusters were identified within the gene set, none of which perfectly correlated with transition from MetScore 1 to 2 to 3, indicating the complexity of the phenomenon. These included an estrogen-receptor (ER)-related gene cluster, weakly associated with MetScores 1-2, and a proliferation cluster.

A small cluster of 13 genes highly associated with MetScore 3 was also detected (Table 1), which included three genes coding for angiogenic factors: VEGFA; adrenomedullin (ADM); and angiopoietin-like 4 (ANGPTL4). Eight of the genes contain binding sites for the hypoxia-induced factor 1 alpha (HIF1A) in their regulatory regions and, as expected, a strong correlation was noted between the mRNA expression of HIF1A and the 'VEGF profile' - defined as the average expression values across all 13 genes. *In situ* hybridization showed that it was the tumor cells that expressed mRNA of the three angiogenic factors, and thus the 13-gene cluster seemed to be related to tumor-cell response to hypoxic conditions. When applied to the 134 primary tumors, the VEGF profile was predictive of relapse-free survival and overall survival, with high expression associated with poor outcome. This was validated in an external series of breast tumors, and also in lung cancer and glioblastoma, further supporting the idea that different tumor types have similar pathways to metastasis. In the breast cancer series (295 patients), the VEGF profile remained an independent prognostic feature in multivariate analysis incorporating classical prognostic features, the molecular subtypes and multiple other expression predictors.

Other factors were associated with the MetScore, including the molecular subtype, a fibroblast signature associated with a low MetScore, and a signature involving the *TWIST* gene and a 'glycolysis profile' associated with a high MetScore, suggesting that the distant metastatic samples not only promote angiogenesis but also survive under anerobic conditions.

A 14-gene set associated with cell proliferation and distant metastasis

The study by Tutt *et al.* [4] addressed the same question using different samples and tools, and the more classical approach of supervised analysis. The aim was to identify an expression signature predictive of distant metastatic relapse after loco-regional treatment alone, without any adjuvant systemic treatment. The authors studied a series of 421 systemically untreated breast carcinomas consisting of a training set of 142 samples and a validation set of 279 samples. The major differences from Hu *et al.* [3] were that all the samples were primary tumors, all ER-positive and lymph-node negative, the patients were homogeneously treated, the mRNAs were extracted from formalin-fixed paraffin-embedded (FFPE) samples, the method used PCR amplification of mRNA from *a priori* selected genes, and the supervised analysis compared tumors without versus tumors with metastatic relapse.

An initial selection of 197 prognosis- and prediction-associated genes was based on four recently published prognostic expression signatures. The gene list went down to 37 after a first supervised analysis of the training set (univariate Cox analysis) and was further reduced to 14 after regression analysis. Nine of these 14 genes (Table 1) are associated with cell proliferation and 10 with the TP53 pathway, as determined by ontology analysis. A metastatic score (MS) was established based on the linear combination of expression values across the 14 genes, and represented the probability of a tumor metastasizing. High MS was associated with an increased risk of distant metastasis and an increased risk of death. Using MS it was possible to separate patients into two groups - low and high risk of metastasis - with different distant metastasis-free survival and overall survival in both training and validation sets. The performances of the predictor were similar in the two sample sets. For example, the hazard ratio for risk of distant metastasis in the high risk group as compared to the low risk group was 4.34 in the training set and 4.71 in the validation set, in univariate analysis. Furthermore, MS remained significant in multivariate analysis after adjustment for classical prognostic factors, whereas the Ki67 index, a marker of proliferation, was no longer significant. Finally, comparison with the histo-clinical Adjuvant! Online predictor showed that the MS provided additional prognostic information.

Table 1

The 27 genes included in the two signatures

Gene symbol	Gene name	Hu *et al.* [3]	Tutt *et al.* [4]	Basal*	16-kinases†	GGI‡
ADM	Adrenomedullin	Yes		Up		
ANGPTL4	Angiopoietin-like 4	Yes				
C14orf58	Chromosome 14 open reading frame 58	Yes		Up		
DDIT4	DNA-damage-inducible transcript 4	Yes		Up		
FABP5	Fatty acid binding protein 5 (psoriasis-associated)	Yes		Up		
GAL	Galanin	Yes		Up		
NDRG1	N-myc downstream regulated gene 1	Yes		Up		
NP	Nucleoside phosphorylase	Yes		Up		
PLOD1	Procollagen-lysine 1,2-oxoglutarate 5-dioxygenase 1	Yes		Up		
RRAGD	Ras-related GTP binding D	Yes		Up		
SLC16A3	Solute carrier family 16 (monocarboxylic acid transporters), member 3	Yes				
UCHL1	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	Yes		Up		
VEGF	Vascular endothelial growth factor	Yes		Up		
BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)		Yes	Up	Yes	Yes
CCNB1	Cyclin B1		Yes	Up		Yes
CENPA	Centromere protein A, 17 kDa		Yes	Up		Yes
DC13	DC13 protein		Yes			Yes
DIAPH3	Diaphanous homolog 3 (Drosophila)		Yes			
MELK	Maternal embryonic leucine zipper kinase		Yes	Up	Yes	
MYBL2	v-Myb myeloblastosis viral oncogene homolog (avian)-like 2		Yes	Up		Yes
ORC6L	Origin recognition complex, subunit 6 like (yeast)		Yes	Up		
PKMYT1	Protein kinase, membrane associated tyrosine/threonine 1		Yes	Up		
PRR11	Proline rich 11		Yes			
RACGAP1	Rac GTPase activating protein 1		Yes			
RFC4	Replication factor C (activator 1) 4, 37 kDa		Yes	Up		
TK1	Thymidine kinase 1, soluble		Yes			
UBE2S	Ubiquitin-conjugating enzyme E2S		Yes	Up		

*Genes upregulated in basal versus luminal A tumors [5]; †16-kinase signature [8]; ‡genomic grade index [7].

Although the list of genes from Tutt *et al.* [4] adds no new information to existing signatures, this study is especially valuable for its use of methods and samples appropriate for routine laboratories, such as FFPE tumors and PCR amplification, for the limited number of genes, and for a broader age range of the patients.

Long-distance call: treating and predicting metastasis

Understanding the biology behind distant metastasis will not only help to design drugs to treat it and, even better, to prevent it, but also provide better ways to detect it and predict it. The two prognostic signatures, related to angiogenesis and proliferation, respectively, confirm the relevance of these biological processes in cancer progression and also the superiority of multigene versus single gene analysis. Indeed, multivariate analysis shows that the signature of Tutt *et al.* [4] provides additional prognostic information compared with the Ki67 proliferation marker. We compared these two signatures with our basal versus luminal A breast cancer signature [5], and found that 10 out of 13 genes from the Hu *et al.* signature [3] and 10 out of 14 genes from the Tutt *et al.* signature [4] were overexpressed in basal breast tumors (Table 1), in agreement with the poorer prognosis of this subtype.

Relevance for treatment

Metastasis is due to a combination of tumor and host factors, with diverse interactions between cancer cells and their microenvironment. One such factor might be the existence of specific cells such as cancer stem cells (CSCs) that fuel the primary tumor. With potential for self-renewal and migration, these cells can leave the primary tumor to colonize distant organs. The study by Hu *et al.* shows that hypoxia may be important in this process, as it might stimulate CSCs to migrate and look for better conditions. Hypoxia also promotes neo-angiogenesis, which offers new routes for CSCs to leave the tumor. Correlation with the 'TWIST' signature is not surprising as *TWIST* is regulated by HIF1A. Some proteins of the VEGF signature, such as ADM and ANGPTL4, represent molecular targets under investigation that could help increase our therapeutic armament against metastatic breast cancer.

The study by Tutt *et al.* [4] shows that proliferation is a marker of breast cancer aggressiveness. This is now well accepted, in particular for ER-positive breast cancer [6]. Proliferative subtypes, such as basal and luminal B cancers, are associated with a poor outcome. The definitions of a genomic grade [7] or mitotic kinase index [8] have strengthened this notion. Five and two genes of the signature of Tutt *et al.* were part of these two signatures, respectively (Table 1). Targeting cell proliferation is a main objective of anticancer therapeutic strategies. Kinases have proved successful targets for therapy and some mitotic kinases of the Tutt 14-gene signature are under investigation as therapeutic targets: MELK, MYT1, TK1 and BUB1.

Relevance for prediction

The two new studies confirm that distant metastasis can be predicted using expression profiles, thus helping physicians to select an appropriate therapy. Three approaches to obtaining gene signatures are in general use. In the first ('top-down'), the expression profiles of two groups of patients are compared to identify genes associated with metastatic relapse without any *a priori* biological hypothesis. Consequently, the signature obtained does not necessarily contain key biological information related to metastasis. This method has produced at least two prognostic signatures in node-negative breast cancer untreated with adjuvant chemotherapy: the Amsterdam 70-gene signature [9] and the Rotterdam 76-gene signature [10]. The second approach ('bottom-up') first identifies a signature associated with a specific biological hypothesis or a phenotypic feature relevant to the metastatic process, and then tests for its correlation with outcome, providing additional insight into the biological mechanisms and possible therapeutic targets. Prognostic signatures associated with wound repair, stem cells, hypoxia or pathological grade [7] have been established this way [2]. The study by Hu *et al.* [3] is an example of this approach based on the initial comparison of non-metastatic versus metastatic samples or metastases. In a similar study, Ramaswamy *et al.* [11] identified a metastasis signature prognostically informative in several tumor types. By supervised analysis, Paik *et al.* [12] identified a multigene predictor of metastatic relapse in ER-positive breast cancer treated with adjuvant hormone therapy. This approach is used by Tutt *et al.* [4] in a series of patients similar to those of Paik *et al.* [12] but not treated with any adjuvant systemic therapy. The clinical interest of this 'pure' prognostic signature may be for low-risk patients, not only to avoid unnecessary adjuvant chemotherapy (as does the Paik signature) but also to avoid hormone therapy and its sometimes troublesome toxicity.

Predicting tumor aggressiveness and metastasis is a crucial step in the management of breast cancer. It is expected that a sensitive and specific molecular barcode will result from this kind of study. The ultimate dream of physicians is to use this barcode to select a drug from the 'inpharmatics' vending machine to treat each particular patient.

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