JCB Journal of Cell Biology

SPOTLIGHT

An in vivo phosphoregulation paradox for focal adhesions

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Focal adhesions (FAs) dynamics regulate single cell migration. In this issue, Xue et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202206078) show that Y118 phosphorylation on Paxilin, a key FA protein, limits migration of cells in vivo. Unphosphorylated Paxilin is necessary for FA disassembly and cell motility. Their findings directly contradict results from in vitro experiments, emphasizing the need for recreating the in vivo complexity to understand how cells behave in their native environments.

Cells use focal adhesions (FAs) as sensory hubs to receive, interpret, and transduce signals from their environment (1–3). FA complexes are composed of hundreds of proteins whose relative abundance, stoichiometry, and architecture influence mechanosensing, migration and regulation of intracellular signaling (1–3). While the interaction between FA dynamics and cell migration has been studied in culture conditions that mimic some aspects of in vivo physiology and tissue architectures (1–3), the influence of FAs on migration of cells in complex, living environments is unclear. In this issue, Xue et al. address an existing knowledge gap in our understanding of the molecular regulation of FAs in cell migration in tissues with complex physicochemical properties in a living animal (4). Using a suite of transgenic zebrafish lines, mouse models, intravital imaging, and molecular tools, the authors found that there is a differential regulation (in vitro vs. in vivo) of tyrosine 118 (Y118) of Paxillin, a key component of FAs (Fig. 1). Reduced phosphorylation of Y118 during adhesion formation resulted in an increase in in vivo single cell migration concomitant with a heightened rate of FA disassembly for zebrafish macrophages and melanoma cells. Examination of co-factors in the signaling cascade revealed downregulation of FAK and an enhanced interaction with CRKII-DOCK180/RacGEF in murine tumors. These results directly contrast findings observed in 2D cell culture models (5), prompting a re-examination of our understanding of the interplay between dimensionality and molecular regulation of in vivo cell migration. Additionally, the zebrafish model the authors generate is an advantageous tool for refining our understanding of in vivo regulation—this system allows for the systematical modulation of signaling components and visualization of subcellular dynamics in multiple tissues. Moreover, the larval zebrafish is amenable to optical techniques that can be used in tandem to decipher how cells traverse complex tissues during immune surveillance and cancer cell escape and transit.

The visualization of FAs in 3D culture and animal models reignited an earlier controversy of the presence and therefore significance of FAs in vivo (2, 3). The study by Xue et al. (4) has made a profound contribution to our understanding of the molecular regulation of FA formation and disassembly for multiple cell types in vivo, thereby strengthening the physiological relevance of FA-mediated single and collective cell migration strategies. Seminal work using fluorescence-based super resolution and quantitative methods such as Number and Brightness determined the spatial organization and dynamics of multiple components of the FA stratum (6, 7). These discoveries have been instrumental in our comprehension of integrin-mediated ECM binding and migration on different 2D environments. Thus, the in vivo findings of differential co-regulation of partners of Paxillin within the signaling hubs necessitate a renewed examination of FA stoichiometry and nanoscale architectures. The logical next step would be to apply these techniques to the zebrafish model for direct comparison. In addition to differences in size, clustering of integrins and the intracellular cytoskeletal interactions may also be differentially regulated, which will have implications for cell contractility and integrin-mediated cell migration.

Cells sense external biophysical cues via FAs to drive cell fate decisions (1, 3). For instance, integrin-mediated mechanosensing regulates cellular outcomes in response to tissue anisotropies that drive direct cell migration, proliferation, and polarity (1, 3). Previous studies have shown that oscillations of traction forces, mediated by distinct actin networks, sense environmental stiffness which influences Paxillin phosphorylation for

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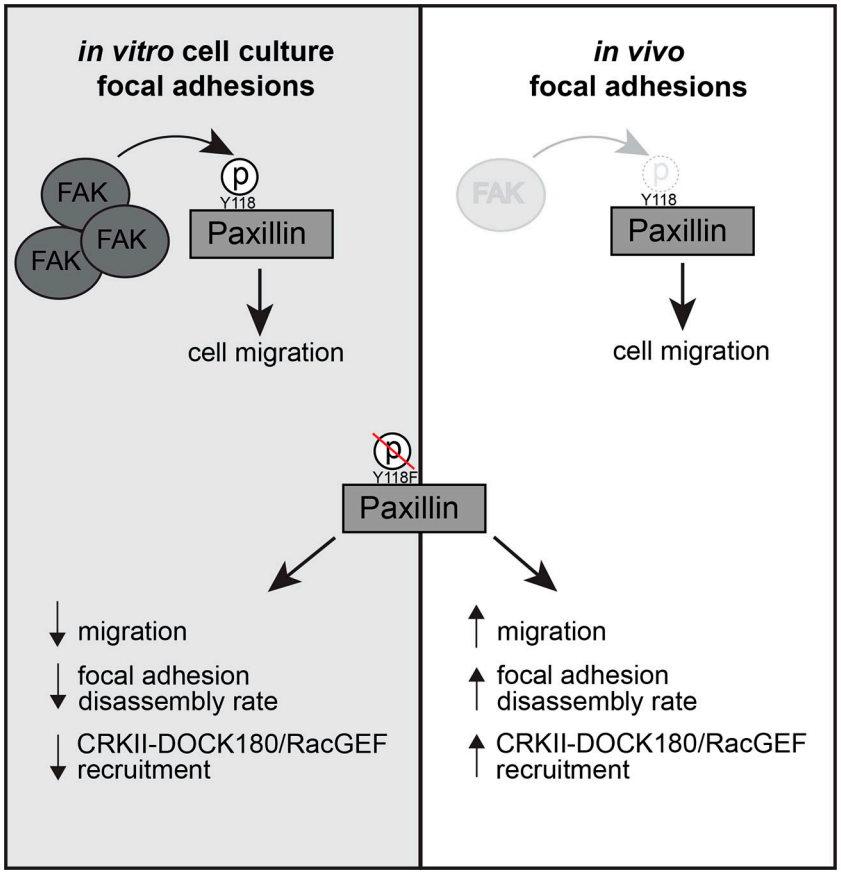

Figure 1. **Working model for how Y118-Paxillin phosphorylation status regulates cell migration in the in vitro cell culture and in vivo conditions.** Top: Under in vitro cell culture conditions, FAK phosphorylates Paxillin on Y118, leading to high levels of Y118-Paxillin phosphorylation in migrating cells. In migrating cells in vivo, FAK levels are low, and Y118-Paxillin lacks phosphorylation. Bottom: Expression of the non-phosphorylatable Y118F-Paxillin leads to reduced cell migration in vitro compared with cells expressing the Y118E-Paxillin phosphomimetic, likely through reduced focal adhesion disassembly rates and reduced CRKII-DOCK180/RacGEF recruitment to Paxillin-positive focal adhesions. However, in vivo, cells expressing the non-phosphorylatable Y118F-Paxillin exhibit increased cell migration, likely through increased focal adhesion disassembly rates, and increased recruitment of CRKII-DOCK180/RacGEF to Paxillin-positive focal adhesions. Figure and legend extracted from Xue et al. (4) with permission of the authors.

mechanotransduction—the stiffer the environment, the more fraction of cells with phosphorylated Paxillin(Y118) (8, 9). However, the authors observed that zebrafish melanoma cells did not show a differential phosphoregulation when cultured on substrata ranging in values of Young's Moduli from 500 Pa to >GPa. One reason for this finding could be the differences in integrin clustering in 2D vs. 3D environments to sense stiffness. A second reason could be that tuning the Young's modulus only does not recreate the full mechanical profile of the tissue in vivo, which additionally exhibits "viscoelastic properties." Finally, the range of values tested in vitro may differ from those that cells experience in the living animal. Hence, translating the in vitro findings to complex environments will require mechanical mapping of tissues in vivo as cells migrate. Thus, to explore FA dynamics in cells migrating through complex environments, Xue et al. (4) used a wound healing assay where the tail is amputated in the zebrafish and visualized macrophage recruitment over several hundreds of microns in different tissue types. Recent optical techniques compatible with intravital imaging, such as optical tweezer active microrheology and Brillouin microscopy, have been used to map microscale tissue mechanical properties in living larval zebrafish (10). One exciting aspect of the model system developed here is its potential to decode FA-mediated mechanosensing in vivo for a plethora of tissue architectures and viscoelasticity.

Furthermore, the work by Xue et al. (4) has implications for human-related disease. Many types of cancers such as breast, prostrate, colorectal, melanoma, and glioblastoma multiform display alterations in the expression, mutation, and phosphorylation of Paxillin (11). These alterations may influence cancer progression due to altered signaling or enhanced ability of cancer cells to migrate, invade, transit, and spread to distal sites. Here, using a two-pronged approach of an allograft model of zebrafish melanoma cell lines in zebrafish and a mouse melanoma model carrying the same genetic mutation implicated in human melanomas, the authors demonstrated that the altered phosphoregulation also affected single cell migration of melanoma cells in vivo. They examined melanoma migration within a specialized region of the zebrafish brain, which may more accurately recapitulate cancer behavior in a metastatic site. It would be powerful to examine the role of Paxillin dynamics as a function of the metastatic cascade, i.e., exit from primary tumor to establish a metastatic lesion. The melanoma model the authors engineered in zebrafish, which displays stepwise progression of cancer with defined timing and is relevant to human disease, can indeed provide such a platform to study transformation from normal to malignant, and the successive stages of escape and colonization.

Overall, the findings reported by Xue et al. (4) can explain how cancer cells adapt to dynamically changing physico-chemical stromal milieu and how detached cells are able to survive and migrate within challenging environments. These insights, combined with the alteration of other dysregulated FA proteins, such as FAK, may provide a druggable target for reducing metastasis-related invasion.

Acknowledgments

This effort was supported by the Intramural Research Program of the National Institutes of Health and the National Cancer Institute.

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