ChatGPT 结果：

Discussion

The mechanical properties of the tumor microenvironment are increasingly recognized as important regulators of cancer cell behavior and therapeutic response. In the present study, we investigated whether extracellular matrix stiffness influences the response of triple-negative breast cancer (TNBC) cells to Compound X, a FAK inhibitor characterized previously. Using MDA-MB-231 and BT-549 cells cultured on collagen-coated substrates with stiffness values of approximately 2 and 20 kPa, we found that increased matrix stiffness enhanced cell spreading, stress-fiber organization, and FAK Tyr397 phosphorylation. Importantly, although Compound X inhibited FAK phosphorylation to a similar relative extent under soft and stiff conditions, its effects on gap closure and focal adhesion organization were substantially greater on the stiff substrate. In contrast, the longer-term inhibition of proliferation showed no significant stiffness-dependent difference. FAK knockdown and treatment with the reference FAK inhibitor defactinib reproduced the stiffness-dependent pattern observed in the migration assay. Collectively, these findings suggest that matrix stiffness can modify the functional consequences of FAK inhibition in TNBC cells, particularly those associated with cell migration and cytoskeletal organization, without necessarily altering the extent of biochemical FAK inhibition itself.

The enhanced FAK activation observed on the stiff substrate is consistent with the established role of FAK as a central component of cellular mechanotransduction. FAK is recruited to integrin-containing focal adhesions and undergoes autophosphorylation at Tyr397 in response to integrin engagement and mechanical forces, thereby providing a signaling platform connecting extracellular matrix cues to cytoskeletal organization, migration, proliferation, and survival [1,2]. Previous studies have demonstrated that breast cancer cells respond strongly to changes in extracellular matrix rigidity and that these responses vary among breast cancer cell lines [3]. Matrix rigidity can also regulate cancer cell growth, metabolism, and protein synthesis [4]. More broadly, increased substrate stiffness promotes mechanotransduction through integrin-associated signaling and other mechanosensitive pathways, including YAP/TAZ signaling [5]. Our observation that stiff substrates increased cell spreading and stress-fiber formation together with the p-FAK Tyr397/FAK ratio, while total FAK abundance remained relatively unchanged, is therefore consistent with increased activation of the focal adhesion machinery rather than increased FAK expression.

A particularly notable finding was that the stiffness dependence of the functional response to Compound X could not be explained simply by differential inhibition of FAK Tyr397 phosphorylation. At 0.5 μM, Compound X produced comparable

relative reductions in p-FAK/FAK on soft and stiff substrates in both TNBC cell lines, and the treatment-by-stiffness interaction for this biochemical endpoint was not significant. Nevertheless, the same concentration produced a substantially greater inhibition of gap closure on the stiff substrate. In MDA-MB-231 cells, gap closure was reduced by approximately 16% relative to vehicle on the soft substrate compared with 45% on the stiff substrate; corresponding values in BT-549 cells were 16% and 42%, respectively. The significant treatment-by-stiffness interactions in both cell lines further support the conclusion that the migratory consequence of FAK inhibition depended on the mechanical environment.

One interpretation of these findings is that increased matrix stiffness creates greater functional dependence on FAK-mediated mechanotransduction. Under stiff conditions, increased integrin-mediated tension and focal adhesion maturation may enhance the contribution of the FAK-associated adhesion–cytoskeleton machinery to cell movement. Consequently, inhibition of a similar f could produce a larger phenotypic effect when cel activated state. In contrast, cells on the soft substra stress-fiber organization, and lower basal FAK phosphorylation, potentially reflecting reduced engagement of this mechanotransduction program. Thus, the magnitude of target inhibition and the magnitude of the resulting cellular phenotype need not exhibit a simple linear relationship. This distinction may be particularly important when evaluating inhibitors of signaling proteins whose functional contribution is determined by the physical microenvironment.

提出“ stiffness增加FAK functional dependence ”的机制解释，但该段未进一步匹配直接支持这一具体机制判断的文献。

The focal adhesion and actin data provide additional support for this interpretation. Compound X caused a substantially greater reduction in vinculin-positive focal adhesion area on the stiff substrate than on the soft substrate in both cell lines. Stress fibers were also visibly disrupted, particularly under stiff conditions. However, total F-actin fluorescence changed by less than 10%, suggesting that the primary effect involved reorganization of the actin cytoskeleton rather than extensive loss of actin itself. Focal adhesions are dynamic structures that mechanically couple the extracellular matrix to actomyosin networks, and FAK participates in their assembly, turnover, and associated signaling [1,2]. Efficient directional migration requires coordinated adhesion formation and disassembly together with cytoskeletal remodeling. The parallel stiffness dependence of focal adhesion disruption and gap-closure inhibition therefore supports a model in which Compound X primarily interferes with the mechani conditions examined here.

机制论述有文献支持，但主要引用综合性FAK文献[1,2]；针对focal adhesion mechanics、adhesion turnover等具体mechanotransduction环节的文献匹配颗粒度相对有限。

Several observations argue against reduced cell number being the principal explanation for the short-term gap-closure phenotype. At the 0.5 μM concentration used in the migration experiments, cell viability remained above 95%, detached cells represented less than 5% of the population, and the proportion of EdU-positive cells did not differ significantly between Compound X and vehicle groups during the 12-h

assay. Similar findings were obtained after FAK knockdown and defactinib treatment. The short duration of the experiment and the absence of detectable changes in EdU incorporation support the interpretation that the observed differences in gap closure predominantly reflect changes in cell movement rather than short-term suppression of proliferation or survival. Nevertheless, gap-closure assays do not directly quantify single-cell migration parameters, and therefore this conclusion should be interpreted within the limitations of the assay.

The longer-term proliferation results further distinguish the stiffness-dependent migratory phenotype from the antiproliferative response. After 48 h of treatment with 1 μM Compound X, EdU incorporation was reduced in both cell lines and under both stiffness conditions. The reduction was similar between soft and stiff substrates, and no significant treatment-by-stiffness interaction was detected. Matrix stiffness itself increased the proportion of proliferating cells, consistent with previous evidence that extracellular matrix rigidity can promote cancer cell growth [4]. Compound X nevertheless produced a broadly comparable proportional antiproliferative response across the two mechanical environments. These results suggest that the effects of FAK inhibition on different cellular outputs need not exhibit the same dependence on extracellular mechanics. Migration requires continuous coordination among matrix adhesion, traction generation, actin remodeling, and adhesion turnover and may therefore be particularly sensitive to changes in substrate mechanics. Proliferation, in contrast, integrates signals from a wider range of pathways, including growth-factor, metabolic, and cell-cycle regulatory networks. FAK itself can interact with multiple downstream pathways, including PI3K/AKT and RAS–MEK–ERK signaling [1,6], potentially allowing its inhibition to affect proliferation through mechanisms that are less tightly coupled to matrix stiffness.

正确指出gap-closure assay的解释局限，但该方法学判断未匹配对应Citation。

The genetic and pharmacological control experiments strengthen the evidence that FAK contributes to the stiffness-dependent migration response. Two independent FAK-targeting siRNAs produced substantial FAK depletion and reproduced the preferential inhibition of gap closure under stiff conditions. The similarity between the two siRNAs reduces the likelihood that this phenotype resulted from a sequence-specific off-target effect. Moreover, defactinib produced a pattern closely resembling that of Compound X, with substantially greater inhibition of gap closure on the stiff substrate while maintaining high cell viability and without detectable short-term suppression of EdU incorporation. Defactinib is an ATP-competitive FAK inhibitor that suppresses FAK phosphorylation and has been investigated extensively in preclinical and clinical settings [6–8]. The convergence of Compound X treatment, FAK knockdown, and reference-inhibitor treatment therefore supports involvement of FAK signaling in the observed response.

At the same time, these comparisons should not be interpreted as demonstrating superiority of Compound X over defactinib. Under the experimental conditions used

here, the two inhibitors produced comparable suppression of FAK phosphorylation and similar stiffness-dependent effects on gap closure. The present experiments were designed primarily to investigate mechanosensitivity rather than comparative pharmacological potency, selectivity, or therapeutic index. Furthermore, kinase inhibitors can have activities beyond their nominal primary targets; for example, defactinib can inhibit PYK2 and additional kinases depending on concentration and assay conditions [6]. Although the FAK-knockdown experiments provide orthogonal support for FAK involvement, comprehensive kinase profiling and rescue experiments would be required to determine how much of the activity of Compound X is exclusively attributable to FAK.

The present results may also have implications for understanding heterogeneity in responses to FAK-targeted therapy. Solid tumors are mechanically heterogeneous, and tumor progression is commonly accompanied by extracellular matrix remodeling, collagen deposition and crosslinking, and changes in tissue stiffness. Because FAK occupies a central position between integrin-mediated adhesion and intracellular signaling, the mechanical state of the extracellular matrix may influence the biological consequences of its inhibition. The present data
engagement alone—for example, reduction of p
the magnitude of a migration-related response. C
target inhibition exhibited markedly different ful
substrate stiffness. This observation raises the possibility that mechanical characteristics of the tumor microenvironment could contribute to variability in responses to FAK-directed interventions. However, establishing matrix stiffness as a predictive biomarker would require considerably more extensive experimental and clinical evidence.

涉及tumor mechanical heterogeneity、ECM remodeling及collagen crosslinking等外部背景论述，但该段未提供对应Citation。

Our results also fit within a broader framework in which mechanotransduction pathways operate as interconnected networks rather than isolated signaling cascades. YAP and TAZ, for example, are major mechanosensitive transcriptional regulators whose nuclear localization and activity respond to extracellular matrix rigidity and cytoskeletal tension [5]. FAK signaling can additionally intersect with Src-family kinases, PI3K/AKT, MAPK, Rho-family GTPases, and other pathways regulating adhesion and cytoskeletal dynamics [1,2,6]. The present experiments do not establish which downstream pathway is responsible for the greater functional sensitivity to FAK inhibition on stiff substrates. It will therefore be important in future studies to determine whether changes in Src activation, RhoA–ROCK-dependent contractility, myosin activity, YAP/TAZ localization, or focal adhesion turnover accompany the stiffness-dependent response to Compound X.

Several limitations should be considered. First, the study used two TNBC cell lines and two discrete substrate stiffness values. Although the consistency between MDA-MB-231 and BT-549 cells strengthens the findings, breast cancers exhibit substantial molecular and mechanical heterogeneity. Additional TNBC models,

including patient-derived cells and organoid systems, would help establish the generalizability of the response. Testing a broader stiffness range would also determine whether the relationship between matrix stiffness and FAK-inhibitor sensitivity is continuous, threshold-dependent, or non-monotonic. Second, the two-dimensional hydrogel system provides controlled manipulation of substrate mechanics but does not reproduce the three-dimensional architecture, ligand organization, matrix remodeling, and mechanical heterogeneity of tumors in vivo. Three-dimensional collagen or engineered matrix models, followed by animal studies, will therefore be necessary to determine whether the observed mechanosensitive response is maintained in more physiologically relevant environments.

Third, although the gap-closure assay was intentionally performed over a short interval and accompanied by EdU and viability measurements, it remains an indirect measurement of migration. Single-cell tracking, transwell migration, traction-force microscopy, or live imaging of focal adhesion turnover could provide more direct mechanistic evidence. Fourth, the present study focused primarily on FAK Tyr397 phosphorylation, vinculin-positive focal adhesions, and F-actin organization. Examination of additional downstream signaling and mechanical outputs would be necessary to define how equivalent biochemical inhibition of FAK is translated into different phenotypic responses. Finally, the current study does not establish the pharmacokinetic behavior, in vivo efficacy, toxicity, or target selectivity of Compound X in the context of stiffness-dependent responses. These issues should be addressed before extrapolating the findings to therapeutic applications.

In conclusion, our findings demonstrate that extracellular matrix stiffness influences the functional response of TNBC cells to FAK inhibition. Stiff substrates enhanced FAK activation, cell spreading, and stress-fiber organization and, importantly, increased the inhibitory effects of Compound X on gap closure and focal adhesion organization. This stiffness dependence was reproduced by FAK knockdown and by the reference inhibitor defactinib, supporting a role for FAK-mediated mechanotransduction. In contrast, Compound X inhibited FAK Tyr397 phosphorylation to a similar proportional extent on soft and stiff substrates, and its longer-term antiproliferative effect was not significantly modified by stiffness. These observations indicate that mechanical context can determine the phenotypic consequences of FAK inhibition even when biochemical target engagement is comparable. Incorporating extracellular mechanics into the evaluation of FAK-targeted compounds may therefore provide a more complete understanding of their effects on tumor-cell behavior.

References

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Reference有所扩充，但针对性覆盖仍有限：由输入的3篇扩充至8篇；新增文献主要覆盖FAK功能及FAK inhibitor/defactinib，部分具体Discussion论点未进一步匹配专门文献。

以上结果来自 **GPT instant 5.6-Sol**