Published OnlineFirst March 24, 2009; DOI: 10.1158/0008-5472.CAN-08-4013

Coordinates in the Universe of Node-Negative Breast Cancer Revisited

Marcus Schmidt,[1] Jan G. Hengstler,[2] Christian von Törne,[3] Heinz Koelbl,[1] and Mathias C. Gehrmann[3]

[1]Department of Obstetrics and Gynecology, Johannes Gutenberg University, Medical School, Mainz, Germany; [2]Leibniz Research Centre for Working Environment and Human Factors at the Technical University of Dortmund (IfADo), Dortmund, Germany; and [3]Siemens Healthcare Diagnostics Products GmbH, Cologne, Germany

Abstract

We present a global picture of the natural history of node-negative breast cancer in which two of three important biological processes have outstanding prognostic consequences. We propose that the transition from slow to fast proliferation of the tumor leads to the most dramatic aggravation of prognosis. Second, immune cell infiltration is of major importance to prevent disease progression in fast-proliferating breast carcinomas, regardless of estrogen receptor status. In the absence of endocrine treatment, steroid hormone receptor expression as a third axis is of limited prognostic importance. Dissecting tumors according to these three major biological axes will allow further understanding of biological processes relevant for tumor progression in patients with node-negative breast cancer. [Cancer Res 2009;69(7):2695–8]

Background

The hunt for "magic" prognostic signatures. In past decades, the search for markers and methods predicting prognosis in patients with node-negative breast cancer repeatedly pointed to two biological motifs, i.e., steroid hormone receptor expression (1) and proliferation (2). Surprisingly, despite overwhelming data analyzing all aspects of these two biological motifs, a widely accepted clear and coherent picture of how they actually relate to each other did not emerge until the advent of microarray technology (3). Simultaneous measurement of multiple proliferation, as well as estrogen receptor (ER)–related transcripts, rediscovered three important points: first, a subgroup of ER-positive tumors might have a similarly poor prognosis to ER-negative tumors; second, the major driving force of a poor outcome is high proliferative activity; and third, although ER-positive tumors vary in proliferative activity, almost all ER-negative tumors are characterized by high proliferation (3–5). Although these interrelations are not novel insights, they have not always been accounted for when new technologies have been used extensively in search for the putative "magic" RNA, protein, or DNA marker or signature. Again, it seemed difficult to "see the wood for the trees." Several different gene expression signatures, containing different genes derived from different methodologies, mainly captured the same biological motifs and, consequently, perform similarly in outcome prediction (6). When several published classifiers were tested in molecular subgroups of breast cancer, all had a similar performance in patients with ER+/HER2− tumors but no single classifier had any prognostic power in ER−/HER2− or HER2+ patients (7). Therefore, the main question still is: what are the major biological factors influencing patient outcome and how do they relate to each other?

Key Findings

Three coordinates in the universe of node-negative breast cancer. In order to develop an understanding of the interrelations of this disease, we performed gene expression profiling in tumors derived from women with node-negative breast cancer who did not receive systemic treatment (Mainz cohort; ref. 8). Following a so-called unsupervised analysis strategy, we investigated tumors in three dimensions after principal component analysis on their respective gene expression profiles. Specifically, we visualized the relative gene expression, as well as clinical variables, e.g., time to metastasis, within the distribution of tumor samples. Because many genes are tightly coregulated, we considered it useful to analyze the normalized median expression of a cluster of highly coregulated genes as a metagene. A "metagene" roughly represents a distinct biological motif as indicated by the genes constituting the respective cluster. Interestingly, ER-coregulated transcripts, proliferation-associated transcripts, and those emanating from B cells and T cells each formed a gradient within the distribution of samples. Thereby, principal component analysis visualization facilitated to grasp the multivariate relation of these individual biological motifs. Visual superposition of time-to-metastasis as a clinically relevant end point allowed the formulation of a number of hypotheses (Supplemental Fig. S1). First, proliferation seemed to be the major prognostic motif in our cohort. Very few tumors from patients who developed a metastasis were observed in a region of low expression of proliferation-associated transcripts. Second, within a region of fast-proliferating tumors, high expression of immune cell–related transcripts were concomitant with an absence of metastasis. Third, the highest expression of ER-related transcripts coincided with the lowest expression of immune-related transcripts. Cox regression analysis, performed in the whole cohort, as well as in subcohorts, split according to low and high proliferation, supported our hypothesis. A clear prognostic association of T cell– and B cell–related transcripts was confined to tumors with high proliferative activity.

In two independent validation cohorts (Rotterdam and TRANSBIG; refs. 5, 9, 10), the orientation of the proliferation, ER, and immune system gene expression gradients were similar to that observed in the Mainz cohort (Supplemental Fig. S1). Furthermore, the B-cell and T-cell metagenes had prognostic relevance within

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Mathias C. Gehrmann, Siemens Healthcare Diagnostic Products GmbH, Nattermann Allee 1, Cologne 50829, Germany. Phone: 49-221-5763603; Fax: 49-221-5763621; E-mail: mathias.gehrmann@siemens.com.

doi:10.1158/0008-5472.CAN-08-4013

Published OnlineFirst March 24, 2009; DOI: 10.1158/0008-5472.CAN-08-4013

both validation cohorts as a whole as well in their fast but not in slow proliferating subcohorts. The B-cell metagene contains almost exclusively tightly coregulated immunoglobulin transcripts like *IGKC* or *IGHG3*. The T-cell metagene contains T cell–related transcripts such as *TRA@, TRB@, CD8A, CD6, CD3Z, CD3D, ITK, GZMK, GZMB*, and other transcripts whose cell type–specific origin is less clear. An analysis of individual genes within this cluster might reveal additional insight about distinct cell types or activation state of cells.

Relevance of the immune system depends on proliferation but not ER expression. A prognostic relevance of immune cell infiltration in breast tumors had been previously observed by histopathologic analysis. In a pioneering work, Aaltomaa and coworkers recognized the particular prognostic role of lymphoid cells in fast-proliferating breast tumors (11). Furthermore, several groups recently discovered a prognostic association of immune cell–related transcripts in specific breast cancer subsets such as ER-negative (12, 13) and ERBB2-positive tumors (14) following gene expression profiling. In contrast to *ESR1* and *ERBB2*, proliferation-associated genes have neither a clear bimodal distribution nor a broad dynamic range of expression values, making it difficult to classify a tumor as fast-proliferating or slow-proliferating. However, when we stratified tumors into two classes according to high and low expression of proliferation-associated genes, almost 90% of all ER-negative and ERBB2-positive tumors were categorized as tumors of high proliferative activity. When we examined ESR1+/ERBB2- tumors displaying high proliferative activity, the B-cell metagene was also associated with good prognosis (8). This observation suggests that the well-known adverse prognostic effect of proliferation is attenuated by the immune system, regardless of ER expression. In summary, the three "coordinates in breast cancer", proliferation, ER, and immune system, facilitate orientation and help to correctly interpret breast cancer biology (Fig. 1; Table 1). Importantly, in fast-proliferating tumors, B-cell transcripts were consistently identified as independently associated with node-negative patient prognosis in the absence of systemic therapy.

Implications

For a long time now, ER expression has confounded our understanding of the relevance of proliferation as a major prognostic motif in node-negative breast cancer. Although the interrelation of the ER and proliferation axis is getting clearer, a more recently rediscovered prognostic motif, i.e., lymphoid infiltration, has not conclusively been put into the context of the other two motifs. Whereas some researchers suggest that the effect on the immune system might depend on ER status (13), we like to add, with caution, that this relation might be the result of

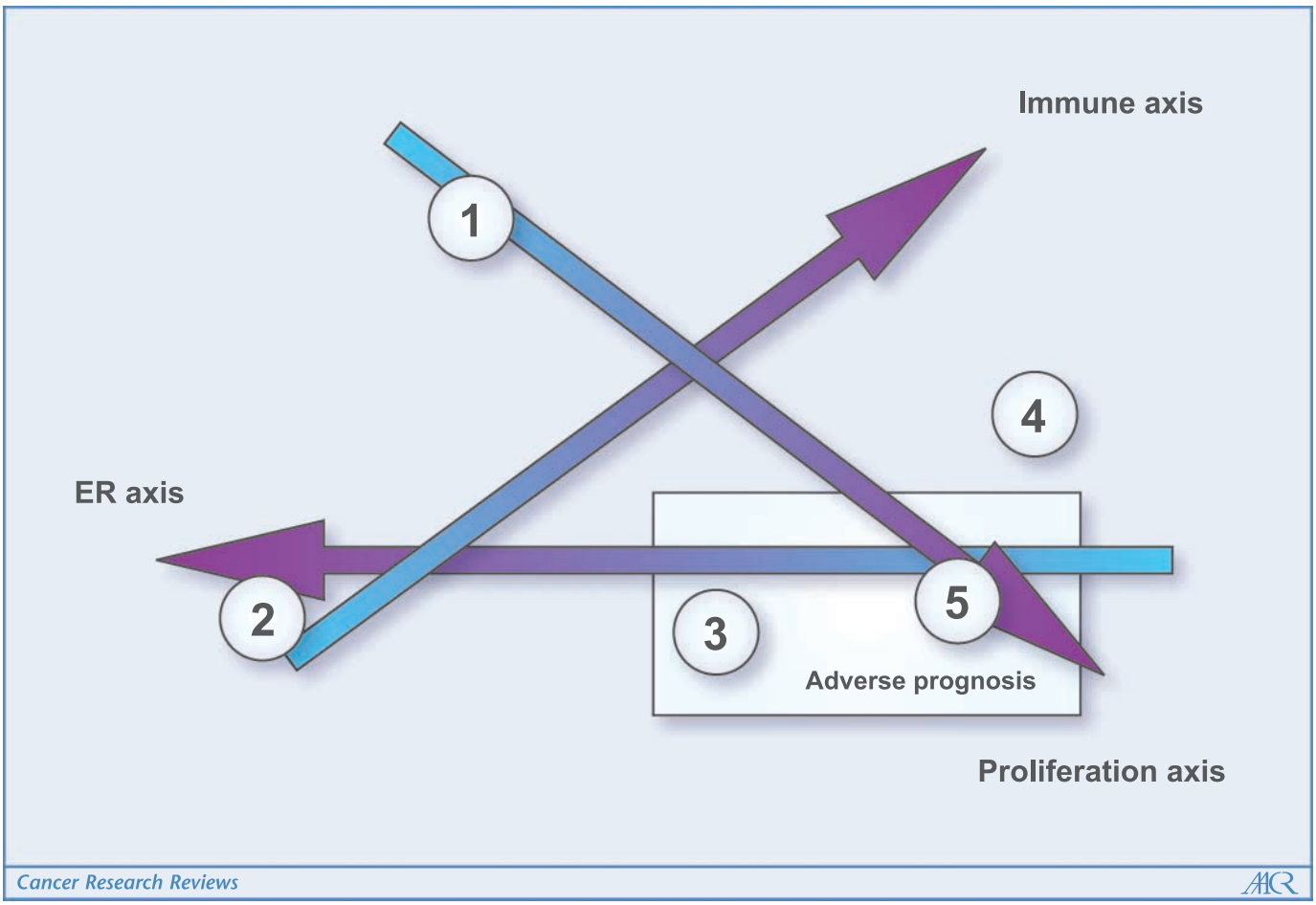

Figure 1. Relation of dominant prognostic motifs in node-negative breast cancer abstracted from principal component analysis of whole genome gene expression data of 788 patients with node-negative breast cancer (Supplemental Fig. S1). High (*purple*) and low (*blue*) expression of genes belonging to their respective axes. Several regions of interest are numbered. *Rectangle,* an area of particular adverse prognosis. Typical characteristics of tumors in selected regions are summarized in Table 1.

Published OnlineFirst March 24, 2009; DOI: 10.1158/0008-5472.CAN-08-4013

Table 1. Characteristics of different regions defined by the proliferation, ER, and immune axes in Fig. 1

Region	Dominant features
1	Small tumors, tubular and lobular histology, grade 1 and 2, expression of basal like marker, ⇒ normal-like subtype
2	Ductal and lobular histology, highest expression of ER, absence of immune-related transcripts, elderly patients, ⇒ luminal A subtype
3	Ductal histology, ER positive and negative, presence and absence of ERBB2 amplification, variable expression of immune-related transcripts, ⇒ luminal B + ER negative but nonbasal-like + ERBB2 subtype
4	Ductal and medullary histology, grade 2 and 3, ERBB2 negative, high expression of basal-like marker, younger patients, high expression of immune-related transcripts ⇒ basal-like A (advantageous prognosis)
5	Ductal histology, grade 2 and 3, ERBB2 negative, high expression of basal-like marker, younger patients, low expression of immune-related transcripts ⇒ basal-like B (bad prognosis)

NOTE: ⇒ tumors within a region are assumed to relate to one or more intrinsic subtypes (3).

confounding factors. Instead, from a biological point of view, we propose that the association of lymphoid infiltration with a good outcome is preserved in fast-proliferating ER-positive tumors. Therefore, the prognostic relevance of lymphocytes is obviously not restricted to ER-negative tumors. It is plausible that the immune system represents an opponent of tumor evolution fueled by the module of proliferation-associated genes. Only fast-proliferating tumors that are not recognized and eliminated by immune cells can progress, form metastases, and finally kill the patient. Therefore, the immune system is one of the major players responsible for the selection aspect in tumor evolution.

The "Janus-faced" immune system. Much is known about the role of T cells eliciting cellular antitumor responses (15). On the contrary, clinical data and experimental animal studies seem to indicate that humoral immune responses initiate protumor effects on developing neoplasms (16, 17). Is the immune system "Janus-faced" in that it promotes metastasis as well and, if so, how? A possible explanation for our contrasting finding that a B-cell response is correlated with good patient outcome might be oligoclonal B-cell responses exerting antitumor effects. Several reports have described oligoclonal expansion of B cells in breast cancer, both in medullary breast cancer and invasive-ductal breast cancer (18, 19). In fact, antigen presentation by B cells might be crucial for T-cell expansion and differentiation (20). Furthermore, early breast cancer patients with a natural humoral response to polymorphic epithelial mucin (*MUC1*) were found to have a better disease-specific survival. The authors speculated that antibodies might control tumor dissemination and outgrowth by aiding the destruction of circulating or seeded tumor cells (21). Thus, the notion that a sustained humoral response has the ability to elicit significant protumor effects (16) should be viewed with caution in node-negative breast cancer. B-cell depletion, as suggested by others (22), might prove counterproductive considering the beneficial role of B cells in this population.

Open issues for future research. Further unanswered questions remain: what is the actual chemotherapy benefit for node-negative breast cancer patients with *versus* without lymphoid infiltrates? Retrospective comparative analysis of formalin-fixed paraffin-embedded tumor tissue of untreated *versus* treated patient cohorts needs to be done in order to resolve this question. In the absence of reliable markers of drug effectiveness, risk information might help to adjust the aggressiveness of chemotherapy. If it is the immune system that controls tumor progression, at which stage does it begin to exert its protective effect, and at which stage of tumor progression do which components of the immune system become crucial? When B cells act primarily as antigen-presenting cells (20), identification of the respective antibody epitopes could provide insight into which tumor antigens are actually processed and ultimately presented in order to stimulate T-cell responses. For this purpose, SEREX (serologic identification of antigens by recombinant expression cloning) technology (23) or protein microarrays (24) can be used to identify potential tumor antigens. Considering that just three biological motifs can create a lot of confusion, and the fact that treatment will add an additional layer of complexity, we might conclude that the hunt for a deeper understanding of more complex interrelations has just begun!

Disclosure of Potential Conflicts of Interest

M.C. Gehrmann, C. von Törne, and M. Schmidt are named as inventors of diagnostic products on patent applications with no ownership interest. The other authors disclosed no potential conflicts of interest.

Acknowledgments

Received 10/16/08; revised 12/23/08; accepted 1/14/09; published OnlineFirst 3/24/09.

References

1. Osborne CK, Yochmowitz MG, Knight WA III, McGuire WL. The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer 1980;46:2884–8.
2. Gentili C, Sanfilippo O, Silvestrini R. Cell proliferation and its relationship to clinical features and relapse in breast cancers. Cancer 1981;48:974–9.
3. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869–74.
4. Sotiriou C, Wirapati P, Harris A, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst 2006;98:262–72.
5. Loi S, Haibe-Kains B, Desmedt C, et al. Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. J Clin Oncol 2007;25:1239–46.
6. Fan C, Oh DS, Wessels L, et al. Concordance among gene-expression-based predictors for breast cancer. N Engl J Med 2006;355:560–9.
7. Desmedt C, Haibe-Kains B, Wirapati P, et al. Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. Clin Cancer Res 2008;14:5158–65.
8. Schmidt M, Böhm D, von Törne C, et al. The humoral immune system has a key prognostic impact in node-negative breast cancer. Cancer Res 2008;68:5405–13.
9. Wang Y, Klijn JG, Zhang Y, et al. Gene-expression

Published OnlineFirst March 24, 2009; DOI: 10.1158/0008-5472.CAN-08-4013

profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 2005; 365:671–9.

10. Desmedt C, Piette F, Loi S, et al. Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. Clin Cancer Res 2007;13:3207–14.

11. Aaltomaa S, Lipponen P, Eskelinen M, et al. Lymphocyte infiltrates as a prognostic variable in female breast cancer. Eur J Cancer 1992;28:859–64.

12. Teschendorff A, Miremadi A, Pinder SE, Ellis IO, Caldas C. An immune response gene expression module identifies a good prognosis subtype in estrogen receptor negative breast cancer. Genome Biol 2007;8:R157.

13. Calabrò A, Beissbarth T, Kuner R et al. Effects of infiltrating lymphocytes and estrogen receptor on gene expression and prognosis in breast Cancer. Breast Cancer Res Treat. Epub ahead of print 2008. doi: 10.1007/s10549-008-0105-3.

14. Alexe G, Dalgin GS, Scanfeld D, et al. High expression of lymphocyte-associated genes in node-negative HER2+ breast cancer correlates with lower recurrence rates. Cancer Res 2007;67:10669–76.

15. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991–8.

16. Tan TT, Coussens LM. Humoral immunity, inflammation and cancer. Curr Opin Immunol 2008;19:209–16.

17. Inoue S, Leitner WW, Golding B, Scott D. Inhibitory effects of B cells on antitumor immunity. Cancer Res 2006;66:7741–7.

18. Hansen MH, Nielsen H, Ditzel HJ. The tumor-infiltrating B cell response in medullary breast cancer is oligoclonal and directed against the autoantigen actin exposed on the surface of apoptotic cancer cells. Proc Natl Acad Sci U S A 2001;98:12659–64.

19. Coronella JA, Telleman P, Kingsbury GA, Truong TD, Hays S, Junghans RP. Evidence for an antigen-driven humoral immune response in medullary ductal breast cancer. Cancer Res 2001;61:7889–99.

20. Crawford A, Macleod M, Schumacher T, Corlett L, Gray D. Primary T cell expansion and differentiation *in vivo* requires antigen presentation by B cells. J Immunol 2006;176:3498–506.

21. von Mensdorff-Pouilly S, Verstraeten AA, Kenemans P, et al. Survival in early breast cancer patients is favourably influenced by a natural humoral immune response to polymorphic epithelial mucin. J Clin Oncol 2000;18:574–83.

22. Johansson M, Denardo DG, Coussens LM. Polarized immune responses differentially regulate cancer development. Immunol Rev 2008;222:145–54.

23. Preuss KD, Zwick C, Bormann C, Neumann F, Pfreundschuh M. Analysis of the B-cell repertoire against antigens expressed by human neoplasms. Immunol Rev 2002;188:43–50.

24. Gnjatic S, Wheeler C, Ebner M, et al. Seromic analysis of antibody responses in non-small cell lung cancer patients and healthy donors using conformational protein arrays. J Immunol Methods 2009;341:50–8.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Coordinates in the Universe of Node-Negative Breast Cancer Revisited

Marcus Schmidt, Jan G. Hengstler, Christian von Törne, et al.

Cancer Res 2009;69:2695-2698. Published OnlineFirst March 24, 2009.