Location, location, location
CD103 demarcates intraepithelial, prognostically favorable CD8⁺ tumor-infiltrating lymphocytes in ovarian cancer

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Although high levels of tumor-infiltrating lymphocytes (TILs) generally correlate with good prognosis in high-grade serous ovarian cancer (HGSC) patients, little is known about the phenotype or specificity of these cells. We have recently demonstrated that TIL expressing the intra-epithelial lymphocyte marker CD103 (official name, integrin α_E, ITGAE) abundantly infiltrate HGSCs, strongly correlating with increased disease-specific survival.

In recent years, an ever growing number of studies has revealed that high levels of tumor-infiltrating lymphocytes (TILs) correlate with a favorable long-term prognosis in patients affected by various epithelial neoplasms.[1] Although TILs comprise a complex mixture of immune cells, CD8⁺ T cells stand out as the TILs associated with the most robust prognostic value in a majority of settings.[2] In ovarian cancer patients, the survival benefit is generally more pronounced when CD8⁺ TILs are located within the neoplastic epithelium rather than in the tumor-associated stroma.[3,4] This epithelial/stromal distinction makes the precise quantification of TILs challenging as the epithelial and stromal areas of the tumor are often in close juxtaposition. Moreover, the molecular mechanisms that are responsible for the epithelial localization of TILs remain poorly defined.

Our group has recently described a subset of CD8⁺ T cells infiltrating human ovarian cancers that express CD103 (official name integrin α_E, ITGAE) on the cell surface.[5,6] CD103 is the α_E subunit of the dimeric α_E/β_7 integrin, which binds to the epithelial cell surface molecule E-cadherin. Adhesive interactions between α_E/β_7 and E-cadherin play an important role in the retention of antigen-specific lymphocytes within epithelial tissues. Accordingly, CD103 is expressed by < 2% of circulating T cells but is widely expressed on intra-epithelial lymphocytes (IELs) of the gut mucosa and skin.[7] CD103 is also expressed by the majority of tissue-infiltrating, alloreactive CD8⁺ T cells in the course of transplant rejection[8] and graft-vs.-host reactions.[9] Furthermore, CD103 is increasingly being recognized as a definitive marker of so-called tissue resident memory CD8⁺ T cells (CD8⁺ T_{RM} cells) in the setting of infectious diseases.[10]

Although prior anecdotal reports have documented the presence of CD103⁺ TILs within epithelial neoplasms, their precise intratumoral location and prognostic significance has been difficult to ascertain, mostly due to the lack of a CD103-specific antibody suitable for use on paraffin-embedded tissue sections. Fortuitously, such a reagent has recently been developed for the detection of hairy cell leukemia cells, which coincidentally also express CD103 on their surface.[11] Using this new antibody, we found that CD103⁺ cells infiltrate all 4 major histological subtypes of ovarian cancer, reaching the highest densities in high-grade serous ovarian cancer (HGSC) lesions.[6] Importantly,

CD103⁺ TILs preferentially localize to the tumor epithelium in both ovarian and breast carcinoma lesions, and although most CD103⁺ TILs turned out to be CD8⁺ T cells, some tumors also contained high amounts of CD103⁺CD56⁺ natural killer (NK) cells. By univariate and multivariate analysis, CD103⁺ TILs were significantly associated with disease-specific survival in HGSC patients, a prognostic effect that was attributable to CD103⁺CD8⁺ T cells rather than to CD103⁺CD56⁺ NK cells.

Given their striking clinical significance, what can be inferred about the functional properties of CD103⁺ TILs? Earlier studies showed that CD8⁺ T cells upregulate CD103 upon antigenic stimulation in the presence of transforming growth factor-β (TGF-β).[9] This requirement for dual antigen/TGF-β co-stimulation suggests that CD103⁺ TILs might be actively responding to tumor-associated antigens (TAAs) in a TGF-β-rich tumor microenvironment. This hypothesis would also be consistent with our finding that CD103⁺ TILs display an activated effector memory phenotype (CD62L⁻CD27⁺CD28⁻HLA-DR^hi) and that the frequency of CD103⁺ TILs in ovarian cancer-associated ascites correlates with the level of TGF-β in the ascites
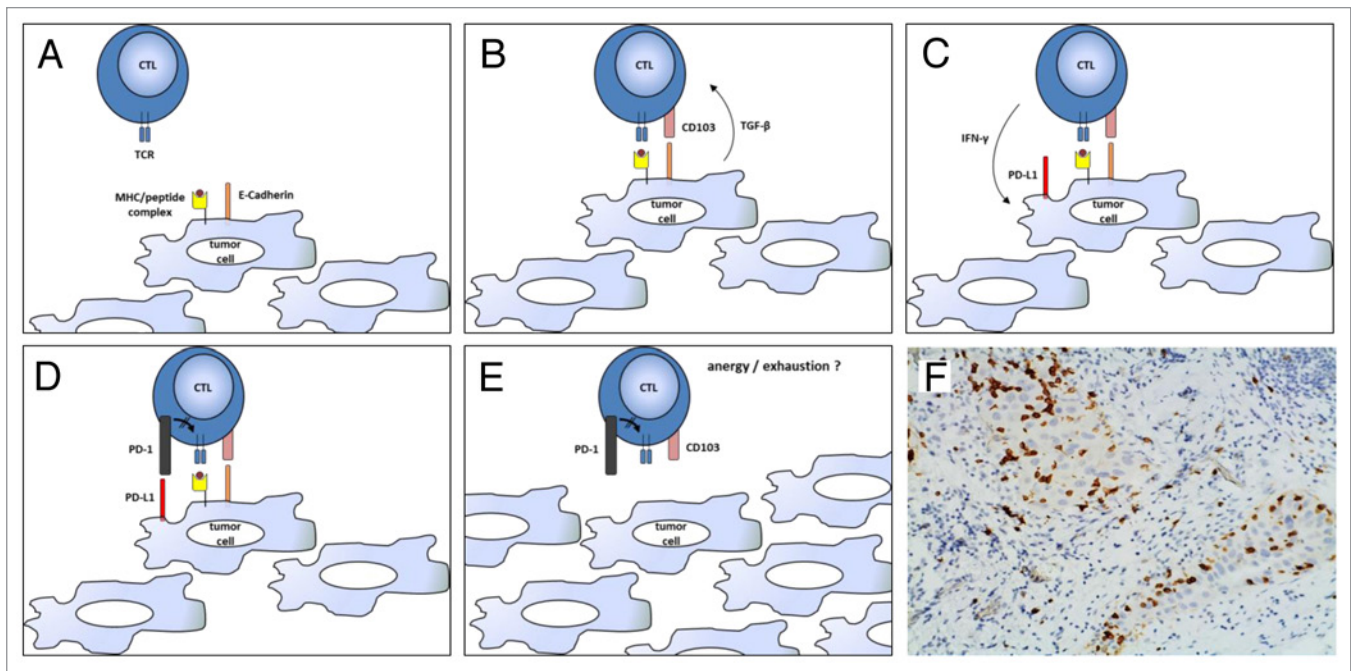
Figure 1. CD103+ lymphocytes infiltrating human ovarian carcinoma. (**A–E**) Ovarian cancer-infiltrating lymphocytes (TILs) express CD103 in response to tumor-associated antigen (TAAs) and transforming growth factor-β (TGF-β). CD103+ tumor-infiltrating lymphocytes (TILs) efficiently control tumor growth for a while, but then become trapped within neoplastic lesions as a consequence of CD103 expression, eventually becoming exhausted (PD-1+) owing to chronic antigen stimulation. (**F**) CD103+ TILs in a high-grade serous ovarian cancer specimen obtained from cytoreductive surgery. The section was stained with an anti-CD103 rabbit monoclonal antibody (Epitomics clone EPR4166[2]), an anti-rabbit horseradish peroxidase-conjugate antibody and diaminobenzidine (DAB). CD103+ TILs (stained in brown) can be seen clustering within epithelial tumor regions. CTL, cytotoxic T lymphocyte; IFNγ, interferon γ; PD-L1, PD-1 ligand 1; TCR, T-cell receptor.

fluid.[5] In addition, virtually all CD103+ TILs express TIA1, a marker of cytolytic activity that also has a high prognostic significance for ovarian cancer patients.[12] Thus, CD103 demarcates a subset of intraepithelial, activated, cytolytic CD8+ T cells exhibiting a highly favorable phenotype.

Despite their desirable phenotype, however, CD103+ TILs failed to impede primary tumor progression, as our patients presented with advanced disease requiring clinical intervention. Thus, we further speculate that tumor-specific CD103+ TILs were functional earlier in the oncogenic process, but have become "trapped" in the tumor bed as a consequence of CD103/E-cadherin adhesive interactions. This would result in a state of chronic antigen stimulation over a period of weeks to months, potentially leading to functional exhaustion. Consistent with this scenario, we have recently found that the vast majority of CD103+ cells infiltrating ovarian cancer lesions express the T-cell

exhaustion marker programmed cell death 1 (PDCD1, best known as PD-1),[6] suggesting that they are under the control of this well-characterized, peripheral immune checkpoint mechanism. To explain the association between CD103+ TILs with favorable disease outcome among ovarian cancer patients, one must remember that these cells are actually removed during surgical de-bulking, implying that they themselves cannot be responsible for improved prognosis. Therefore, we propose that CD103+ TILs act as a surrogate indicator of either a highly immunogenic tumor, or a patient with residual anti-tumor immunity in the periphery post-surgery, or both. Antitumor immunity could be successfully re-engaged upon cytoreductive surgery or chemotherapy and could impede tumor re-growth for some period, until the above described exhaustion scenario eventually repeats itself. Looking ahead, the fact that most CD103+ TILs express PD-1 raises the encouraging prospect that the functional

activity of these cells could be enhanced by PD-1-blocking interventions. Indeed, based on results from our group and others, CD103 warrants investigation as a predictive biomarker for clinical responses to checkpoint-blocking approaches. CD103 might also prove useful to isolate specific TIL subsets for functional studies, antigen discovery, or adoptive cell transfer-based therapeutic interventions.

In conclusion, our findings strongly suggest that CD103 is a definitive marker of intraepithelial, tumor-specific TILs in ovarian cancer, paving the way to the discovery of novel TAAs and perhaps the development of novel therapeutic strategies. We also find that intraepithelial CD103+ TILs are present in a small proportion of breast cancers, implying that CD103 may be a universal feature of TIL in epithelial cancers. If this were indeed the case, CD103+ cells might constitute prominent targets for the development of novel therapeutic regimens against these dreadful neoplasms. (**Fig. 1**)

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