Stem cell-like T cells have a specific entry gate to the tumor

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Abstract

Tumor-infiltrated T cells with stem cell-like properties are important to determine the response to immunotherapy. In this issue of *Cancer Cell*, Asrir and colleagues show that their entry requires specialized tumor-associated endothelial cells resembling immature and inflamed lymph node vessels, and that immunotherapy enhances the recruitment capacity of these endothelial cells.

Main text

Immune checkpoint blockade (ICB) has the potential to mediate complete and durable cancer remission. Although this intervention shows considerable efficacy in patients with metastatic melanoma, the clinical response rates for most other cancer types are still low. Treatment failures have been attributed to both tumor- and lymphocyte-associated features, including a paucity of tumor-infiltrating T cells. While many tumor-associated T cells are bystanders, tumor-reactivity is enriched among T cells that express inhibitory receptors, such as PD-1. Among the latter, the response to immunotherapy chiefly depends on a subset of cells with stem cell-like properties (Siddiqui et al., 2019) (Miller et al., 2019). In this issue of *Cancer Cell*, Asrir et al. report the characterization of the tumor vasculature and find that the entry of stem-cell like T cells into tumors depends on a specific subset of tumor-associated vessels whose efficacy increases in response to ICB (Asrir et al., 2022).

The work of Asrir et al. builds on the understanding of lymphocyte recirculation and recruitment to normal and inflamed lymph nodes (LNs). Specialized vessels, so-called high endothelial venules (HEVs) allow the continuous recruitment of naïve $CD62L^+$ T cells from the blood into lymph nodes (LN), where antigen encounters induce primary immune responses. HEV-like vessels have previously been observed in some murine tumor models (Hindley et al., 2012) as well as in human tumors (Martinet et

al., 2011). The current study shows that a minority of the tumor-associated endothelial cells has HEV features (referred to as TA-HECs) including MECA79$^+$ sialomucin (peripheral node addressin (PNAd)) expression. Using homing assays and elegant *in vivo* imaging analyses the authors show that TA-HECs represent the critical site in the tumor microcirculation where T cells extravasate and enter into the tumor parenchyma (**Figure 1**).

Asrir et al. next compare the transcriptomes of TA-HEC to that of MECA79$^-$ TA-ECs and inflamed and non-inflamed LN-HECs. They find that TA-HECs combine features of both normal and inflamed LN-HECs. Notably, TA-HECs express MECA79$^+$ sialomucins, which are critical for the entry of CD62L$^+$ naïve or central memory CD8$^+$ T cells. In addition, they also express P- and E-selectins (CD62P/E-selectins) characteristic of inflamed LN-HEC, which mediate the recruitment of PSGL-1$^+$ effector or effector memory CD8$^+$ T cells. Notwithstanding, TA-HEC are immature based on the low expression of target genes of the lymphotoxin beta receptor (LTβR) pathway, which play a central role in orchestrating the mature phenotype of LN-HECs.

The authors further investigate whether the activity of TA-HECs is impacted by ICB. They first establish that a combination of anti-CTLA4 and anti-PD-1 antibodies is necessary to induce tumor stasis in their subcutaneous sarcoma model. Indeed, ICB considerably enhances the capacity of TA-HEC to recruit the phenotypically distinct T cell subtypes discussed above. As most of these cells are bystanders (Simoni et al., 2018), the authors further focus on tumor-reactive T cells that can be identified by the expression of inhibitory receptors, such as PD-1. More specifically, they scrutinize a subset of PD-1$^+$ CD8$^+$ T cells that expresses the transcription factor Tcf1 and that has stem cell-like properties (Siddiqui et al., 2019) (Miller et al., 2019). In response to ICB, intratumoral PD1$^+$ Tcf1$^+$ CD8$^+$ cells expand and self-renew or differentiate into effector-like PD-1$^+$ Tcf1$^-$ CD8$^+$ T cells that have more limited proliferative capacity but acquire lytic potential (Siddiqui et al., 2019) (Miller et al., 2019) (**Figure1**). As the stem-like T cells arise in local lymph nodes (Schenkel et al., 2021), Asrir et al define the mechanisms mediating their influx into tumors. They find that stem-like CD8$^+$ T cells are recruited into tumors via TA-HECs and that ICB considerably improves the efficacy of this process (**Figure 1**).

Increased T cell recruitment is accompanied by regression of TA-ECs, while the TA-HEC abundance (relative to the size of the tumor) remains unaltered. The authors suggest that the ratio of TA-HECs relative to TA-ECs is an important predictor and determinant of T cell recruitment in response to ICB. In addition, based on elevated PNAd (MECA79$^+$ sialomucins) levels the authors suggest that ICB

improves the maturation of TA-HEC. This provides an explanation for the improved influx of stem-like $CD8^+$ T cells, which express the PNAd receptor CD62L (Siddiqui et al., 2019). Thus, in addition to the known expansion and differentiation of intratumoral stem-like $CD8^+$ T cells, the authors show that ICB enhances anti-tumor effects by facilitating the access of stem-like $CD8^+$ T cells to tumors via a subset of blood vessels.

So how is the potency of TA-HECs regulated? The authors provide three pieces of information, which will be useful to guide further investigation. First, the capacity of TA-HECs to recruit T cells improves upon anti-CTLA4/PD-1 therapy. As there is no evidence that ICB impacts TA-HEC function directly, the effects are likely indirect and mediated by T cells. Indeed, $CD4^+$ T cells are shown to be critical for TA-HEC maturation in response to ICB. In addition, depletion of $CD4^+$ cells reduces the abundance of TA-HECs. Conversely, treatment with an agonistic anti-LTβR antibody not only increases TA-HEC maturation but also their abundance. Thus, TA-HEC function and abundance involves $CD4^+$ T cells and LTβR signaling. Indeed, combining agonistic anti-LTβR and anti-PD-1/CTLA4 antibodies further enhances the maturity and abundance of TA-HEC, increases the presence of intratumoral stem-like $CD8^+$ T cells and results in tumor regression (Asrir et al., 2022). Together with earlier studies (Allen et al., 2017) (Johansson-Percival et al., 2017), the results of Asrir et al. underscore the therapeutic potential of HEV-promoting therapies.

Even though ICB has revolutionized the care of certain cancer patients, many patients do not respond or respond only transiently. The holy grail of cancer immunotherapy is to understand and to predict, which patients will benefit from a given therapeutic intervention. Asrir et al. thus address whether the presence of TA-HECs can predict patient survival in response to ICB. To that end, they score the abundance of MECA-79^+ TA-HECs in melanoma lesions from 94 patients. Patients showing a clinical response to ICB have a significantly higher pre-treatment TA-HEC score compared to non-responder patients in the overall cohort. Moreover, TA-HEC scores are significantly higher in patients treated with anti-CTLA and anti-PD-1 compared to patients treated with anti-PD-1 alone. Most importantly, patients with high pre-treatment TA-HECs scores have better overall and progression-free survival than those with low scores upon treatment with the combination ICB. In contrast to the TA-HEC scores, the pre-treatment density of $CD8^+$ T cells does not predict survival. In keeping with the preclinical model, it would be of interest to determine whether survival correlates with an increased density of tumor-specific

and/or stem cell-like $CD8^+$ T cells. Irrespectively, the authors identify TA-HECs as a biomarker predicting better response and survival of metastatic melanoma patients upon ICB treatment.

In summary, the work by Asrir et al. further challenges the paradigm of viewing the tumor vasculature as a homogenous “one size fits all” entity and highlights the importance of characterizing tumor endothelial heterogeneity. These new results raise exciting further questions. The transcriptome profiling suggested that TA-HECs arise in postcapillary venules. What initiates their emergence in specific locations in tumor-associated vessels and is there a defined TA-HEC progenitor cell? What is the precise role of $CD4^+$ T cells in sustaining the TA-HEC function and abundance and are other immune populations involved? Why are TA-HECs inherently immature in untreated tumors and what are the cellular targets of activating LTβR signaling? TA-HEC maturation in response to ICB correlates with a reduced presence of TA-ECs. Do TA-ECs counteract the maturation of TA-HECs? How does loss of TA-ECs observed in this study relate to vascular normalization induced by antiangiogenic treatments, which prune tumor-associated immature capillaries (de Palma et al., 2017)? Both types of interventions reduce TA-EC/immature capillaries and may thereby enrich for TA-HECs and induce their maturation. Thus, further work is needed to characterize the interplay between vessel normalization, immune cell recruitment and HEV-like vascular specialization. Deciphering the molecular mechanisms regulating the efficacy of TA-HECs is thus a promising avenue to further improve the efficacy of tumor immune therapy.

DECLARATION OF INTERESTS

T.V.P. receives research support from Roche. The remaining authors declare no competing interests.

REFERENCES

Allen, E., Jabouille, A., Rivera, L. B., Lodewijckx, I., Missiaen, R., Steri, V., Feyen, K., Tawney, J., Hanahan, D., Michael, I. P., and Bergers, G. (2017). Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation. Science Translational Medicine *9*.

Asrir, A., Tardiveau, C., Coudert, J., Laffont, R., Blanchard, L., Bellard, E., Veerman, K., Bettini, S., Lafouresse, F., Vina, E.*, et al.* (2022). Tumor-associated high endothelial venules mediate lymphocyte entry into tumors and predict response to PD-1 plus CTLA-4 combination immunotherapy. Cancer Cell *this issue*.

de Palma, M., Biziato, D., and Petrova, T. V. (2017). Microenvironmental regulation of tumour angiogenesis. Nat Rev Cancer *17*, 457-474.

Hindley, J. P., Jones, E., Smart, K., Bridgeman, H., Lauder, S. N., Ondondo, B., Cutting, S., Ladell, K., Wynn, K. K., Withers, D.*, et al.* (2012). T-Cell Trafficking Facilitated by High Endothelial Venules Is Required for Tumor Control after Regulatory T-Cell Depletion. Cancer Research *72*, 5473-5482.

Johansson-Percival, A., He, B., Li, Z. J., Kjellen, A., Russell, K., Li, J., Larma, I., and Ganss, R. (2017). De novo induction of intratumoral lymphoid structures and vessel normalization enhances immunotherapy in resistant tumors. Nature Immunology *18*, 1207-+.

Martinet, L., Garrido, I., Filleron, T., Le Guellec, S., Bellard, E., Fournie, J. J., Rochaix, P., and Girard, J. P. (2011). Human Solid Tumors Contain High Endothelial Venules: Association with T- and B-Lymphocyte Infiltration and Favorable Prognosis in Breast Cancer. Cancer Research *71*, 5678-5687.

Miller, B. C., Sen, D. R., Al Abosy, R., Bi, K., Virkud, Y. V., LaFleur, M. W., Yates, K. B., Lako, A., Felt, K., Naik, G. S.*, et al.* (2019). Subsets of exhausted CD8(+) T cells differentially mediate tumor control and respond to checkpoint blockade. Nat Immunol *20*, 326-336.

Schenkel, J. M., Herbst, R. H., Canner, D., Li, A., Hillman, M., Shanahan, S. L., Gibbons, G., Smith, O. C., Kim, J. Y., Westcott, P.*, et al.* (2021). Conventional type I dendritic cells maintain a reservoir of proliferative tumor-antigen specific TCF-1(+) CD8(+) T cells in tumor-draining lymph nodes. Immunity *54*, 2338-+.

Siddiqui, I., Schaeuble, K., Chennupati, V., Fuertes Marraco, S. A., Calderon-Copete, S., Pais Ferreira, D., Carmona, S. J., Scarpellino, L., Gfeller, D., Pradervand, S.*, et al.* (2019). Intratumoral Tcf1(+)PD-1(+)CD8(+) T Cells with Stem-like Properties Promote Tumor Control in Response to Vaccination and Checkpoint Blockade Immunotherapy. Immunity *50*, 195-211 e110.

Simoni, Y., Becht, E., Fehlings, M., Loh, C. Y., Koo, S. L., Teng, K. W. W., Yeong, J. P. S., Nahar, R., Zhang, T., Kared, H.*, et al.* (2018). Bystander CD8(+) T cells are abundant and phenotypically distinct in human tumour infiltrates. Nature *557*, 575-579.

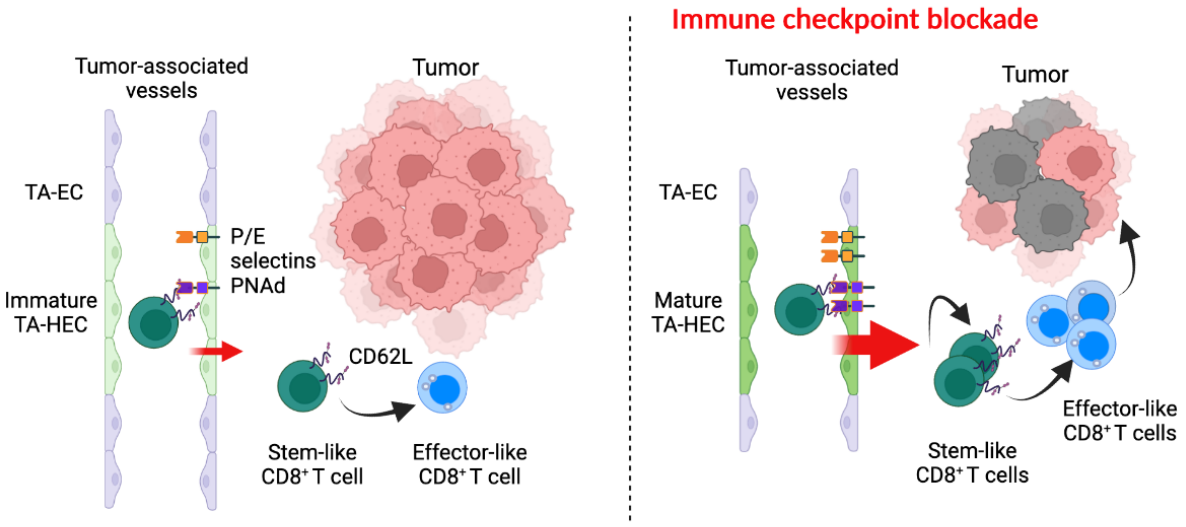

Figure 1

Left panel: A subset of tumor-associated endothelial cells (TA-ECs) in untreated tumors resemble lymph node high endothelial venules (HEVs) as they express PNAd (MECA79$^+$ sialomucins). Tumor-associated endothelial cells with HEVs features (TA-HEC) also express P/E-selectins, and represent the critical site of entry of CD62L$^+$ T cells, including tumor-reactive PD-1$^+$ Tcf1$^+$ CD8$^+$ T cells that have stem cell-like properties. In the tumor, stem-like CD8$^+$ T cells differentiate into effector-like PD-1$^+$ Tcf1$^-$ CD8$^+$ T cells, that have cytolytic potential.

Right panel: Immune checkpoint blockade, using anti-PD-1 plus anti-CTLA4 treatment, prunes MECA79$^-$ TA-ECs and increases the maturation of TA-HECs as judged by elevated levels of P/E-selectins and PNAd. This enhances their efficacy to recruit T cells, including CD62L$^+$ stem-like T cells, to the tumor (red arrow). In addition, intratumoral stem-like CD8$^+$ T cells expand and self-renew or differentiate into effector-like CD8$^+$ T cells, which mediate tumor control. Created with BioRender.com