DeepSeek 结果：

Discussion

In this study, we investigated whether substrate stiffness modulates the efficacy of a small-molecule FAK inhibitor (Compound X) in triple-negative breast cancer (TNBC) cells. Our principal finding is that stiffness selectively amplifies the anti-migratory activity of FAK inhibition: Compound X reduced gap closure substantially more on stiff substrates while its anti-proliferative effect remained comparable across stiffness conditions. This differential response was corroborated by FAK knockdown and a reference FAK inhibitor, and was associated with stiffness-dependent changes in focal adhesion and actin organization. These results suggest that the therapeutic impact of FAK-targeted agents in TNBC may depend critically on the mechanical context of the tumor microenvironment, with stiffness amplifying inhibition of migration-related FAK functions but not proliferation.

The observation that basal FAK activation was higher on stiff gels (approximately 2-fold for both cell lines) is consistent with the established role of FAK as a mechanotransduction hub. Matrix stiffness promotes integrin clustering and FAK autophosphorylation at Tyr397, which in turn engages downstream effectors including Src, Rho/ROCK, and ERK to drive cytoskeletal remodeling and motility . Studies in breast cancer models have shown that increased collagen density and ECM rigidity activate a FAK-ERK signaling axis that promotes invasive phenotypes . The stiffness-dependent differences in cell spreading and stress fiber formation we observed prior to treatment align with FAK activity on stiff substrates reflects catalytic inhibition.

具体mechanotransduction及invasive phenotype论述后未标注可识别Citation；文末虽列有高度相关的Provenzano 2009，但正文未建立明确Citation – Reference对应关系。

A notable finding was the dissociation between migration and proliferation outcomes. Despite comparable p-FAK inhibition at 0.5 μM across stiffness conditions, Compound X preferentially suppressed gap closure on stiff gels (approximately 45% inhibition versus 16% on soft gels). The short-term EdU results (12 h) ruled out a contribution from altered proliferation or viability, and this dissociation persisted even at 48 h with 1 μM treatment, where relative proliferation inhibition was equivalent (~29% for both stiffnesses in MDA-MB-231). This suggests that FAK's contribution to proliferation is either saturated at the basal mediated through a pathway that does not sub role in migration is specifically sensitized by with this, FAK knockdown reproduced the stiffness-selective effect on gap closure, supporting an on-target mechanism for Compound X rather than an off-target artifact.

"ruled out"表述过强：12 h EdU无显著差异及高viability可降低短期增殖/存活变化解释gap-closure差异的可能性，但不足以完全排除其贡献。

The effects on focal adhesions provide a mechanistic link between FAK inhibition and the stiffness-dependent motility phenotype. Compound X reduced focal adhesion area more substantially on stiff gels (48% versus 19% in MDA-MB-231), and stress fiber

organization was more clearly disrupted on stiff substrates, while total F-actin fluorescence remained largely unchanged. This distinction between actin organization and actin quantity is important: it suggests th
spatial assembly and maturation of adhesion
the actin cytoskeleton. On stiff substrates, w
abundant at baseline, the consequences of th
consequential for traction force generation and directed migration. The significant treatment-stiffness interaction for focal adhesion area supports this interpretation. The finding that defactinib produced a similar pattern reinforces the generality of this phenotype among FAK inhibitors, though we did not observe Compound X to be superior to defactinib under the conditions tested.

These results have implications for interpreting
inhibitors in breast cancer. Previous work has de
suppress tumor growth and non-angiogenic vasc
and that FAK activation correlates with resistance to mTORC1 inhibition in breast cancer . Our data suggest that the anti-metastatic potential of FAK inhibitors may be most pronounced in stiff, fibrotic tumor regions that characterize many breast cancers, particularly high-grade TNBC. Conversely, the modest inhibition of migration on soft substrates suggests that FAK inhibition may have limited efficacy in preventing dissemination from regions of the tumor with lower mechanical stiffness. This mechanopharmacological perspective may help explain heterogeneity in therapeutic responses and argues for incorporating stiffness into preclinical screening and patient stratification strategies.

Several limitations should be acknowledged. First, our experiments used 2D hydrogel systems with defined stiffness, which captur
replicate the fibrillar architecture and interst
we examined two TNBC cell lines, and altho
generalization to other breast cancer subtype
requires further study. Third, we focused on short-term (6–12 h) cytoskeletal and migration readouts; the long-term consequences of stiffness-modulated FAK inhibition on invasion, metastasis, and adaptive resistance in vivo remain unknown. Fourth, although FAK knockdown and defactinib support on-target effects, we cannot exclude contributions from other FAK family kinases or off-target activities of Compound X at the concentrations used.

In conclusion, this study demonstrates that matrix stiffness selectively enhances the ability of FAK inhibition to suppress TNBC cell migration, without proportionally affecting proliferation. This phenotype is associated with stiffness-dependent differences in focal adhesion dynamics and is reproduced by genetic and pharmacological FAK targeting. These findings position substrate stiffness as a critical variable in the therapeutic response to FAK inhibitors and support further investigation of mechanopharmacological approaches to targeting TNBC metastasis.

仅比较Compound X与一种reference inhibitor，不足以将共同response pattern推广为FAK inhibitors整体的“generality”。

由两种细胞系的2D gap-closure结果进一步推至anti-metastatic potential及tumor dissemination疗效；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patient stratification，缺少患者治疗反应及临床验证证据。

References

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Reference扩充有效，但Citation－Reference映射不完整：新增3篇真实且相关的文献，但正文多处文献性论述未标注可识别Citation，读者仍需自行反查具体论述对应哪篇Reference。

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“找到了相关文献，但没有建立清晰Citation－Reference对应关系。”