

Avemar, a lyophilised fermented wheat germ extract inhibits breast cancer cell proliferation and invasion *in vitro*

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AIM OF THE STUDY

Avemar, a fermented wheat germ extract has been demonstrated to inhibit metastatic tumor spread and prolong survival in colorectal cancer and melanoma patients.^{1,2} In the present study, the antiproliferative and antimigratory effects of a freeze-dried wheat germ extract (Avemar lyophilisate) have been investigated in breast cancer cells using a 3D-carcinoma-lymphendothelial-cell co-culture model.

METHODS

MCF-7 estrogen-receptor expressing and HCC-1937, MDA-MB-231 and MDA-MB-468 estrogen-receptor negative breast cancer cells were incubated with increasing concentrations of Avemar. Cell cycle phase distribution was determined by flow cytometry. Induction of Caspase-dependent apoptosis was analyzed by determination of the activity of effector caspases 3/7 and 8. Inhibition of tumor cell invasion was quantified using the ORIS Cell Invasion kit. To elucidate the antiinvasive effects of Avemar, a 3D co-culture model of MCF-7 tumor cell spheroids and lymphendothelial cells was utilized.³ The expression of motility-associated proteins was analyzed by Western blotting.

RESULTS

Avemar arrested luminal-type MCF-7 cells in the S phase of the cell cycle, whereas basal type breast cancer cells underwent a G0/G1 arrest in a dose-dependent manner after treatment with 100-400 µg/ml Avemar (Figure 1). Induction of apoptosis was mediated by caspase 3/7 in HCC-1937, MDA-MB-231 and MDA-MB-468, whereas in caspase-3 negative MCF-7 cells, caspase 8 was clearly activated (Figure 2). Invasive capacity of breast cancer cells was inhibited by Avemar lyophilisate in all three cancer cell lines in a dose-dependent manner (Figure 3). In a 3D co-culture model, Avemar significantly inhibited lymphendothelial motility, reducing tumor spheroid induced gap size by 43% (Figure 4). Western blotting revealed regulation of several proteins involved in cell motility, such as NFκB and paxillin (Figure 5).

CONCLUSIONS

Avemar exerts differential effects in luminal and basal type breast cancer cells and is able to inhibit cellular processes involved in tumor cell invasion and lymphatic spread. Therefore, further *in vivo* studies investigating the antitumor effects of this natural compound with particular emphasis on basal-like breast cancer in animal models are necessary.

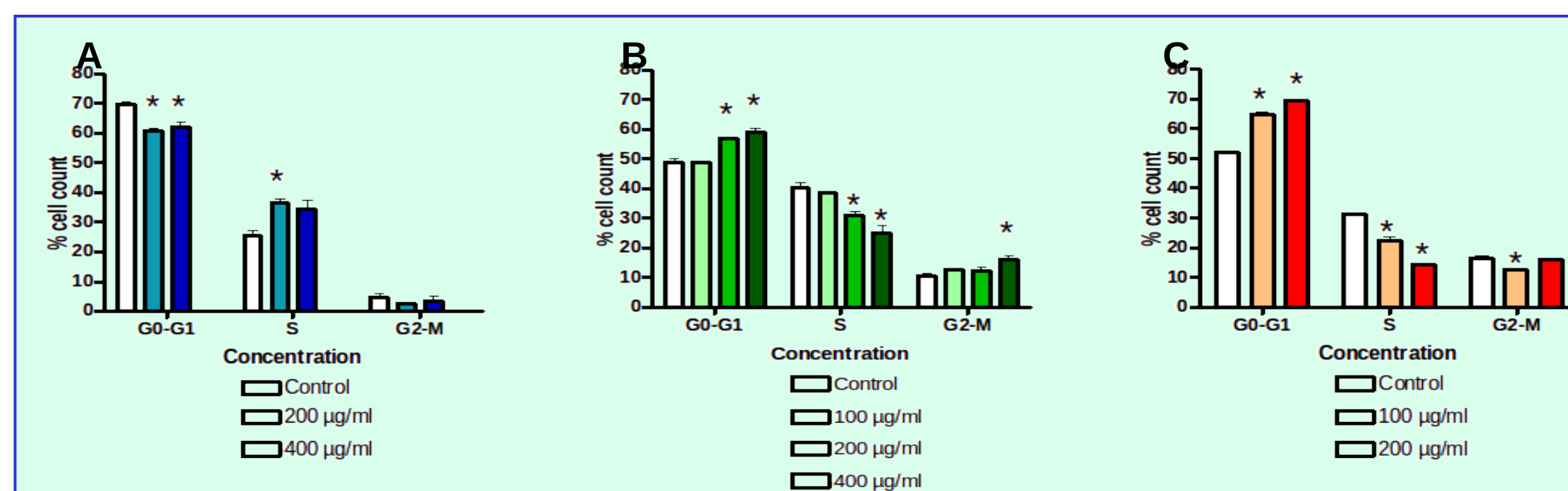


Figure 1. Perturbations in cell cycle phase distribution in MCF-7 (A), HCC-38 (B) and MDA-MB-231 (C) cells after treatment with Avemar lyophilisate for 24 h

Values significantly different from control are marked with an asterisk*

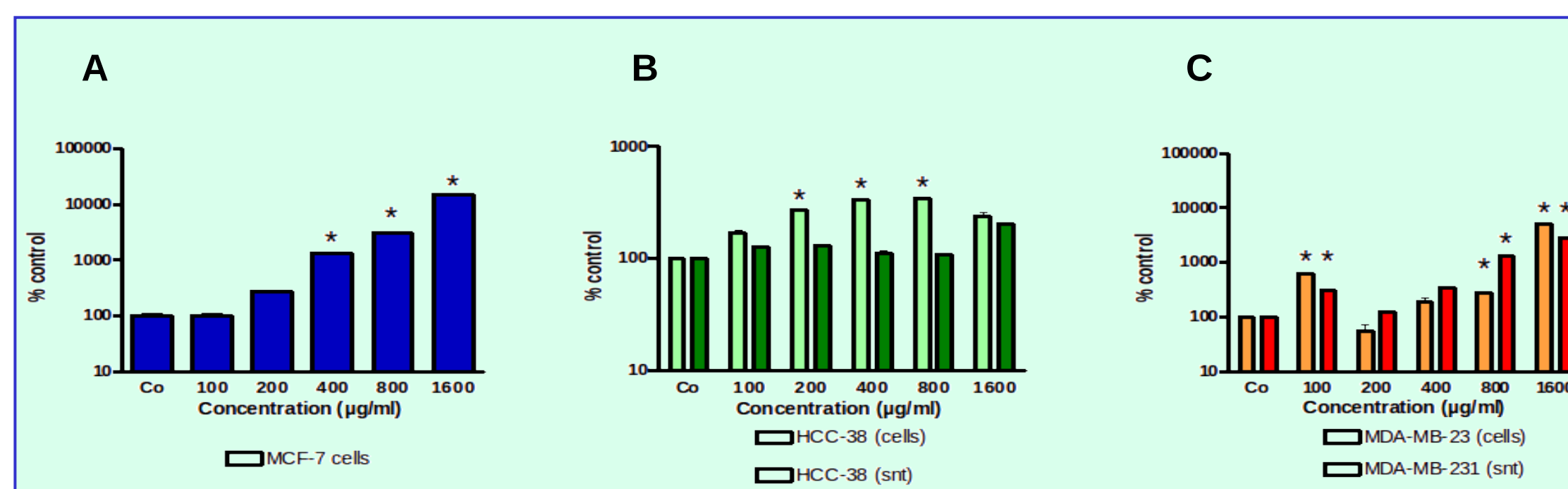


Figure 2. Activation of caspase 8 in MCF-7 (A) and caspase 3/7 HCC-38 (B) and in MDA-MB-231 (C) cells and supernatant (snt) after treatment with Avemar lyophilisate (24 h)

Values significantly different from control are marked with an asterisk*

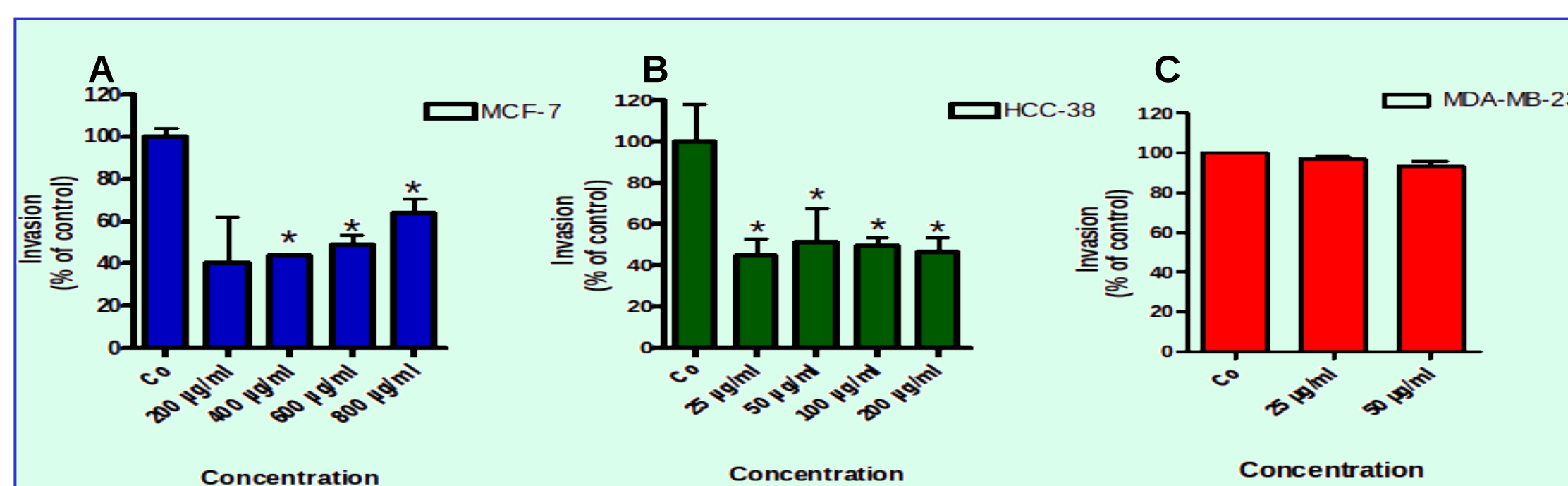


Figure 3. Inhibition of tumor cell invasion by Avemar lyophilisate in MCF-7 (A), HCC-38 (B) and MDA-MB-231 (C) breast cancer cells after 4 days of incubation

Values significantly different from control are marked with an asterisk*

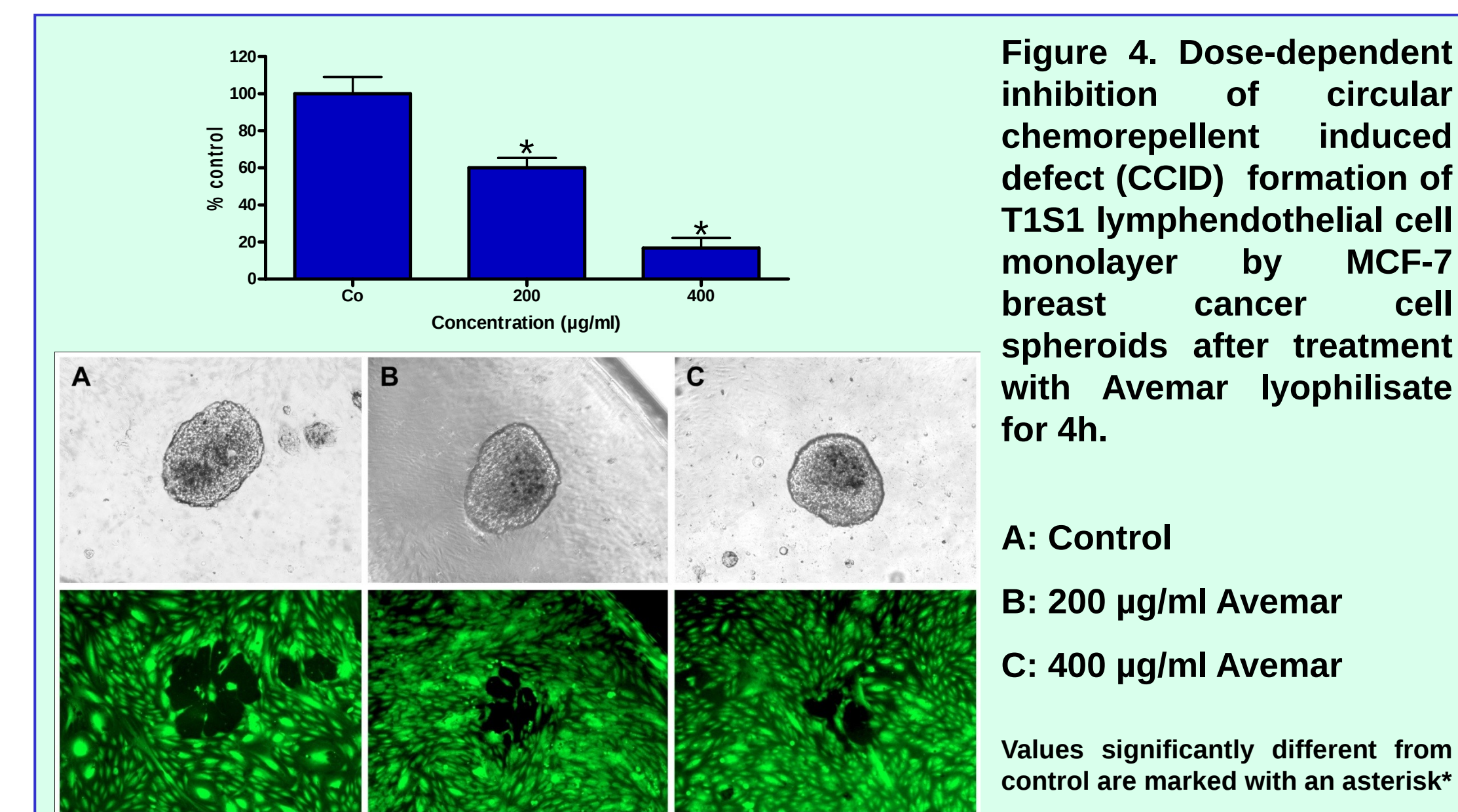


Figure 4. Dose-dependent inhibition of circular chemorepellent induced defect (CCID) formation of T1S1 lymphendothelial cell monolayer by MCF-7 breast cancer cell spheroids after treatment with Avemar lyophilisate for 4h.

A: Control

B: 200 µg/ml Avemar

C: 400 µg/ml Avemar

Values significantly different from control are marked with an asterisk*

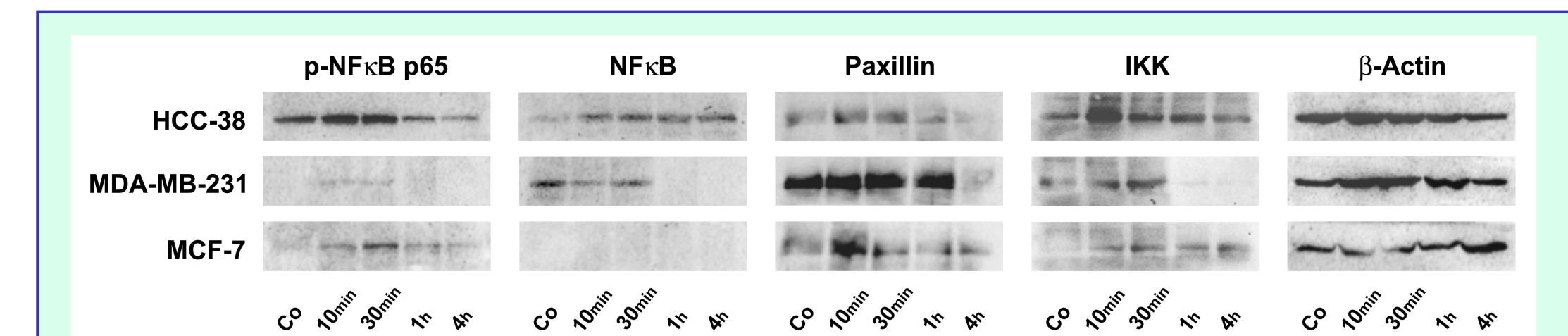


Figure 5. Western blotting of HCC-38 (A), MDA-MB-231 (B) MCF-7 (A) and MCF-7 (C) cells after treatment with 400 µg/ml Avemar lyophilisate

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