LetPub 结果：

The principal finding of this in vitro study is that matrix stiffness selectively amplified the migration-related consequences of FAK pathway inhibition in MDA-MB-231 and BT-549 TNBC cells, despite comparable proximal biochemical suppression of p-FAK Tyr397 across stiffness conditions. Stiff gel culture established a more mechanosensitive phenotype in both cell lines, characterized by increased spreading, stress fiber prominence, and elevated FAK Tyr397 phosphorylation. Compound X suppressed p-FAK Tyr397 to a similar degree on soft and stiff gels, yet its inhibition of 12 h gap closure was substantially greater on stiff gels, a pattern accompanied by larger reductions in focal adhesion area and more evident reorganization of actin architecture with little change in total F-actin fluorescence. The 12 h gap-closure effect is most consistent with altered migration-related behavior, given that short-term EdU labeling and viability were not meaningfully changed, whereas 48 h proliferation inhibition—though present—was not stiffness-dependent. Concordant results obtained with FAK knockdown and defactinib support the interpretation that matrix stiffness increases dependence on FAK-linked migratory machinery in these cells, rather than simply altering general drug sensitivity.

The baseline morphological and signaling responses observed on stiff matrices align with the broader understanding that ECM stiffening reinforces mechanotransduction programs supporting motile and invasive cancer phenotypes. Tumor ECM stiffening drives aberrant signaling through integrin-mediated and adhesion-linked pathways that promote malignant cell behavior [1], and the coordination between cell adhesion, cytoskeletal interactions, and matrix mechanics is recognized as a key determinant of cancer cell invasion potential [2]. ECM stiffness has accordingly been identified as a therapeutically relevant regulator of tumor progression through its effects on mechanotransducers [3]. Within this framework, the stronger inhibition of 12 h gap closure by Compound X on stiff gels is consistent with the concept that cells in mechanically reinforced states become more reliant on adhesion-linked signaling for movement. Prior work has demonstrated that breast cancer cell lines, including MDA-MB-231, can exhibit differential migration and proliferation responses across matrix rigidities [4], supporting the biological plausibility of stiffness-dependent functional differences. The present study extends this concept by demonstrating that the stiffness-dependent difference is not limited to baseline behavior but also manifests as a differential response to pharmacologic FAK inhibition in TNBC cells.

The observation that Compound X suppressed p-FAK Tyr397 comparably on soft and stiff gels, yet produced stronger functional and cytoskeletal consequences on stiff matrices, suggests that the downstream adhesion and cytoskeletal context—not the degree of proximal kinase inhibition—determines the magnitude of the migratory response. Integrin-based adhesions function as a molecular clutch that couples ECM mechanics to the actin cytoskeleton, and the transmission of forces across these adhesions establishes a mechanical reciprocity between matrix viscoelasticity and

围绕focal adhesion – cytoskeleton mechanotransduction的不同机制环节匹配针对性文献，而非仅以综合性FAK文献笼统支撑。

cellular tension [5]. Focal adhesion composition undergoes force-dependent maturation that regulates migration through mechanisms extending well beyond a single proximal phosphorylation event [6]. Distinct protein modules within focal adhesions separately control mechanosensing and downstream mechano-responses such as protrusion and migration; notably, the turnover of structural adhesion proteins is directly influenced by substrate stiffness, whereas FAK and paxillin turnover rates are not [7]. Cellular responses to mechanical cues depend on multiple downstream effectors whose activity is shaped by context [8], and FAK itself possesses both kinase-dependent and scaffolding functions that contribute to its roles in adhesion, migration, and survival signaling [9]. These considerations provide a plausible explanation for the present results: on stiff matrices, where adhesions and stress fiber organization were more developed at baseline, FAK inhibition may disproportionately destabilize the traction-supporting adhesion and cytoskeletal architecture even when the immediate reduction in p-FAK Tyr397 appears similar. The stronger reduction in focal adhesion area and reorganization of actin architecture, with minimal change in total F-actin fluorescence, is consistent with altered adhesion organization and force transmission rather than bulk actin loss. However, p-FAK Tyr397/FAK may not capture all signaling changes relevant to migration, and the downstream pathways responsible for the stiffness-dependent functional divergence were not directly measured.

The parallel between reduced focal adhesion area, altered actin organization, and reduced 12 h gap closure on stiff gels is biologically coherent because cell migration on rigid substrates depends on traction generation through adhesion-cytoskeletal coupling. Matrix stiffness regulates tumor cell migration speed and traction force polarization, establishing a direct link between substrate mechanics and motility-related behavior [10]. Cytoskeletal reorganization and reduced focal adhesions have been associated with impaired mechanical strength and altered motility in cancer cells [11], and ECM mechanics influence focal adhesion assembly, force development, and migration through transcriptional and structural mechanisms [12]. FAK is mechanically coupled to focal adhesions through multiple anchoring sites and is subject to stretching forces generated by actomyosin contraction during migration [13], while phosphorylation-dependent focal adhesion modules are required for downstream processes including Rac1-driven protrusion and cell movement [7]. These findings support the interpretation that stiff matrices increase reliance on force-bearing focal adhesion architecture, thereby making migration more vulnerable to FAK pathway inhibition. The observation that the main visible change was in adhesion structure and actin arrangement rather than bulk F-actin abundance fits this interpretation. Nonetheless, the data do not establish a complete causal chain from focal adhesion remodeling to gap closure reduction, and additional mediators likely contribute to the observed phenotype.

The interpretation that the 12 h gap-closure reduction reflects a migration-associated effect is supported by the absence of obvious short-term changes in EdU labeling or

针对gap-closure assay的解释边界主动匹配方法学文献，并区分migration与proliferation对实验readout的贡献。

viability during the same assay window. Gap closure assays are widely used tools for studying collective cell migration [14,15], but they inherently integrate both cell movement and proliferation [16]. The short-term EdU and viability controls in this study reduce the likelihood that the differential gap-closure response on stiff gels was driven by acute antiproliferative or cytotoxic effects, but they do not fully eliminate that possibility. The 12 h time frame further limits the proliferative contribution, yet some residual ambiguity remains inherent to this assay format. Accordingly, the present data most strongly su assay window, but do not constitut on, nor do they extend to broader claim

严格控制证据强度：EdU/viability仅降低增殖或细胞毒性解释的可能性，并未表述为完全排除，也未将gap closure直接等同于invasion/metastasis。

The contrast between the stiffness-dependent migration phenotype and the stiffness-independent 48 h proliferation response sharpens the central interpretation. Compound X reduced EdU-positive fractions at 48 h in both cell lines, but the relative magnitude of this antiproliferative effect did not differ between soft and stiff gels. Matrix stiffness has been reported to influence cancer cell proliferation in other systems [17], and ECM mechanics can modulate both proliferative and metastatic phenotypes [3,18]. Migration behavior, however, appears particularly sensitive to matrix mechanics and traction-related signaling [10]. The present results suggest that, in these two TNBC cell lines, matrix stiffness preferentially modified the migration-related consequences of FAK targeting rather than uniformly sensitizing cells to all effects of the inhibitor. This phenotype-selective modulation argues against a simple model in which stiff matrices globally enhance drug response, and instead points to a more specific role for mechanical context in shaping adhesion- and traction-dependent outputs. This conclusion is limited to the measured 48 h proliferation readout in MDA-MB-231 and BT-549 cells and does not exclude the possibility that stiffness modulates proliferative responses to FAK inhibition in other models or over different time scales.

The convergence of FAK knockdown, defactinib, and Compound X data materially strengthens the inference that FAK pathway disruption contributes to the stiffness-dependent gap-closure phenotype. Both independent siRNAs and the reference inhibitor defactinib reproduced the pattern of stronger gap-closure reduction on stiff gels, with preserved short-term viability and EdU labeling. The use of complementary pharmacologic and genetic perturbations to support on-target inference is an established strategy in FAK-related biology [19], and the concordance observed here increases confidence that the functional phenotype involves FAK-dependent signaling. However, siRNA approaches have recognized implementation limitations that prevent genetic concordance alone from completing causal proof [20]. FAK operates within broader integrin-adhesion signaling networks of considerable complexity [21], and its kinase-dependent and scaffolding functions can contribute to cellular phenotypes through multiple mechanisms [9]. The present concordance therefore supports FAK involvement in the stiffness-dependent anti-migratory response but does not prove that all observed effects are exclusively

准确限定验证实验的证据边界：knockdown与defactinib结果支持FAK参与，但不进一步推为FAK唯一介导，也不宣称Compound X优于reference inhibitor。

FAK-mediated. Equally important, Compound X and defactinib produced similar response profiles, and the experiments did not establish superiority of Compound X over the reference inhibitor.

These findings have potential relevance to TNBC biology because stromal remodeling, collagen crosslinking, and ECM-linked signaling are recognized features of aggressive TNBC microenvironments. Stromal remodeling and fibrillar collagen production by cancer-associated fibroblasts create a niche that supports chemoresistance and metastatic phenotypes in TNBC [22], and ECM remodeling through lysyl oxidase-mediated collagen crosslinking activates FAK/Src signaling pathways that contribute to chemoresistance in this disease context [23]. ECM stiffness and mechanotransducers have been identified as potential therapeutic targets in cancer [3], and stiffness is increasingly recognized as a therapeutically relevant feature of the tumor microenvironment [24]. Mechanotransduction provides a broad framework for understanding how stiffness-responsive signaling networks shape cellular behavior in cancer [25]. Within this context, the present data raise the possibility that mechanically stiff tumor regions may display greater dependence on FAK-linked migratory machinery and therefore greater vulnerability to FAK pathway inhibition at the level of movement-related behavior. This implication is hypothesis-generating: the current study used in vitro soft and stiff gel conditions and therefore infers, rather than demonstrates, relevance to in vivo tumor microenvironments. The full complexity of stromal interactions, immune components, vascular architecture, and three-dimensional matrix organization was not modeled, and no in vivo dissemination or treatment response data were obtained.

Strengths and limitations

明确区分体外结果与临床转化证据：将肿瘤微环境意义限定为hypothesis-generating，并主动指出缺少in vivo dissemination及treatment-response数据。

A strength of this stud ochemical, functional, and validation data ac support a coherent mechanobiological int hibitor response. The use of complementary perturbation strategies—Compound X, defactinib, and two independent FAK siRNAs—provides internal consistency that strengthens the central conclusion. Several limitations constrain the scope of inference. Only two TNBC cell lines were studied on soft and stiff gels, which limits generalizability across breast cancer subtypes and models. Gap closure assays carry inherent ambiguity regarding the relative contributions of migration and proliferation; the short-term EdU and viability controls reduce but do not eliminate this concern. The reliance on p-FAK Tyr397/FAK as the principal biochemical readout may not capture all downstream or parallel signaling changes that contribute to the stiffness-dependent functional response. The short assay windows for migration and imaging capture early responses but do not establish longer-term effects on invasion or metastatic behavior. Future work should test additional breast cancer models, employ longer-term invasion or dissemination-relevant assays such as three-dimensional matrix invasion or transwell systems, incorporate more comprehensive downstream signaling and

adhesion-dynamics measurements, and use in vivo or organotypic systems that better capture stromal and matrix complexity.

Conclusion

In these TNBC cell models, matrix stiffness did not materially alter proximal inhibition of p-FAK Tyr397 by Compound X, but it amplified the migration-related and adhesion/cytoskeletal consequences of FAK pathway inhibition. The divergence between stiffness-dependent effects on gap closure and stiffness-independent effects on 48 h proliferation supports a selective role for mechanical context in shaping FAK-linked motility responses. These results suggest that matrix stiffness is an important modifier of anti-migratory FAK-targeting effects in vitro and provide a rationale for testing mechanobiological context as a variable in future preclinical models of TNBC.

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