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FOLATE-FUNCTIONALIZED MESOPOROUS SILICA NANOPARTICLES FOR TARGETED DELIVERY OF MEBENDAZOLE IN FR⁺ BREAST CANCER CELLS

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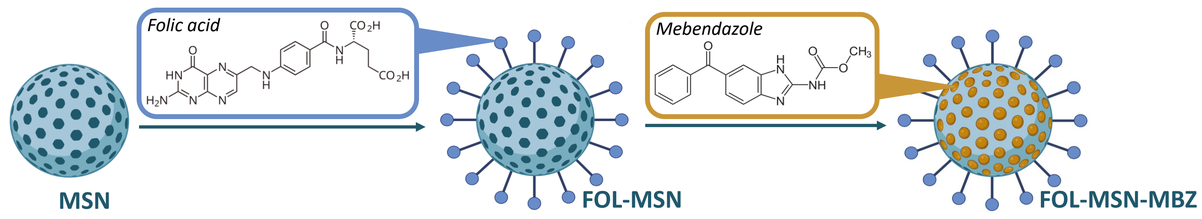
INTRODUCTION & AIM

Breast cancer is the most frequently diagnosed cancer among women worldwide. Triple-negative breast cancer (TNBC) and tamoxifen-resistant (TamR) breast cancer remain major clinical challenges due to limited therapeutic options and poor prognosis. In recent years, mebendazole (MBZ), an orally available FDA-approved anthelmintic drug, has gained attention as a repurposing candidate in oncology due to its anticancer activity across multiple tumor types, including breast cancer; however, its clinical translation is limited by low oral bioavailability.

To overcome this limitation and enhance tumor targeting, we developed a folate-functionalized mesoporous silica nanoparticle system (FOL-MSN-MBZ) for the delivery of MBZ to folate receptor-positive (FR⁺) breast cancer models, including TamR and TNBC cells.

METHODS

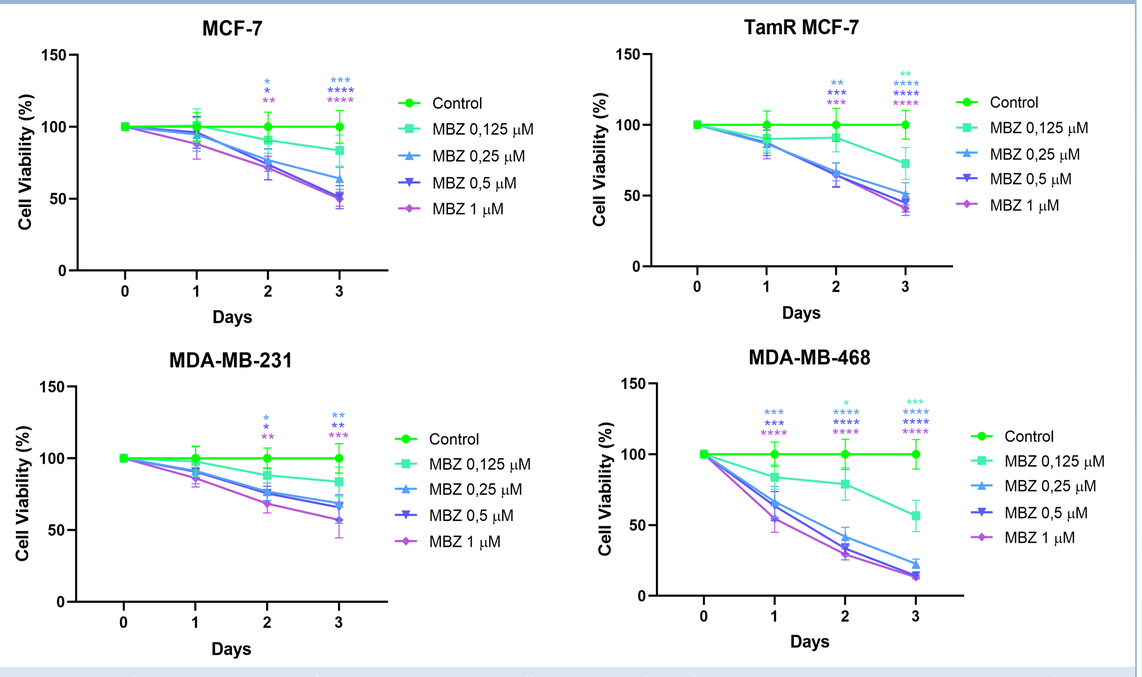
FOL-MSN-MBZ was synthesized by covalently grafting folic acid (FOL) onto the surface of MSNs, followed by MBZ loading through a two-step impregnation process (drug loading: 8.80%). The cytotoxic activity of free MBZ was evaluated using MTT assays. The selective efficacy of FOL-MSN-MBZ was assessed in FR⁺ breast cancer cell lines—MCF-7, TamR MCF-7, TamR T47D, MDA-MB-231, and MDA-MB-468—and compared with FR⁻ normal cells. Cell proliferation was assessed after 72 h treatment using Trypan Blue exclusion assays, while long-term growth inhibition was evaluated by clonogenic assays. In 3D breast cancer spheroids, treatment efficacy was assessed by measuring spheroid size and metabolic activity (Alamar Blue assay). Free MBZ was used as a reference control.



RESULTS & DISCUSSION

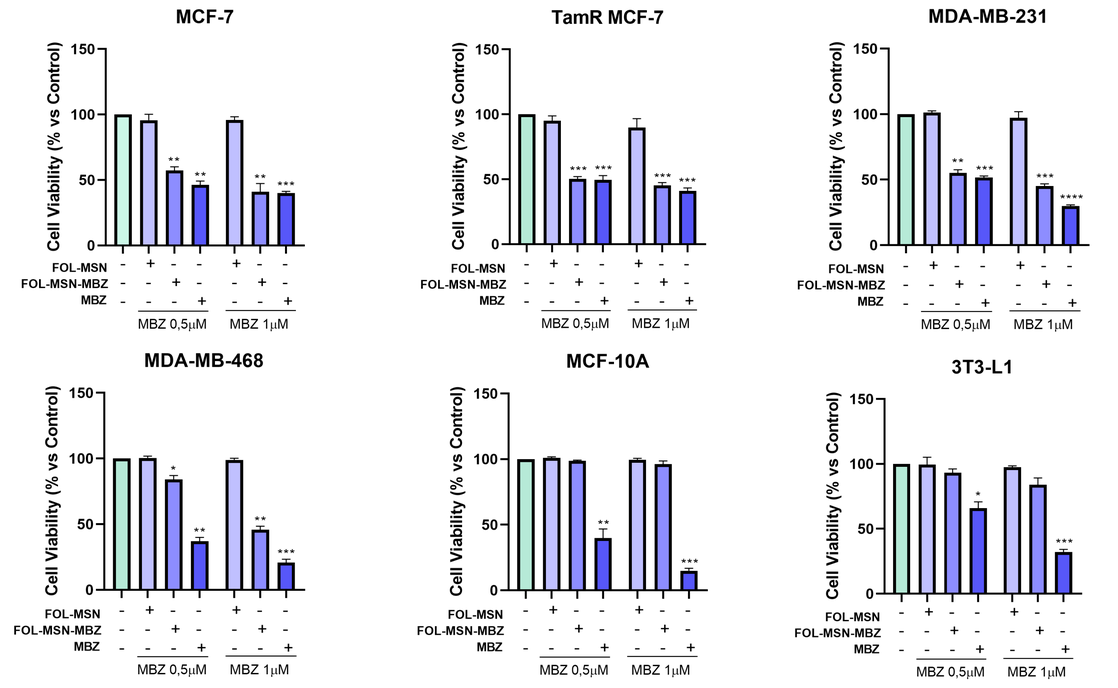
Preliminary experiments with free MBZ confirmed its dose- and time-dependent antiproliferative activity in breast cancer cells, identifying 0.5 and 1 µM as the most effective concentrations after 72 h of treatment. These concentrations were therefore selected for the evaluation of the nanosystem. Our data demonstrate that FOL-MSN-MBZ effectively induces selective cell death in all FR⁺ breast cancer models, including TamR and TNBC cell lines, while exhibiting limited effects on non-tumoral FR⁻ cells (MCF-10A and 3T3-L1). In contrast, free MBZ demonstrated cytotoxic effects in both cancerous and non-cancerous cells, suggesting reduced selectivity. The nanosystem also impaired clonogenic potential and decreased spheroid size and viability in 3D breast cancer models, with effects comparable to those observed for free MBZ, indicating that nanoencapsulation preserves the antitumor activity of the drug while improving its selectivity.

Fig.1 MBZ EFFECTIVELY REDUCES VIABILITY IN CANCER CELL LINES



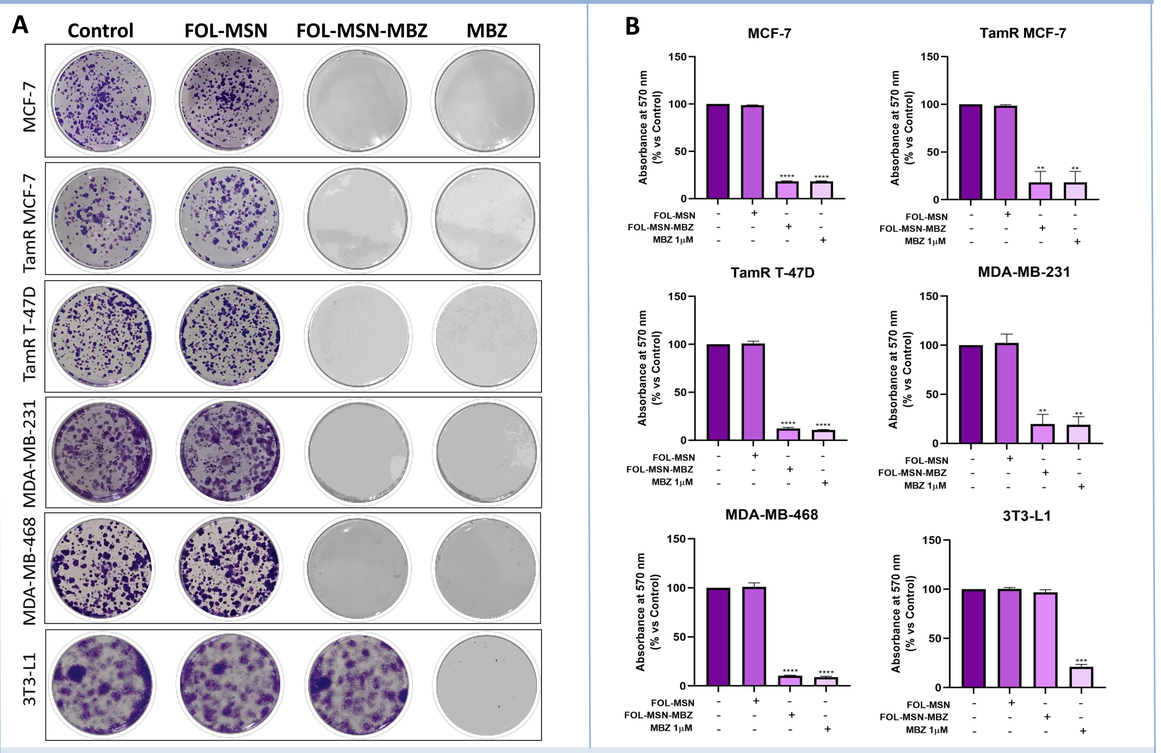
To evaluate the cytotoxicity of MBZ on cancer cells, parental and TamR MCF-7, MDA-MB-231, and MDA-MB-468 cell lines were treated with increasing concentrations of MBZ. Cell viability was assessed at 24, 48, and 72 hours using the MTT assay, revealing higher cell death at 72 hours. Values represent the mean ± SD of three independent experiments performed in sextuplicate. Statistical significance (vs Control): *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig.2 FOL-MSN-MBZ INDUCES DEATH IN FR⁺ CANCER CELLS WHILE SPARING FR⁻ NORMAL CELLS



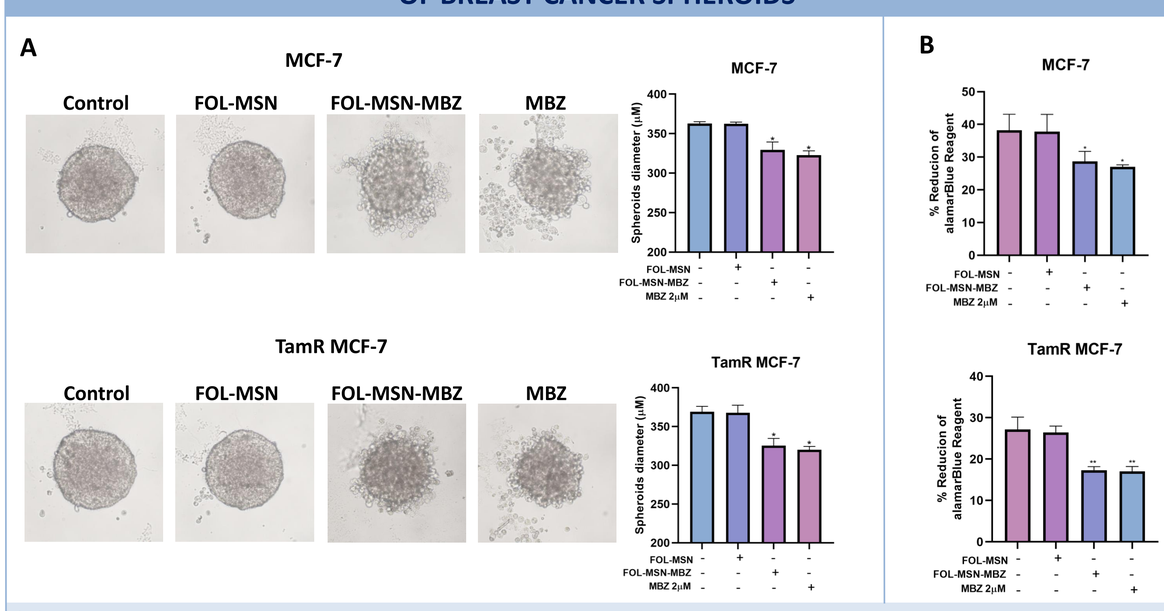
FOL-MSN-MBZ induces death in FR+ parental MCF-7, TamR MCF-7, MDA-MB-231 and MDA-MB-468 cancer cells lines, while having minimal impact on normal 3T3-L1 cells and almost no effect on non-cancerous MCF10A cells. Cells were treated with FOL-MSN-MBZ (at two MBZ concentrations) or left untreated (Control); FOL-MSN was used as a negative control, and free MBZ (0.5 and 1 µM) as a positive control. Cell viability was assessed after 72 hours using the Trypan Blue exclusion test. Values represent the mean ± SD of two independent experiments performed in triplicate. Statistical significance (vs Control): *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig.3 FOL-MSN-MBZ IMPAIRS COLONY FORMATION IN BREAST CANCER CELLS



(A) Representative images of Crystal Violet-stained colonies after 14 days of treatment under the same experimental conditions used for the cell proliferation assay. (B) Quantification of colony formation by Crystal Violet absorbance at 570 nm. FOL-MSN-MBZ suppressed clonogenic survival in all breast cancer models but not in 3T3-L1 cells, while free MBZ significantly reduced colony formation in both cancerous and non-cancerous cells. Values represent the mean ± SD of three independent experiments performed in triplicate. Statistical significance (vs Control): **p < 0.01; ***p < 0.001; ****p < 0.0001.

Fig.4 FOL-MSN-MBZ REDUCES 3D GROWTH AND VIABILITY OF BREAST CANCER SPHEROIDS



(A) Representative images and diameter measurements of parental and TamR MCF7 spheroids after 72 h of treatment (2 µM MBZ equivalent). FOL-MSN-MBZ and free MBZ reduced spheroid diameter and compactness, resulting in irregular, frayed borders. (B) Spheroid viability assessed by Alamar Blue assay after 72 h of treatment. Both FOL-MSN-MBZ and free MBZ significantly reduced the metabolic activity of parental and TamR MCF7 spheroids. Values represent the mean ± SD of two independent experiments performed in sextuplicate. Statistical significance (vs Control): *p < 0.05; **p < 0.01.

CONCLUSIONS

Our results indicate that FOL-MSN-MBZ enables targeted delivery of MBZ, inducing cytotoxicity specifically in FR⁺ tumor cells while sparing normal cells, suggesting that the nanosystem can reproduce the antitumoral effects of the free drug with enhanced tumor specificity. These findings support the potential of folate-targeted mesoporous silica nanoparticles as a strategy to enhance the therapeutic profile of MBZ for the treatment of aggressive and therapy-resistant breast cancer subtypes.

ACKNOWLEDGMENT

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