LetPub 结果：

The mechanical properties of the extracellular matrix provide a biologically relevant context for studying breast cancer behavior. Breast cancer cells encounter microenvironments with distinct physical features, and substrate stiffness has been discussed as one of several cues that can influence progression- and metastasis-related phenotypes [1]. Primary work using populations derived from MDA-MB-231 breast cancer cells showed that cellular responses to matrix rigidity can vary and that proliferation and migration were increased on matrices whose rigidities corresponded to tissues in which metastasis was observed, supporting the concept that rigidity-sensitive behavior is not uniform across breast cancer models [2]. These observations place matrix rigidity alongside biochemical composition and architecture as a component of the tumor microenvironment that can shape cell growth and motility at a functional level [1]. Because migration depends on cells that can adhere to, generate force against, and remodel their interaction with the matrix, stiffness provides a relevant experimental variable when evaluating motility-associated phenotypes in breast cancer cells.

具体研究结论紧跟对应Citation，论述与文献对应清晰。

Within this mechanical context, focal adhesion kinase (FAK) is a plausible node linking adhesion-based sensing to migratory behavior. Integrin-mediated adhesions connect extracellular matrix ligands to the F-actin cytoskeleton and transmit mechanical forces through focal adhesion proteins, producing biochemical signals that regulate cellular mechanics and longer-term cellular responses [3]. Focal adhesions also undergo compositional and structural changes that support traction generation and signaling during cell migration [4]. FAK is a cytoplasmic nonreceptor tyrosine kinase activated downstream of integrins and growth factor receptors in multiple cancers, and its kinase-dependent and scaffolding functions have been linked to cancer cell signaling, progression, and metastasis-related processes [5]. In mesenchymal triple-negative breast cancer cells, FAK activation has been reported to participate in IGF1R-mediated regulation of EMT-associated features, migration, and invasion [6]. Additional breast cancer work has connected focal adhesion regulation and phosphorylated FAK with tumor aggressiveness-associated phenotypes [7]. Consistent with this disease-specific rationale, prior studies of FAK inhibition in malignant breast cancer have reported effects on cell morphology, migration, FAK phosphorylation, and related vascularization models [8].

The possibility that matrix stiffness could influence therapeutic response creates a practical gap for FAK-directed approaches in breast cancer. Matrix rigidity can affect growth-related biology: cancer cells cultured on softer substrates showed longer cell-cycle progression, reduced metabolic activity, lower ATP levels, and altered protein synthesis compared with cells on stiffer substrates in a polyacrylamide gel system [9]. These observations indicate that stiffness can influence cellular states that may affect interpretation of drug response assays, particularly when motility, proliferation, and viability are measured in parallel. More broadly, mechanical

abnormalities in the tumor microenvironment, including extracellular matrix composition and stiffness, have been proposed to affect therapeutic responses through mechanotransduction programs linked to proliferation, survival, apoptosis, and phenotype switching [10]. Reviews of extracellular matrix remodeling similarly describe the ECM as a structural and signaling context tha progression and treatment response [11], and the interplay and cancer therapeutics has been highlighted as a source o Against the broader background of FAK inhibitor development [13], Compound X was previously reported as a potent small-molecule inhibitor of FAK [14]. Whether defined stiffness conditions alter the cellular response to this inhibitor in TNBC models remains an unresolved question.

从已有研究自然收束至明确Research Gap，突出本研究要解决的问题。

A stiffness-controlled experimental design can address this question by combining biochemical, structural, and functional readouts. Prior breast cancer rigidity-response work supports comparing cell behavior across matrices with defined mechanical properties rather than treating substrate stiffness as a neutral culture condition [2]. In this setting, phosphorylation of FAK at Tyr397 is a relevant readout because force and FAK localization, as well as force and Y397-FAK phosphorylation, have been shown to couple at individual focal adhesions under stiffness-dependent conditions [15]. Focal adhesion composition and dynamics provide complementary information, as distinct focal adhesion protein modules have been reported to control different aspects of mechanotransduction, with structural proteins such as vinculin showing stiffness-sensitive behavior [16]. Vinculin is also linked to actin-dependent focal adhesion mechanotransduction, and disruption of vinculin–actin interaction affects stiffness-dependent regulation of vinculin behavior [17]. These considerations support measuring p-FAK, focal adhesion organization, and actin architecture alongside gap closure, proliferation, and viability. In the present study, MDA-M TNBC cells were used on soft and stiff collagen-coated gels to ev substrate stiffness changes the response to Compound X.

针对p-FAK、focal adhesion和vinculin等具体实验readout匹配对应文献，而非笼统堆砌Citation。

This study builds on a literature in which FAK has been repeatedly linked to breast cancer migration-related phenotypes while focusing specifically on the role of matrix stiffness in inhibitor response. Pharmacologic inhibition of FAK with defactinib has been reported to reduce FAK phosphorylation and alter migration-related behavior in malignant breast cancer models, with siRNA-mediated FAK knockdown producing similar effects in that prior work [8]. Genetic and regulatory studies also support the relevance of FAK to motility-associated phenotypes: FAK-dependent signaling was implicated in migration and invasion of mesenchymal TNBC cells [6], and miR-7–mediated reduction of FAK expression suppressed migration and invasion in aggressive breast cancer cells, with FAK rescue reversing effects on migration and invasion [18]. However, these findings do not by themselves determine whether the mechanical environment modifies responses to a FAK inhibitor. Accordingly, this study investigates whether soft versus stiff matrix conditions alter Compound X effects on FAK phosphorylation, gap closure, focal adhesion and actin organization,

结尾聚焦Research Gap与研究目的，未提前报告本研究主要结果。

proliferation, and viability in MDA-MB-231 and BT-549 cells, with FAK knockdown and a reference FAK inhibitor included to contextualize FAK involvement.

References

[1] Brandon D. Riehl; Eunju Kim; Tasneem Bouzid; Jung Yul Lim. The Role of Microenvironmental Cues and Mechanical Loading Milieus in Breast Cancer Cell Progression and Metastasis. Frontiers in Bioengineering and Biotechnology. 2021. 8: 608526-608526. https://doi.org/10.3389/fbioe.2020.608526.
[2] Ana Kostić; Christopher D. Lynch; Michael P. Sheetz. Differential Matrix Rigidity Response in Breast Cancer Cell Lines Correlates with the Tissue Tropism. PLoS ONE. 2009. 4(7): e6361. https://doi.org/10.1371/journal.pone.0006361.
[3] Zhiqi Sun; Shengzhen Guo; Reinhard Fässler. Integrin-mediated mechanotransduction. The Journal of Cell Biology. 2016. 215(4): 445-456. https://doi.org/10.1083/jcb.201609037.
[4] Jean‐Cheng Kuo. Mechanotransduction at focal adhesions: integrating cytoskeletal mechanics in migrating cells. Journal of Cellular and Molecular Medicine. 2013. 17(6): 704-712. https://doi.org/10.1111/jcmm.12054.
[5] Yuling Tai; Lih‐Chyang Chen; Tang‐Long Shen. Emerging Roles of Focal Adhesion Kinase in Cancer. BioMed Research International. 2015. 2015: 1-13. https://doi.org/10.1155/2015/690690.
[6] LaTonia D Taliaferro-Smith; Elaine M. Oberlick; Tongrui Liu; Tanisha Z. McGlothen; Tiffanie Y. Alcaide; Rachel S. Tobin; Siobhan Donnelly; Rachel Commander; Erik R. Kline; Ganji Purnachandra Nagaraju; Lauren S. Havel; Adam I. Marcus; Rita Nahta; Ruth M O'Regan. FAK activation is required for IGF1R-mediated regulation of EMT, migration, and invasion in mesenchymal triple negative breast cancer cells. Oncotarget. 2015. 6(7): 4757-4772. https://doi.org/10.18632/oncotarget.3023.
[7] Jie Shen; Beibei Cao; Yatao Wang; Chenshen Ma; Zhuo Zeng; Liang Liu; Xiaolan Li; Deding Tao; Jianping Gong; Daxing Xie. Hippo component YAP promotes focal adhesion and tumour aggressiveness via transcriptionally activating THBS1/FAK signalling in breast cancer. Journal of Experimental & Clinical Cancer Research. 2018. 37(1): 175-175. https://doi.org/10.1186/s13046-018-0850-z.
[8] Misato Masuyama; Masafumi Shimoda; Ikumi Seto; Kaori Kikumori; Kaori Abe; Nanae Masunaga; Chieko Mishima; Masami Tsukabe; Tetsuhiro Yoshinami; Yoshiaki Sota; Tomohiro Miyake; Tomonori Tanei; Kenzo Shimazu. Targeting focal adhesion kinase inhibits cell migration and non-angiogenic vascularization in malignant breast cancer. Breast Cancer. 2025. 33(1): 188-199. https://doi.org/10.1007/s12282-025-01792-6.
[9] Robert W. Tilghman; Edik Matthew Blais; Catharine Cowan; Nicholas E. Sherman; Pablo Rafael Grigera; Erin D. Jeffery; Jay W. Fox; Brett R. Blackman; Daniel J. Tschumperlin; Jason A. Papin; J. Thomas Parsons. Matrix Rigidity Regulates Cancer Cell Growth by Modulating Cellular Metabolism and Protein Synthesis. PLoS ONE. 2012. 7(5): e37231. https://doi.org/10.1371/journal.pone.0037231.

[10] Maria Kalli; Matthew D. Poskus; Triantafyllos Stylianopoulos; Ioannis K. Zervantonakis. Beyond matrix stiffness: targeting force-induced cancer drug resistance. Trends in cancer. 2023. 9(11): 937-954. https://doi.org/10.1016/j.trecan.2023.07.006.
[11] Zhennan Yuan; Yingpu Li; Sifan Zhang; Xueying Wang; He Dou; Yu Xi; Zhiren Zhang; Shanshan Yang; Min Xiao. Extracellular matrix remodeling in tumor progression and immune escape: from mechanisms to treatments. Molecular Cancer. 2023. 22(1): 48-48. https://doi.org/10.1186/s12943-023-01744-8.
[12] Jai Prakash; Yuval Shaked. The Interplay between Extracellular Matrix Remodeling and Cancer Therapeutics. Cancer Discovery. 2024. 14(8): 1375-1388. https://doi.org/10.1158/2159-8290.cd-24-0002.
[13] Xiaojing Pang; Xiujuan Liu; Yuan Liu; Wenbo Liu; Yin-Ru Li; Guang-Xi Yu; Xinyi Tian; Yanbing Zhang; Jian Song; Cheng‐Yun Jin; Sai‐Yang Zhang. Drug Discovery Targeting Focal Adhesion Kinase (FAK) as a Promising Cancer Therapy. Molecules. 2021. 26(14): 4250-4250. https://doi.org/10.3390/molecules26144250.
[14] Smith, Y; Cohen, H; Chen, X; Wang, J; Galileo, M. Discovery and characterization of Compound X, a potent small-molecule inhibitor of focal adhesion kinase. Chemical Biology & Drug Design. 2024. 12(3): 145-158.
[15] Dennis W. Zhou; Marc Fernández; Elijah N. Holland; Andrés F. García; Nicolas Castro; Eric B. O'Neill; Jeroen Eyckmans; Christopher S. Chen; Jianping Fu; David D. Schlaepfer; Andrés J. García. Force-FAK signaling coupling at individual focal adhesions coordinates mechanosensing and microtissue repair. Nature Communications. 2021. 12(1): 2359-2359. https://doi.org/10.1038/s41467-021-22602-5.
[16] Ben Stutchbury; Paul Atherton; Ricky Tsang; De‐Yao Wang; Christoph Ballestrem. Distinct focal adhesion protein modules control different aspects of mechanotransduction. Journal of Cell Science. 2017. 130(9): 1612-1624. https://doi.org/10.1242/jcs.195362.
[17] Tomohiro Omachi; Takafumi Ichikawa; Yasuhisa Kimura; Kazumitsu Ueda; Noriyuki Kioka. Vinculin association with actin cytoskeleton is necessary for stiffness-dependent regulation of vinculin behavior. PLoS ONE. 2017. 12(4): e0175324-e0175324. https://doi.org/10.1371/journal.pone.0175324.
[18] Xiangjun Kong; Gaopeng Li; Yan Yuan; Yan He; Xiaoli Wu; Weijie Zhang; Zhengsheng Wu; Tingting Chen; Wenyong Wu; Peter E
MicroRNA-7 Inhibits Epithelial-to-Mesenchymal Tran
Cancer Cells via Targeting FAK Expression. PLoS ON
https://doi.org/10.1371/journal.pone.0041523.

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