JCB Journal of Cell Biology

SPOTLIGHT

An unexpected role for PD-L1 in front–rear polarization and directional migration

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Programmed cell death-ligand 1 (PD-L1)–mediated T cell inhibition through PD-1 is a key checkpoint frequently exploited by tumors to evade immunity. In this issue, Wang et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202108083) reveal an unexpected role for PD-L1 in promoting tumor cell front–rear polarity and directionally persistent cell migration, independently of PD-1.

Programmed cell death-ligand 1 (PD-L1) was characterized in the early 2000s as part of a specific immune checkpoint mechanism through its recognition by the programmed cell death protein 1 (PD-1) receptor in T cells, which propagates an inhibitory signal suppressing proliferation and cytotoxic activity (1). While the physiological roles of this checkpoint mechanism—which underlies autoimmune disorders such as diabetes, systemic lupus erythematosus, or psoriasis—are still being characterized, its identification as a major resource for tumor evasion from immunosurveillance has focused much research (1, 2). Many tumors exhibit upregulated PD-L1 expression, creating an immunosuppressive microenvironment that significantly contributes to disease progression, and the intervention of this mechanism is one of the current spearheads in antitumor therapy for several cancer types (3; Fig. 1 A).

Increased PD-L1 expression in tumors is associated with higher aggressiveness and mortality. But is the engagement of PD-1–mediated suppression of T cell activity the only mechanism involved? Recently, PD-L1 has been proposed to modulate pro-survival and growth-promoting signaling networks intrinsically in tumor cell lines expressing this receptor, although the potential precise mechanisms have not been dissected in detail (4, 5).

In this issue of the *Journal of Cell Biology*, Wang et al. (6) report the impact of selectively disrupting the expression of PD-L1 on the motility and migration of different tumor cell lines in vitro. PD-L1 depletion significantly attenuated directionally persistent migration in all studied cell models, and this effect was rescued by a PD-L1–EGFP expression construct in the absence of detectable levels of PD-1, implying a specific, cell-autonomous effect (6). Wang et al. show that PD-L1 accumulates at the cell rear, where it interacts with β4-integrin, and is required for the formation of retraction fibers and migrasomes—extracellular membrane-bound structures forming from the rear of migrating cells that can in turn transmit information to other cells. Importantly, the authors provide substantial evidence indicating that PD-L1 depletion impacts plasma membrane (PM) organization and front–rear polarity normally displayed by migrating cells. First, other polarity markers such as a $PIP_3/PI_{(3,4)}P_2$ biosensor (derived from the pleckstrin homology domain of protein kinase B (PKB/AKT), which should accumulate at the leading edge) and Thr567 phospho-ezrin (which accumulates at the cell rear) were evenly distributed in PD-L1^{KO} cells. Second, the decreasing tension gradient associated with active cell migration was dissipated upon PD-L1 depletion, a pattern correlating with reduced caveolae accumulation at the cell rear as inferred by direct immunostaining and cholera toxin B binding (7). Further, hyposmotic swelling, increasing cell tension at the rear region of wild type cells, was sufficient to reduce β4 integrin accumulation to levels similar to those in PD-L1^{KO} cells. The authors propose an interesting model whereby reduced PM tension at the cell rear is required for β4-integrin accumulation and further rear retraction (potentially through the accumulation of caveolar domains), and this tension gradient requires PD-L1 expression.

What are the molecular mechanisms involved? While PD-L1 accumulates at the cell rear and interacts there with β4 integrin, downstream mechanisms could be directly responsible for polarized PM organization. PD-L1 expression correlates with enhanced mechanistic Target of Rapamycin (mTOR) signaling and WAS/WASL-interacting protein family member 1 (WIP)/β-catenin activation in tumor cells (4, 5). WIP is a well-established regulator of actin cytoskeleton dynamics; mTORC2 can, among several outputs, promote polarized cell migration. Indeed, RhoA activity is engaged in migrating cells in a PD-L1–dependent manner (6). However, a non-palmitoylable PD-L1^{C272A} mutant was unable to rescue front–rear polarity in PD-L1^{KO} cells, indicating that PD-L1 does need to efficiently reach the PM to exert its organizing effect,

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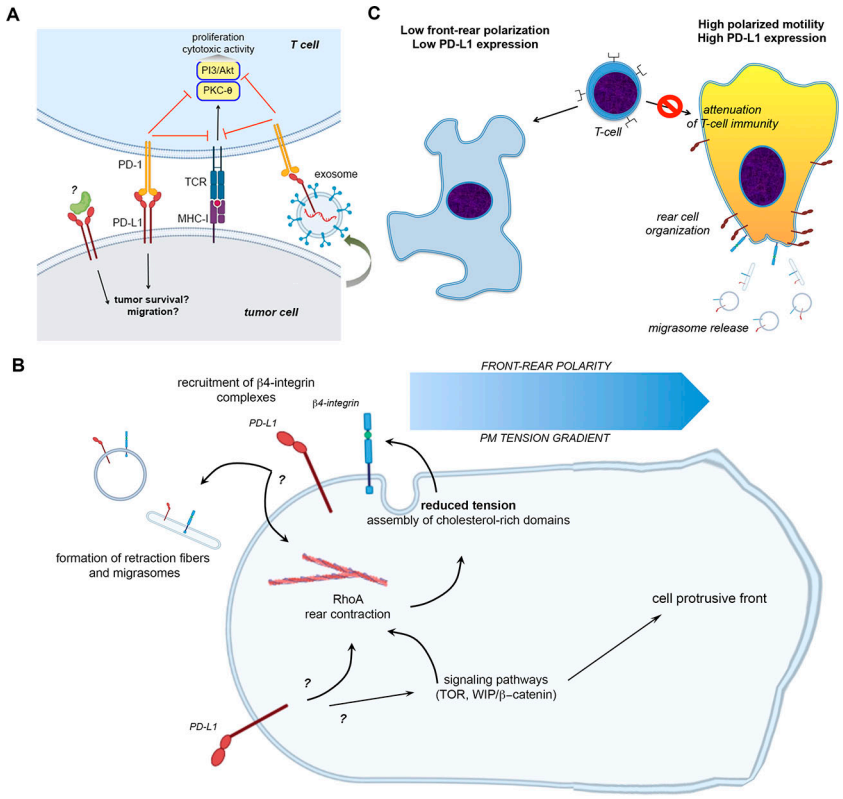

Figure 1. **PD-L1 has cell-intrinsic roles beyond the immune checkpoint. (A)** Main known features of the PD-1/PD-L1 immune checkpoint. **(B)** PD-L1 is a key regulator of mechanisms organizing cell rear retraction and migrasome generation through the organization of PM domains at this region of lower tension. These mechanisms contribute to the sustaining of front–rear polarity and directional migration. **(C)** Tumor cell phenotypes of marked front–rear polarity and directional migration would thus potentially be associated or coordinated with the efficient engagement of PD-L1–dependent immunosuppression.

potentially arguing for a more direct influence on cell polarity. PD-1 expression in the isolated in vitro systems used was undetectable; whether an alternative PD-L1 ligand acts in an autocrine/paracrine fashion to promote the novel positive regulation of directional migration described here is unknown. Studies on PD-L1 interactomes (particularly of the cytoplasmic region of this receptor), comparing conditions of polarized migration to backgrounds suppressing cell polarity (for example, Cdc42 depletion), might constitute a strategy to reveal candidate molecular partners (apart from β4 integrin) associating with PD-L1 to promote this activity, with higher detail.

A non-exclusive mechanism could consist on a capacity of PD-L1 to bias the composition of PM subdomains, for example by promoting the assembly of caveolae, which spatially coincide with the rear cell zone where PD-L1–β4 integrin interaction takes place. Indeed, caveolar absence (by knocking out its main component caveolin-1) shows a phenotype uncannily similar to PD-L1^{KO} phenotypes, in terms of loss of persistent migration and increased PM tension at the cell rear (7–9). Cholesterol organization is key for the assembly of specialized PM domains, and their roles in cell migration (8). Of note, apart from constituting a target for type I interferon responses, PD-L1 is a gene that is highly responsive to cholesterol homeostasis surveillance mechanisms (10), which in turn are part of networks involved in tumor cell survival and migration. These mechanisms could hypothetically establish positive loops contributing to sustain front–rear polarity, rear retraction, and cell motility with persistent direction (Fig. 1 B).

Further research will be required to unravel the relevance of these mechanisms in the tumor microenvironment in vivo. PD-L1 expression might constitute a hub integrating tumor cell migration and related cell activities (communication through migrasomes or extracellular matrix sensing) in a PD-1–independent manner, and the protection from immune surveillance through PD-1–dependent attenuation of T-cell activity, thus coordinating tumor cell migration and immune escape (Fig. 1 C). These relationships might also provide a novel conceptual ground, beyond the strict dynamics of the two components of the PD-1/PD-L1 immune checkpoint, to interpret associations between patient cholesterol metabolism (and concomitant interventions, such as through statins) and the response to immunotherapy regimes (10). As such, the study from Mercurio and colleagues encourages revisiting PD-L1 biology and its impact on tumor behavior, and the assessment of therapeutic interventions beyond tumor immunomodulation.

Acknowledgments

Open-source features from the BioRender platform were used to prepare Fig. 1, A and B.

M. Sánchez-Álvarez acknowledges support from the Tec4Bio consortium (of which M.Á. del Pozo is co-coordinator; ref. S2018/NMT4443, Actividades de I+D entre Grupos de Investigación en Tecnologías, Comunidad Autónoma de Madrid/Federación Española de enfermedades raras, Spain) and a Centro Nacional de Investigaciones Cardiovasculares (CNIC) International Postdoctoral Programme COFUND Marie Skłodowska-Curie Actions fellowship, and is currently a recipient of a Ramón y Cajal research contract from the Spanish Ministry of Science and Innovation (RYC2020-029690-I). Research at M.Á. del Pozo's lab is supported by grants from the Spanish Ministry of Science and Innovation (PID2020-118658RB-I00, SAF2017-83130-R), La Caixa Health Research Programme (HR20-00075, AtheroConvergence), La Marató TV3 (201936-30-31), and the Asociación Española Contra el Cáncer (PROYE20089DELP). The CNIC is supported by the Instituto de Salud Carlos III, the Ministerio de Ciencia e Innovación, and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MICIN/AEI/10.13039/501100011033).

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