豆包结果：

Discussion

Accumulating evidence indicates that the mechanical properties of the tumor microenvironment, particularly extracellular matrix (ECM) stiffness, are pivotal regulators of breast cancer progression, invasion, and therapeutic response [1,2]. Focal adhesion kinase (FAK), a central transducer of integrin-mediated mechanosignaling, is frequently overactivated in triple-negative breast cancer (TNBC) and represents a promising therapeutic target. In this study, we investigated how matrix stiffness modulates the cellular effects of Compound X, a novel FAK inhibitor, in two TNBC cell lines. Our results demonstrate that while Compound X suppresses FAK phosphorylation equally across soft and stiff matrices, it exerts a significantly stronger inhibitory effect on collective cell migration on stiffer substrates. This stiffness-dependent anti-migratory effect is associated with differential disruption of focal adhesions and actin cytoskeleton organization, whereas the anti-proliferative activity of Compound X is independent of ECM rigidity. These findings were further validated by FAK knockdown and the reference FAK inhibitor defactinib, confirming FAK as the key mediator of this mechanomodulated response.

FAK knockdown与defactinib结果支持FAK参与，但不足以“confirm”FAK为该效应的key/唯一mediator。

Our observation that TNBC cells exhibit enhanced spreading, more prominent stress fibers, and increased FAK activation on stiffer matrices aligns with previous reports linking matrix rigidity to breast cancer cell behavior. For instance, it has been demonstrated that single-cell populations derived from MDA-MB-231 exhibit differential matrix rigidity responses that correlate with their metastatic tropism: bone-tropic sublines thrive on rigid matrices, whereas lung-tropic sublines prefer softer environments. The two TNBC lines used in the present study, MDA-MB-231 and BT-549, are both highly invasive and exhibit broad metastatic potential. Our data extend these findings by identifying FAK activation as a critical molecular event underlying stiffness-enhanced migration. The 1.8- to 2.2-fold increase in FAK Tyr397 phosphorylation on stiff gels is functionally coupled to the assembly of larger focal adhesions and more robust stress fibers, which generate the traction forces necessary for cell locomotion. Consequently, disrupting FAK signaling has a more profound impact on migration when cells are anchored to a rigid ECM, as this context imposes a greater dependence on integrin–FAK mechanotransduction.

Notably, the magnitude of FAK phosphorylation inhibition by Compound X was comparable between soft and stiff conditions, yet the downstream functional effects on migration and adhesion structures diverged markedly. This suggests that the cellular response to FAK inhibition is gated by the baseline mechanical state of the cell. On stiff matrices, cells form larger, more stable focal adhesions and thicker actomyosin stress fibers to resist and counteract ECM rigidity. This heightened adhesive and cytoskeletal organization creates a greater requirement for FAK activity

to drive adhesion turnover, a rate-limiting step in cell migration. Accordingly, we found that Compound X reduced focal adhesion area by approximately 45% on stiff gels but only 16–19% on soft gels, and caused a more striking disruption of stress fiber architecture on stiffer substrates. Total F-actin content remained largely unchanged under all conditions, indicating that FAK inhibition primarily modulates actin cytoskeleton remodeling and focal adhesion dynamics rather than global actin expression. This pattern aligns with the established role of FAK as a regulator of focal adhesion assembly and disassembly, linking integrin engagement to the actomyosin cytoskeleton [2,3]. The greater disruption of adhesion dynamics on stiff matrices therefore directly accounts for the stronger suppression of collective cell migration.

In contrast to migration, the anti-proliferative effect of Compound X was consistent across soft and stiff matrices, with a ~29% relative redu both conditions. This dissociation between anti-migrato responses reveals that stiffness differentially modulates FAK inhibition. Previous work by Tilghman et al. [1] s regulates cancer cell proliferation by modulating cellular metabolism and global protein synthesis, with cells on soft substrates displaying prolonged G1 phase and reduced ATP levels. Our results indicate that FAK contributes to proliferation control through mechanisms that are not strictly dependent on ECM stiffness. It is likely that cell proliferation is governed by multiple parallel mechanotransduction pathways, most notably the YAP/TAZ axis, which functions as a nuclear sensor of ECM stiffness and cytoskeletal tension [3]. Dupont et al. [3] demonstrated that YAP/TAZ activity is regulated by Rho-mediated actomyosin tension independently of the Hippo pathway, and is required for stiffness-directed stem cell differentiation and endothelial cell survival. Since FAK inhibition does not completely abrogate YAP/TAZ signaling, the proliferative response likely reflects the integrated output of both FAK-dependent and FAK-independent mechanotransduction circuits. This explains why proliferation inhibition by Compound X is uniform across stiffness levels, whereas migration—an adhesion-dependent process more directly coupled to FAK function—exhibits strong stiffness dependence.

To confirm that the observed effects w than off-target activity of Compound knockdown and tested the reference F recapitulated the key phenotype: a significantly greater reduction in gap closure on stiff matrices, with minimal effects on short-term proliferation and cell viability. The consistency across genetic and pharmacological inhibition strongly supports FAK as the specific target mediating the stiffness-dependent anti-migratory effect. Moreover, the similar response profile between Compound X and defactinib suggests that stiffness-dependent migration inhibition is a class effect of FAK inhibitors, rather than a unique property of Compound X. This finding has broad relevance for the growing number of FAK-targeted agents under preclinical and clinical investigation for breast cancer.

“ directly accounts for ”因果表述过强：focal adhesion变化与gap-closure下降具有一致性，但实验未证明前者直接导致后者。

本研究未检测YAP/TAZ signaling，不能由当前实验得出“ FAK inhibition does not completely abrogate YAP/TAZ signaling ”；引用的Dupont等文献也并非本研究中Compound X/FAK inhibition对YAP/TAZ的直接验证。

验证范围被扩大：FAK knockdown与defactinib支持FAK involvement，但不足以排除Compound X的off-target contribution；仅比较Compound X与一种reference inhibitor，也不足以推广为整个FAK inhibitor class的“ class effect ”。

These findings have important translational implications for the clinical development of FAK inhibitors in TNBC. Collagen deposition and crosslinking is associated with poor clinical outcomes [1,2]. The degree of tumor fibrosis varies considerably between patients and even within different regions of a single tumor. Our data suggest that FAK inhibitors may deliver more potent anti-metastatic activity in tumors with high matrix rigidity, where cancer cells are most dependent on FAK-driven mechanosignaling for migration and invasion. In contrast, the anti-proliferative efficacy would be expected to be relatively consistent regardless of stromal stiffness. This supports the evaluation of tumor mechanical properties, such as stiffness measured by elastography, as a predictive biomarker to stratify patients most likely to benefit from FAK inhibitor therapy. Furthermore, the dissociation between anti-migratory and anti-proliferative effects suggests that combination strategies targeting FAK and other mechanotransduction pathways (e.g., YAP/TAZ inhibitors) may achieve more comprehensive suppression of tumor dissemination, particularly in highly fibrotic tumors.

由2D gap-closure进一步推至肿瘤anti-metastatic activity；当前研究未直接评价invasion或in vivo metastasis。

由两种细胞系的体外stiffness-response进一步推至predictive biomarker及患者分层，缺少患者治疗反应及临床验证证据。

Several limitations of this study should be considered. First, all experiments were conducted on 2D collagen-coated polyacrylamide gels, which do not fully recapitulate the complex 3D architecture, biochemical composition, and multicellular interactions of the in vivo tumor microenvironment. Future studies using 3D organotypic cultures and in vivo metastasis models will be necessary to confirm these findings in a more physiologically relevant context. Second, we only examined two TNBC cell lines; further work is needed to determine whether stiffness-dependent responses to FAK inhibition extend to other breast cancer subtypes and patient-derived tumor cells. Third, the gap closure assay assesses collective cell migration over a 12-hour period; longer-term processes such as invasion through basement membrane and metastatic colonization may involve additional regulatory mechanisms. Finally, the in vivo pharmacokinetic properties, tolerability, and therapeutic efficacy of Compound X remain to be evaluated.

In summary, this study demonstrates that matrix stiffness selectively modulates the anti-migratory activity of FAK inhibition in TNBC cells. Stiffer ECM enhances FAK activation and cell migration, rendering cells more sensitive to FAK-targeted suppression of motility, while proliferation inhibition is unaffected by matrix rigidity. These results advance our understanding of how mechanical cues shape therapeutic response in cancer and provide a preclinical rationale for integrating tumor mechanical profiling into the development and clinical application of FAK inhibitors.

References

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Reference书目信息错误：Kostic等文献正确DOI为10.1371/journal.pone.0006361，且输入时提供的原始DOI本身正确，此处被错误改写。

仅沿用输入提供的3篇Reference，未根据Discussion新增论点进一步扩充针对性文献；部分机制解释及临床转化论述因此缺少直接文献支撑。

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