International Journal of Biological Macromolecules 149 (2020) 600–608

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Fucoidan from *Laminaria japonica* exerts antitumor effects on angiogenesis and micrometastasis in triple-negative breast cancer cells

Wen-Jing Hsu [a,b,1], Mei-Hsiang Lin [c,1], Tai-Chih Kuo [a,b], Chih-Ming Chou [a,b], Fwu-Long Mi [a,b], Chia-Hsiung Cheng [a,b,*], Cheng-Wei Lin [a,b,d,e,*]

[a] Department of Biochemistry and Molecular Cell Biology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[b] Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan
[c] Graduate Institute of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan
[d] Center for Cell Therapy and Regeneration Medicine, Taipei Medical University, Taipei, Taiwan
[e] Cell Physiology and Molecular Image Research Center, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 20 October 2019
Received in revised form 22 January 2020
Accepted 25 January 2020
Available online 28 January 2020

Keywords:
Angiogenesis
Fucoidan
Invasion
Metastasis
Triple-negative breast cancer

ABSTRACT

Fucoidan is a fucose-rich polysaccharide that has gained attention for its various anticancer properties. However, the effect and underlying mechanism of fucoidan on triple-negative breast cancer (TNBC) are still unknown. Herein, we investigated the anticancer potential of fucoidan from *Laminaria japonica*. We found that fucoidan showed modest antiproliferative activity against TNBC cells, while it effectively reduced migratory and invasive capacities. Mechanistically, fucoidan suppressed activation of MAPK and PI3K followed by inhibition of AP-1 and NF-κB signaling in TNBC. Additionally, fucoidan downregulated expressions of proangiogenic factors in TNBC cells, and fucoidan blocked tumor-elicited tube formation by human umbilical vascular endothelial cells (HUVECs). We also observed that fucoidan blocked tumor adhesion and invasion towards HUVECs. Surprisingly, fucoidan robustly suppressed tube formation on HUVECs. Moreover, fucoidan inhibited *in vivo* angiogenesis and micrometastasis in a transgenic zebrafish model. Together, *L. japonica* fucoidan exhibits potent antitumor effects by its attenuation of invasiveness and proangiogenesis in TNBC.

1. Introduction

Breast cancer is the most commonly occurring cancer in women and the second leading cause of death among all cancers. Approximately 2 million new cases are diagnosed with 0.5 million deaths *per annum* worldwide [1]. Triple-negative breast cancer (TNBC), which is determined by negative expressions of the estrogen receptor, progesterone receptors, and human epidermal growth factor receptor 2, accounts for 10%~20% of all breast cancers [2]. TNBC is considered to be more aggressive and has a poorer prognosis than other subtypes of breast cancer, mainly because TNBC is more likely to spread, as it shows a poor response to hormone therapy and often recurs after treatment. Molecular targeting inhibitors and immunotherapy are currently undergoing investigation, and adjuvants combined with chemotherapy or radiotherapy are considered effective treatments for TNBC [3,4].

Angiogenesis, the recruitment of new blood vessels is required for invasive tumor growth and metastasis. These vessels supply nutrients for tumor growth and provide the principle route for tumor cells to invade the vascular basement membrane and move into the bloodstream (intravasation) [5]. Metastatic tumor cells travel through the circulatory system and interact with platelets, leukocytes, and endothelial cells that further contribute to tumor adhesion, extravasation, and colonization at metastatic sites [6]. Tumor angiogenesis is regulated by the production of proangiogenic factors including vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), insulin-like growth factor (IGF), and matrix metalloproteinases (MMPs) [7,8]. The potential use of angiogenic inhibitors or natural components as anticancer drugs is currently under intensive investigation and has shed new light on cancer treatment.

Fucoidan is a fucose-rich polysaccharide isolated from a marine brown alga that has gained attention for its various bioactivities including anti-inflammatory, antioxidant, anticoagulant, antivirus, immunomodulatory, and antitumor properties [9,10]. The broad bioactivities of fucoidans differ with their structure and molecular weight [11,12],

Abbreviations: AP, activator protein; CM, conditioned medium; ERK, extracellular-regulated protein kinase; HUVEC, human umbilical vascular endothelial cell; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; PCR, polymerase chain reaction; PI3K, phosphoinositide 3-kinase; TNBC, triple-negative breast cancer; VEGF, vascular endothelial growth factor.

* Corresponding authors.
E-mail addresses: chcheng@tmu.edu.tw (C.-H. Cheng), cwlin@tmu.edu.tw (C.-W. Lin).

[1] Wen-Jing Hsu and Mei-Hsiang Lin contributed equally to this work.

https://doi.org/10.1016/j.ijbiomac.2020.01.256
0141-8130/

and the best-studied fucoidan is extracted from *Fucus vesiculosus* [13]. Additionally, we previously reported that fucoidan-based nanoformulation exhibits multifunction including antiangiognesis, antibacterial, and enhancing drug delivery [14–16]. There are several lines of evidence that fucoidans from different species show various antitumor activities including growth inhibition, apoptotic induction, and suppression of tumor aggressiveness [17–19]. Studies have shown that low-molecular-weight fucoidan (LMWF) lowered the viability of breast cancer cells and induced mitochondrial-dependent apoptosis in those cells [20,21], and fucoidan inhibited the growth of mouse breast cancer 4T1 cells *via* downregulating Wnt/β-catenin signaling [22]. Moreover, conditioned serum from rats treated with fucoidan inhibited the growth and promoted apoptosis of MCF-7 breast cancer cells [23]. However, clarifying the inhibitory activity and underlying regulatory mechanism of fucoidan requires additional study. Herein, we investigated the antitumor activity of L. *japonica* fucoidan in TNBC, and our study identified that L. *japonica* fucoidan inhibited the *in vitro* invasive and proangiogenic capacities of TNBC cells, and fucoidan suppressed *in vivo* angiogenesis and micrometastasis.

2. Materials and methods

2.1. Cell culture and reagents

The MDA-MB-231 and HCC1806 human breast cancer cell lines were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). MDA-MB-231 cells were cultured in Dulbecco's modified Eagle medium (DMEM), and HCC1806 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 media. All culture media were supplemented with 7% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), GlutaMAX (Gibco), and penicillin-streptomycin (Gibco). Human umbilical vein endothelial cells (HUVECs) were purchased from Promo Cell and were cultured in the Endothelial Cell Growth Medium 2 with Growth Medium 2 Supplement Mix (Promo Cell). Fucoidan from *Laminaria japonica* (34% of ester sulfate, Mw: 80 kDa) was obtained from NOVA Pharma & Liposome Biotech Co., Ltd. (Kaohsiung, Taiwan) and dissolved in phosphate buffer saline (PBS) as described previously [24].

2.2. RNA isolation and real-time quantitative polymerase chain reaction

Total RNA was extracted with an RNA extraction kit and reverse-transcribed with a high-capacity cDNA conversion kit (Invitrogen, Carlsbad, CA, USA). cDNA was amplified using EvaGreen Master Mix (Biotium, Hayward, CA, USA) using a StepOne Plus Real-Time PCR system (Applied Biosystems, Darmstadt, Germany) with specific primers as following: bFGF, 5′-AGGAGTGTGTGCTAACCGTTAC-3′ (sense) and 5′-ACTCATCCGTAACACATTTAGAA-3′ (antisense); IGF-I, 5′-AGGAAGTACATTTGAAGAACGCAAGT-3′ (sense) and 5′-CCTGCGGTGGCATGTCA-3′ (antisense); VEGF-A, 5′-CCTTGCTGCTCTACCTCCAC-3′ (sense) and 5′-ATGATTCTGCCCTCCTCCTT-3′ (antisense); MMP-2, 5′-AGATGCCTGGAATGCCAT-3′ (sense) and 5′-GGTTCTCCAGCTTCAGGTAAT-3′ (antisense); MMP-9, 5′-TACTGTGCCTTTGAGTCCG-3′ (sense) and 5′-TTGTCGGCGATAAGGAAG-3′ (antisense); and 18S rRNA, 5′-GCAATTATTCCCCATGAACG-3′ (sense) and 5′-GGGACTTAATCAACGCAAGC-3′ (antisense). Results were calculated using the ΔΔCT equation and are expressed as multiples of change relative to a control sample [25].

2.3. Cell viability

MDA-MB-231 and HCC1806 cells were seeded at a density of 1.5×10^4 cells/well in a 24-well plate until they reached confluence. Cells were refreshed with appropriate medium containing 0.7% FBS and were treated with fucoidan at indicated concentrations for 24 and 48 h. Additionally, HUVECs (1.5×10^4 cells/well) were seeded into a 96-well plate and treated with fucoidan for 24 h. After treatment, cells were incubated with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (MTT; 10 mg/ml) for 1 h. The MTT solution-containing medium was removed after incubation, and isopropanol was added to solubilize the formazan crystals for analysis. Cell viability was determined by detecting the absorbance at 570 nm and was expressed as the percentage of MTT reduction, assigning 100% to the value of the absorbance of control cells.

2.4. Live/dead staining assay

MDA-MB-231 and HCC1806 cells were seeded at a density of 1.5×10^4 cells/well in a 24-well plate and treated with fucoidan at the indicated concentration for 48 h. After incubation, cells were stained with Calcein-AM/propidium iodide (PI) in phosphate-buffered saline (PBS) for 15 min. Stained cells were observed using an inverted microscope, and quantification was carried out using Image J software.

2.5. Western blotting

Cells were lysed in ice-cold radioimmunoprecipitation assay (RIPA) buffer supplemented with a protease and phosphatase inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Cell extracts were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). After Western transfer, membranes were probed with antibodies against Akt (GeneTex GTX59640; San Antonio, TX, USA), phospho-Akt (pS473) (Cell Signaling #4060; Danvers, MA, USA), mammalian target of rapamycin (mTOR; Cell Signaling #2983), phospho-mTOR (pS2448) (Cell Signaling #2971), phospho-70S6K(pT389) (Cell Signaling #9234), extracellular signal-regulated kinase (ERK; Cell Signaling #4695), phospho-ERK (Cell Signaling #9101), c-Jun N-terminal kinase (JNK; Cell Signaling #9252), phospho-JNK (Cell Signaling #9251), p38 (Cell Signaling #9212), phospho-p38 (Cell Signaling #9211), NF-κB (Cell Signaling #4764), phospho-NF-κB (Cell Signaling #3033), c-JUN (Cell Signaling #9165), c-FOS (Cell Signaling #2250), and GAPDH (GeneTex GTX100118). Bands were detected with an enhanced chemiluminescence system (Millipore, Temecula, CA, USA). Western blotting was performed at least three times, and representative experiments are shown. Quantification was carried out using Image J software.

2.6. Wound-healing assay

MDA-MB-231 and HCC1806 cells were seeded into six-well plates and incubated overnight to form a monolayer. The next day, cells were gently scratched with a pipette tip across the center of the well. After scratching, the well was washed with medium to remove any detached cells. Cell medium was refreshed with medium containing 0.7% FBS, and cells were treated with different concentrations of fucoidan as indicated for 24 h. After incubation, cells were washed with PBS and photographed under an inverted microscope. The distance of the gap was quantitatively evaluated using Image J software.

2.7. Transwell migration and invasion assay of TNBC cells

MDA-MB-231 and HCC1806 cells were treated with fucoidan for 24 h before the assays. For the cell-migration assay, 10^5 cells were seeded into the upper chamber of a Transwell insert (8-μm pore size). For the cell-invasion assay, 4×10^5 cells were seeded into the upper chamber of a Matrigel (BD Biosciences, Franklin Lakes, NJ, USA)-coated transwell insert. The lower wells were filled with complete medium which served as a chemoattractant. After 24 and 48 h, cells were fixed with methanol and stained with crystal violet. Matrigel and any unmigrated cells were removed using cotton swabs prior to analysis.

2.8. Transwell migration assay for HUVECs

MDA-MB-231 and HCC1806 cells (2×10^5) were seeded into the lower wells of a transwell plate and treated with fucoidan for 24 h. The next day, cells in the lower well were refreshed with culture medium containing 1% FBS as a chemoattractant. Then, 2.5×10^4 HUVECs were seeded into the upper well of the transwell and incubated for another 24 h. HUVECs were fixed with methanol and stained with crystal violet. Unmigrated HUVECs were removed using a cotton swab, and cells that had migrated were observed under an inverted microscope.

2.9. Tube-formation assay

HUVECs were seeded at a density of 1.5×10^4 cells/well in a 96-well plate precoated with basement membrane extract (BD Biosciences). Cells were grown in HUVEC growth medium and treated with fucoidan for 18 h. In the tumor conditioned medium (CM)-induced tube-formation assay, MDA-MB-231 and HCC1806 cells were treated with fucoidan for 24 h, and tumor cells were washed with culture medium and collected after another 24 h. HUVECs were seeded and incubated with tumor CM for 18 h, and formation of capillary-tube structures was observed under an inverted microscope.

2.10. Adhesion assay and extravasation assay

For the tumor-adhesion assay, 10^5 HUVECs were seeded in a 24-well plate and incubated overnight to form a confluent monolayer. After incubation, HUVECs were treated with fucoidan for 2 h, and 2×10^5 green fluorescent protein (GFP)-expressing MDA-MB-231 cells were seeded into the well covered with pretreated HUVECs for another 4 h. Unattached tumor cells were gently washed with PBS, and attached tumor cells were imaged under a fluorescent microscope. For the extravasation assay, 5×10^4 HUVECs were seeded into the upper chambers of a transwell for 24 h until they had formed a confluent monolayer. HUVECs were then treated with fucoidan for 6 h. GFP-expressing MDA-MB-231 cells (5×10^4) in serum-free medium were seeded into the transwell insert, and the lower chamber was supplemented with culture medium containing 3.5% FBS as a chemoattractant. After 24 h of incubation, all cells were counterstained with DAPI, and trans-migratory tumor cells were observed with a fluorescent microscope.

2.11. Zebrafish assay

Zebrafish (*Danio rerio*) embryos were obtained from the Zebrafish Core Laboratory of Taipei Medical University and maintained at 28 °C. All animal procedures were approved by the Animal Care and Use Committee or Panel (IACUC/IACUP) (protocol #LAC-2019-0249). Methods were carried out in accordance with approved guidelines. The migration assay of cancer cells in zebrafish was carried out according to a previous report [26]. For the angiogenesis assay, embryos of transgenic zebrafish *Tg*(*fli1:EGFP*), which expressed enhanced GFP (EGFP) in the vasculature, were incubated with different doses of fucoidan in E3 buffer in a 28 °C incubator. After incubation for 48 h, the formation and development of vessels were imaged with a fluorescent microscope. Percentage of embryos with impaired vessels was evaluated. For the metastasis assay, GFP-expressing MDA-MB-231 cells were pretreated with fucoidan for 24 h, and approximately 50 tumor cells were injected into the perivitelline cavity of wild-type zebrafish embryos. Two days after the injection, embryos were imaged, and distant micrometastases were analyzed.

2.12. Statistical analyses

Data are presented as the mean ± standard error (SE) of three independent experiments. Statistical significance was determined by an unpaired, two-tailed Student's *t*-test unless stated otherwise. * Indicates a p value of <0.05; ** indicates a p value of <0.01; *** indicates a p value of <0.001. Statistical analyses were carried out using GraphPad Prism software.

3. Results

3.1. Fucoidan suppresses the migratory and invasive capacities on TNBC cells

To test the effect of fucoidan on cell viability, we treated the MDA-MB-231 and HCC1806 TNBC cell lines with fucoidan (0, 0.125, 0.25, 0.5, 1, and 2 mg/ml) for 24 and 48 h. Results of the MTT assay showed modest inhibitory activity against the viability of both MDA-MB-231 and HCC1806 cells (Fig. 1A). We further stained TNBC with Calcein-AM and PI, which respectively label viable and dead cells. Incubation with 2 mg/ml fucoidan for 48 h elicited approximately 8% cell death in MDA-MB-231 cells, whereas no obvious cell death was observed in HCC1806 cells (Fig. 1B). These results showed that fucoidan preferentially reduced cellular growth rather than inducing cell death in TNBC. We further tested the effect of fucoidan on tumor aggressive properties including wound healing and transwell migration and invasion assays. Results showed that treatment with 2 mg/ml fucoidan in MDA-MB-231 and HCC1806 significantly increased wound area by 5.3 folds and 4.2 folds, respectively (Fig. 1C). Moreover, results of transwell analysis showed that fucoidan significantly suppressed 68% migration and 83% invasion in MDA-MB-231 cells (Fig. 1C, D), and similar results were obtained in HCC1806 cells (Fig. 1C, D). Together, these data suggest that fucoidan suppresses tumor aggressiveness without causing cell death.

3.2. Fucoidan suppresses MAPK and PI3K/AKT followed by downregulating the AP-1 and NF-κB signaling pathways

We further deciphered the inhibitory mechanism of fucoidan in TNBC. MDA-MB-231 and HCC1806 cells were treated with fucoidan (0, 1, and 2 mg/ml) for 24 h, and results of Western blotting showed that fucoidan suppressed phosphorylation of ERK and JNK, whereas phosphorylation of p38 was not affected in the presence of fucoidan (Fig. 2A). Additionally, we also tested the effect of fucoidan on PI3K/AKT signaling. Results showed that fucoidan effectively downregulated AKT phosphorylation. Moreover, downstream targets of AKT, including mTOR and p70S6K, were both suppressed by fucoidan in MDA-MB-231 and HCC1806 cells (Fig. 2B). We further examined downstream signaling of MAPK and PI3K/AKT, including AP-1 and NF-κB which play crucial roles in tumor growth and invasiveness. AP-1 is composed of a heterodimer of c-Jun and c-Fos, and we observed that treatment with fucoidan significantly downregulated expression of c-Jun but not c-Fos (Fig. 2C). In addition to the AP-1 family, we also identified that activation of NF-κB, as determined by phosphorylation of the p65 subunit, was significantly suppressed by fucoidan (Fig. 2C). Taken together, these data indicate that fucoidan downregulates MAPK and PI3K/AKT followed by inhibiting the AP-1 and NF-κB signaling pathways in TNBC.

3.3. Fucoidan exerts antiangiogenic activity in vitro

To further investigate the effect of fucoidan on the antiangiogenic potential, we treated MDA-MB-231 and HCC1806 cells with 1 mg/ml fucoidan for 24 h. Results of the qPCR showed that fucoidan significantly inhibited expressions of proangiogenic factors, including VEGF-A, IGF-I, bFGF, MMP-2, and MMP-9 (Fig. 3A). We further tested TNBC-elicited tube formation in the presence of fucoidan. HUVECs were incubated in CM from fucoidan-treated TNBC cells for 24 h to induce endothelial cell tube formation. Interestingly, we found that CM from 1 mg/ml fucoidan effectively suppressed TNBC-elicited capillary-like structures

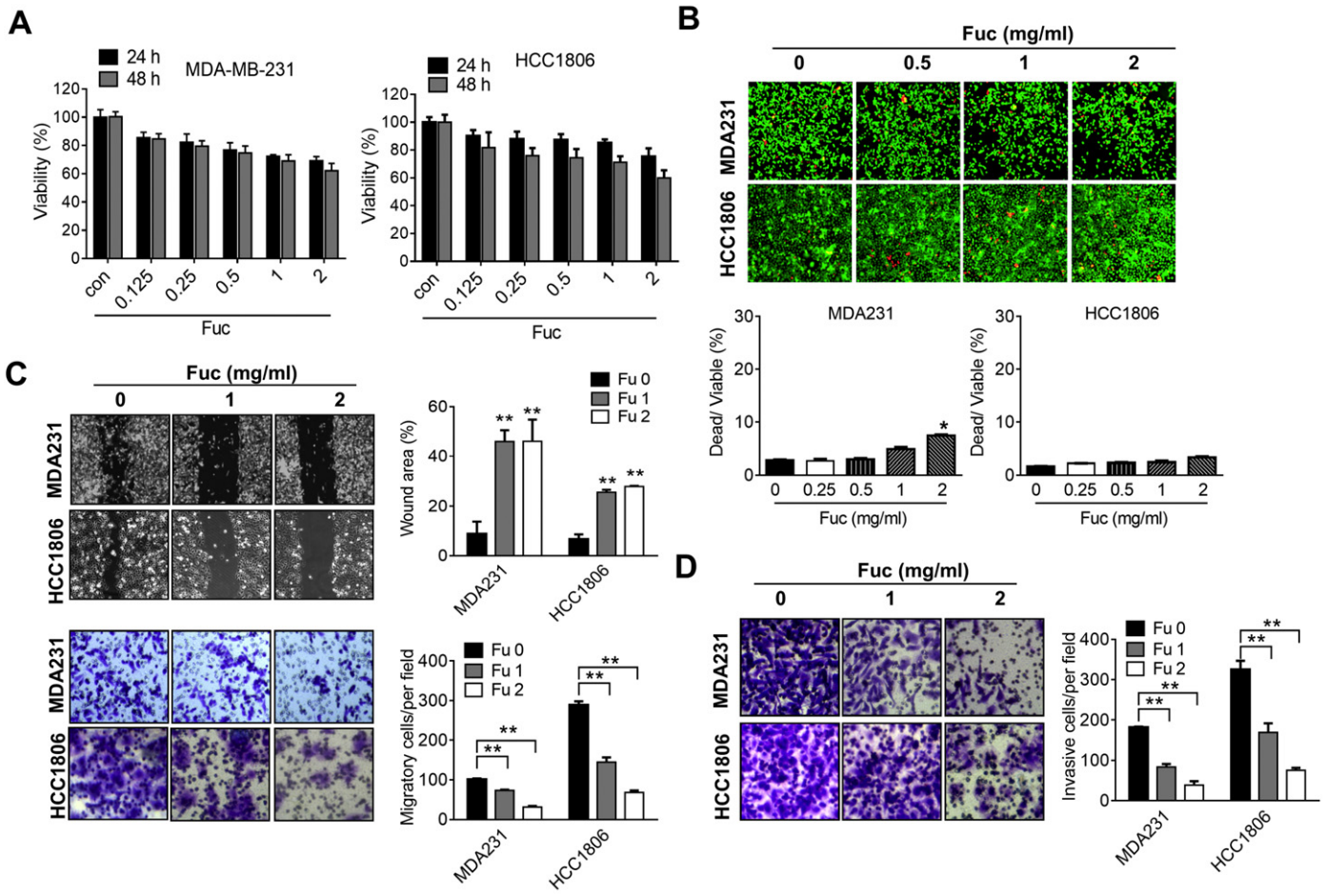

Fig. 1. The effect of fucoidan on cell viability and the aggressiveness capacity of breast cancer cells. (A) MDA-MB-231 and HCC1806 cells were treated with various concentrations of fucoidan (Fuc: 0, 0.125, 0.25, 0.5, 1, and 2 mg/ml) for 24 and 48 h, and cell viability was measured by an MTT assay. Data are expressed as a percentage of the control. Values are expressed as the mean ± standard error. (B) Calcein-AM/PI staining of fucoidan-treated MDA-MB-231 and HCC1806 cells. The upper panel shows representative images from fucoidan (0, 0.5, 1, and 2 mg/ml)-treated cells (upper panel), and quantitative data are shown in the lower panel. * $p < 0.05$ by an unpaired *t*-test. (C and D) Fucoidan suppresses the migratory and invasive capacities of TNBC cells. MDA-MB-231 and HCC1806 cells were treated with fucoidan (Fuc: 0, 1, and 2 mg/ml) for 24 h and then seeded in 6-well plates. Cells that had migrated into the wound area were photographed under an inverted light microscope at 200× magnification (C, upper left panel). Quantitative data show closing of the wound area (C, upper right panel). The inhibitory effects of fucoidan on breast cancer migration (C, lower panel) and invasion (D) were assessed by a transwell assay. Quantification was obtained from three random fields of three experimental replicates. Values are expressed as the mean ± standard error. ** $p < 0.01$ and * $p < 0.05$, unpaired *t*-test.

in HUVECs (Fig. 3B). In addition, a transwell assay confirmed that tumor cell-induced migration of HUVECs was significantly impeded in the presence of fucoidan (Fig. 3C). These data indicate that fucoidan downregulates tumor-induced proangiogenic activity. We further tested the direct inhibitory effect of fucoidan on HUVECs. Surprisingly, treatment with a low concentration of fucoidan at 0.1 mg/ml drastically suppressed tube formation in HUVECs (Fig. 3D); however, no obvious cytotoxic activity was detected in the presence of fucoidan (Fig. 3E). These data suggest that fucoidan shows potent antiangiogenic activity.

Fucoidan was reported to block several membrane molecules such as P-selectin, and P-selectin expression by the activated vasculature facilitates tumor metastasis by supporting tumor rolling and adhesion. Therefore, we tested the effect of fucoidan on TNBC adhesion to and invasion of endothelial cells. HUVECs were treated with fucoidan 2 h before seeding with MDA-MB-231/GFP cells. Results showed that fucoidan treatment significantly inhibited adhesion of MDA-MB-231/GFP cells to HUVECs (Fig. 4A). Moreover, results of a transwell assay showed that MDA-MB-231/GFP cells which had invaded through HUVECs were suppressed in the presence of fucoidan (Fig. 4B). These data suggest that fucoidan not only suppresses TNBC-elicited proangiogenesis but also blocks adhesion and extravasation of tumors to vascular endothelial cells.

3.4. Fucoidan exerts antiangiogenic and anti-micrometastatic activities in an in vivo zebrafish model

To monitor the *in vivo* antiangiogenic activity of fucoidan, we applied a transgenic zebrafish *Tg*(*fli1:EGFP*) expressing EGFP in the vasculature. Zebrafish embryos were incubated with fucoidan (0.1, 1, and 2 mg/ml) for 48 h, and results showed that percentages of zebrafish embryos with impaired vascular development were 20% (4 of 20), 50% (10 of 20), and 75% (15 of 20), respectively, as the concentration of fucoidan increased (Fig. 5A). We observed that no lethality occurred after 48 h of incubation with 0.1, 1, and 2 mg/ml fucoidan. However, embryonic lethality occurred with 4 mg/ml fucoidan (data not shown). To further monitor the inhibitory effect of fucoidan on tumor micrometastasis, fucoidan-treated MDA-MB-231-GFP cells were injected into the perivitelline cavity of wild-type zebrafish embryos for 2 days, and quantitative data showed that 60% of control and 18% of fucoidan-treated tumor cells exhibited micrometastasis (Fig. 5B), suggesting that fucoidan significantly attenuates the metastatic capability of TNBC cells.

4. Discussion

TNBC is the most devastating malignancy because of its highly aggressive capacity and the lack of targetable molecules, and radiotherapy

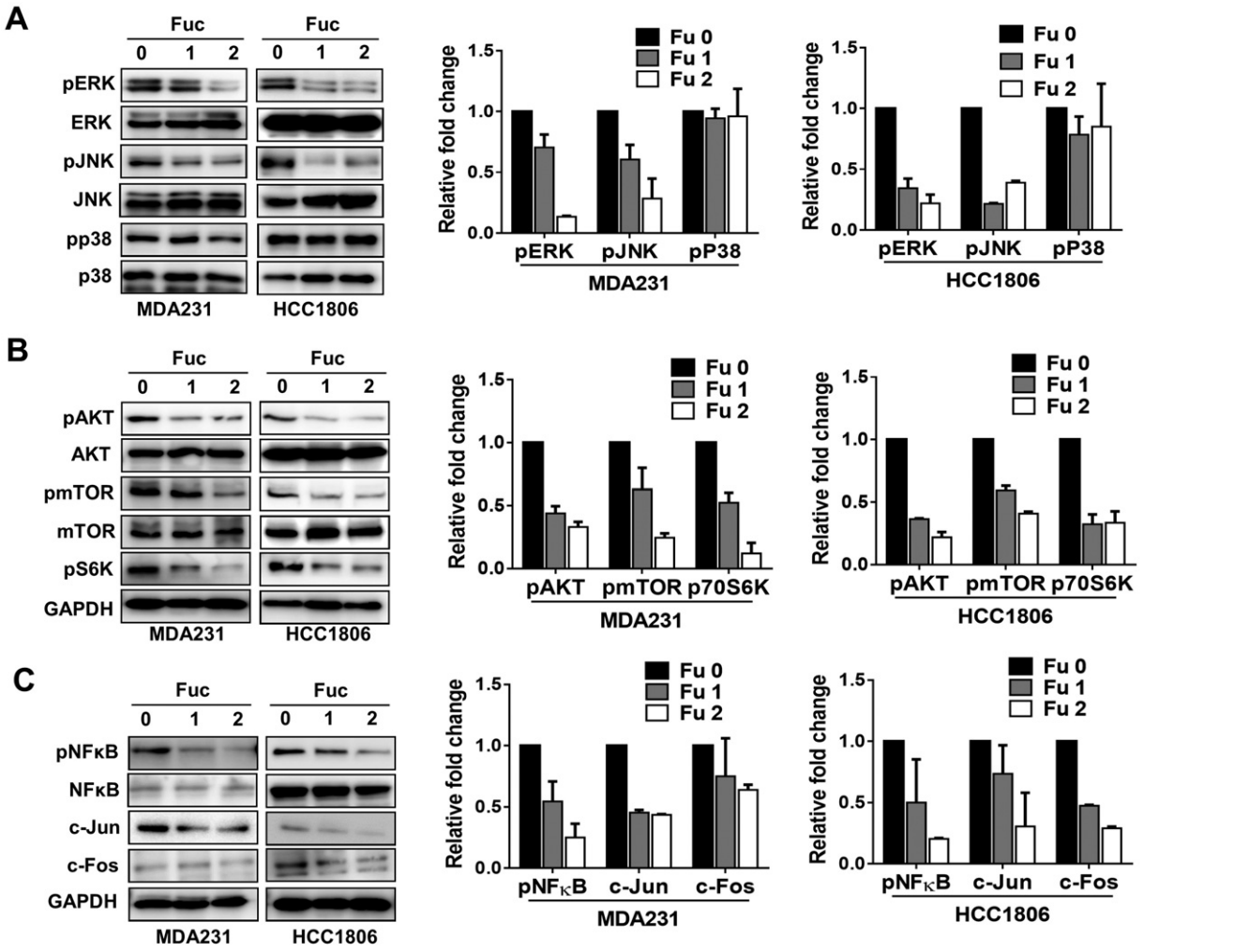

Fig. 2. Fucoidan suppresses MAPK and PI3K/AKT signaling pathways. (A and B) MDA-MB-231 and HCC1806 cells were treated with fucoidan (0, 1, and 2 mg/ml) for 24 h, and protein lysates were subjected to Western blot analysis using specific antibodies against MAPK family (A) or PI3K/AKT family members (B). GAPDH was used as a loading control. (C) Protein lysates from fucoidan-treated MDA-MB-231 and HCC1806 cells were subjected to Western blot analysis using specific antibodies against AP-1 and NF-κB. Quantitative data were obtained from at least three independent experiments and analyzed using ImageJ software.

or chemotherapy combined with neoadjuvant remains the most reliable treatment so far. Several lines of evidence have reported various antitumor activities of fucoidan, which is considered a potential adjuvant for cancer treatment. In the present study, we identified that fucoidan from L. *japonica* exhibited potent antitumor activities *via* attenuating the invasive, proangiogenic, and adhesive abilities of TNBC cells. We revealed that fucoidan suppressed MAPK and AKT signaling cascades followed by inhibiting AP-1 and NF-κB, as a result in downregulating expressions of proangiogenic and metastatic genes. Using a zebrafish experimental model, we confirmed that fucoidan effectively blocked *in vivo* angiogenesis and micrometastasis.

The high proliferative rate is one of the hallmarks of cancer, and the literature reports that fucoidans from different species of brown seaweed exhibit antiproliferative activity against various types of cancer. However, the effect of fucoidan on TNBC is still unclear. Recently, Lu et al. [20] showed that low-molecular-weight fucoidan (LMWF) extracted from New Zealand *Undaria pinnatifida* inhibited breast cancer growth. Their data showed that treatment with 300 μg/ml LMWF suppressed the growth of MCF-7 and MDA-MB-231 breast cancer cells by 20% and 30%, respectively. Additionally, Wu et al. [27] found that 200 μg/ml of fucoidan from *Sargassum hemiphyllum* slightly attenuated tumor viability but significantly suppressed the migration and invasion of MDA-MB-231 cells. In our present study, 1 mg/ml of fucoidan from L. *japonica* drastically suppressed migration and invasion of MDA-MB-231 and HCC1806 cells. Our data also showed that 1 mg/ml fucoidan modestly reduced the cell viability but did not obviously induce cytotoxicity. On the contrary, Hsu and colleagues [28] found that treatment with 400 μg/ml fucoidan effectively induced apoptosis in lung cancer cells. These data suggest that the effect of fucoidan on TNBC may by specifically mediated by attenuating tumor mobility but not by cytotoxicity.

Tumor metastasis is the leading cause of death due to cancer, especially in TNBC patients. TNBC is more likely to metastasize than other subtypes, with 40% of patients presenting with positive lymph nodes at diagnosis. Fucoidan was reported to suppress the migration and invasion of lung, liver, and breast cancer cells [29–31], but the underlying mechanism is still unclear. Hsu et al. [31] reported that fucoidan decreased the expression of the epithelial-mesenchymal transition and reduced *in vivo* metastasis of MDA-MB-231 cells by suppressing transforming growth factor (TGF)-β and Smad signaling. In the present study, we found that fucoidan decreased activation of the MAPK and AKT signaling cascade, accompanied by reductions in AP-1 and NF-κB. We previously identified that activation of MAPK/AP-1 and AKT/NF-κB signaling is crucial for phorbol ester TPA-elicited invasiveness in breast cancer cells [32]. In the present study, we found that treatment with inhibitors of MAPK and AKT significantly suppressed migration of MDA-MB-231 cells (Fig. S1A). Additionally, results of Western blot analysis showed that AKT inhibitor attenuated expressions of Jun and Fos, whereas phosphorylation of NF-κB was not affected. However,

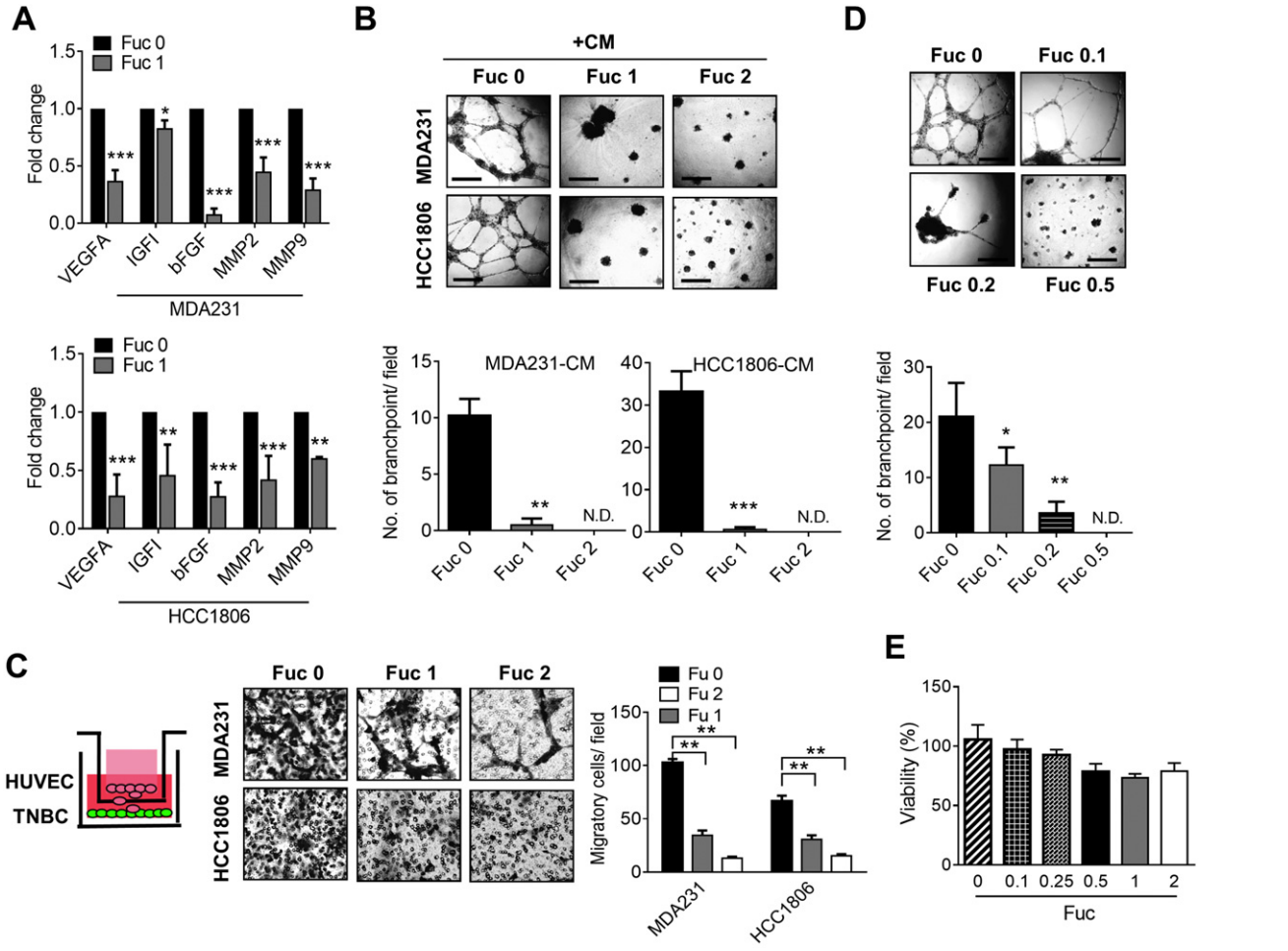

Fig. 3. Fucoidan suppresses TNBC-mediated angiogenesis *in vitro*. (A) MDA-MB-231 and HCC1806 cells were treated with 1 mg/ml of fucoidan, and expressions of proangiogenic factors were measured by a real-time PCR. Values are expressed as the mean ± standard error. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, unpaired *t*-test. (B) HUVECs were incubated with conditioned medium (CM) from fucoidan-pretreated MDA-MB-231 and HCC1806 cells for 24 h and photographed with an inverted microscope (upper panel). Quantification of the formation of tube-like structures is presented in the lower panel. Scale bar = 100 μm. ** $p < 0.01$ and *** $p < 0.001$, unpaired *t*-test. N.D.: not detected. (C) MDA-MB-231 and HCC1806 cells were pretreated with fucoidan (0, 1, and 2 mg/ml) for 24 h followed by refreshing the culture medium. HUVECs were then seeded and placed onto a transwell insert for another 24 h. Images of the migration of HUVECs were stained and photographed with an inverted microscope (upper panel), and numbers of migratory cells were quantified (right panel). (D) HUVECs were treated with fucoidan (0, 0.1, 0.2, and 0.5 mg/ml) for 18 h. Formation of tube-like structures was photographed with an inverted microscope (upper panel), and quantitative data are presented in the lower panel. Scale bar = 100 μm. ** $p < 0.01$, unpaired *t*-test. N.D.: not detected. (E) Cell viability of fucoidan-treated HUVECs, as determined by an MTT assay. Data are expressed as a percentage of the control. Values are expressed as the mean ± standard error.

treatment with inhibitors of ERK and JNK downregulated both AP-1 and NF-κB (Fig. S1B). These data indicate that inhibition of MAPK and AKT, accompanies by downregulating AP-1 and NF-kB may participate in fucoidan-mediated inhibitory effect.

A previous study reported that fucoidan inhibited angiogenesis by downregulating hypoxia-inducing factor (HIF)/VEGF in bladder cancer cells [33], and that fucoidan suppressed expressions of stroma-derived factor (SDF)-1 and CXCR4 in coculture with osteosarcoma and blood-derived endothelial cells [34]. Oliveira et al. recently identified that fucoidan from *F. vesiculosus* suppressed *in vitro* tube formation and *in vivo* angiogenesis using a chick embryo chorioallantoic membrane assay [35]. Our data showed that fucoidan downregulated expression of proangiogenic genes to different extent (Fig. 3A). We also found that the expressions of VEGF-A and MMP-9 were significantly suppressed in the presence of MAPK and AKT inhibitors (Supplemental Fig. S1C). In Fig. 2A and B, we identified that fucoidan suppressed activation of MAPK and AKT; however, treatment with inhibitors of MAPK and AKT had less effect on mRNA expressions of IGF-I, bFGF, and MMP-2 (data not shown), suggesting that other signaling mechanism may participate in fucoidan-mediated inhibitory effect. A previous study reported that fucoidan suppressed tumor growth and angiogenesis in prostate cancer cells, possibly through downregulation of JAK-STAT signaling cascade [36]. These data indicate that downregulation of VEGF-A and MMP-9 involves in fucoidan-mediated anti-angiogenesis at least in part by blocking MAPK and AKT signaling cascade.

The ability of tumor cells to adhere to endothelial cells and leave the bloodstream is a critical step during metastasis. In the present study, fucoidan blocked tumor attachment and invasion on HUVECs. Moreover, the *in vivo* zebrafish model showed that fucoidan significantly suppressed formation of vasculature, and that fucoidan-treated tumor cells had a significantly reduced ability to metastasize to distant organs. Several studies have reported that fucoidan from different species of blown alga exhibited anti-angiogenesis and anti-metastasis in various cancer models [34,35,37]. However, the effect of fucoidan on endothelial cells remains controversial. Liu et al. reported that fucoidan extracted from *Undaria pinnatifida* inhibited angiogenesis of HUVECs [38], whereas LMWF from *Ascophyllum nodosum* was shown to enhance migration and angiogenesis of HUVECs [39]. In our present study, we identified that fucoidan from *Laminaria japonica* robustly suppressed tube formation of HUVECs by the concentration of 0.2 mg/ml, we also found that conditioned medium from 1 mg/ml fucoidan-treated TNBC substantially suppressed tube formation of HUVECs. Interestingly, about 50%

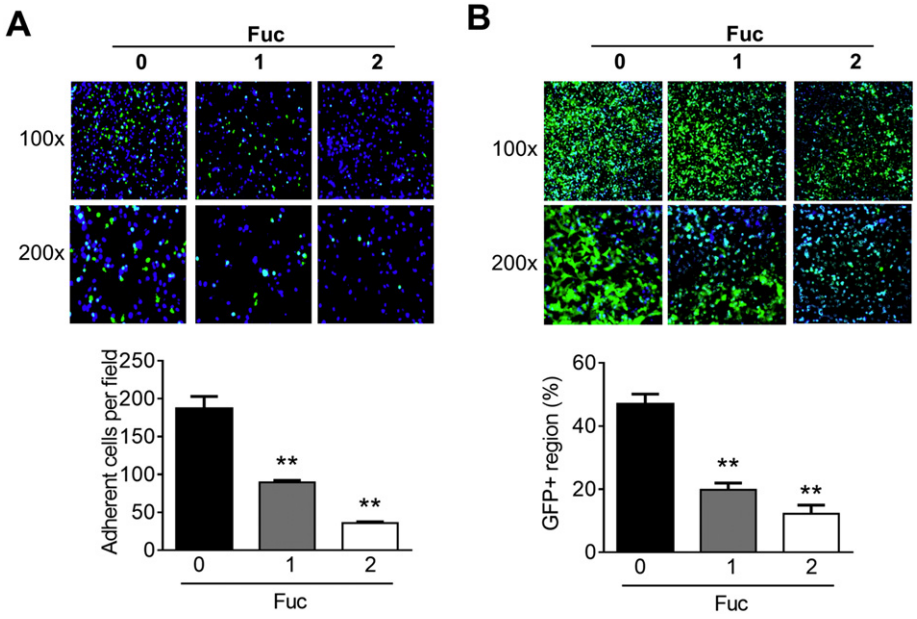

Fig. 4. Fucoidan blocks tumor adhesion and invasion on HUVECs. (A) Images of MDA-MB-231/GFP cells attached to HUVECs in the presence of fucoidan (0, 1, and 2 mg/ml) were pictured under 100× and 200× magnification by an inverted fluorescent microscope (upper panel), and quantitative data are presented in the lower panel. (B) MDA-MB-231/GFP cells were seeded onto fucoidan-treated HUVECs in a transwell insert for another 24 h. Invasion of MDA-MB-231/GFP cells is pictured under 100× and 200× magnification by an inverted fluorescent microscope (upper panel), and quantitative data are presented in the lower panel. ** $p < 0.01$ and * $p < 0.05$, unpaired *t*-test.

of tumor migration/invasion and adhesion were inhibited by 1 mg/ml fucoidan. Our data indicate that *Laminaria japonica* fucoidan shows different inhibitory extent to both TNBC and HUVECs. Although several studies have reported on cytotoxic activity by fucoidan, the working concentration differs to species of seaweed and tumor type [40–42]. Moreover, the underlying mechanism remains unclear. Recently, fucoidan from *Fucus vesiculosus* was reported to induce apoptosis *via* induction of reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress in liver and ovarian cancer [37,40], and fucoidan induced p53-independent apoptosis in colon cancer [43]. These data suggest that different origin of fucoidan may have diverse biological function, but more evidence needs to be exploited.

A recent study reported that 0.5 mg/ml fucoidan reduced angiogenesis in an onplanted breast tumor by chicken embryo chorioallantoic membrane (CAM) assay [35]. Our present study showed that 0.1 mg/ml fucoidan robustly suppressed tube formation of HUVECs, and 1 mg/ml fucoidan blocked vessel formation in Tg zebrafish model. A recent study reported on immunomodulatory activity of BALB/c mice by 1000 mg/kg fucoidan [44]. Another study reported that 1000 mg/kg fucoidan shows no significant toxicological change in

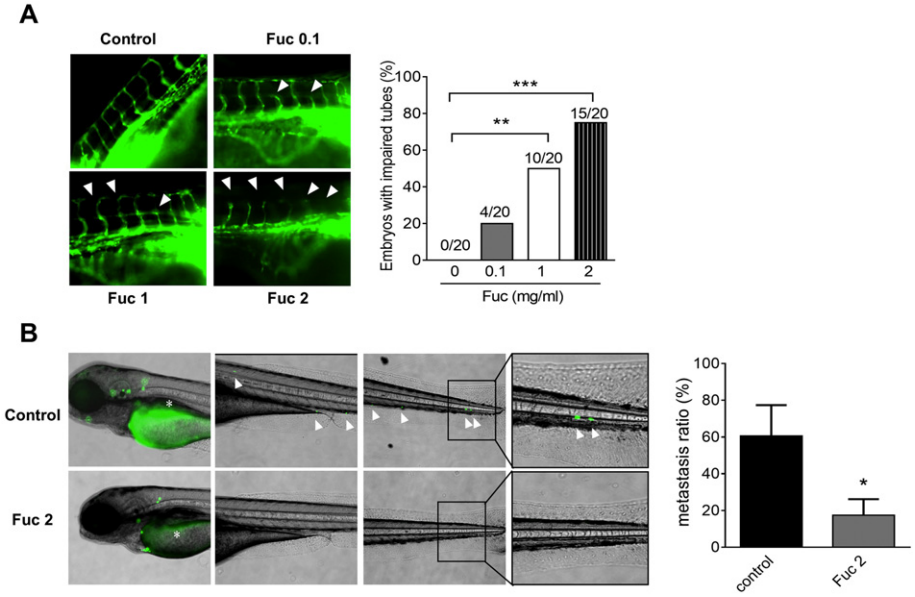

Fig. 5. Fucoidan exerts antiangiogenic and antimetastatic capacities *in vivo*. (A) Representative images of the trunk vasculature in *Tg(fli1:EGFP)* zebrafish embryos incubated with fucoidan (0, 0.1, 1, and 2 mg/ml) for 48 h (left panel). Arrows indicate impaired vasculature. Quantitative analysis presents percentages of fish embryos with defective vasculature (right panel). ** $p < 0.01$ and *** $p < 0.001$, unpaired *t*-test. (B) MDA-MB-231/GFP cells were treated with fucoidan (0 and 2 mg/ml) for 24 h and then injected into the pericardial space of wild-type zebrafish embryos for 48 h. Representative images show GFP-positive tumor cells distributed throughout the fish body. Arrows indicate metastatic tumor cells. An asterisk denotes the fluorescent background. Quantitative analysis presents the percentage of fish embryos with micrometastasis.

Sprague-Dawley rats, as determined by biological and hematological examinations [45]. Several studies have reported that *in vivo* working concentrations of fucoidan against xenograft tumors are about 20–150 mg/kg [31,36,46]. These data together support the effectiveness and safety of fucoidan as a therapeutic antitumor agent. Collectively, we identify the potentiality of fucoidan from *Laminaria japonica* in suppressing TNBC-mediated proangiogenesis and metastasis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2020.01.256.

CRediT authorship contribution statement

Wen-Jing Hsu: Conceptualization, Methodology, Data curation, Software, Formal analysis, Writing - original draft, Writing - review & editing. **Mei-Hsiang Lin:** Data curation, Resources, Formal analysis, Investigation. **Tai-Chih Kuo:** Resources, Formal analysis, Investigation. **Chih-Ming Chou:** Resources, Formal analysis, Investigation. **Fwu-Long Mi:** Resources, Formal analysis, Investigation. **Chia-Hsiung Cheng:** Conceptualization, Writing - review & editing, Investigation, Project administration. **Cheng-Wei Lin:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition.

Acknowledgements

This study was supported by grants from the Ministry of Science and Technology, Taiwan (MOST107-2320-B-038-052-MY3 to C.W. Lin, MOST108-2320-B-038-014 to C.H. Cheng, and MOST102-2320-B-038-014 to M.H. Lin).

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, CA Cancer J. Clin. 69 (1) (2019) 7–34.
[2] K. Aysola, A. Desai, C. Welch, J. Xu, Y. Qin, V. Reddy, R. Matthews, C. Owens, J. Okoli, D.J. Beech, C.J. Piyathilake, S.P. Reddy, V.N. Rao, Triple negative breast cancer - an overview, Hereditary Genet 2013 (Suppl. 2) (2013).
[3] H.A. Wahba, H.A. El-Hadaad, Current approaches in treatment of triple-negative breast cancer, Cancer Biol Med 12 (2) (2015) 106–116.
[4] J.M. Lebert, R. Lester, E. Powell, M. Seal, J. McCarthy, Advances in the systemic treatment of triple-negative breast cancer, Curr. Oncol. 25 (Suppl. 1) (2018) S142–S150.
[5] J. Folkman, Role of angiogenesis in tumor growth and metastasis, Semin. Oncol. 29 (6 Suppl 16) (2002) 15–18.
[6] G. Doglioni, S. Parik, S.M. Fendt, Interactions in the (pre)metastatic niche support metastasis formation, Front. Oncol. 9 (2019) 219.
[7] V. Loizzi, V. Del Vecchio, G. Gargano, M. De Liso, A. Kardashi, E. Naglieri, L. Resta, E. Cicinelli, G. Cormio, Biological pathways involved in tumor angiogenesis and Bevacizumab based anti-angiogenic therapy with special references to ovarian cancer, Int. J. Mol. Sci. 18 (9) (2017).
[8] Y. Zhao, A.A. Adjei, Targeting angiogenesis in cancer therapy: moving beyond vascular endothelial growth factor, Oncologist 20 (6) (2015) 660–673.
[9] S. Palanisamy, M. Vinosha, T. Marudhupandi, P. Rajasekar, N.M. Prabhu, Isolation of fucoidan from Sargassum polycystum brown algae: structural characterization, in vitro antioxidant and anticancer activity, Int. J. Biol. Macromol. 102 (2017) 405–412.
[10] J.H. Fitton, D.N. stringer, S.S. Karpiniec, therapies from fucoidan: an update, Mar Drugs 13 (9) (2015) 5920–5946.
[11] G. van Weelden, M. Bobinski, K. Okla, W.J. van Weelden, A. Romano, J.M.A. Pijnenborg, Fucoidan structure and activity in relation to anti-cancer mechanisms, Mar Drugs 17 (1) (2019).
[12] R.V. Usoltseva, N.M. Shevchenko, O.S. Malyarenko, S.D. Anastyuk, A.E. Kasprik, N.V. Zvyagintsev, S.P. Ermakova, Fucoidans from brown algae Laminaria longipes and Saccharina cichorioides: structural characteristics, anticancer and radiosensitizing activity in vitro, Carbohydr. Polym. 221 (2019) 157–165.
[13] F. Atashrazm, R.M. Lowenthal, G.M. Woods, A.F. Holloway, J.L. Dickinson, Fucoidan and cancer: a multifunctional molecule with anti-tumor potential, Mar Drugs 13 (4) (2015) 2327–2346.
[14] L.C. Tsai, C.H. Chen, C.W. Lin, Y.C. Ho, F.L. Mi, Development of mutlifunctional nanoparticles self-assembled from trimethyl chitosan and fucoidan for enhanced oral delivery of insulin, Int. J. Biol. Macromol. 126 (2019) 141–150.
[15] C.H. Chen, Y.S. Lin, S.J. Wu, F.L. Mi, Mutlifunctional nanoparticles prepared from arginine-modified chitosan and thiolated fucoidan for oral delivery of hydrophobic and hydrophilic drugs, Carbohydr. Polym. 193 (2018) 163–172.
[16] S.H. Yu, S.J. Wu, J.Y. Wu, D.Y. Wen, F.L. Mi, Preparation of fucoidan-shelled and genipin-crosslinked chitosan beads for antibacterial application, Carbohydr. Polym. 126 (2015) 97–107.
[17] K. Teruya, Y. Kusumoto, H. Eto, N. Nakamichi, S. Shirahata, Selective suppression of cell growth and programmed cell death-ligand 1 expression in HT1080 fibrosarcoma cells by low molecular weight fucoidan extract, Mar Drugs 17 (7) (2019).
[18] I. Niyonizigiye, D. Ngabire, M.P. Patil, A.A. Singh, G.D. Kim, In vitro induction of endoplasmic reticulum stress in human cervical adenocarcinoma HeLa cells by fucoidan, Int. J. Biol. Macromol. 137 (2019) 844–852.
[19] W. Blaszczak, M.S. Lach, W. Barczak, W.M. Suchorska, Fucoidan exerts anticancer effects against head and neck squamous cell carcinoma in vitro, Molecules 23 (12) (2018).
[20] J. Lu, K.K. Shi, S. Chen, J. Wang, A. Hassouna, L.N. White, F. Merien, M. Xie, Q. Kong, J. Li, T. Ying, W.L. White, S. Nie, Fucoidan extracted from the New Zealand Undaria pinnatifida-physicochemical comparison against five other Fucoidans: unique low molecular weight fraction bioactivity in breast cancer cell lines, Mar Drugs 16 (12) (2018).
[21] M. Xue, X. Ji, C. Xue, H. Liang, Y. Ge, X. He, L. Zhang, K. Bian, L. Zhang, Caspase-dependent and caspase-independent induction of apoptosis in breast cancer by fucoidan via the PI3K/AKT/GSK3beta pathway in vivo and in vitro, Biomed. Pharmacother. 94 (2017) 898–908.
[22] M. Xue, Y. Ge, J. Zhang, Y. Liu, Q. Wang, L. Hou, Z. Zheng, Fucoidan inhibited 4T1 mouse breast cancer cell growth in vivo and in vitro via downregulation of Wnt/beta-catenin signaling, Nutr. Cancer 65 (3) (2013) 460–468.
[23] X. He, M. Xue, S. Jiang, W. Li, J. Yu, S. Xiang, Fucoidan promotes apoptosis and inhibits EMT of breast cancer cells, Biol. Pharm. Bull. 42 (3) (2019) 442–447.
[24] K.Y. Lu, R. Li, C.H. Hsu, C.W. Lin, S.C. Chou, M.L. Tsai, F.L. Mi, Development of a new type of multifunctional fucoidan-based nanoparticles for anticancer drug delivery, Carbohydr. Polym. 165 (2017) 410–420.
[25] H.C. Lin, M.H. Lin, J.H. Liao, T.H. Wu, T.H. Lee, F.L. Mi, C.H. Wu, K.C. Chen, C.H. Cheng, C.W. Lin, Antroquinonol, a ubiquinone derivative from the mushroom Antrodia camphorata, inhibits colon cancer stem cell-like properties: insights into the molecular mechanism and inhibitory targets, J. Agric. Food Chem. 65 (1) (2017) 51–59.
[26] J. Ren, S. Liu, C. Cui, P. Ten Dijke, Invasive behavior of human breast cancer cells in embryonic zebrafish, J. Vis. Exp. 122 (2017).
[27] S.Y. Wu, A.T. Wu, K.S. Yuan, S.H. Liu, Brown seaweed fucoidan inhibits cancer progression by dual regulation of mir-29c/ADAM12 and miR-17-5p/PTEN axes in human breast Cancer cells, J. Cancer 7 (15) (2016) 2408–2419.
[28] H.Y. Hsu, T.Y. Lin, C.H. Hu, D.T.F. Shu, M.K. Lu, Fucoidan upregulates TLR4/CHOP-mediated caspase-3 and PARP activation to enhance cisplatin-induced cytotoxicity in human lung cancer cells, Cancer Lett. 432 (2018) 112–120.
[29] H. Teng, Y. Yang, H. Wei, Z. Liu, Z. Liu, Y. Ma, Z. Gao, L. Hou, X. Zou, Fucoidan suppresses hypoxia-induced lymphangiogenesis and lymphatic metastasis in mouse hepatocarcinoma, Mar Drugs 13 (6) (2015) 3514–3530.
[30] H. Lee, J.S. Kim, E. Kim, Fucoidan from seaweed Fucus vesiculosus inhibits migration and invasion of human lung cancer cell via PI3K-Akt-mTOR pathways, PLoS One 7 (11) (2012) e50624.
[31] H.Y. Hsu, T.Y. Lin, P.A. Hwang, L.M. Tseng, R.H. Chen, S.M. Tsao, J. Hsu, Fucoidan induces changes in the epithelial to mesenchymal transition and decreases metastasis by enhancing ubiquitin-dependent TGFbeta receptor degradation in breast cancer, Carcinogenesis 34 (4) (2013) 874–884.
[32] W.T. Lee, T.H. Lee, C.H. Cheng, K.C. Chen, Y.C. Chen, C.W. Lin, Antroquinonol from Antrodia Camphorata suppresses breast tumor migration/invasion through inhibiting ERK-AP-1- and AKT-NF-kappaB-dependent MMP-9 and epithelial-mesenchymal transition expressions, Food Chem. Toxicol. 78 (2015) 33–41.
[33] M.C. Chen, W.L. Hsu, P.A. Hwang, T.C. Chou, Low molecular weight fucoidan inhibits tumor angiogenesis through downregulation of HIF-1/VEGF signaling under hypoxia, Mar Drugs 13 (7) (2015) 4436–4451.
[34] F. Wang, H. Schmidt, D. Pavleska, T. Wermann, A. Seekamp, S. Fuchs, Crude fucoidan extracts impair angiogenesis in models relevant for bone regeneration and osteosarcoma via reduction of VEGF and SDF-1, Mar Drugs 15 (6) (2017).
[35] C. Oliveira, S. Granja, N.M. Neves, R.L. Reis, F. Baltazar, T.H. Silva, A. Martins, Fucoidan from Fucus vesiculosus inhibits new blood vessel formation and breast tumor growth in vivo, Carbohydr. Polym. 223 (2019) 115034.
[36] X. Rui, H.F. Pan, S.L. Shao, X.M. Xu, Anti-tumor and anti-angiogenic effects of Fucoidan on prostate cancer: possible JAK-STAT3 pathway, BMC Complement. Altern. Med. 17 (1) (2017) 378.
[37] H. Bae, J.Y. Lee, C. Yang, G. Song, W. Lim, Fucoidan derived from Fucus vesiculosus inhibits the development of human ovarian cancer via the disturbance of calcium homeostasis, endoplasmic reticulum stress, and angiogenesis, Mar Drugs 18 (1) (2020).
[38] F. Liu, J. Wang, A.K. Chang, B. Liu, L. Yang, Q. Li, P. Wang, X. Zou, Fucoidan extract derived from Undaria pinnatifida inhibits angiogenesis by human umbilical vein endothelial cells, Phytomedicine 19 (8–9) (2012) 797–803.
[39] C. Bouvard, I. Galy-Fauroux, F. Grelac, W. Carpentier, A. Lokajczyk, S. Gandrille, S. Colliec-Jouault, A.M. Fischer, D. Helley, Low-molecular-weight fucoidan induces endothelial cell migration via the PI3K/AKT pathway and modulates the transcription of genes involved in angiogenesis, Mar Drugs 13 (12) (2015) 7446–7462.
[40] A. P, A. K, S. L, M. M, M. K, Anticancer effect of fucoidan on cell proliferation, cell cycle progression, genetic damage and apoptotic cell death in HepG2 cancer cells, Toxicol. Rep. 6 (2019) 556–563.
[41] S. Palanisamy, M. Vinosha, M. Manikandakrishnan, R. Anjali, P. Rajasekar, T. Marudhupandi, R. Manikandan, B. Vaseeharan, N.M. Prabhu, Investigation of antioxidant and anticancer potential of fucoidan from Sargassum polycystum, Int. J. Biol. Macromol. 116 (2018) 151–161.
[42] S.N. Somasundaram, S. Shanmugam, B. Subramanian, R. Jaganathan, Cytotoxic effect of fucoidan extracted from Sargassum cinereum on colon cancer cell line HCT-15, Int. J. Biol. Macromol. 91 (2016) 1215–1223.

[43] H.Y. Park, S.H. Park, J.W. Jeong, D. Yoon, M.H. Han, D.S. Lee, G. Choi, M.J. Yim, J.M. Lee, D.H. Kim, G.Y. Kim, I.W. Choi, S. Kim, H.S. Kim, H.J. Cha, Y.H. Choi, Induction of p53-independent apoptosis and G1 cell cycle arrest by fucoidan in HCT116 human colorectal carcinoma cells, Mar Drugs 15 (6) (2017).
[44] M. Tomori, T. Nagamine, T. Miyamoto, M. Iha, Evaluation of the immunomodulatory effects of fucoidan derived from Cladosiphon Okamuranus Tokida in mice, Mar Drugs 17 (10) (2019).
[45] H.J. Chung, J. Jeun, S.J. Houng, H.J. Jun, D.K. Kweon, S.J. Lee, Toxicological evaluation of fucoidan from Undaria pinnatifidain vitro and in vivo, Phytother. Res. 24 (7) (2010) 1078–1083.
[46] F. Atashrazm, R.M. Lowenthal, G.M. Woods, A.F. Holloway, S.S. Karpiniec, J.L. Dickinson, Fucoidan suppresses the growth of human acute promyelocytic leukemia cells in vitro and in vivo, J. Cell. Physiol. 231 (3) (2016) 688–697.