OncoImmunology 3, e28727; April 2014; © 2014 Landes Bioscience

Meta-analysis and metagenes

CXCL-13-driven signature as a robust marker of intratumoral immune response and predictor of breast cancer chemotherapeutic outcome

Davide Bedognetti[1,*], Ena Wang[1,2], and Francesco M Marincola[1,2]

[1]Infectious Disease and Immunogenetics Section; Department of Transfusion Medicine; Clinical Center and trans-NIH Center for Human Immunology; National Institutes of Health; Bethesda, Maryland USA; [2]Research Branch; Sidra Medical and Research Centre; Doha, Qatar

Keywords: immunotherapy, gene-expression, breast cancer, CXCL-13, melanoma, biomarkers, immunotherapy, neoadjuvant chemotherapy, gene signature

Abbreviations: CTLA-4, cytotoxic T lymphocyte-associated protein 4; ER, endoplasmic reticulum; GNLY, granulysin; IDO, Indoleamine-pyrrole 2,3-dioxygenase; IFN-γ, interferon-γ; IL-2, interleukin 2; IRF-1, interferon regulatory factor-1; mAb, monoclonal antibody; PD-1, programmed cell death 1; PD-L1, PD ligand 1; PRF1, perforin; Tfh, T follicular helper; Th-1, T helper 1

Integrative gene-expression analysis applied to the study of human samples has defined shared themes invariably associated with immune-mediated tissue destruction. Such themes define an in situ T helper 1 (Th-1)-like immune response characterized by the coordinate expression of interferon-γ (IFN-γ)/interferon regulatory factor-1 (IRF-1)-induced transcripts, mRNA's encoding CXCR3/CCR5 chemokine ligands (i.e., CXCL9–11, CCL3–5), and those encoding immune-effector functional molecules (e.g., granzymes, granulysin [GNLY], and perforin [PRF1]). We refer to these collectively as the immunologic constant of rejection (ICR) pathways.[1-4] Their upregulation has been observed in a plethora of different immune-related conditions, spanning from allograft rejection to flares of autoimmunity.[1-3] In the context of cancer immunotherapy, an efficient induction of these molecular pathways early after treatment correlates with achievement of favorable clinical response later.[5,6] Moreover, patients bearing metastatic tumors that display this polarized immunophenotype respond better to various forms of immunity-related manipulations, including interleukin 2 (IL-2) treatment,[5] adoptive-therapy,[7] vaccines,[4] and anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) monoclonal antibody (mAb).[6] In addition, the presence of largely overlapping gene-signatures has been convincingly associated with favorable prognosis of melanoma, colon, breast, and ovarian cancers, as recently reviewed elsewhere.[3] Observations from immune-checkpoint inhibitor trials (e.g., anti-CTLA-4 and anti-programmed cell death 1 [PD-1] mAbs[8]) have also revealed that this inflammatory status is accompanied by the concomitant counteractivation of immunosuppressive mechanisms (i.e., indoleamine-pyrrole 2,3-dioxygenase [IDO] and PD ligand 1 [PD-L1]),[6,9] which likely reflect ongoing immune-escape processes. This suppressed immune response could eventually be reverted by the administration of immune checkpoint inhibitors. However, tumors lacking these 2 features are relatively resistant to immunotherapeutic manipulations.[5-7,9] Because of the pivotal role of the Th-1 like response in mediating tumor rejection, targeted therapy aimed at reprogramming the tumor microenvironment by inducing ICR pathways are the object of effervescent investigations.[3,10,11]

In the meta-analysis recently published in *OncoImmunology* by Stoll et al.,[12] investigators determined whether robust gene-signatures (metagenes) are predictive of beneficial response to neoadjuvant chemotherapy among breast cancer patients. Authors employed a hypothesis-driven approach based on a strong rationale. Rather than testing the expression of the whole transcriptome, they focused on the identification of metagenes (i.e., modules of genes strongly correlated) underlying phenomena conducive to tumor rejection. Gene expression levels evaluated to build metagenes consisted of a wide range of transcripts reflecting intratumoral immune responses, as well as genes induced in response to local stress (i.e., endoplasmic reticulum [ER] stress-, autophagy-, and lysosome-associated transcripts). In fact, as demonstrated by previous works,[13] the induction of ER stress and autophagy by certain chemotherapeutic agents can drive tumor cells toward an immunogenic form of apoptosis (known as immunogenic cell death) conducive to the elicitation local immune response.[14] For each category, metagenes were delineated by exploring The Cancer Genome Atlas breast cancer data set. Their reproducibility was assessed using 6 additional cancer data sets including colorectal, head and neck, and other breast cancer samples.

*Corresponding author: Davide Bedognetti; Email: davide.bedognetti@nih.gov
Submitted: 03/01/2014; Accepted: 03/01/2014; Published Online: 04/09/2014
Citation: Bedognetti D, Wang E, Marincola FM. Meta-analysis and metagenes: CXCL-13-driven signature as a robust marker of intratumoral immune response and predictor of breast cancer chemotherapeutic outcome. OncoImmunology 2014; 3:e28727; http://dx.doi.org/10.4161/onci.28727

Among these were 3 breast cancer data sets for which information in regards to clinical neoadjuvant chemotherapy response was available that were used to test metagenes predictive capabilities. The reproducibility of stress-related metagenes was found to be generally poor—perhaps suggesting a lack of coordinated and persistent stress-related events in pre-treatment tumor deposits—such that no association between these metagenes and clinical outcome was detected. However, the immune-related metagene driven by the C-X-C motif chemokine ligand, *CXCL13* transcript bore the highest reproducibility across data sets and was strongly associated with the achievement of complete pathological response. Genes embraced by the CXCL13 metagenes[12] largely overlap with those associated with favorable cancer prognosis and response to immunotherapy.[3,4] They include classical ICR genes such as ligands for the chemokine receptors CXCR3 and CCR5 (*CXCL9–10,* and *CCL5* transcripts, respectively), immune-effector genes (e.g., *PRF1*, granzymes), and Th-1 related genes (*IFNG* and *CD8B*).[12] To explain the favorable predictive role of Th-1 like gene signatures in the setting of cancer immunotherapy, it has been proposed that immune-manipulation could restore a naturally occurring, though insufficient, host's immune response by enhancing its effector functions and, thus, its predictive significance.[4] Similarly, it is tempting to hypothesize that the immunogenic cell death, eventually induced by antineoplastic drugs, requires the presence of an ongoing intratumoral response to exert its immune-adjuvant effect.

A number of investigations have reported a correlation between T-cell infiltrates, Th-1 related genes, and achievement of complete response following neoadjuvant chemotherapy in breast cancer patients.[15] By defining the *CXCL13*-meta gene, Stoll et al. added molecular precision to previous observations.[15,16] In fact, only recently, has CXCL13 emerged as critical modulator of intratumoral response.[17-19] This chemokine, which binds CXCR5, is physiologically highly expressed in the follicles of secondary lymphoid organs, where it can be secreted by follicular dendritic cells and T follicular helper (Tfh) cells. In this context, CXCL13 mediates migration of high-affinity CXCR5+ Tfh cells and B cells into B-cell concentrated areas. While a number of studies have reported the presence of tertiary lymphoid structure in a considerable proportion of cancers,[20] it was only last year that the presence of CXCL13+ Tfh cells were demonstrated in solid tumors.[18] By analyzing breast cancer samples, Gu-Trantien et al.[17] showed that the presence of tumor-infiltrating CXCL13+ Tfh cells, localizing primarily in peritumoral tertiary lymphoid structures, was associated with improved disease outcome. In parallel, the presence of Tfh cells were shown to correlate with abundance of Th1 cells and B cells within the neoplastic bed.[17] Similar conclusions were recently independently reached by Bindea et al.[19] via analysis of colorectal tumor specimens. Interestingly, authors showed that tumor cells also expressed CXCL13 and that genetic deletion of *CXCL13* markedly lowers the density of B cells and Tfh cells in invasive margins.[19] It remains, however, to be fully elucidated whether (and how) the genetic makeup of the host, somatic cancer cell genetic or epigenetic aberrations, or environmental factors, such as lifetime exposure to commensale microbiota,[21] may interact to influence the development of a favorable cancer immune phenotype. We believe that assessing these critical questions using integrated high-throughput approaches will allow the development of innovative targeted therapy that may dramatically impact therapeutic outcome in the near future.[22]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Spivey TL, Uccellini L, Ascierto ML, Zoppoli G, De Giorgi V, Delogu LG, Engle AM, Thomas JM, Wang E, Marincola FM, et al. Gene expression profiling in acute allograft rejection: challenging the immunologic constant of rejection hypothesis. J Transl Med 2011; 9:174; PMID:21992116; http://dx.doi.org/10.1186/1479-5876-9-174
2. Bedognetti D, Wang E, Sertoli MR, Marincola FM. Gene-expression profiling in vaccine therapy and immunotherapy for cancer. Expert Rev Vaccines 2010; 9:555-65; PMID:20518712; http://dx.doi.org/10.1586/erv.10.55
3. Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity 2013; 39:11-26; PMID:23890060; http://dx.doi.org/10.1016/j.immuni.2013.07.008
4. Wang E, Bedognetti D, Marincola FM. Prediction of response to anticancer immunotherapy using gene signatures. J Clin Oncol 2013; 31:2369-71; PMID:23715576; http://dx.doi.org/10.1200/JCO.2013.49.2157
5. Weiss GR, Grosh WW, Chianese-Bullock KA, Zhao Y, Liu H, Slingluff CL Jr., Marincola FM, Wang E. Molecular insights on the peripheral and intratumoral effects of systemic high-dose rIL-2 (aldesleukin) administration for the treatment of metastatic melanoma. Clin Cancer Res 2011; 17:7440-50; PMID:21976537; http://dx.doi.org/10.1158/1078-0432.CCR-11-1650
6. Ji RR, Chasalow SD, Wang L, Hamid O, Schmidt H, Cogswell J, Alaparthy S, Berman D, Jure-Kunkel M, Siemers NO, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother 2012; 61:1019-31; PMID:22146893; http://dx.doi.org/10.1007/s00262-011-1172-6
7. Bedognetti D, Spivey TL, Zhao Y, Uccellini L, Tomei S, Dudley ME, Ascierto ML, De Giorgi V, Liu Q, Delogu LG, et al. CXCR3/CCR5 pathways in metastatic melanoma patients treated with adoptive therapy and interleukin-2. Br J Cancer 2013; 109:2412-23; PMID:24129241; http://dx.doi.org/10.1038/bjc.2013.557
8. Galluzzi L, Vacchelli E, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Zucman-Rossi J, Zitvogel L, Kroemer G. Trial Watch: Monoclonal antibodies in cancer therapy. Oncoimmunology 2012; 1:28-37; PMID:22720209; http://dx.doi.org/10.4161/onci.1.1.17938
9. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, Chen S, Klein AP, Pardoll DM, Topalian SL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med 2012; 4:127ra37; PMID:22461641; http://dx.doi.org/10.1126/scitranslmed.3003689
10. Tomei S, Wang E, Delogu LG, Marincola FM, Bedognetti D. Non-BRAF-targeted therapy, immunotherapy, and combination therapy for melanoma. Expert Opin Biol Ther 2014; (Forthcoming); PMID:24625306; http://dx.doi.org/10.1517/14712598.2014.890586
11. Pescatori M, Bedognetti D, Venturelli E, Ménard-Moyon C, Bernardini C, Muresu E, Piana A, Maida G, Manetti R, Sgarrella F, et al. Functionalized carbon nanotubes as immunomodulator systems. Biomaterials 2013; 34:4395-403; PMID:23507086; http://dx.doi.org/10.1016/j.biomaterials.2013.02.052
12. Stoll G, Enot D, Mlecnik B, Galon J, Zitvogel L, Kroemer G. Immune-related gene signatures predict the outcome of neoadjuvant chemotherapy. OncoImmunology 2014; 3:e27884
13. Zitvogel L, Galluzzi L, Smyth MJ, Kroemer G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. Immunity 2013; 39:74-88; PMID:23890065; http://dx.doi.org/10.1016/j.immuni.2013.06.014
14. Vacchelli E, Galluzzi L, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Kroemer G. Trial watch: Chemotherapy with immunogenic cell death inducers. Oncoimmunology 2012; 1:179-88; PMID:22720239; http://dx.doi.org/10.4161/onci.1.2.19026
15. Ignatiadis M, Singhal SK, Desmedt C, Haibe-Kains B, Criscitiello C, Andre F, Loi S, Piccart M, Michiels S, Sotiriou C. Gene modules and response to neoadjuvant chemotherapy in breast cancer subtypes: a pooled analysis. J Clin Oncol 2012; 30:1996-2004; PMID:22508827; http://dx.doi.org/10.1200/JCO.2011.39.5624
16. Issa-Nummer Y, Loibl S, von Minckwitz G, Denkert C. Tumor-infiltrating lymphocytes in breast cancer: A new predictor for responses to therapy. OncoImmunology 2014; 3:e27926

17. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, Ravoet M, Le Buanec H, Sibille C, Manfouo-Foutsop G, et al. CD4+ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest 2013; 123:2873-92; PMID:23778140; http://dx.doi.org/10.1172/JCI67428
18. Gu-Trantien C, Willard-Gallo K. Tumor-infiltrating follicular helper T cells: The new kids on the block. Oncoimmunology 2013; 2:e26066; PMID:24244900; http://dx.doi.org/10.4161/onci.26066
19. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Fredriksen T, Lafontaine L, Berger A, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 2013; 39:782-95; PMID:24138885; http://dx.doi.org/10.1016/j.immuni.2013.10.003
20. Goc J, Fridman WH, Sautès-Fridman C, Dieu-Nosjean MC. Characteristics of tertiary lymphoid structures in primary cancers. Oncoimmunology 2013; 2:e26836; PMID:24498556; http://dx.doi.org/10.4161/onci.26836
21. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, Molina DA, Salcedo R, Back T, Cramer S, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013; 342:967-70; PMID:24264989; http://dx.doi.org/10.1126/science.1240527
22. Wang E, Bedognetti D, Tomei S, Marincola FM. Common pathways to tumor rejection. Ann N Y Acad Sci 2013; 1284:75-9; PMID:23651198; http://dx.doi.org/10.1111/nyas.12063