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Letter to the Editor

Basement membrane promotes tumor development by attenuating T cell activation

Dear Editor,

T cells recognize and eliminate cancer cells. Prolonged survival of cancer patients is associated with not only intratumoral T cell infiltration but also the functional status of infiltrated T cells (Galon and Bruni, 2020). The immune contexture, including the number and functionality of T cells, is modulated by the tumor microenvironment (Joyce and Fearon, 2015; Galon and Bruni, 2020). The tumor microenvironment regulates the recruitment and accumulation of T cells, as well as the local activation, replication, and viability of T cells within tumors (Joyce and Fearon, 2015). Dynamic remodeling of the extracellular matrix (ECM) microenvironment, including basement membranes (BMs) underneath epithelial and endothelial cells, is one of the characteristics of cancer. ECM regulates immune cell adhesion and migration, and thus modulates inflammation in the tumor microenvironment, in addition to regulating cancer cell proliferation, migration, and invasion. T cells migrate poorly in dense collagen matrix areas surrounding tumor nests (Salmon et al., 2012; Joyce and Fearon, 2015). The resultant T cell trapping in the collagen matrix and exclusion from the tumor mass attenuate antitumor immunity and the efficacy of immune therapies (Salmon et al., 2012; Joyce and Fearon, 2015). Reduction of the collagen matrix density increased intratumoral T cell infiltration (Mariathasan et al., 2018; Peng et al., 2020; Nicolas-Boluda et al., 2021). However, it had minimal effect on tumor growth but improved the response to anti-PD-1 therapy (Mariathasan et al., 2018; Peng et al., 2020; Nicolas-Boluda et al., 2021), suggesting that infiltrated T cells were not properly functioning. Other factors, including the intratumoral ECM, may modulate T cell functionality in tumors.

BMs mediate tissue compartmentalization and transduce microenvironmental signals to cells that support tumor initiation and progression. We hypothesize that BMs may regulate T cell functionality in tumors. Analyses of the expression of BM proteins in The Cancer Genome Atlas (TCGA) pan-cancer atlas indicated strong negative correlation between the BM gene set variation analysis (GSVA) enrichment score and $CD8^+$ cytotoxic T cell infiltration score across diverse cancer types (Figure 1A). In addition, BM GSVA enrichment scores negatively correlated with the tumor-suppressive type 1 helper T cell (Th1)-type immune response, whereas they positively correlated with the tumor-promotive Th2-type immune response (Figure 1A). Matrigel, a reconstituted BM extracted from the Engelbreth–Holm–Swarm mouse sarcoma, is routinely coimplanted with cancer cells in xenograft tumor studies to facilitate xenograft tumor development (Fridman et al., 1990). We next sought to investigate whether Matrigel facilitates tumor formation by modulating tumor immunity. Highly malignant murine breast cancer 4T1 cells formed xenograft tumors (Figure 1B–E; Supplementary Figure S1A). While few $CD3^+$ T cells were detected in the 4T1 xenograft tumors with Matrigel, much more $CD4^+$ and $CD8^+$ T cells were detected in the tumors without Matrigel (Figure 1B and C).

Tumor-infiltrating T cells play critical roles in regulating tumor initiation and progression. In line with reduced T cell numbers in the presence of Matrigel, 4T1 cells coimplanted with Matrigel efficiently formed xenograft tumors in immunocompetent BALB/c mice, whereas 4T1 cells were less tumorigenic in the absence of Matrigel in the same immunocompetent mice, with delayed tumor onset and slowly growing xenograft tumors (Figure 1D and E; Supplementary Figure S1A). At Day 7 after implantation, 4T1 xenograft tumors were significantly larger in the presence of Matrigel (Supplementary Figure S1B), which could not be explained by the moderate promoting effects of Matrigel on cancer cell proliferation *in vitro* (Supplementary Figure S1C). In addition, Matrigel did not significantly accelerate the onset of 4T1 xenograft tumors (Figure 1D and E), but moderately promoted 4T1 xenograft tumor growth in immunodeficient nude mice (Figure 1D and E; Supplementary Figure S1A). Matrigel similarly accelerated tumor onset of murine colon adenocarcinoma MC-38 cells in immunocompetent C57BL/6 mice, but not in the nude mice (Supplementary Figure S2A and B). These data collectively suggest that BMs may promote tumor initiation mainly via modulating tumor immunity.

Laminins, key components of BMs, were reported to directly reduce T cell chemotaxis and thus result in T cell exclusion (Li et al., 2021). Matrigel or laminin-111 did not affect T cell transmigration (Supplementary Figure S3A). Cancer cells recruit T cells via secreted chemokines. 4T1 cell-conditioned media robustly increased T cell chemotaxis, regardless of whether 4T1 cells were cultured on Matrigel or not (Supplementary Figure S3B).

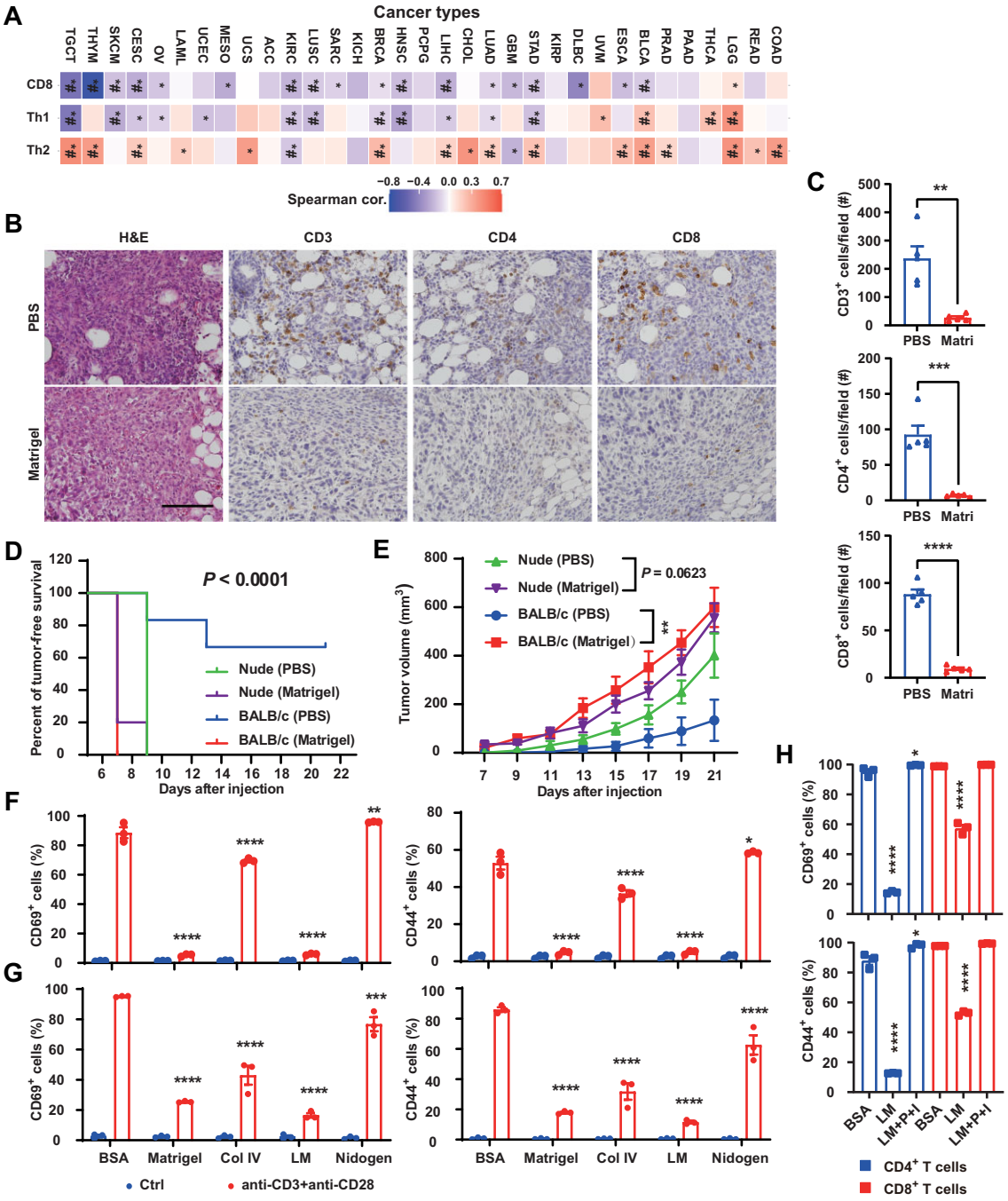

Figure 1 BM proteins reduce T cell activation and promote tumor growth. (**A**) Correlation of BM signature GSVA enrichment score to $CD8^+$ T, Th1, and Th2 cell infiltration score in TCGA pan-cancer atlas. TGCT, testicular germ cell tumors; THYM, thymoma; SKCM, skin cutaneous melanoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; OV, ovarian serous cystadenocarcinoma; LAML, acute myeloid leukemia; UCEC, uterine corpus endometrial carcinoma; MESO, mesothelioma; UCS, uterine carcinosarcoma; ACC, adrenocortical carcinoma; KIRC, kidney renal clear cell carcinoma; LUSC, lung squamous cell carcinoma; SARC, sarcoma; KICH, kidney chromophobe; BRCA, breast invasive carcinoma; HNSC, head and neck squamous cell carcinoma; PCPG, pheochromocytoma and paraganglioma; LIHC, liver hepatocellular carcinoma; CHOL, cholangiocarcinoma; LUAD, lung adenocarcinoma; GBM, glioblastoma multiforme; STAD, stomach adenocarcinoma; KIRP, kidney renal papillary cell carcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; UVM, uveal melanoma; ESCA, esophageal carcinoma; BLCA, bladder urothelial carcinoma; PRAD, prostate adenocarcinoma; PAAD, pancreatic adenocarcinoma; THCA, thyroid carcinoma; LGG, brain lower grade glioma; READ, rectum adenocarcinoma; COAD, colon adenocarcinoma. (**B**) CD3, CD4, or CD8 immunohistochemical staining on 4T1 xenograft tumor sections implanted in BALB/c mice with phosphate-buffered saline (PBS) or Matrigel.

The tumor microenvironment is the major site of clonal expansion of cancer-specific T cells (Thompson et al., 2010; Joyce and Fearon, 2015). Coimplantation with Matrigel significantly reduced the percentage of functional IFN-γ^+ $CD4^+$ and $CD8^+$ T cells in MC-38 xenograft tumors (Supplementary Figure S2C), suggesting that T cell adhesion to Matrigel in the tumor mass may directly affect T cell activation and function. Upon activation, T cells express the cell surface molecules CD69 and CD44, which reflect the activation status of T cells and contribute to further T cell activation. Matrigel completely abolished $CD4^+$ T cell activation (Figure 1F; Supplementary Figure S4A and B) and reduced $CD8^+$ T cell activation *in vitro* (Figure 1G; Supplementary Figure S4A and C). Type IV collagens, laminins, and nidogens are the major BM components (Pozzi et al., 2017). Type IV collagens and laminins self-assemble into independent networks that are interconnected by nidogens to form the BMs (Pozzi et al., 2017). We next sought to investigate which components in the BMs inhibit T cell activation. Nidogen had minimal effect on $CD4^+$ and $CD8^+$ T cell activation, while type IV collagen partially inhibited $CD4^+$ and $CD8^+$ T cell activation (Figure 1F and G; Supplementary Figure S4). Laminin-111, the major laminin in Matrigel, inhibited $CD4^+$ and $CD8^+$ T cell activation to an extent comparable with Matrigel (Figure 1F and G; Supplementary Figure S4). Laminin-111 also significantly reduced the percentage of functional IFN-γ^+ $CD4^+$ and $CD8^+$ T cells (Supplementary Figure S5). In addition to affecting T cell activation and function, laminin-111 significantly reduced the percentage of $Ki67^+$ proliferating $CD4^+$ and $CD8^+$ T cells (Supplementary Figure S6A and B) and increased the percentage of apoptotic $CD4^+$ and $CD8^+$ T cells (Supplementary Figure S6C and D). Thus, laminins are the major Matrigel components regulating T cell activation, functionality, proliferation, and survival.

Laminins are heterotrimeric glycoproteins composed of one α, one β, and one γ chain. Five α, four β, and three γ chains form at least 16 laminin molecules. Laminins affect leukocyte adhesion, migration, proliferation, and survival. Besides laminin-111, laminin-211, laminin-221, laminin-332, laminin-411, laminin-421, and laminin-511 partially or completely inhibited $CD4^+$ T cell activation (Supplementary Figure S7A and B). Laminin-332 is essential for tumor initiation and the maintenance of tumor cell proliferation. Laminin γ2 chain overexpression results in T cell exclusion and attenuates the response of patients to anti-PD-1 therapy (Li et al., 2021). Laminin-332, however, did not robustly regulate $CD8^+$ T cell activation. Laminin-111, laminin-211, laminin-221, and laminin-411, but not other laminins, inhibited $CD8^+$ T cell activation (Supplementary Figure S7C and D).

To explore the mechanism of how laminins inhibit T cell activation, T cell receptor (TCR) signaling in $CD4^+$ and $CD8^+$ T cells was analyzed. T cell activation requires phosphorylation of ZAP70 and PLCγ to initiate antigen-specific TCR signaling. Such proximal TCR signaling was diminished upon laminin treatment in both $CD4^+$ and $CD8^+$ T cells (Supplementary Figure S8A). Treatment with phorbol 12-myristate 13-acetate (PMA) and ionomycin efficiently restored $CD4^+$ and $CD8^+$ T cell activation (Figure 1H; Supplementary Figure S8B and C).

BMs mediate tissue compartmentalization and transduce microenvironmental signals to cells. Rather than just a structural component of tissues, BMs have been recognized as an important regulator of cell behavior. BM components are critical in tumor initiation. Besides its well-documented functions in promoting cancer cell proliferation, protecting cancer cells from chemotherapy-induced apoptosis, and promoting tumor neoangiogenesis, BMs have also been proposed to regulate tumor immunity. BMs, in particular laminins, not only modulate T cell adhesion and migration (Li et al., 2021) but also directly regulate T cell activation, functionality, proliferation, and survival. Laminin-111 does not regulate T cell migration and is a potent inhibitor of T cell activation and proliferation. However, the laminin γ2 chain modulates T cell adhesion and migration, which results in T cell exclusion (Li et al., 2021), but not $CD8^+$ T cell activation. Thus, different laminins may differentially participate in regulating the recruitment of T cells or the local activation, replication, and viability of T cells within tumors, and then synergistically regulate immune contexture. Deregulated BM expression and remodeling thus directly participate in immunosurveillance during tumor initiation and progression.

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Figure 1 *(Continued)* Scale bar, 100 μm. (**C**) Quantitation of intratumoral T cell numbers per field in 4T1 xenograft tumor sections implanted in BALB/c mice with PBS or Matrigel (Matri) ($n = 5$). (**D**) Kaplan–Meier tumor-free survival analysis of BALB/c and nude mice implanted with 4T1 cells with PBS or Matrigel ($n = 5$; except for the group of BALB/c (PBS), $n = 6$). (**E**) Growth kinetics of 4T1 xenograft tumors implanted in BALB/c or nude mice with PBS or Matrigel ($n = 5$; except for the group of BALB/c (PBS), $n = 6$). (**F** and **G**) Naïve $CD4^+$ (**F**) and $CD8^+$ (**G**) T cells were stimulated with anti-CD3 and anti-CD28 antibodies in the presence of bovine serum albumin (BSA), Matrigel, type IV collagen (Col IV), laminin-111 (LM), and nidogen for 48 h. Percentages of $CD69^+$ and $CD44^+$ T cells were analyzed by flow cytometry. (**H**) Naïve $CD4^+$ and $CD8^+$ T cells were stimulated with anti-CD3 and anti-CD28 antibodies with or without PMA/ionomycin (P+I) in the presence of BSA or LM for 48 h. Percentages of $CD69^+$ and $CD44^+$ T cells were analyzed by flow cytometry. Data are mean ± SEM. Statistical analyses were performed with log-rank test (**C**), two-way ANOVA followed by Sidak's multiple comparison test (**D**), or two-way ANOVA followed by Dunnett's multiple comparison test (**E**–**G**). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. $^{\#}$false-discovery rate <0.05.

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