Clinical Cancer Research

Tumor Landscapes: β-Catenin Drives Immune Desertification

Denarda Dangaj, David Barras, and George Coukos

Immune checkpoint blockade therapy requires a pre-established activated immune landscape. Understanding tumor-intrinsic mechanisms that lead to T-cell desertification is key to resensitizing them to such therapies. The WNT/β-catenin tumor-intrinsic signaling is emerging as an immune exclusion pathway that holds high promise to counteract resistance to immunotherapy.

See related article by Luke et al., p. 3074

In this issue of *Clinical Cancer Research*, Luke and colleagues tested the universality of the tumor-intrinsic WNT/β-catenin pathway in driving immune desertification in cancer by mining The Cancer Genome Atlas (TCGA) pan-cancer database (1). Efficient immune recognition can indeed control tumor outgrowth both in animal models and in human patients. On the basis of the presence of preexisiting T-cell immune responses, tumors have been categorized as immunogenic, inflamed, or hot tumors and nonimmunogenic, noninflamed, or cold tumors. Definitive proof for the ability of tumor-infiltrating T cells (TIL) to eliminate human tumors is given by the clinical responses following adoptive transfer of *ex vivo*–expanded autologous TILs, and by the numerous clinical responses in patients treated with antibodies neutralizing the programmed death 1 (PD-1) pathway.

What leads to the establishment and persistence of spontaneous antitumor T-cell responses remains partly unclear. Recent literature suggests that tumorigenic cancer cell–intrinsic pathways can shape and regulate TIL infiltration. For example mutations in the DNA repair pathway, microsatellite instability, and *POLE* mutations, which generate genomically unstable tumors, are characterized by a higher T-cell content. Tumors with mismatch-repair deficiencies respond better to immune checkpoint blockade (ICB) comparing to mismatch-proficient tumors. On the contrary, gain-of-function alterations of the WNT/β-catenin or MYC pathway, and loss-of-function mutations in *PTEN* or loss of the LKB1 pathway restrain T-cell recruitment and generate non-inflamed tumors. Inhibition of the above oncogenic pathways via genetic manipulation in mouse models has sensitized tumors to ICB therapy. Thus, understanding the mechanisms that are responsible for the presence of noninflamed tumors is of paramount importance and manipulating those can broaden the efficacy of ICB and immunotherapy in general.

Luke and colleagues analyzed data from 9,244 samples of 31 solid tumor types and categorized every tumor into T-cell–inflamed, intermediate, or non-T-cell–inflamed tumors using a 160-gene expression signature. Across all tumors, around one-third exhibited high levels of the signature, while one other third exhibited low levels and were categorized as non-T-cell–inflamed tumors. Tumor types like lung cancer, kidney cancer, and mesothelioma had the highest proportion of T-cell–inflamed phenotype, consistent with a substantial clinical activity of PD-1 checkpoint blockade, while tumor types resistant to immunotherapy like paraganglioma, uveal melanoma, and adrenocortical tumors had the highest proportion of non-T-cell–inflamed cases. Metastatic melanoma, with an approximate 40% objective response rate to ICB, displayed a similar proportion of hot tumors. Importantly, comparing matched tumor and normal adjacent tissues they found that cold tumors of most tumor types analyzed (except kidney cancer) displayed lower levels of the T-cell–inflamed signature compared with healthy tissues, suggesting that T-cell exclusion is the result of an active process taking place during carcinogenesis.

Prompted by their previous seminal findings in melanoma, where gain of function in the WNT/β-catenin pathway drives exclusion of CD103+ dendritic cells and antigen-specific T cells (2), they next assessed cold solid tumors for alterations in the WNT/β-catenin pathway. Fifteen percent of the cold tumors and 10% of the hot tumors were enriched for gain-of-function mutations and copy number alterations. Although this difference in enrichment was faint, it was found to be statistically significant. The vast majority of these alterations were dominated by activating mutations in *CTNNB1* and *APC* loss from colorectal cancers. These results suggested that DNA alterations in WNT pathway are not frequently implicated in mediating immunosuppression. Then, they computed β-catenin activity scores using gene expression signatures of known cancer type–dependent CTNNB1 transcriptional targets and evaluated their enrichment across hot and cold tumors. Approximately half of the cancer types had high β-catenin scores within cold tumors. Cancers like clear cell kidney carcinoma, adrenocortical, sarcoma, and ovarian cancers had the highest β-catenin enrichment difference (at least 47%) between noninflamed and inflamed tumors. However, these differences often only involved a few patients. Colorectal cancer and melanoma had an extensive overlap between inflamed and noninflamed tumors in the β-catenin gene expression score. Finally, CTNNB1 protein levels anticorrelated with the T-cell–inflamed score in 65% of cancer types. In summary, 90% of cancer types displayed higher β-catenin activity in cold tumors, which could be attributed to either (i) DNA alterations

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Ludwig Institute for Cancer Research and Department of Oncology, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland.

Note: D. Dangaj and D. Barras contributed equally to this article.

Corresponding Author: George Coukos, CHUV, Rue du Bugnon 46, Lausanne BH09-701, Switzerland. Phone: 41 21 3140627; E-mail: george.coukos@chuv.ch

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of WNT-related genes, (ii) hyperactivation of the WNT/β-catenin pathway, or (iii) CTNNB1 protein overexpression. All three parameters could be identified in approximately 29% of cases, and the molecular basis of WNT/β-catenin–mediated exclusion of T cells in cold tumors is illustrated in Fig. 1.

Transcriptional profiling of bulk tumors combined with bioinformatic analyses permits the assessment of both the tumor and stroma components of cancer and has been widely used to unravel predictive gene expression signatures. Most methods computing gene signature scores utilize gene-set

enrichment analysis (GSEA), utilize gene-set variation analysis (GSVA), or compute the mean or median expression of individual genes. Luke and colleagues performed a score categorization for the expression of each gene (i.e., −1 for downregulated, 0 for nonvarying, and +1 for upregulated) and further categorized their resulting sum. This signature correlated tightly to established scores reflecting T-cell cytotoxicity. However promising, the lack of standardization of reported signatures with respect to gene content and how they are computed has made their clinical application challenging so far.
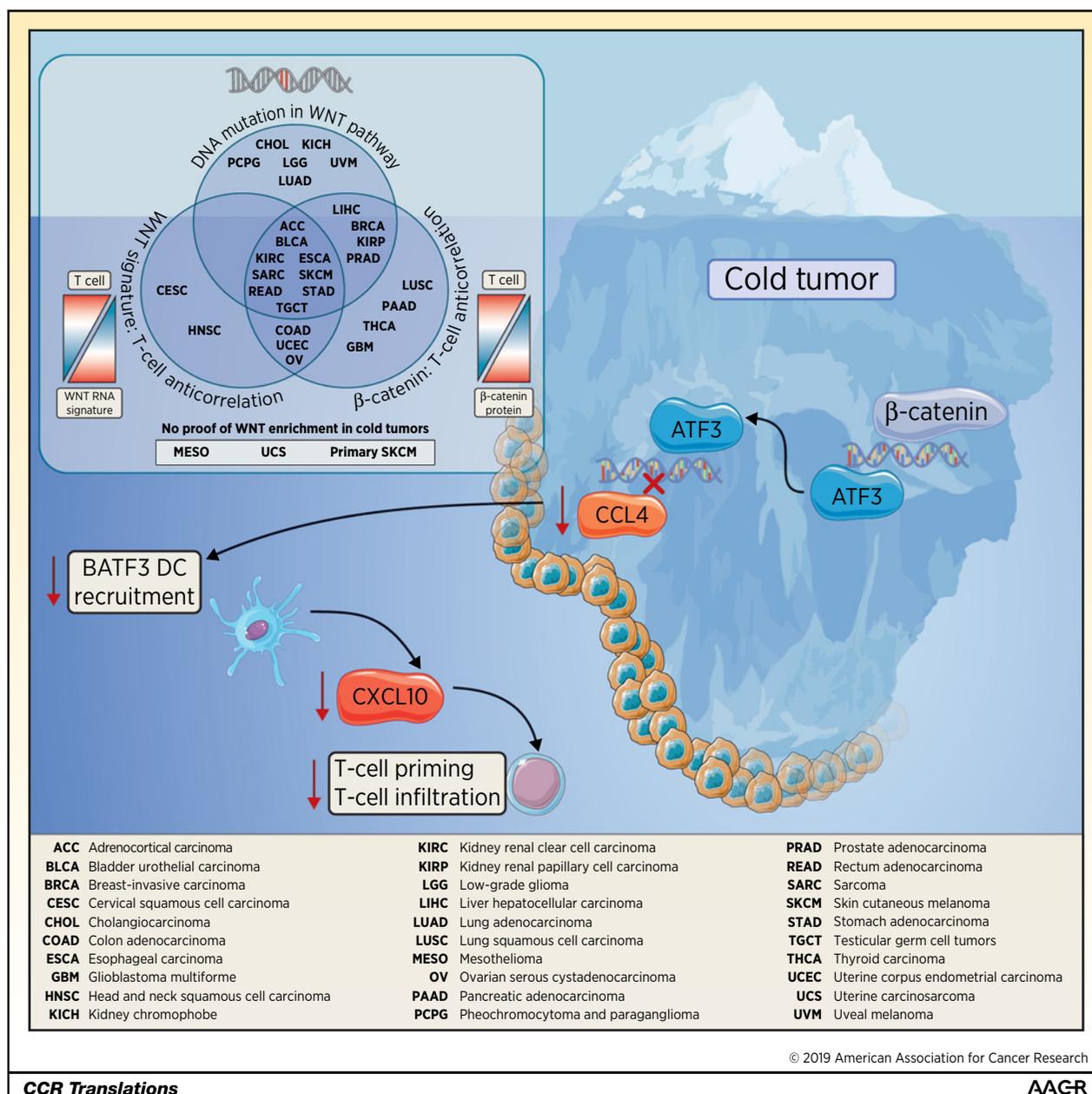

Figure 1.
This figure summarizes the results of Luke and colleagues (1) and shows which alterations/characteristics of the WNT pathways are enriched in non-T-cell–inflamed (cold) tumors of the TCGA. The molecular mechanisms underlying β-catenin–induced immune desertification in cold tumors as unraveled by Spranger and colleagues (2) is also illustrated here. DC, dendritic cell.

Although purely *in silico* and lacking experimental validation across tumors, the work by Luke and colleagues introduces an important concept, that of identifying cold tumors by measuring the activity of tumor-intrinsic pathways such as WNT/β-catenin. However, how universally active and dominant the WNT/β-catenin pathway is in noninflamed tumors requires further investigation. The authors show that only a proportion of non-inflamed tumors display activation of the WNT/β-catenin pathway, suggesting that other tumor cell–intrinsic pathways could contribute to immune desertification. Additional parameters such as the presence of immunogenic epitopes and the level of DNA damage might, for example, be also at play, diluting the effect of the WNT/β-catenin pathway on immune desertification.

It is well established that the metastatic process requires WNT/β-catenin activity for epithelial–mesenchymal transition, cell proliferation, and angiogenesis. Thus, the results of Luke and colleagues, may underestimate the actual prevalence of the WNT/β-catenin pathway, because the authors mainly analyzed TCGA data, which almost exclusively contains primary tumor data. Tumors with high mutational load and preserved truncal mutations are more likely to respond to ICB than highly heterogeneous tumors. Furthermore, intratumoral heterogeneity (ITH) can lead under continuous pressure by local factors, the immune attack, and applied therapies to important clonal divergence. One could hypothesize that more than one tumor-intrinsic pathway can thus emerge stochastically in distinct metastatic sites, independently leading to immune desertifica-tion. The aspect of ITH is often overlooked and cannot be accurately addressed by the TCGA as its data are derived from single tumor sites. The study by Jiménez-Sánchez and collea-gues (3), looking at multiple metastases of ovarian cancer before and after chemotherapy suggests that indeed WNT but also the MYC pathway seem to dominate the immune desert-ification process at metastatic sites, further underscoring the significance of the findings by Luke and colleagues.

In conclusion, understanding the causal relationship between the genetic makeup of tumors and immune landscape has impor-tant implications for patient stratification for immunotherapy, understanding resistance to it, and for prioritizing pathways for development of drug combinations.

Disclosure of Potential Conflicts of Interest

Acknowledgments

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