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RAPID COMMUNICATION

Myosin regulates intracellular force and guides collective cancer cell migration via the FAK-Rho/ROCK feedback loop

Breast cancer has been one of the biggest killers of women due to its susceptibility and high metastasis. Pathological observations show that malignant cancer cells frequently invade the surrounding normal tissue in collective rather than individual cell migration.[1,2] For individual cell migration, it has been found that the Rho/ROCK signaling is up-regulated and correlates with disease progression.[3] Meanwhile, Rho activates myosin-II and the actomyosin-mediated contraction creates tension within the cells.[4,5] However, the roles of the activation of the Rho/ROCK signal pathway in collective cell migration and the precise mechanisms by which myosin-II fine-tunes the contractility of the cells to allow for the reorganization of the cytoskeleton that drives collective cell migration, remain unclear. This study investigated whether the high cellular contractility could activate the inside-out signal transductions and how did the intracellular force contribute to the collective cell migration.

To address the involvement of NM-II in the collective migration of cancer cells, we constructed three stably transfected cell lines: WT (wild-type), NM-II-T18D/S19D (myosin-activated), and NM-II-T18A/S19A (myosin-inactivated) (Fig. S1A–E). The MCF-7 represents a typical epithelial cell line with strong cell–cell junctions. We found that activated myosin leads to a decrease in wound healing rate, speed, persistence, and directionality (Fig. 1A–E). Moreover, cells in the myosin-inactivated group were more polarized (Fig. S2A, B). The results indicated that high NM-II significantly blocks MCF-7 collective cell migration.

Cell migration is controlled by adhesion maturation. Hence, we investigated whether NM-II affects adhesions. Focal adhesions were stained with paxillin, and we counted the adherent spots in the cells according to their size into mature ($>1\ \mu m^2$) and immature ($\leq 1\ \mu m^2$) fractions. It was found that activated myosin caused cells to produce an increased number of larger and more mature adherent spots (Fig. 1F–H). These adhesions, not only are detrimental to cell migration but also make it necessary for the cells to overcome the contractile force which drives individual cell migration. We further examined the mechanism by which NM-II mediated cell adhesion (Fig. 1I). We found that p-FAK in the T18D/S19D (myosin-activated) group was significantly stronger than that in wild-type cells; similar results were obtained by western blotting (Fig. 1J, K). We also investigated FAK activity by FRET, which labels FAK in cells and emits fluorescence of different intensities depending on their spatial distance. FRET in the phosphorylated mutant myosin was low, indicating that the high cellular stress stretched the structure of FAK and increased its phosphorylation (Fig. S3A, B).

To further investigate whether the activation of FAK is due to the activated myosin directly or indirectly, we verified FAK activation by light-activated reversible inhibition by assembled trap (LARIAT). When LARIAT is activated in specific cells, it will specifically aggregate myosin, resulting in the loss of GFP-NM-II function (Fig. 1L). We found that the optogenetic inhibition of myosin resulted in a significant decrease in intracellular p-FAK, Rho, and ROCK activity (Fig. 1M), which indicates that the myosin could directly affect upstream FAK-Rho-ROCK. We also revealed that the phosphorylated myosin mutant group had higher Rho and ROCK activity by antibody staining (Fig. S4A, B). Western blot experiments confirmed the findings (Fig. S4C, D). Based on the above results, it is suggested that the high intracellular stress leads to the activation of FAK-Rho-ROCK by optogenetics in a feedback way.

We also conducted the *in vivo* assay to confirm the roles of myosin. Tumors in the myosin-activated group were found to be significantly smaller, which indicated that cell

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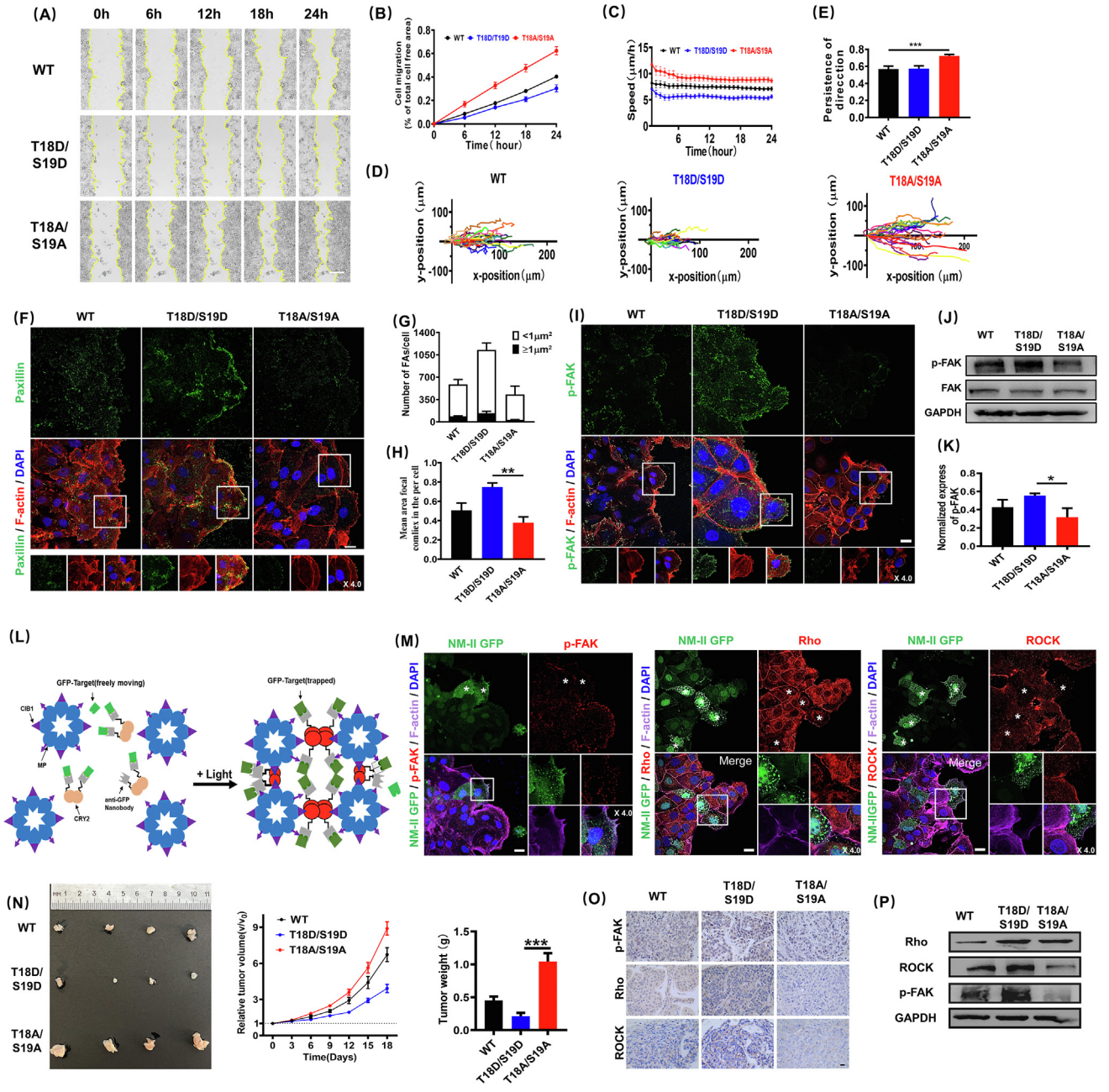

Figure 1 Myosin-mediated intracellular force regulates collective cancer cell migration via the feedback loop of FAK-Rho-ROCK signal pathway. **(A)** Wound-healing assay of wild-type NM-II, NM-II-T18D/S18D, and NM-II-T18A/S19A. Scale bar = 150 μm. **(B, C)** Quantification of the relative closure of scratch (B) and speed (C). **(D)** A histogram reporting cell persistence of directions with different levels of phosphorylation of NM-II, respectively. **(E)** Quantifications of direction persistance. ***$P < 0.001$. **(F)** Micrographs of paxillin captured by confocal microscopy. Scale bar = 20 μm. The white boxed areas were enlarged. **(G)** Quantifications of focal adhesions (FAs) number. $n = 30$. **(H)** Mean area per focal adhesion for the three representative images ($n = 20$). **$P < 0.01$. **(I)** Micrographs of p-FAK captured by confocal microscopy. Scale bar = 20 μm. The white boxed areas were enlarged. **(J, K)** The expression and quantification of the phosphorylated FAK by western blotting. *$P < 0.05$. **(L)** Schematic of GFP-protein trapping by CRY-2 anti-GFP nanobody. **(M)** The use of visible light for activating the GFP-proteins trapping system (* means the activated cells). The white boxed areas were enlarged. Scale bar = 20 μm. **(N)** Tumor growth curves (normalized to initial volume) and tumor weight. **(O)** Immunofluorescence staining of p-FAK, Rho, and ROCK from tumors. Scale bar = 40 μm. **(P)** Proteins from BALB/c mice tumor tissues were verified by western blotting for Rho, ROCK, and p-FAK.

expression-activated myosin had less malignancy (Fig. 1N). The results of immunohistochemistry and tissue western blotting revealed that over-activated myosin significantly increased the activity of p-FAK-Rho/ROCK (Fig. 1O, P).

In summary, the study indicates that myosin-activated cells display increased actomyosin contractility, which translates into slower collective migration with less malignancy. Myosin activation could activate the upstream FAK thus regulating the Rho-ROCK pathway, and further strengthening the NM-II phosphorylation. FAK could further induce the focal adhesion paxillin formation to strengthen the adhesion force between the cell and extracellular matrix, which acts as a principal factor mediating collective cell migration (Fig. S5). Our work highlights that myosin-mediated cell adhesion is the key factor for collective cell migration, which provides a new research direction for probing the mechanism of breast cancer cell collective migration.

Author contributions

X.Q. conceived and designed the research, Y.H. and Y.Z. performed experiments and analyzed the results. X.Q. and Y.H. drafted the manuscript. S.L., T.L., and F.Y. discussed the data. Y.L. supervised the project. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no potential conflict of interests in this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.02.037.

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