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Review

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Integration of Host-Related Signatures with Cancer Cell–Derived Predictors for the Optimal Management of Anticancer Chemotherapy

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Abstract

Current cancer management aims to integrate molecular signatures into the design of personalized therapies. Recent advances in "omics" done on tumor specimens have led to the identification of factors that either recognize cancers of dismal prognosis or pinpoint "druggable" signaling pathways, which can be interrupted by targeted therapies. However, accumulating evidence underscores the biological and clinical significance of immune predictors in several compartments (blood, serum, tumor) in a variety of malignancies. An additional aspect that has been overlooked is the bidirectional, tumor-host interaction during therapeutic intervention, suggesting that dynamic molecular, biochemical, and metabolic signatures should be developed in the future. We review immune parameters of prognostic or predictive value during cancer therapy, and highlight existing "descriptive-prognostic" and "functional-therapeutic" molecular signatures, with the hindsight of designing appropriate compensatory therapies. *Cancer Res; 70(23); 9538–43. ©2010 AACR.*

The host immune response can have an impact on cancer incidence, cancer growth, prognosis, and response to therapy. The development and progression of cancer may be counteracted by innate immune effectors [such as dendritic cells (DC) or natural killer (NK) cells] and cognate immune responses (mediated by antibodies or tumor-specific $CD4^+$ and $CD8^+$ T lymphocytes) in three phases: elimination, equilibrium, and evasion (1–3). During the oncogenic process, dedifferentiation can induce (a) overexpression of tumor-associated antigens or neomutations that elicit specific T-cell responses, (b) exposure of stress-induced danger signals (such as ligands of the NK receptor NKG2D) that elicit anticancer immunosurveillance through critical receptors expressed by immune effectors, and/or (3) tumor cell death and/or senescence pathways that are associated with the production of chemokines and consequent attraction leukocytes. In specific cases, chemotherapeutic agents restore the immunologic equilibrium or even the elimination phase of tumors that have escaped from immunosurveillance, either by "debulking" tumor burdens or through direct or indirect effects on the immune system (reviewed in ref. 4).

Immune Predictors Negatively Influencing Clinical Outcome in Cancer

Lymphopenia is clearly associated with poor survival in various malignancies (Supplementary Table S1). Moreover, expression of PD-L1 by tumor cells has a negative impact on overall survival in clear-cell renal cell, breast, esophageal, pancreatic, non–small cell lung (NSCLC), and hepatocellular (HCC) carcinomas (Supplementary Table S1). PD-L1 expression was inversely correlated with the number of tumor-infiltrating lymphocytes (TIL), specifically $CD8^+$ T cells, and positively associated with infiltration by $Foxp3^+$Tregs. Blocking PD-L1 together with gemcitabine could restore T-cell infiltrates and promote tumor regression in preclinical models of pancreatic cancer (5). By reducing the surface expression of human leukocyte antigens (HLA) class I molecules, tumor cells can evade immunosurveillance by cytotoxic T cells (which recognize tumor antigens presented by HLA class I), yet become ultimately recognizable by NK cells (which recognize the absence of HLA class I). Therefore, changes in the expression of HLA class I molecules have a major impact on patient prognosis (Supplementary Table S1). Defects in HLA class I expression are caused by multiple mechanisms including loss of heterozygosity at chromosome 6p, β2 microglobulin, HLA-class I mutations, or defective expression of components of the antigen-processing machinery (6, 7). By regulating HLA class II and cochaperone expression, interferon-γ (IFN-γ), whose expression is often linked with TIL infiltrates, plays a prognostic role, as shown in several cancers. Thus, patients

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with breast cancers with a particular HLA expression pattern ($DR^+Ii^+DM^-$) have markedly decreased recurrence-free and disease-specific survival as compared with $DR^+Ii^+DM^+$ tumors [which contain increased levels of IFN-γ, interleukin (IL)-2, and IL-12 mRNA]. HLA-DR^+/cochaperone expression is associated with high $CD3^+$, $CD4^+$, and $CD8^+$ T-cell infiltrates (but not B cells) and constitutes an independent predictor of survival in breast cancer (8), whereas IFN-γ expression is an independent prognostic factor in ovarian cancers (9). Total loss of HLA class I by breast cancer cells independently predicted improved patient survival (10), suggesting a potential role of NK cells in the control of breast cancer (11).

Immune Predictors Positively Influencing Clinical Outcome in Cancer

Although controversial for decades, the notion that the quality and contexture of immune infiltrates invading tumors can influence prognosis has become increasingly accepted, as reviewed in (12) and summarized in Supplementary Table S2. Infiltration by cytotoxic memory effector cells (positive for CD3, CD8, granzyme B, and CD45RO) around and within the cancerous lesions predicted lower recurrence risk in colorectal cancer (CRC; ref. 13). Although many CRC tumors express IFN-γR, some (72 out of 402) with good prognosis exhibit a nuclear localization of STAT1, correlating with TIL infiltration. Expression of MICA, a ligand for NKG2D, TIL infiltration, and major histocompatibility complex (MHC) class I expression, also constitutes independent positive prognostic markers in CRC (14–17). Together, these examples illustrate the potential utility of immune parameters as prognostic biomarkers.

Large-scale Screening for the Identification of Immune Biomarkers

Numerous systematic studies relying on the use of mRNA expression arrays and systematic single nucleotide polymorphism (SNP) analyses have led to the identification of immune parameters that can influence the prognosis of cancer patients.

Non-Hodgkin lymphoma

The number of karyotypic abnormalities correlates with dismal prognosis in follicular non-Hodgkin lymphoma (FNHL). However, gene expression signatures of nonmalignant tumor-infiltrating immune cells (including T cells, macrophages, and DCs) appeared as the strongest predictors of survival in FNHL (18–20). Good prognosis of FNHL was associated with the expression of genes encoding T-cell–relevant proteins (such as CD7 and CD8b1, but not CD4 or CD2), whereas bad prognosis was marked by the expression of genes specific for myeloid cells (19). Confirming the immune control of FNHL, gene polymorphisms (14 SNPs) in cytokine genes and related immune regulation genes can influence the fate of FNHL patients. Cerhan and colleagues (21) identified SNPs in four genes (*IL-8*, *IL-2*, *IL-12B*, and *IL1RN* coding for the IL-1R antagonist) that influenced patient survival individually, but even more so when combined into a common carrier model that summed the number of deleterious genotypes. Combining the SNPs with clinical and demographic factors increased the predictive ability of the model (21). Similarly, evaluation of the multi-SNP risk score in diffuse large B-cell lymphoma (DLBL) led to the identification of an IL-10 haplotype as well as four SNP genotypes [in IL-1A, IL-8RB, IL-4R, and tumor necrosis factor (TNF)] that were deleterious for patient survival in univariate and multivariate analyses. Patients with four deleterious genotypes were six times more likely to die compared with patients with zero deleterious genotypes (22).

Hepatocarcinoma

A 17-gene signature of noncancerous hepatic tissues predicted both survival and local recurrence of HCC, and was superior to other clinical variables in the multivariate Cox regression model (23). The metastases-inclined microenvironment (MIM) expression profile, which was associated with a 7.9-increased risk of recurrence (either by venous metastasis or local recurrence), was associated with a Th2 cytokine switch (elevated IL-4, 5, 8, 10) and increased expression of MHC class I–related genes as well as platelet proteoglycan (PRG1) and annexin A1 (ANXA1), correlating with elevated hepatocyte and serum CSF1 (23). Immunohistochemistry confirmed that the metastases-averse microenvironment (MAM), which is antinomic to MIM, was associated with reduced infiltration by HLA-DR^+ and $CD68^+$ cells [antigen-presenting cell (APC) other than Kuppfer cells], induction of inflammatory cytokines (such as IFN-γ and TNF-α), elevated expression of the inflammatory marker NOS2 by noncancerous hepatocytes, and an increased proportion of $CD4^+$ and $CD8^+$ T cells (23). Other studies have corroborated that molecular signatures of noncancerous liver tissues predict late recurrence of HCC (24, 25). An innate inflammatory signature (composed of TNF-α, IL-6, and CCL2) was found to correlate with patient survival in 61 HCC patients, TNF being an independent predictor of survival in multivariate analyses. NK (characterized by the expression of the *NCR3* gene and by $CD3^-CD56^+$ infiltrates in immunohistochemistry) and $CD8^+$ T-cell infiltrates correlated with tumor apoptosis and were the main proliferating lymphocytes in tumors of favorable prognosis (24, 25).

Colorectal cancers

Galon and colleagues (13) showed, for CRC, that good prognosis was associated with CD3ζ, CD8, granulysin, granzyme B, as well as the expression of T helper 1 (Th1)–associated genes such as T-box transcription factor 21, interferon regulatory factor 1, and IFN-γ. Applying the same immune signature to small retrospective cohorts of patients with different kinds of cancer, Hsu and colleagues (26) found that the Th1 adaptive immunity-related pattern was associated with good prognosis in prostate cancers, in early diagnosed breast cancers, but not in NSCLC. Surprisingly, inflammation- and immunosuppression-related clusters were associated with favorable clinical outcome in lymphoma patients. In glioblastoma multiforme, the inflammation-related genes dictated a more favorable prognosis (26). Unfortunately, in this study (26), none of these signatures was analyzed using the multivariate Cox regression model.

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Stage IV cutaneous melanoma

An unsupervised hierarchical clustering of global gene expression data from 57 stage IV melanomas led to the identification of four subgroups of patients with distinct prognosis: the high immune responses (HIR), proliferative, pigmentation, and normal-like subgroups (27). As compared with the other subgroups, HIR was associated with overexpression of LCK, IFNGR1, HLA class I and II, CXCL12, and IL1R1, as well as a brisk infiltration of $CD3^+$ T lymphocytes, determined by immunohistochemistry, hypermethylation of the p16INK4A gene, and gains in CGH arrays. The HIR subgroup also exhibited prolonged overall survival under dacarbazine therapy (DTIC; ref. 27). This subgroup classification has been validated, to some extent, on publicly available melanoma microarray databases (such as the GO:0002429 database), confirming the idea that tumors associated with a low expression of immune-relevant genes have a dismal prognosis (27). These data corroborate previous correlations between favorable prognosis and infiltration by mature DCs and activated T cells in primary cutaneous melanoma (28, 29).

Breast cancer

The prognostic impact of lymphocyte infiltration (LI) in breast cancer is being debated (Supplementary Table S2). Microarray analyses done on whole-tumor specimens suggested several immunologically relevant genes (such as genes encoding interferons or markers relevant to B- or T-lymphocyte functions). However, gene expression analyses done on breast cancer cell lines of the basal cell subtype can express such immune response–related genes (30, 31), shedding doubts on the possibility that these profiles, indeed, reflect an immune response. However, laser-captured microdissection of stromal components led to the identification of a signature associated with Th1 immune responses that predicted favorable clinical outcome (32).

Numerous prognostic molecular signatures have been reported to predict the clinical outcome of breast cancer (33). In particular, these signatures identify a subgroup of favorable prognosis, that is, the low-proliferating estrogen receptor $(ER)^+$ tumors (34), whereas the signatures tend to assign a bad prognosis to all of the ER^- tumors and the highly proliferating ER^+ tumors. Importantly, the LCK metagene, which is thought to reflect the abundance of T cells, may separate the ER^- group into two subgroups with opposite clinical outcome (35). Rody and colleagues (35) identified several clusters of immune response–related metagenes by large-scale microarray analysis and found that the level of expression of the LCK metagene (T cells) and the IgG metagene (B cells) correlated among each other, suggesting a parallel recruitment or intratumor proliferation of B and T lymphocytes. High expression of the LCK metagene predicted improved disease-free survival of patients with ER^-, as well as in HER2-overexpressing ER^+, cancers and outperformed all standard parameters in multivariate analysis (35). This finding contrasts with another report, in which the IgG metagene outperformed the LCK metagene as a favorable prognostic factor in highly proliferating specimens, as determined on a series of 200 lymph node–negative breast cancers treated by surgical resection only (36). Another study identified an immune response–related signature that correlated with TIL (verified by hematoxylin and eosin staining) and predicted favorable clinical outcome among HER2-overexpressing breast cancers (37). Teschendorff and colleagues (38) analyzed three microarray data sets and identified a cluster of ER^- breast cancers displaying a high expression of six immune response–related genes that was associated with improved prognosis (38). On the basis of a previously published 368-gene expression signature, Sabatier and colleagues (39) reported that immune response–related genes are predictors identifying a subgroup of basal cell carcinomas [breast cancers (mostly ER^-)] with favorable prognosis (39). Accordingly, a meta-analysis of breast cancer–related molecular signatures strongly indicated that including immune modules ameliorated the prognostic performance of microarray signatures (40).

Together, the inclusion of immune response–related genes into microarray analyses may refine the prognostic stratification of ER^- or HER2-overexpressing ER^+ tumors that were traditionally considered as a homogeneous group with bad prognosis. It should be noted that this may hold true for other tumor types as well. Thus, a 23-prognostic gene classifier (encoding 21 genes related to cellular proliferation plus two immune response–related genes including IFN-γR1) has been validated for its prognostic impact in 17 out of 20 independent tumor cohorts across several solid tumor types of various grades (41).

Integrating the Host-Tumor Interaction in Predictive Models

Recent investigations have aimed at analyzing the predictive value of immune parameters including TIL, the ratio between regulatory versus effector T lymphocytes, Th1 profiles, functional T- or NK-cell assays, loss of function SNPs, microarray-based gene profiling, or serum autoantibody responses (Supplementary Table S3). How may the immune system influence the therapeutic response? One response to this question may reside in how tumor cells die in response to cytotoxic chemotherapeutics. Preclinical studies indicate that, when cancer cells succumb to an immunogenic cell death modality, they may elicit a therapeutic anticancer immune response, which then contributes to the eradication of residual tumor cells (42). Conversely, when cancer cells succumb to a nonimmunogenic death modality, they fail to elicit such a protective immune response (4, 43, 44). One form of immunogenic cell death is characterized by the early cell surface exposure of chaperones including calreticulin (CRT) and/or heat shock proteins, which determine the uptake of tumor antigens and/or affect DC maturation. The later release of the protein high mobility group box 1 (HMGB1), which acts on Toll-like receptor 4 (TLR4) on the surface of DCs, is required for optimal presentation of antigens from dying tumor cells. Moreover, ATP secreted from dying tumor cells stimulates purinergic P2RX7 receptors on the surface of DCs to stimulate the production of essential cytokines. Hence, CRT,

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HMGB1, and ATP compose some of the "dents" of the "key" that unlocks the immune response (4, 45). On the basis of these premises, it can be speculated that anticancer therapies can modify the host-tumor interaction, which, in turn, may affect the therapeutic success. Optimal molecular predictors should hence integrate factors that affect the host-tumor dialogue during the cytotoxic insult.

Single nucleotide polymorphisms in TLR4 and P2RX7 as predictors in breast cancer therapy

As shown in preclinical models, the dialogue between dying cells and immune effectors (according to the "key-lock paradigm") involves two receptors present on DCs, namely TLR4 and P2RX7, which recognize two soluble molecules released from the dying tumor cells, HMGB1 and ATP, respectively. In the absence of TLR4 or P2RX7, the immune system fails to mount an antitumor immune response after chemotherapy, and tumors implanted in $TLR4^{-/-}$ or $P2RX7^{-/-}$ mice fail to respond to anticancer therapy in conditions in which tumors growing on wild-type mice can be controlled by chemotherapy (46, 47). Similarly, a loss-of-function SNP in *tlr4* (rs4986790) has been associated with poor prognosis in breast cancer treated with anthracyclines (46). We investigated the prognostic value of a loss-of-function SNP, *p2rx7* (rs3751143), that reduces the affinity of P2RX7 to ATP and reduces the ATP-dependent IL-1β release from monocytes (48). We analyzed 225 sporadic breast cancer patients, all with the normal TLR4 allele, which were stratified according to their *P2 × 7* genotype [normal (64%) versus variant (36%) *P2 × 7*]. Although no significant differences in classical prognostic factors between normal and variant groups of patients were found, the *P2 × 7* loss-of-function allele had a significant negative prognostic impact on metastatic disease-free survival (Log rank test; $P = 0.02$). A multivariate Cox regression model confirmed a significant effect for tumor grade and for *P2 × 7* genotype (47). Together, these data suggest that selective immune defects can compromise the response to anticancer radiotherapy and chemotherapy, at least in N^+ breast cancer treated with adjuvant anthracyclines.

Extension of the key-lock concept to other tumor subtypes

A variety of different agents including anthracyclines, oxaliplatin, ionizing irradiation (45), and vinblastine elicit immunogenic cell death (49). We analyzed whether the loss-of-function SNP in the *tlr4* gene (rs4986790) would predict time to progression in oxaliplatin-treated colon cancers. In the FFCD 2000 to 2005 randomized trial, 334 patients with metastatic colon cancer were treated with 5-fluorouracil and folinic acid (FuFol) until failure, followed by combination of FuFol + oxaliplatin versus FuFol alone. In this cohort, patients with at least one *tlr4* loss-of-function allele (16.5%) did not differ from those carrying two normal *tlr* alleles with respect to age, sex, number of disease sites, the World Health Organization classification, alkaline phosphatase serum levels, or other laboratory parameters. However, CRC patients bearing the *tlr4* Asp299Gly polymorphism had a shorter time to progression than patients with normal *tlr4* alleles. In stark contrast, the *tlr4* genotype had no influence on progression-free or disease-free survival of a cohort of stage II CRC patients ($n = 258$) who were treated by surgical removal of the primary tumor without any adjuvant chemotherapy. This result suggests that *tlr4*Asp299Gly is not a prognostic but rather a predictive factor of the response to oxaliplatin (50).

Together, these results provide a preliminary validation of the concept that immune parameters can influence the efficacy of anticancer chemotherapies (51–54). It will be important to investigate the possibility to predict therapeutic outcome by assessing dynamic variables such as changes in the frequency, composition, activation status and repertoire of TIL, the expression of immune-relevant metagenes (in repeated lymph node biopsies), or the generation of tumor-specific antibodies (in patient sera) after chemotherapy.

Conclusions

To provide accurate and standardized treatment recommendations, clinicians will be guided in the future by increasingly sophisticated logarithms that integrate clinical, histopathologic, and molecular parameters (Fig. 1). So far, algorithms or nomograms aimed at predicting therapeutic responses have integrated mostly tumor cell–intrinsic parameters, yet have neglected stromal or immune parameters. We believe that the host-tumor equilibrium is modified during the course of successful chemotherapies according to the "key-lock paradigm," and that the stigmata of this paradigm should be considered as candidate biomarkers to detect "nonresponders," which would be candidates for compensatory immune stimulation. Prospective studies examining the added value of such new immune predictors for the clinical outcome, as well as randomized trials aimed at compensating immunologic defects, will confirm or invalidate these predictions.

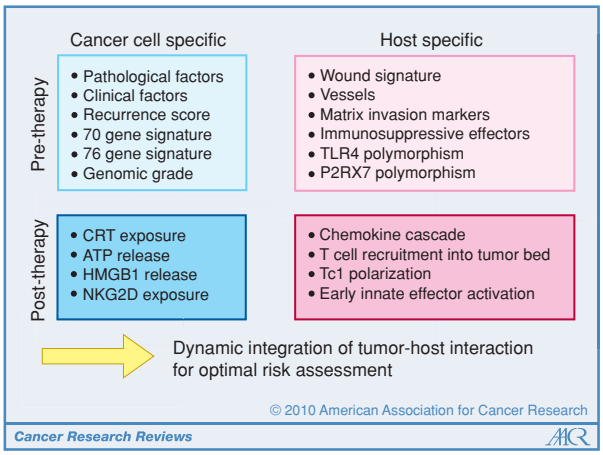

Figure 1. Integration of multiple parameters for the molecular profiling of cancer. Multiple cancer cell and host-specific pre- and post-therapeutic parameters might be used for a personalized and dynamic integration of the tumor-host interaction for optimal risk assessment before and during treatment. Please consult the main text for further details.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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