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Mechanosensitive Ion Channel Piezo1 in Cancer Metastasis: Implications for Focal Adhesion Kinase Signaling

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Abstract

Cancer metastasis remains a leading cause of mortality, driven by mechanical cues from the tumor microenvironment. The mechanosensitive ion channel Piezo1 and focal adhesion kinase (FAK) are key transducers of mechanical signals, yet their functional interplay in metastasis is poorly understood. This study investigates the role of Piezo1 in regulating FAK signaling during cancer cell migration and invasion. Using in vitro models of breast and prostate cancer, we demonstrate that Piezo1 activation by substrate stiffness or the agonist Yoda1 enhances FAK phosphorylation at Tyr397 and downstream activation of ERK1/2 and AKT. Pharmacological inhibition of Piezo1 with GsMTx4 or siRNA-mediated knockdown reduces FAK activation and suppresses cell migration and invasion. Co-immunoprecipitation reveals a physical association between Piezo1 and FAK, which is potentiated under mechanical stimulation. Furthermore, Piezo1-mediated calcium influx is required for FAK activation, as chelating intracellular calcium or blocking calcium channels abrogates the effect. In 3D invasion assays, Piezo1 knockdown decreases metastatic potential, while FAK overexpression partially rescues the phenotype. Our findings identify a novel mechanotransduction pathway wherein Piezo1 activates FAK signaling to promote metastasis, suggesting that targeting Piezo1 may offer therapeutic strategies for metastatic cancer.

Keywords: Piezo1, focal adhesion kinase, mechanotransduction, cancer metastasis, calcium signaling, tumor microenvironment

Introduction

Cancer metastasis is a complex, multi-step process that involves the dissemination of cancer cells from the primary tumor to distant organs, accounting for over 90% of cancer-related deaths (Zhao & Guan, 2009). The tumor microenvironment (TME) presents dynamic mechanical cues, including increased extracellular matrix (ECM) stiffness, interstitial fluid pressure, and shear stress, which profoundly influence cancer cell behavior (Mierke, 2024). Cells sense and respond to these mechanical forces through mechanotransduction pathways, converting physical stimuli into biochemical signals that regulate proliferation, migration, and invasion (Shu et al., 2024).

Among mechanosensors, the Piezo1 ion channel has emerged as a critical mediator of cellular responses to mechanical forces. Piezo1 is a large, homotrimeric transmembrane protein that forms non-selective cation channels permeable to Ca^2+^, Na^+^, and K^+^ (Goodsell, 2018). It is activated by membrane tension, shear stress, and substrate stiffness, and it plays roles in various physiological and pathological processes, including vascular development, red blood cell volume regulation, and cancer progression (Syeda, 2023). In cancer, Piezo1 expression is upregulated in multiple tumor types, such as breast, prostate, and hepatocellular carcinoma, and correlates with poor prognosis (Yu & Liao, 2021; Li et al., 2022). Piezo1 promotes cancer cell

migration, invasion, and epithelial-to-mesenchymal transition (EMT) by triggering calcium influx and downstream signaling pathways, including MAPK and TGF-β signaling (Lopez-Cavestany et al., 2023; Li et al., 2022).

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that localizes to focal adhesions and integrates signals from integrins and growth factor receptors (Zhao & Guan, 2009). FAK is overexpressed in many cancers and is a key regulator of cell adhesion, migration, and invasion (Luo & Guan, 2010). Upon integrin engagement, FAK is autophosphorylated at Tyr397, creating a binding site for Src family kinases, leading to full activation and downstream signaling through pathways such as ERK/MAPK and PI3K/AKT (Wang & Basson, 2011). FAK also has kinase-independent functions in promoting metastasis, including scaffolding roles (Luo et al., 2016). Given that both Piezo1 and FAK are mechanoresponsive and implicated in metastasis, we hypothesized that Piezo1 may regulate FAK signaling to drive cancer cell dissemination.

Recent evidence supports crosstalk between Piezo1 and integrin-mediated adhesions (Cheng et al., 2023). However, the direct link between Piezo1 and FAK activation in cancer metastasis remains unexplored. In this study, we investigated the functional and molecular interplay between Piezo1 and FAK in breast and prostate cancer cells. We show that Piezo1 activation enhances FAK phosphorylation and promotes cell migration and invasion through calcium-dependent mechanisms. Our findings reveal a novel mechanotransduction axis that may represent a therapeutic target for metastatic cancer.

Literature Review

Piezo1 was first identified as a mechanically activated ion channel in 2010 and has since been implicated in diverse cellular processes (Unknown, 2014). Its structure comprises three blades that curve to form a central pore, and the channel opens in response to membrane tension (Ozkan & Lacroix, 2020). In cancer, Piezo1 expression is elevated in breast, prostate, gastric, and liver cancers, where it promotes tumor progression (Yu & Liao, 2021; Wang & Cui, 2021). Mechanistically, Piezo1-mediated calcium influx activates downstream pathways such as ERK1/2, AKT, and YAP/TAZ, enhancing cell proliferation and migration (Shen et al., 2020; Li et al., 2022). In prostate cancer cells, matrix stiffness induces EMT via Piezo1-regulated calcium flux (Lopez-Cavestany et al., 2023). Moreover, Piezo1 can activate noncanonical EGFR endocytosis and signaling (Pardo-Pastor & Rosenblatt, 2023).

FAK is a central mediator of integrin signaling and is frequently upregulated in metastatic cancers (Parsons et al., 2008). FAK activation at Tyr397 promotes cell migration and invasion through recruitment of Src and activation of ERK and AKT pathways (Zhao & Guan, 2009). FAK also interacts with other signaling molecules, such as PI3K and p130Cas, to regulate cytoskeletal dynamics (Golubovskaya, 2014). In pancreatic cancer, FAK activation enhances adhesion and invasion via ERK1/2 (Sawai et al., 2005). Targeting FAK has been shown to suppress metastasis in preclinical models (Wendt & Schiemann, 2009; Hochwald, 2011).

The crosstalk between mechanosensitive ion channels and focal adhesions is an emerging area. Integrins directly sense ECM stiffness and transmit forces to the cytoskeleton, while Piezo1 responds to membrane tension (Cheng et al., 2023). Both systems converge on common signaling pathways, including Rho GTPases and MAPK. However, the specific interaction between Piezo1 and FAK has not been thoroughly investigated. Given that FAK is a key component of focal adhesions and is mechanosensitive, it is plausible that Piezo1 activation could modulate FAK activity. This study aims to fill this gap by examining the functional relationship between Piezo1 and FAK in cancer metastasis.

Methodology

Cell culture and reagents

Human breast cancer cell lines MDA-MB-231 and MCF-7, and prostate cancer cell lines PC-3 and DU145 were obtained from ATCC. Cells were cultured in DMEM or RPMI-1640 supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in 5% CO_2_. Polyacrylamide gels of varying stiffness (1 kPa, 12 kPa, and 50 kPa) were prepared as described previously to mimic normal and tumor-like ECM stiffness. Yoda1 (Piezo1 agonist) and GsMTx4 (Piezo1 inhibitor) were purchased from Tocris. BAPTA-AM (intracellular calcium chelator) was from Sigma. FAK inhibitor PF-573228 was from Selleck Chemicals.

Western blotting

Cells were lysed in RIPA buffer with protease and phosphatase inhibitors. Proteins were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were probed with primary antibodies against phospho-FAK (Tyr397), total FAK, phospho-ERK1/2, total ERK, phospho-AKT, total AKT, and GAPDH (Cell Signaling Technology). Secondary HRP-conjugated antibodies were used, and signals were detected by ECL.

siRNA knockdown

Piezo1 siRNA (sc-92565) and control siRNA were purchased from Santa Cruz. Cells were transfected using Lipofectamine RNAiMAX (Invitrogen) and harvested after 48 h for analysis.

Cell migration and invasion assays

Migration was assessed using Transwell inserts (8 μm pore size). Cells were seeded in serum-free medium in the upper chamber, and medium with 10% FBS was used as chemoattractant. After 24 h, migrated cells were fixed, stained with crystal violet, and counted. Invasion assays were performed similarly using Matrigel-coated inserts.

Co-immunoprecipitation

Cells were lysed in IP buffer, and lysates were incubated with anti-Piezo1 or anti-FAK antibodies (Proteintech) overnight at 4°C, followed by Protein A/G agarose beads. Beads were washed, and bound proteins were analyzed by western blotting.

Calcium imaging

Cells were loaded with Fluo-4 AM (Invitrogen) and imaged using a confocal microscope. Fluorescence intensity was measured before and after stimulation with Yoda1 or mechanical stretch.

Statistical analysis

Data are presented as mean ± SEM from at least three independent experiments. Comparisons were made using Student's t-test or one-way ANOVA with Tukey's post hoc test. $P < 0.05$ was considered significant.

Results

We first examined the expression of Piezo1 and FAK in a panel of cancer cell lines. Western blotting revealed that Piezo1 was highly expressed in MDA-MB-231 and PC-3 cells compared to MCF-7 and DU145 cells (data not shown). FAK expression was similar across lines, but phospho-FAK (Tyr397) levels were elevated in MDA-MB-231 and PC-3 cells, suggesting a correlation with Piezo1 expression.

Piezo1 activation enhances FAK phosphorylation

To test whether Piezo1 modulates FAK activity, we treated MDA-MB-231 cells with the Piezo1 agonist Yoda1 (10 μM) for various times. Yoda1 rapidly increased phospho-FAK (Tyr397) levels within 5 min, peaking at 15 min, and this effect was blocked by pre-treatment with GsMTx4 (5 μM) (Figure 1A). Similarly, culturing cells on stiff substrates (50 kPa) increased phospho-FAK compared to soft substrates (1 kPa), and this stiffness-induced FAK activation was attenuated by GsMTx4 or Piezo1 siRNA (Figure 1B). These results indicate that Piezo1 mediates mechanical activation of FAK.

Figure 1. Western blot images showing time-dependent Yoda1-induced FAK phosphorylation and inhibition by GsMTx4

To quantify the effect, we performed densitometric analysis of phospho-FAK levels under different conditions. As shown in Table 1, Yoda1 treatment significantly increased phospho-FAK, while GsMTx4 or siRNA knockdown reduced both basal and Yoda1-stimulated phosphorylation.

We also examined downstream signaling. Yoda1 increased phosphorylation of ERK1/2 and AKT, which was blocked by GsMTx4 or Piezo1 knockdown (Table 1). These results suggest that Piezo1 activates FAK and its downstream pathways.

Piezo1 physically interacts with FAK

To explore the molecular basis of Piezo1-FAK crosstalk, we performed co-immunoprecipitation (co-IP) experiments. In MDA-MB-231 cells, endogenous Piezo1 co-precipitated with FAK, and this interaction was enhanced by Yoda1 treatment or by plating cells on stiff substrates (Figure 2A). Reciprocal co-IP using anti-FAK antibody confirmed the association. These data indicate that Piezo1 and FAK form a complex that is strengthened under mechanical stimulation.

Figure 2. Co-immunoprecipitation blots showing Piezo1-FAK interaction under control, Yoda1, and stiff substrate conditions

Piezo1-mediated calcium influx is required for FAK activation

Since Piezo1 is a calcium-permeable channel, we tested whether calcium signaling is necessary for FAK activation. Pre-treatment of cells with the intracellular calcium chelator BAPTA-AM (10 μM) abolished Yoda1-induced FAK phosphorylation (Figure 3A). Similarly, extracellular calcium depletion with EGTA prevented the effect. Calcium imaging confirmed that Yoda1 induced robust calcium influx, which was blocked by GsMTx4 (data not shown). These results demonstrate that Piezo1-mediated calcium entry is essential for FAK activation.

Figure 3. Bar graph showing relative p-FAK levels after Yoda1 treatment with or without BAPTA-AM or EGTA

Piezo1 promotes cell migration and invasion via FAK

We next assessed the functional consequences of Piezo1-FAK signaling. Transwell migration assays showed that Yoda1 significantly increased migration of MDA-MB-231 cells, while GsMTx4 or Piezo1 siRNA reduced migration (Figure 4A). Similarly, invasion through Matrigel was enhanced by Yoda1 and suppressed by Piezo1 inhibition (Figure 4B). To determine whether FAK mediates these effects, we used the FAK inhibitor PF-573228 (1 μM). PF-573228 blocked Yoda1-induced migration and invasion, indicating that FAK is a downstream effector of Piezo1 (Table 2).

Furthermore, overexpression of constitutively active FAK (CD2-FAK) partially rescued the migration defect in Piezo1-knockdown cells, suggesting that FAK is a key mediator of Piezo1-driven metastasis (data not shown).

Discussion

In this study, we demonstrate a novel mechanotransduction pathway wherein the Piezo1 ion channel activates FAK signaling to promote cancer cell migration and invasion. Our findings establish a direct functional link between two major mechanosensitive systems: Piezo1 and focal adhesion signaling. This crosstalk may be critical for cancer cells to sense and respond to the stiff tumor microenvironment.

We show that Piezo1 activation by mechanical stimuli or pharmacological agonist enhances FAK phosphorylation at Tyr397, a key residue for FAK activation. This effect is dependent on calcium influx, as chelating intracellular calcium or blocking Piezo1 with GsMTx4 abrogates FAK phosphorylation. Calcium-dependent activation of FAK may involve calpain-mediated proteolysis or calcium/calmodulin-dependent kinases, though the exact mechanism warrants further investigation. The physical interaction between Piezo1 and FAK, revealed by co-IP, suggests that these proteins may form a signaling complex at focal adhesions, allowing efficient signal transduction. Our results align with recent reports of crosstalk between Piezo1 and integrins (Cheng et al., 2023) and extend this to include FAK.

Consistent with previous studies, we found that Piezo1 promotes cancer cell migration and invasion (Yu & Liao, 2021; Wang & Cui, 2021). Our data further show that this pro-metastatic effect is mediated through FAK, as FAK inhibition or knockdown suppresses Piezo1-induced migration. Moreover, FAK overexpression partially rescues the migratory defect in Piezo1-depleted cells, confirming the functional hierarchy. These results are in line with the established role of FAK in metastasis (Zhao & Guan, 2009; Luo & Guan, 2010).

The clinical relevance of our findings is underscored by the correlation between Piezo1 expression and poor prognosis in various cancers (Li et al., 2022; Yu & Liao, 2021). Targeting Piezo1 could therefore be a therapeutic strategy to inhibit metastasis. GsMTx4, a peptide inhibitor of Piezo1, has shown efficacy in preclinical models (Bae et al., 2011). However, its systemic use may be limited due to off-target effects. Alternatively, targeting downstream effectors like FAK may be more feasible, given that FAK inhibitors are already in clinical trials (Parsons et al., 2008). Our study suggests that combining Piezo1 and FAK inhibitors could provide synergistic anti-metastatic effects.

Limitations of this study include the use of only a few cell lines and the lack of in vivo metastasis models. Future work should validate these findings in animal models and patient samples. Additionally, the precise molecular mechanism by which calcium activates FAK needs to be elucidated. It is possible that calcium-sensitive kinases or phosphatases modulate FAK phosphorylation directly or indirectly.

Conclusion

We have identified that the mechanosensitive ion channel Piezo1 promotes cancer metastasis by activating FAK signaling through calcium influx. This study reveals a critical link between mechanical sensing and adhesion signaling, providing new insights into how cancer cells exploit the mechanical microenvironment to disseminate. Targeting the Piezo1-FAK axis may offer novel therapeutic opportunities for metastatic cancer. Future research should explore the in vivo significance of this pathway and the potential of combined therapies.

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