

Rogaratinib Suppresses Breast Cancer Invasion and Survival in a Microfluidic Tumor-on-Chip Model and Patient-Derived Organoids

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Research Article

Keywords: Breast cancer, Microfluidic chip, invasion, Rogaratinib

Posted Date: May 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9597983/v1>

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Additional Declarations: The authors declare no competing interests.

Abstract

Purpose

Fibroblast growth factor receptor (FGFR) signaling plays a critical role in breast cancer progression by promoting tumor cell proliferation, survival, and invasion, particularly under microenvironmental stimulation. However, conventional in vitro models inadequately reproduce the chemotactic gradients that regulate FGFR-driven invasion. This study aimed to evaluate the therapeutic potential of the pan-FGFR inhibitor rogaratinib using complementary preclinical models, including a microfluidic tumor-on-a-chip platform and patient-derived organoids.

Methods

MDA-MB-231 triple-negative breast cancer cells were treated with rogaratinib (0.01–100 μM) for 24–72 h, and viability was assessed by MTT assay. Directional invasion was evaluated using a gradient-generating microfluidic device under basal, serum (10% and 20% FBS), and bFGF (50 ng/mL) conditions, with or without rogaratinib (0.1 μM). Patient-derived organoids from three breast cancer subtypes (ER⁺/PR⁺/HER2⁻, HER2⁺, and triple-negative) were treated with rogaratinib for three days, and viability was assessed by Calcein-AM/PI live-dead staining.

Results

Rogaratinib significantly reduced MDA-MB-231 proliferation in a dose- and time-dependent manner, with an IC_{50} of approximately 0.1 μM at 72 h. In the microfluidic invasion platform, rogaratinib markedly suppressed directional invasion toward bFGF and 20% FBS gradients, while exerting minimal effects under basal or 10% FBS conditions, indicating ligand-dependent FGFR signaling. Rogaratinib significantly reduced viability in two of three patient-derived organoid models.

Conclusion

Rogaratinib suppresses breast cancer proliferation, invasion, and survival in a context-dependent manner, with greatest efficacy under ligand-rich, pro-metastatic conditions. These findings support biomarker-guided application of rogaratinib and highlight the value of microfluidic platforms for functional drug evaluation.

Introduction

Breast cancer remains the most commonly diagnosed malignancy and a leading cause of cancer-related mortality among women worldwide [1]. Among its subtypes, triple-negative breast cancer (TNBC) is particularly aggressive, characterized by the absence of estrogen receptor (ER), progesterone receptor

(PR), and HER2 expression, and associated with early recurrence and a high risk of visceral metastasis [2, 3]. Increasing evidence indicates that tumor progression is driven not only by intrinsic cancer cell properties but also by dynamic interactions within the tumor microenvironment (TME). This complex ecosystem—comprising stromal cells, immune components, extracellular matrix (ECM), and soluble growth factors—actively promotes tumor growth, invasion, immune evasion, and therapeutic resistance [4]

Among the signaling pathways mediating tumor–stromal crosstalk, the fibroblast growth factor (FGF)/FGF receptor (FGFR) axis plays a central role. Cancer-associated fibroblasts (CAFs) secrete FGFs, including FGF2, which activate FGFR signaling in tumor cells and promote proliferation, epithelial-to-mesenchymal transition (EMT), migration, and metastasis. Metin girmek için buraya tıklayın veya dokununuz. In TNBC, FGFR1–4 are frequently dysregulated through receptor amplification or ligand overexpression, leading to activation of downstream PI3K/AKT and MAPK/ERK pathways that enhance survival and therapeutic resistance [7–9]. These findings identify FGFR signaling as a critical mediator of pro-metastatic microenvironmental cues and a promising therapeutic target.

Rogaratinib is a selective pan-FGFR (FGFR1–4) inhibitor that blocks FGFR-dependent ERK phosphorylation and tumor cell proliferation. Preclinical studies have demonstrated antitumor activity in FGFR-altered malignancies, including breast cancer, with response correlating to FGFR mRNA expression [10–12]. However, heterogeneous clinical responses underscore the need for improved preclinical systems capable of modeling microenvironmental signaling and identifying contexts in which FGFR inhibition is most effective [13, 14].

Traditional two-dimensional culture systems fail to replicate tumor architecture, biochemical gradients, and stromal interactions. Although spheroids and patient-derived xenografts provide partial improvements, they lack precise control over dynamic microenvironmental cues. Microfluidic tumor-on-a-chip platforms overcome these limitations by recreating physiologically relevant three-dimensional matrices with stable chemotactic gradients, enabling quantitative analysis of directional invasion under defined biochemical conditions [15–19]. These findings confirm that invasion and resistance are extrinsic traits driven by stromal cues. However, dissecting the specific contribution of soluble factors, such as FGF gradients, requires a reductionist system that can isolate these chemical signals from physical cell-cell contact.

In this study, we evaluated whether rogaratinib suppresses TNBC invasion and survival under defined microenvironmental conditions using a gradient-generating microfluidic platform. By establishing stable bFGF and serum gradients, we modeled chemotactic drivers of invasion to determine the impact of FGFR blockade on directional migration. To assess translational relevance, we further tested rogaratinib sensitivity in patient-derived organoids representing TNBC, HER2+, and luminal subtypes. This combined bioengineered and patient-derived approach enables functional interrogation of FGFR inhibition within controlled microenvironmental contexts.

Materials and Methods

Cell Culture

The human triple-negative breast cancer cell line MDA-MB-231 was obtained from the Pasteur Institute (Tehran, Iran). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37°C in a humidified incubator with 5% CO₂. Cells were passaged at 70–80% confluence using 0.025% trypsin–0.01% EDTA and used within low passage numbers for experiments.

PDMS microfluidic devices were mounted directly into six-well tissue-culture plates by gently pressing the devices against the plate surface to form sealed microchannels. Geltrex (2 µL) was injected into each hydrogel channel using a micropipette and confined between microposts by capillary action. Devices were incubated at 37°C for 8 min in a humidified chamber to allow gel polymerization, thereby isolating the central cell-culture channel from the signaling channels.

Prewarmed culture medium was added to the reservoirs to fill the channels and prevent gel dehydration, and devices were equilibrated in the incubator prior to cell seeding. For cell loading, medium was removed from the culture-channel reservoirs, and a cell suspension (5×10^6 cells/mL) was added to one reservoir, allowing pressure-driven flow to uniformly seed cells along the channel. Devices were incubated at 37°C for 2 h to permit cell attachment, after which reservoirs were replenished with fresh medium. Devices were then maintained under standard culture conditions and monitored daily.

cell viability assay

Rogaratinib was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution and stored according to the manufacturer's instructions. Working concentrations were freshly prepared by dilution in complete culture medium, and vehicle controls received equivalent concentrations of DMSO. Vehicle control groups received DMSO at the same final concentration used in rogaratinib-treated samples. Cell viability was assessed using the MTT assay. MDA-MB-231 cells were seeded into 96-well plates and allowed to adhere overnight, followed by treatment with rogaratinib (100, 10, 1, 0.1, and 0.01 µM) for 24, 48, or 72 h. MTT reagent was then added, and after incubation, formazan crystals were solubilized, and absorbance was measured using a microplate reader. Cell viability was expressed as a percentage of vehicle-treated controls, and IC₅₀ values were calculated using nonlinear regression analysis.

Microfluidic Device Design and Fabrication

The microfluidic invasion chip used in this study was based on our previously developed gradient-generating platform (previously described [20]). The device consists of a central cell-culture channel flanked by four parallel signaling channels, separated by hydrogel regions retained by arrays of microposts. The micropost structures prevent hydrogel leakage while permitting diffusion of soluble

factors, enabling the formation of stable and reproducible chemoattractant gradients across the hydrogel matrix.

Microfluidic devices were fabricated using standard soft-lithography techniques. Briefly, polydimethylsiloxane (PDMS) was cast onto an SU-8–patterned silicon master and cured. After curing, individual PDMS devices were carefully removed from the master, and inlet and outlet ports were created using biopsy punches. The devices were placed directly into wells of a six-well tissue-culture plate for cell-culture experiments. Prior to use, devices were sterilized, rinsed with sterile phosphate-buffered saline, and equilibrated with culture medium under standard cell-culture conditions.

Microfluidic invasion assay

The microfluidic invasion assay was performed using MDA-MB-231 cells to evaluate directional invasion under defined chemoattractant gradients. The assay consisted of two sequential components: (1) validation of gradient establishment and device functionality, and (2) evaluation of invasion under pharmacologic and growth factor stimulation.

1. Validation of chemoattractant gradient formation

To validate the formation of stable and functional gradients within the microfluidic device, an asymmetric serum-gradient experiment was conducted. MDA-MB-231 cells were seeded into the central culture channel in medium containing 5% fetal bovine serum (FBS). Signaling channels 1 and 2 were supplied with medium containing 20% FBS, while channels 3 and 4 were filled with serum-free medium. This configuration enabled assessment of directional migration toward a defined high-serum chemoattractant source. Media in all reservoirs were refreshed daily to maintain stable gradients. After 3 days of culture under standard conditions (37°C, 5% CO₂), cell invasion into adjacent hydrogel regions was evaluated by phase-contrast microscopy to confirm preferential migration toward high-serum channels, thereby validating the invasion model.

2. Evaluation of rogaratinib and growth factor–mediated invasion

Following validation, invasion assays were performed under defined biochemical conditions. Chemoattractant gradients were established by supplying signaling channels with either serum-free medium (control), medium containing 10% or 20% FBS, or recombinant basic fibroblast growth factor (bFGF; 50 ng/mL; PeproTech, Rocky Hill, NJ, USA). The central culture channel was maintained in medium containing 5% FBS. For each chemoattractant condition, parallel devices were treated with either rogaratinib (0.1 µM) or an equivalent volume of dimethyl sulfoxide (DMSO) as vehicle control. Media were refreshed daily to preserve gradient stability. Cell invasion into the hydrogel matrix was monitored over a 3-day period using an inverted phase-contrast microscope. Images of predefined hydrogel regions were acquired at specified time points. Quantitative analysis was performed using ImageJ software. Invasion was calculated as the percentage of cells located within a defined hydrogel

area relative to the total number of cells in the field of view. Multiple fields were analyzed per device, and data were obtained from at least three independent experiments.

Patient-derived breast cancer organoid culture

Breast tumor specimens were obtained from three patients with distinct receptor profiles (ER⁺/PR⁺/HER2⁻, ER⁻/PR⁻/HER2⁺, and triple-negative). This study was conducted in accordance with the ethical standards of the institutional research committee under ethics approval code IR.MU.MED.REC.1400.240. All participants provided written informed consent and had complete medical records available prior to inclusion in the study. Tumor tissues were mechanically minced into small fragments and dissociated to generate single cells and small cell clusters. The resulting suspension was passed through a cell strainer to remove undigested tissue fragments. Organoids were generated using the hanging-drop method. Briefly, defined volumes of the cell suspension were dispensed as droplets and cultured to allow cell aggregation and organoid formation. After two days of culture, formed organoids were treated with rogaratinib for an additional three days. Organoid viability was evaluated using Calcein-AM and propidium iodide (PI) live–dead staining. Fluorescence images were acquired following live–dead staining. ImageJ software was used to quantify fluorescence intensity. Viability was calculated as the ratio of green fluorescence to total fluorescence (green / [green + red]). Data were normalized to untreated controls.

Statistical analysis

All data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using R software (version 4.3.2). Comparisons between treated and control groups were conducted using Student's t-test, and a p-value < 0.05 was considered statistically significant. All experiments were performed with at least three biological replicates unless otherwise stated.

Results

Rogaratinib inhibits the proliferation of MDA-MB-231 triple-negative breast cancer cells

To evaluate the antiproliferative effect of Rogaratinib, MDA-MB-231 cells were treated with five concentrations of the drug (100, 10, 1, 0.1, and 0.01 μ M) for 24, 48, and 72 h, and cell viability was measured using the MTT assay (Fig. 1). Rogaratinib reduced cell viability in both a concentration- and time-dependent manner. At the 72-h time point, the half-maximal inhibitory concentration (IC₅₀) was approximately 0.1 μ M. At this IC₅₀ concentration, cell viability decreased progressively over time, reaching 73.4% at 24 h, 57.7% at 48 h, and 47.8% at 72 h compared with vehicle-treated controls (Fig. 1). These findings indicate that Rogaratinib significantly inhibits the proliferation of MDA-MB-231 cells in a dose- and time-dependent manner.

Microfluidic device design

The microfluidic invasion device consists of a central culture channel flanked by signaling channels separated by hydrogel barriers (Fig. 2). The PDMS structure confines the hydrogel within defined regions, allowing the formation of stable and reproducible chemoattractant gradients across the hydrogel matrix. This configuration enables quantitative assessment of directional cell invasion under controlled biochemical gradients. To confirm the formation of stable and functional chemoattractant gradients within the microfluidic platform, MDA-MB-231 cells were exposed to asymmetric serum conditions. Cells preferentially invaded toward hydrogel regions adjacent to signaling channels containing 20% FBS, whereas minimal invasion was observed toward serum-free channels after 3 days of culture (Fig. 3). This directional migration demonstrates that the device successfully establishes stable biochemical gradients capable of guiding chemotactic invasion. These findings validate the microfluidic system as a reliable platform for quantitative analysis of growth factor- and serum-driven breast cancer cell invasion.

Rogaratinib reduces bFGF-stimulated invasion of MDA-MB-231 cells

Representative images of the hydrogel region illustrate MDA-MB-231 cell migration along the channel edges toward chemoattractant sources (Fig. 4a). Quantitative analysis using ImageJ (percentage of cells within a defined hydrogel area) showed that invasion increased over 3 days in both bFGF-treated and untreated groups (Fig. 4b). Exposure to bFGF markedly enhanced invasion into the hydrogel matrix; concurrent treatment with Rogaratinib reduced both the percentage of invading cells and the apparent invasion front (Fig. 4c). The reduction associated with Rogaratinib in the bFGF condition approached significance ($p \approx 0.05$, $n = 3$), suggesting that FGFR inhibition attenuates bFGF-driven invasion in triple-negative breast-cancer cells. Data are presented as mean $\pm$ SD.

Rogaratinib inhibits invasion under strong serum stimulation

Under high-serum conditions (20% FBS in signaling channels), MDA-MB-231 cells exhibited robust invasion into the hydrogel over 3 days. Representative images and ImageJ-based quantification demonstrate that treatment with Rogaratinib significantly decreased the percentage of invading cells compared with vehicle-treated controls ($p < 0.05$, $n = 3$) (Fig. 5a–c). These findings indicate that Rogaratinib effectively limits serum-stimulated invasive behavior in this highly invasive breast-cancer cell line.

Rogaratinib effect under moderate (10% FBS) and basal (serum-free) conditions

Under moderate chemoattractant stimulation (10% FBS), MDA-MB-231 cells exhibited intermediate invasion into the hydrogel region after 3 days of culture. Quantitative ImageJ analysis showed a

reduction in the percentage of invading cells in Rogaratinib-treated samples compared with controls; however, this decrease did not reach statistical significance ($p > 0.05$, $n = 3$) (Fig. 6a–c). In contrast, when cultured in basal media lacking FBS or growth factors, MDA-MB-231 cells showed minimal invasion into the hydrogel. Rogaratinib treatment did not further alter this already low invasion level ($n = 3$) (Fig. 7a–c). Together, these findings indicate that Rogaratinib's inhibitory effect on invasion becomes evident only under conditions that include external pro-migratory cues, such as serum or growth-factor stimulation.

Rogaratinib decreases viability in short-term patient-derived organoids

To evaluate the translational relevance of FGFR inhibition, organoids were established from breast-tumor tissues of three patients with distinct receptor profiles: one ER⁺/PR⁺/HER2⁻, one ER⁻/PR⁻/HER2⁺, and one triple-negative (ER⁻/PR⁻/HER2⁻). After two days of culture, organoids were treated with Rogaratinib for three days and assessed by Calcein-AM/propidium-iodide (PI) live-dead staining. ImageJ analysis of fluorescence intensity (green / [green + red]) was used to calculate viability. Two of the three patient-derived organoid samples showed a significant reduction in calculated viability and a corresponding increase in PI signal following Rogaratinib treatment compared with untreated controls ($p < 0.05$, $n = 3$ per condition). The third sample showed no marked change (Fig. 8A–B). Overall, Rogaratinib reduced organoid viability in the majority of patient samples, mirroring its anti-invasive activity observed in the microfluidic assay.

Discussion

In this study, we investigated the therapeutic potential of the pan-FGFR inhibitor rogaratinib in complementary preclinical models of breast cancer, with a particular focus on triple-negative breast cancer (TNBC). Rogaratinib potently suppressed proliferation of MDA-MB-231 TNBC cells in a time- and dose-dependent manner and markedly reduced invasion in a microfluidic model that recapitulates pro-metastatic microenvironmental conditions. Notably, inhibition of invasion was most pronounced in the presence of strong chemoattractant gradients, such as serum and basic FGF (FGF2), whereas minimal effects were observed under basal conditions, indicating a context-dependent role for FGFR signaling in invasive behavior. Extending these findings to clinically relevant systems, rogaratinib also reduced viability in the majority of patient-derived breast cancer organoids representing ER⁺/PR⁺/HER2⁻, HER2⁺, and TNBC subtypes. Collectively, these results support a central role for aberrant FGF/FGFR signaling in promoting breast cancer cell proliferation, survival, and invasion, and support FGFR inhibition as a context-dependent therapeutic strategy, particularly in microenvironmentally stimulated settings. In MDA-MB-231 cells, FGFR1 overexpression drives MAPK/ERK and PI3K/AKT signaling [21–23]. Rogaratinib blocks FGFR autophosphorylation at nanomolar concentrations [10]. Our observed IC₅₀ (0.1 μ M) is clinically achievable, as peak plasma levels reach 2–5 μ M [24]. Compared to erdafitinib or pemigatinib, rogaratinib shows similar potency but distinct selectivity [25]. This context-dependent activity suggests that ligand stimulation, not baseline FGFR expression alone, determines sensitivity.

In our microfluidic invasion model, rogaratinib strongly reduced MDA-MB-231 cell migration toward serum and FGF2 gradients, indicating that FGFR signaling is required for invasion under pro-metastatic conditions. These findings align with observations reported by Li et al. [26], who demonstrated in a tumor-on-chip system that early tumor cell migration is strongly influenced by stromal-derived growth factors and fibroblast-mediated signaling. In their study, bFGF enhanced cancer cell migration, while rogaratinib suppressed invasion predominantly in the presence of stromal activation, with minimal effects under basal conditions. Similarly, in our 3D invasion-on-chip model, rogaratinib nearly abrogated invasion induced by bFGF and serum-derived chemoattractants, while only minimal inhibition was observed in unstimulated conditions.

Consistent with this model, MDA-MB-231 cells rely on aberrant FGFR signaling for both proliferation and motility, frequently overexpressing FGFR1 and exhibiting sensitivity to FGFR inhibition through suppression of ERK and Akt pathways [27, 28]. Comparable growth-inhibitory effects have been reported with other FGFR inhibitors in basal-like and triple-negative breast cancer models, where FGFR blockade induces G1 arrest and reduces cell viability [27]. Importantly, our invasion data reveal a ligand-dependent role for FGFR signaling in directional migration: rogaratinib markedly suppressed invasion in the presence of FGF2-containing chemoattractant gradients, while exerting minimal effects under unstimulated conditions. This indicates that FGFR signaling primarily governs chemotactic guidance rather than intrinsic motility, likely by enabling tumor cells to sense stromal- and flow-mediated cues. Consistent with prior studies showing that FGF2 derived from cancer-associated fibroblasts drives TNBC migration via FGFR1–MAPK signaling and that FGF2 neutralization abolishes CAF-induced chemotaxis[29–31], these findings support the conclusion that rogaratinib is most effective at suppressing FGFR-driven invasion under pro-metastatic conditions.

The broader antitumor effects observed in patient-derived organoids (PDOs) indicate that FGFR dependency is not limited to TNBC. Rogaratinib decreased viability in organoids derived from ER⁺/PR⁺ and HER2⁺ tumors as well, suggesting that FGFR signaling provides survival advantages across molecular subtypes. Moreover, organoids represent a clinically relevant ex vivo model that preserves intratumoral heterogeneity, thereby strengthening the translational significance of our findings. Consistent with our findings, prior studies have reported subtype-specific sensitivity to FGFR inhibition in patient-derived systems, including growth suppression of FGFR4-high luminal organoids by FGFR4-selective inhibitors[29], and responsiveness of FGFR1/2-altered HR⁺ models to rogaratinib-based strategies [32]. Moreover, preclinical xenograft and patient-derived models have demonstrated that the magnitude of FGFR expression or amplification predicts response to FGFR inhibition [10]. Collectively, these observations support the concept that FGFR signaling functions as a context-dependent survival axis across ER⁺, HER2⁺, and TNBC tumors.

Despite promising preclinical activity, rogaratinib faced clinical challenges. The phase II/III FORT-1 trial in FGFR1/3 mRNA-positive urothelial carcinoma failed to improve overall survival [14]. In breast cancer, a phase I combination trial showed only modest efficacy [33]. These failures highlight that FGFR mRNA expression alone poorly predicts response. Our observation that rogaratinib suppresses invasion only

under ligand-stimulated conditions supports a context-dependent model requiring active microenvironmental signaling.

This study has several limitations. First, invasion experiments were performed using a single TNBC cell line, which does not capture the molecular heterogeneity of breast cancer. Second, although the microfluidic platform models defined biochemical gradients, it lacks additional tumor microenvironment components such as immune cells and vascular structures. Third, patient-derived organoid analysis was limited to a small sample set ($n = 3$), restricting generalizability. Finally, mechanistic pathways downstream of FGFR inhibition and potential resistance mechanisms were not explored.

Several limitations warrant consideration. First, only one TNBC cell line (MDA-MB-231) was used; future studies should include additional lines representing molecular subtypes. Second, the microfluidic platform lacks cancer-associated fibroblasts, which are major FGF2 sources [19]. Third, the organoid cohort was limited to three patients; larger cohorts with molecular profiling are needed. Fourth, acquired resistance mechanisms (FGFR gatekeeper mutations, alternative RTK activation, EMT) were not assessed [34, 35]. Finally, organoid viability was assessed after only three days of treatment, capturing acute cytotoxicity but not long-term suppression of tumor-regenerative capacity. Extended culture and organoid passaging following drug washout would provide insight into durable responses.

Future studies should evaluate additional breast cancer models to account for intertumoral heterogeneity and identify molecular predictors of FGFR inhibitor sensitivity. Incorporation of stromal, immune, and vascular components into microfluidic systems may further enhance physiological relevance. Expanding patient-derived organoid cohorts and integrating molecular profiling approaches will be important for defining biomarkers of response and resistance to FGFR-targeted therapy.

Conclusion

In conclusion, our study demonstrates that FGFR signaling plays a context-dependent but critical role in breast cancer progression, regulating tumor cell proliferation, survival, and chemotactic invasion in response to pro-metastatic microenvironmental cues. Using complementary in vitro, microfluidic, and patient-derived organoid models, we show that rogaratinib is most effective under ligand-rich conditions that mimic stromal activation, while exerting minimal effects on basal motility. These findings provide mechanistic insight into the role of FGF/FGFR signaling as a microenvironment-responsive pathway and support the development of biomarker-guided FGFR-targeted therapies for breast cancer.

Declarations

Acknowledgments

We are deeply grateful to Isfahan University of Medical Sciences, Deputy of Research, for their support (Grant No 199635)

References

1. Zhang S, Jin Z, Bao L, Shu P (2024) The global burden of breast cancer in women from 1990 to 2030: assessment and projection based on the global burden of disease study 2019. *Front Oncol* 14
2. Jie H, Ma W, Huang C, Diagnosis (2025) Prognosis, and Treatment of Triple-Negative Breast Cancer: A Review. *Breast Cancer: Targets Therapy Volume* 17:265–274
3. Le Du F et al (2025) [Triple negative breast cancer: Current status and perspectives]. *Bull Cancer* 112:82–99
4. Rodríguez-Bejarano OH, Parra-López C, Patarroyo MA (2024) A review concerning the breast cancer-related tumour microenvironment. *Crit Rev Oncol Hematol* 199:104389
5. Dou T et al (2024) The cellular composition of the tumor microenvironment is an important marker for predicting therapeutic efficacy in breast cancer. *Front Immunol* 15
6. Li Y, Wang C, Huang T, Yu X, Tian B (2023) The role of cancer-associated fibroblasts in breast cancer metastasis. *Front Oncol* 13
7. Tuokkola JE, Schwertfeger KL (2026) Breast Cancer Progression by the FGF/FGFR Axis: A Metabolic Perspective. *J Mammary Gland Biol Neoplasia* 31:2
8. Zhang P et al (2024) Targeting FGFR for cancer therapy. *J Hematol Oncol* 17:39
9. Chen J, Wang Q, Wu H, Huang X, Cao C (2025) Therapies targeting triple-negative breast cancer: a perspective on anti-FGFR. *Front Oncol* 14
10. Grünewald S et al (2019) Rogaratinib: A potent and selective pan-FGFR inhibitor with broad antitumor activity in FGFR-overexpressing preclinical cancer models. *Int J Cancer* 145:1346–1357
11. Krook MA et al (2021) Fibroblast growth factor receptors in cancer: genetic alterations, diagnostics, therapeutic targets and mechanisms of resistance. *Br J Cancer* 124:880–892
12. Politz O et al (2017) Abstract 1079: Preclinical activity of the FGFRinhibitor BAY 1163877 alone or in combination with antihormonal therapy in breast cancer. *Cancer Res* 77:1079–1079
13. Schuler M et al (2019) Rogaratinib in patients with advanced cancers selected by FGFR mRNA expression: a phase 1 dose-escalation and dose-expansion study. *Lancet Oncol* 20:1454–1466
14. Sternberg CN et al (2023) FORT-1: Phase II/III Study of Rogaratinib Versus Chemotherapy in Patients With Locally Advanced or Metastatic Urothelial Carcinoma Selected Based on FGFR1 / 3 mRNA Expression. *J Clin Oncol* 41:629–639
15. Wan L, Neumann CA, LeDuc PR (2020) Tumor-on-a-chip for integrating a 3D tumor microenvironment: chemical and mechanical factors. *Lab Chip* 20:873–888
16. Frankman ZD, Jiang L, Schroeder JA, Zohar Y (2022) Application of Microfluidic Systems for Breast Cancer Research. *Micromachines (Basel)* 13:152
17. Truong D et al (2016) Breast Cancer Cell Invasion into a Three Dimensional Tumor-Stroma Microenvironment OPEN. 10.1038/srep34094. Nature Publishing Group <https://doi.org/>

18. Zhai J et al (2024) Drug screening on digital microfluidics for cancer precision medicine. *Nat Commun* 15:4363
19. Lugo-Cintrón KM et al (2020) Breast Fibroblasts and ECM Components Modulate Breast Cancer Cell Migration through the Secretion of MMPs in a 3D Microfluidic Co-Culture Model. *Cancers (Basel)* 12:1173
20. Samandari M, Rafiee L, Alipanah F, Sanati-Nezhad A, Javanmard SH (2021) A simple, low cost and reusable microfluidic gradient strategy and its application in modeling cancer invasion. *Sci Rep* 11:89635
21. Babina IS, Turner NC (2017) Advances and challenges in targeting FGFR signalling in cancer. *Nat Rev Cancer* 17:318–332
22. Braun M, Piasecka D, Sadej R, Romanska HM (2024) FGFR4-driven plasticity in breast cancer progression and resistance to therapy. *Br J Cancer* 131:11–22
23. Peng Y, Zhang P, Mei W, Zeng C (2024) Exploring FGFR signaling inhibition as a promising approach in breast cancer treatment. *Int J Biol Macromol* 267:131524
24. Schuler M et al (2019) Rogaratinib in patients with advanced cancers selected by FGFR mRNA expression: a phase 1 dose-escalation and dose-expansion study. *Lancet Oncol* 20:1454–1466
25. Grünewald S et al (2019) Rogaratinib: A potent and selective pan-FGFR inhibitor with broad antitumor activity in FGFR-overexpressing preclinical cancer models. *Int J Cancer* 145:1346–1357
26. Li C et al (2021) On-Chip Replication of Extremely Early-Stage Tumor Behavior. *ACS Appl Mater Interfaces* 13:19768–19777
27. Sharpe R et al (2011) FGFR Signaling Promotes the Growth of Triple-Negative and Basal-Like Breast Cancer Cell Lines Both In Vitro and In Vivo. *Clin Cancer Res* 17:5275–5286
28. Suh J, Kim D, Lee Y, Jang J, Surh Y (2020) Fibroblast growth factor-2, derived from cancer-associated fibroblasts, stimulates growth and progression of human breast cancer cells via FGFR1 signaling. *Mol Carcinog* 59:1028–1040
29. Chew NJ et al (2021) Evaluation of FGFR targeting in breast cancer through interrogation of patient-derived models. *Breast Cancer Res* 23:82
30. Suh J et al (2022) Nuclear Localization of Fibroblast Growth Factor Receptor 1 in Breast Cancer Cells Interacting with Cancer Associated Fibroblasts. *J Cancer Prev* 27:68–76
31. Hu D et al (2022) Cancer-associated fibroblasts in breast cancer: Challenges and opportunities. *Cancer Commun* 42:401–434
32. Martínez-Jáñez N et al (2025) Co-clinical trial targeting ER, FGFR and CDK4/6 in resistant hormone-positive breast cancer with FGFR alterations. *NPJ Precis Oncol* 9:343
33. Martínez-Jáñez N et al (2024) Abstract PO4-05-09: Phase I study of the FGFR inhibitor rogaratinib, fulvestrant and palbociclib in advanced hormone receptor-positive (HR+) breast cancer (BC) with FGFR1/2 amplification and/or overexpression (FGFR1/2+). *Cancer Res.* 84, PO4-05-09-PO4-05-09

34. Shyamsunder S, Lu Z, Takiar V, Waltz SE (2025) Challenges and resistance mechanisms to EGFR targeted therapies in head and neck cancers and breast cancer: Insights into RTK dependent and independent mechanisms. *Oncotarget* 16:508–530

35. Brown WS, Akhand SS, Wendt M (2016) K. FGFR signaling maintains a drug persistent cell population following epithelial-mesenchymal transition. *Oncotarget* 7:83424–83436

Figures

Figure 1:

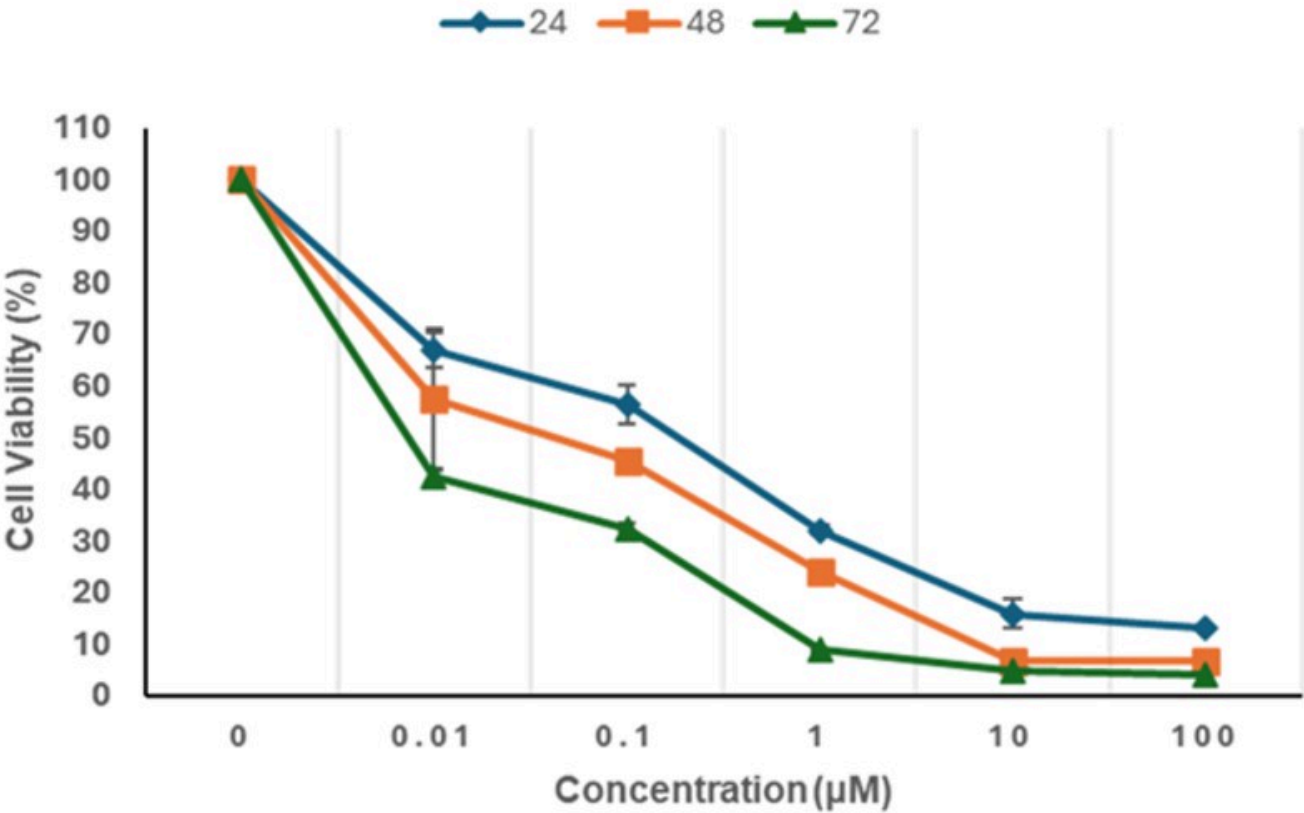


Figure 1

Rogaratinib inhibits proliferation of MDA-MB-231 triple-negative breast cancer cells. Dose–response curve of MDA-MB-231 cell viability after for 24, 48, and 72 h treatment with Rogaratinib at indicated concentrations (100, 10, 1, 0.1, and 0.01 µM), as measured by MTT assay. The calculated IC₅₀ value was approximately 0.1 µM. Data are expressed as percentage of vehicle-treated control. Values represent mean ± SEM from three independent experiments (n = 3).

Figure 2:

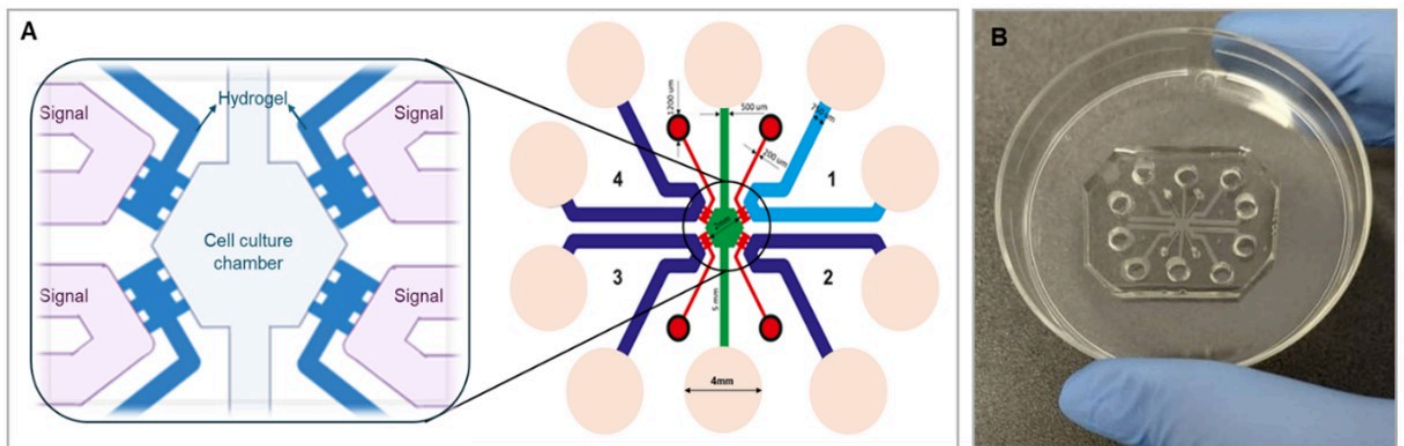


Figure 2

Design of the microfluidic invasion chip. (A) Schematic representation of the previously developed microfluidic device used to study breast cancer cell invasion. The chip consists of one central cell culture channel flanked by four signaling channels, separated by hydrogel barriers retained by micro-posts. The hydrophobic PDMS structure confines the hydrogel within defined regions, allowing controlled diffusion of soluble factors to create chemoattractant gradients. (B) The device is formed by direct mounting of the Polydimethylsiloxane (PDMS) microfluidic chip on the polystyrene (PS)-based culture dishes or plates.

Figure 3:

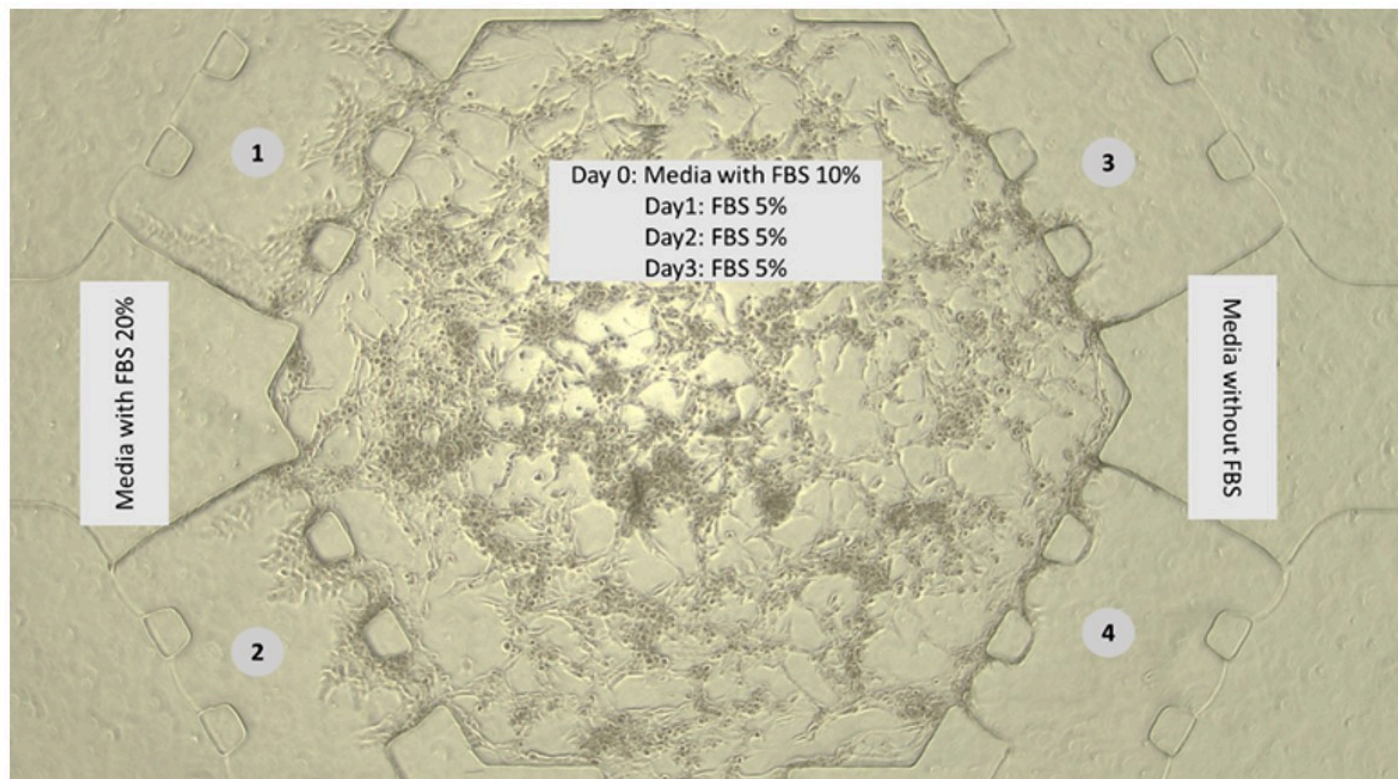


Figure 3

Validation of the microfluidic invasion assay using FBS gradients. MDA-MB-231 cells were seeded in the central culture chamber with FBS 5%, exposed to 20% FBS media in two signaling channels (1 and 2) and to serum-free media in channels 3 and 4. After 3 days, cells invaded preferentially toward the 20% FBS regions, confirming the establishment of stable chemoattractant gradients and validating the microfluidic model as an invasion assay.

Figure 4:

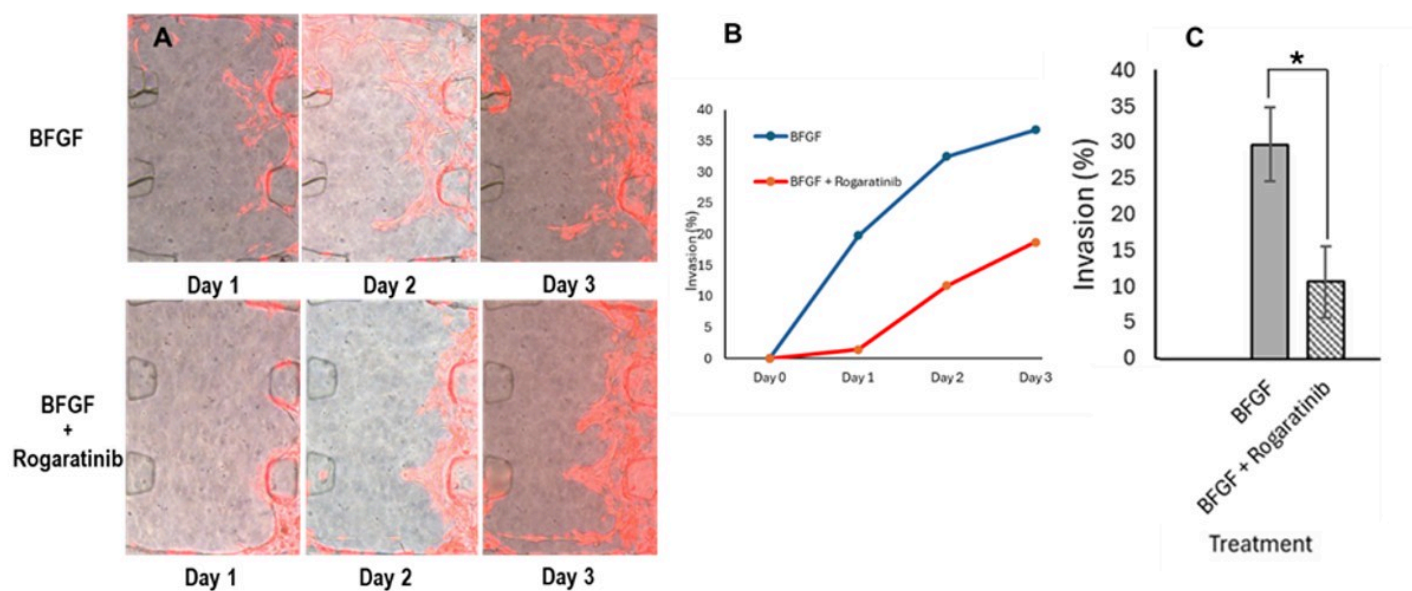


Figure 4

Effect of Rogaratinib on invasion stimulated by basic fibroblast growth factor (bFGF). (A) Representative images of the hydrogel region illustrate MDA-MB-231 cell invasion, showing how cells preferentially migrate along the edges toward the chemoattractant source. (B) Quantitative analysis indicates that MDA-MB-231 cell invasion increased over 3 days post-seeding in both the bFGF-treated and untreated groups. (C) Exposure to bFGF markedly enhanced cell invasion into the hydrogel matrix, whereas concurrent treatment with Rogaratinib reduced both the invasion distance and the number of invading cells. This reduction showed a trend toward significance ($p \approx 0.05$, $n = 3$), suggesting that FGFR inhibition may attenuate bFGF-driven invasion. Data represent mean $\pm$ SD.

Figure 5:

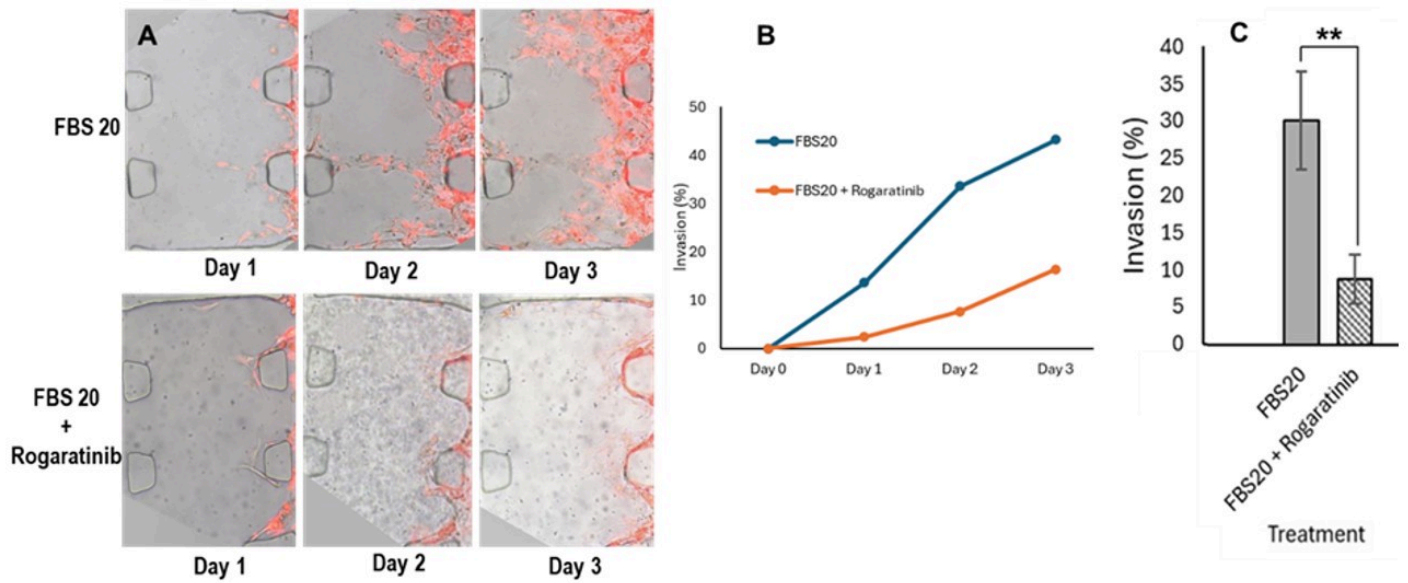


Figure 5

Effect of Rogaratinib under 20% FBS stimulation. (A) Representative images of the hydrogel region illustrate MDA-MB-231 cell invasion, showing how cells preferentially migrate along the edges toward the FBS 20%. (B) Quantitative analysis indicates that MDA-MB-231 cell invasion increased over 3 days post-seeding in both group with and without Rogaratinib. (C) Representative phase-contrast images and quantitative analysis showing that treatment with Rogaratinib markedly suppresses MDA-MB-231 cell invasion toward 20% FBS compared with untreated controls. Data represent mean $\pm$ SD from $n = 3$ independent experiments; $p < 0.05$ versus control.

Figure 6:

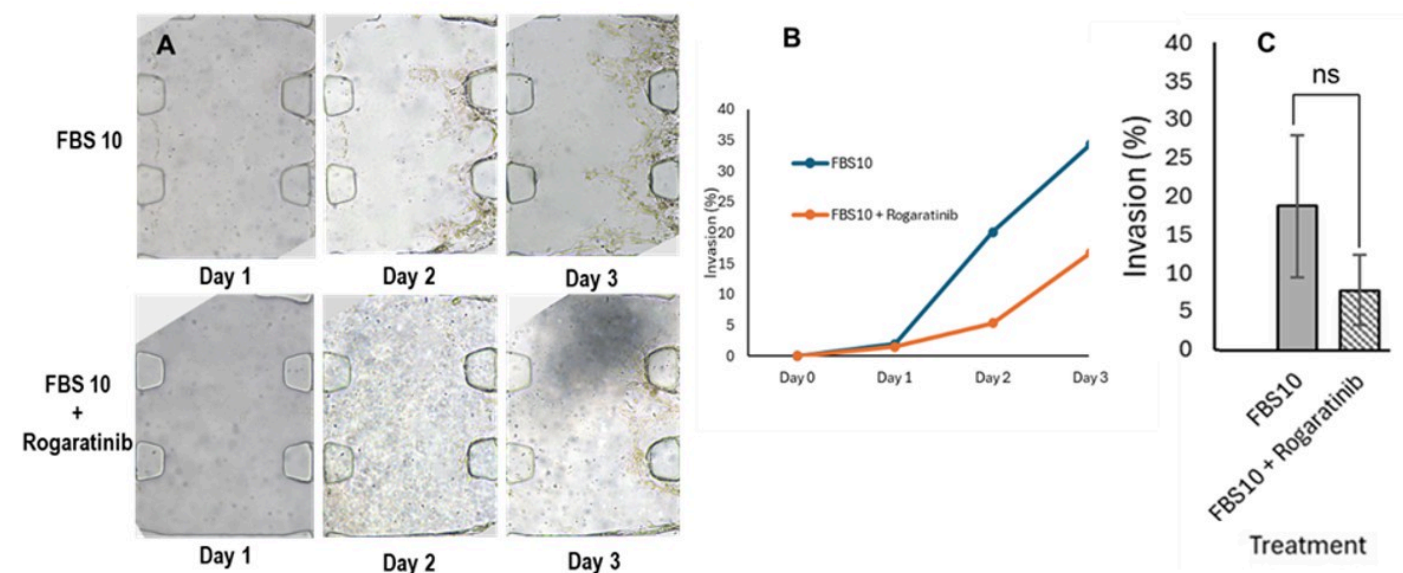


Figure 6

Effect of Rogaratinib under 10% FBS stimulation. (A) Representative images of the hydrogel region illustrate MDA-MB-231 cell invasion. (B) Quantitative analysis indicates that MDA-MB-231 cell invasion increased over 3 days post-seeding in both group with and without Rogaratinib. (C) Representative micrographs showing MDA-MB-231 invasion with and without Rogaratinib in 10% FBS conditions. Although invasion decreased in the presence of Rogaratinib, the reduction did not reach statistical significance. Data are mean $\pm$ SD, n = 3; ns = not significant.

Figure 7:

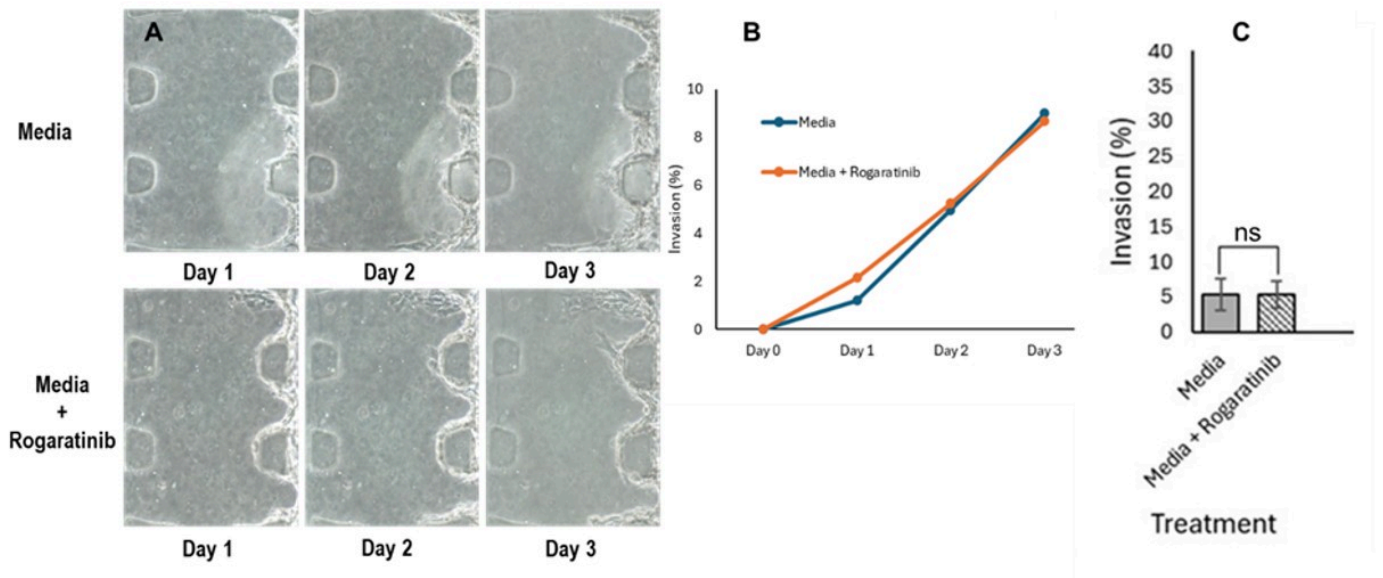


Figure 7

Effect of Rogaratinib under basal media conditions. (A) Representative images of the hydrogel region illustrate MDA-MB-231 cell invasion. (B) Quantitative analysis indicates that MDA-MB-231 cell invasion increased over 3 days post-seeding in both group with and without Rogaratinib. (C) Under normal media (no FBS or growth factors), MDA-MB-231 cells exhibited minimal invasion. Rogaratinib treatment did not further alter this baseline behavior, confirming that invasion inhibition is specific to stimulated conditions.

Figure 8:

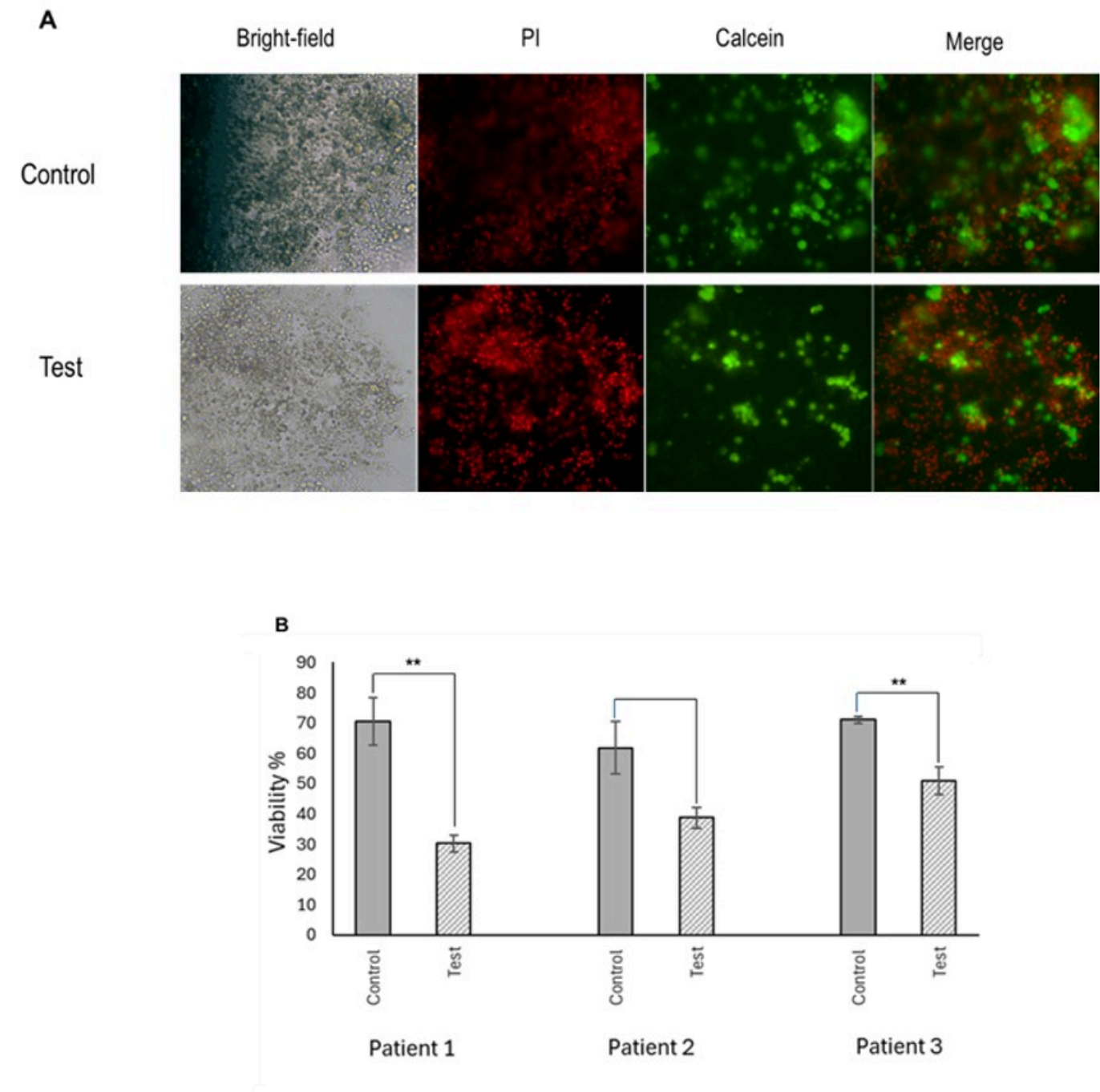


Figure 8

Effect of Rogaratinib on short-term patient-derived breast organoids. (A) Organoids generated from three patient breast tissue samples were cultured for 5 days and treated with Rogaratinib. Live/dead staining was performed using Calcein-AM (live cells) and propidium iodide (PI, dead cells) to assess viability after treatment. (B) Quantitative analysis revealed that organoids from two of the three patients showed a significant reduction in viability and an increase in the proportion of dead cells in response to Rogaratinib compared with untreated controls, indicating decreased organoid viability upon FGFR inhibition.