Osteopontin promotes cell pelletizing and angiogenesis in triple negative breast cancer by activating the PI3k/Akt/mTOR pathway to regulate GPX4-mediated anti-lipid peroxidation levels

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Research Article

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Abstract

Background

Triple negative breast cancer (TNBC) features high aggressiveness, metastasis rate, drug resistance as well as poor prognosis. Osteopontin (OPN) is a key protein in the process of osteogenesis and has been found to be a new tumor marker in recent years. This study will discuss the regulatory role that OPN plays in TNBC as well as its unconcealed mechanism.

Methods

The cell viability was tested utilizing CCK-8 while Transwell as well as wound healing was adopted to test cell invasive and migrative capabilities. The pellet size of each group was detected by tumor-sphere formation assay. HUVEC tube formation assay was used to measure the ability of tumor cells to form blood vessels. Western blot was applied for the estimation of globule-forming-, angiogenesis-, PI3k/Akt/mTOR signaling pathway-related proteins as well as OPN. The levels of oxidative stress-related markers were assessed with the help of ELISA. Through transfection, OPN in MD-MB-436 cells was overexpressed and PI3k/Akt/mTOR pathway inhibitor LY294002 was employed for the administration of cells. After that, a series of functional experiments were implemented to investigate the mechanism of tumor cells. Finally, in vitro experiment of tumor-bearing mice were performed to verify.

Results

The proliferative, invasive and migrative capabilities as well as angiogenesis of TNBC cells were conspicuously ascended in contrast with non-TNBC cell lines. OPN expression in TNBC cells was dramatically enhanced. OPN upregulation rapidly elevated cell proliferative, invasive and migrative capabilities as well as angiogenesis, which may be achieved by activating PI3k/Akt/mTOR to regulate GPXX4-mediated anti-lipid peroxidation.

Conclusion

OPN can promote cell pelletizing and angiogenesis in TNBC by activating the PI3k/Akt/mTOR pathway to regulate GPX4-mediated anti-lipid peroxidation levels.

Introduction

Being a kind of breast cancer, triple negative breast cancer (TNBC) features negative contents of estrogen receptor, progesterone receptor as well as human epidermal growth factor receptor [1]. TNBC has strong aggressiveness and rapid metastasis, along with poor prognosis. TNBC, which has low 5-year survival

rate, has the highest mortality among all kinds of breast cancer [2]. Therefore, the investigation of TNBC mechanism and possible targeted therapy has become a research hotspot.

Osteopontin (OPN) is a calcium-binding phosphorylated protein associated with malignant transformation, which plays a role in promoting cell adhesion chemotaxis and tumor metastasis in vivo [3]. That is to say, OPN is a key factor involved in the regulation of malignant behavior of tumor cells, and can impart tremendous influence on the advancement of tumor. Abundant OPN expression in blood indicates lower survival rate and higher recurrence rate of patients [4]. At present, the influence of OPN on the pathogenesis and development of tumors and its molecular mechanism have become a research hotspot. The study of OPN on tumor malignant behavior and its molecular mechanism is expected to provide a novel angle for exploring the treatment of tumors. A case of previous study has shown that the increased expression of OPN in TNBC can be used as a predictive biomarker of anti-epidermal growth factor therapy in TNBC [5]. However, the specific molecular regulatory mechanism and signal pathway through which OPN regulates in TNBC have not been studied too much.

Angiogenesis has decisive role in aggressive tumor growth. Tumor angiogenesis is divided into endothelial cell activation, proliferation, migration, vascular growth factor production in tumor and surrounding tissues as well as tissue infiltration of endothelial cells [6]. Therefore, the study of tumor-induced dysangiogenesis helps to develop new anti-angiogenesis therapies. As a pivotal angiogenic factor, OPN can promote vascular endothelial growth factor (VEGF) secretion in breast cancer cells [7] and prostate cancer cells [8], and affect vascular formation through VEGF. Therefore, in this paper, we will discuss the effects of OPN on TNBC cell pelletization and angiogenesis.

It was discovered that PI3K/AKT/mTOR is correlated with the expression of GPX4 and can affect its high expression [9]. GPX4 is a key factor in enhancing cellular antioxidant capacity, ferroptosis, and iron autophagy (a type of autophagy that is generally considered to promote cell death) [10]. In other words, the high expression of PI3k/Akt/mTOR may make tumor cells resistant to death caused by lipid oxidative stress by activating GPX4 activity, and oxidative stress may promote the secretion of related pro-tumor factors. In summary, mTOR promotes the development of TNBC by activating GPX4 expression, reducing oxidative stress and ferroptosis damage in cells. Moreover, OPN may induce malignant phenotype of cells by activating the PI3K/AKT/mTOR pathway, contributing to enhanced cell proliferative and invasive capabilities, metastasis, angiogenesis as well as undesirable treatment efficacy [11]. In acute myeloid leukemia, the mRNA expressions of AKT1 and mTOR decreased with the increase of downregulation OPN [12]. Therefore, we speculated that OPN might regulate GPX4-mediated anti-lipid peroxidation level by regulating PI3K/AKT/mTOR, thus affecting cell pellet formation and angiogenesis in TNBC.

Therefore, in this paper, we will discuss the effect of OPN on TNBC cell pelletization and vascular formation as well as its mechanism. Our paper provides a theoretical basis for the exploration of TNBC pathogenesis and possible targeted therapy.

Materials And Methods

Cell culture

MCF-10A、MCF-7 and MDA-MB-436 cell lines that provided by Cell Bank of the Chinese Scientific Academy were cultivated in DMEM with 10% FBS. Cells were seeded in a six-well plate (10^5 cells per well). Upon reaching 80% confluence, cells were transfected with overexpression plasmid using Dharmafect 1 (Dharmacon). OPN and its control was cloned into pCMV3-C-his vector (OPN-OE or vector, Sino Biological). After 48h, RT-qPCR was utilized to test transfection efficiency . LY294002 was provided by Selleck.

RT-qPCR

Total RNA was harvested by Trizol reagent (Gibco, Grand Island, NY, USA) in light of the recommended protocol. Reverse transcriptase reaction was operated with Brilliant II Fast SYBR green QPCR master mix (Agilent Technologies, Santa Clara, CA, USA). RT-qPCR was operated utilizing SYBR Premix Ex Taq TM (Takara Bio, Inc., Otsu, Japan) and measured by 7500 FAST Real-Time PCR System (Bio- Rad Co., USA). The mRNA expressions were valued by $2^{-\Delta\Delta Ct}$ method and GAPDH was used to normalize the data[13].

CCK8

The level of cell viability was assayed employing a CCK8-Kit in light of standard protocol. The OD values were assessed at 450 nm.

Wound healing

The inoculation of cells (2×10^5cells/well) into 6-well plates was carried out. When cells reached 90% confluence, a 200 μl plastic tip was used to make a straight line in the monolayer, and plates rinsed by PBS. The cells were observed under an inverted microscope coupled to a camera (Leica DMI 4000) at 25°C, and this time was designated at 0 h. Afterwards, cells were maintained in an incubator. The migrative cells were evaluated applying Image J (v 1.5.1, National Institutes of Health, USA).

Transwell

Cells were suspended in serum-free medium and then seeded into the upper chamber of a transwell with an 8-μm pore (Corning, Inc.). The upper chambers were pre-coated with (Transwell invasion assay) or without (Transwell migration assay) Matrigel (1 mg/ml), while lower chambers were decorated with medium carrying 10% FBS. The chambers were adopted for cell cultivation. The cells were subsequently exposed to 4% polyoxymethylene fixation as well as 1% crystal violet staining. Cells were visualized employing a light microscope (Leica DM 4000).

Tumor-Sphere Formation Assay

The cells were inoculated into 6-well plates with 1000 cells per well after corresponding treatment. After 14 d cultivation of cells, tumor spheres were formed. The tumor spheres were observed and documented

under an optical microscope (DM4M, Leica, Solms, Germany) at a magnification of 200 ×.

Western blot

The lysis of cells was carried out employing RIPA buffer (Shanghai Ruji Biotechnology Development Co., Ltd.). Subsequently, lysates were centrifuged, the supernatant was recovered and used for protein quantification by a BCA kit（Beyotime）. Then 30 μg of protein were loaded onto PVDF membranes (Merck Millipore) which were impeded by 5% milk in PBS-Tween 20 and cultivated with corresponding primary antibodies, after which was the exposure to secondary antibodies HRP-linked anti-rabbit IgG (1:5000, Abcam). The relative density of each band was decided employing ImageJ software. Then the relative protein expression was presented as the ratio of the density values of bands between experimental and control samples.

In vitro HUVEC tube formation assay

To solidify the gel, 300 μL of growth factor-reduced Matrigel (BD Biosciences, USA) was added into precooled 48-well plates and incubated for 30-60 min at 37 °C. Subsequently, human umbilical vein endothelial cells (HUVECs) that inoculated into a 48-well plate (300 μL/well) were suspended in breast cell-derived conditioned medium carrying 10% FBS for cultivation. The tube forming ability of cells was captured applying an inverted light microscope.

Cell transfection

The overexpression and corresponding controls of OPN (OPN-OE#1, OPN-OE#2 and Vector) were synthesized by GenePharma Co. 69 (Shanghai, China). After that, the transfection of these vectors into cells was conducted adopting Lipofectamine 3000 reagent (Beijing Ya'anda Biotechnology Co., Ltd.) in light of recommended protocol.

Mice xenograft models

Nude mice were intramuscularly injected with MDA-MB-231 cells in the right hind thigh at the density of 2×10^6 per ml. The transplanted nude mice were separated into 3 groups at random: Vector, OPN-OE and OPN-OE+ LY294002, $n = 5$. OPN-OE overexpression plasmid was injected into the tail vein of mice in OPN-OE and OPN-OE+ LY294002 group. Vector group was injected with blank control plasmid via tail vein. Control mice were left untreated. Mice in OPN-OE+ LY294002 group were injected with 10 μ LY294002 once every 4 days. All mice were examined every 3 days and sacrificed 21 days after tumor inoculation.

Immunohistochemistry (IHC)

Tissue sections were prepared and subjected to immunohistochemical analysis. Anti-VWF was used as primary antibody. HRP-conjugated secondary Ab served as secondary antibody. Photographs were captured employing an Olympus-IX71 microscope at 40 × 10 magnification.

Measurement of MDA conten and GSH in tumor tissue

The enzymatic activities of MDA (Cat.no.A003-1-2) and GSH (Cat.no. A005-1-2) were assessed with different commercial assay kits which were supplied by Nanjing Jiancheng Bioengineering Institute.

Statistical Analysis

All data that displayed in the form of mean ± SD got analyzed utilizing SPSS 20.0 software. Student's *t*-test and one-way ANOVA were applied for the demonstration of comparisons. $P < 0.05$ meant that the experimental figures exhibited statistical significance.

Results

Cell pellet-forming and angiogenesis abilities of MCF-10A, MCF-7 and MDA-MB-436 cells

CCK8 was employed for the estimation of cell proliferative ability. Compared with MCF-10A, the proliferative ability of MDA-MB-436 and MCF-7 cells were rapidly enhanced (Fig. 1A). Transwell (Fig. 1B and C) as well as wound healing (Fig. 1D and E) was applied for the evaluation of cell invasive and migrative abilities, and it was discovered that in comparison with MCF-10A, the invasive and migrative abilities of MCF-7 and MDA-MB-436 cells were markedly elevated, particularly in MDA-MB-436 cells. Cell pellet-forming experiments showed that MDA-MB-436 cells pellet-forming ability was stronger than MCF-10A and MCF-1 cells (Fig. 1F). Western blot was adopted for the assessment of pellet-related proteins CD44, CD24 and ALDH1, and results in Fig. 1G exhibited that the contents of CD44, CD24 and ALDH1 in MDA-MB-436 and MCF-7 were remarkably ascended. Tubule formation assay detected the effect of tumor cells on endothelial cell tubule formation ability. The results showed a remarkable increase in tubular structure formation in HUVECs cultured with MDA-MB-436 medium compared with that in HUVECs cultured with control (Fig. 2A and B). As Fig. 2C depicted, the levels of VWF and CD31 in MDA-MB-436 medium group were conspicuously enhanced when compared to the Control group.

Opn Regulates Cell Pelletizing And Vascular Formation By Activating The Pi3k/akt/mtor Signaling Pathway In Tnbc

Subsequently, the contents of OPN and PI3K/AKT/mTOR pathway-related proteins were assessed and it was discovered that in comparison with MCF-10A, the contents of OPN, p-PI3K, p-Akt, mTOR as well as GPX4 were dramatically ascended (Fig. 3). We then constructed an OPN overexpression vector and transfected it into MDA-MB-436 cells. RT-qPCR was used to detect the transfection efficiency (Fig. 4A). In addition, PI3K/AKT/mTOR signaling pathway inhibitor LY294002 was also added, and cells were separated into control, Vector, OPN-OE and OPN-OE + LY294002 groups. Results from CCK8 displayed that the activity in OPN-OE group was rapidly enhanced when compared to the Vector group, while the

activity in OPN-OE + LY294002 group was reversed compared with the OPN-OE group (Fig. 4B). Transwell (Fig. 4C and D) and wound healing (Fig. 4E and F) experiments showed that the invasive and migrative abilities of OPN-OE group were greatly increased compared with the Vector group, and those of OPN-OE + LY294002 group were tremendously reversed. The pellet-forming experiment results showed that the pellet-forming rate of MDA-MB-436 cells was increased after overexpressing OPN, which was subsequently declined to some extent after inactivating PI3K/AKT/mTOR signaling pathway (Fig. 4G and H). Results in Fig. 4I exhibited that the contents of CD44, CD24 and ALDH1 were elevated in MDA-MB-436 cells after overexpressing OPN, which were reversed after blocking PI3K/AKT/mTOR pathway. Tubule formation experiments as well as Western blot results demonstrated that in comparison with the Vector group, HUVECs in OPN-OE group were markedly elevated tubular structure formation (Fig. 5A and B), and the contents of VWF and CD31 were rapidly ascended (Fig. 5C). In comparison with OPN-OE group, the tubular structure formation of HUVECs in OPN-OE + LY294002 group was greatly diminished, and the contents of VWF and CD31 were remarkably descended.

OPN regulates GPX4-mediated anti-lipid peroxidation by activating PI3K/AKT/mTOR signaling pathway to affect cell pelletizing and vascular formation in TNBC

As Fig. 6A demonstrated, MDA activity in cells was conspicuously cut down while reductive GSH expression was elevated after overexpressing OPN. However, after the administration with LY294002, MDA expression was rapidly ascended and reductive GSH expression was descended. Western blot was adopted for the evaluation of OPN as well as pathway-related proteins. It was discovered that the contents of OPN, p-PI3K, p-Akt, mTOR as well as GPX4 were remarkably enhanced in OPN-OE group in comparison with Vector group. After further administration with LY294002, contents of these proteins in cells were reversed (Fig. 6B). The above findings implied that OPN could activate GPX4 expression, reduce the anti-lipid peroxidation level of cells, and promote the development of TNBC by activating PI3K/AKT/mTOR pathway.

Animal experiments verifies that OPN regulates GPX4-mediated anti-lipid peroxidation by activating PI3K/AKT/mTOR signaling pathway to affect cell pelletogenesis and vascular formation in TNBC

Finally, the tumor cells were cultured and inoculated subcutaneously, and then the mice were photographed and the tumor size was observed. We found that overexpression of OPN greatly ascended body weight and tumor size in mice. In comparison with OPN-OE, body weight and tumor were conspicuously reduced in OPN-OE + LY294002 group (Fig. 7A-E). Results obtained from IHC demonstrated that VWF level was markedly enhanced in OPN-OE group in comparison with Vector group. When compared to OPN-OE group, VWF level in OPN-OE + LY294002 group was rapidly descended (Fig. 7F). Oxidative stress level in tumor tissues was shown in Fig. 7G. After overexpressing OPN, MDA activity in tumor tissues was dramatically cut down, while GSH activity was enhanced (Fig. 7G). It was also discovered that the contents of OPN, p-PI3K, p-Akt, mTOR as well as GPX4 in tumor tissues of mice in OPN-OE group were rapidly elevated in comparison with the vector group. In contrast with OPN-OE group,

the levels of OPN, p-PI3K, p-Akt, mTOR as well as GPX4 were greatly declined in the OPN-OE + LY294002 group (Fig. 7H and I).

Discussion

TNBC, which accounts for 10 to 17 percent of all breast cancers, differs from other traditional breast cancers. Studies have evidenced that in comparison with non-TNBC patients, TNBC patients have a higher risk of postoperative recurrence [14, 15]. Moreover, the tumor diameter of TNBC, which belongs to a poorly differentiated tumor, is larger than that of non-TNBC patients. Cell proliferation activity, histological grade and mitotic count were rapidly ascended [16]. In our experiment, it was found that the proliferation capacity of TNBC cell line MDA-MB-436 was greatly enhanced in comparison with non-TNBC cell line MCF-7 and normal cell line MCF-10A. In addition, it displayed that TNBC has stronger invasion and metastasis abilities, and is easy to invade into blood vessels, thus elevating the recurrence rate [17]. Our experimental results also showed that the invasive as well as migrative abilities of MDA-MB-436 cells were higher than that of MCF-7 and MCF-10A. In addition, the pellet formation and angiogenesis abilities of MDA-MB-436 cells were also higher than that of MCF-7 and MCF-10A.

OPN is a multifunctional tumor regulatory protein [18]. Multiple studies in human and tumor-bearing mice have shown that the increased expression of OPN in breast, gastric, lung, as well as colon cancers has close relation with the occurrence, development and metastasis of tumors [19–22]. Moreover, OPN level is considered to be able to evaluate the metastatic potential of some tumors [23, 24]. As a multifunctional protein, OPN plays an important role in determining the oncogenic potential of various tumors, especially in highly invasive tumors. Previous study has shown that elevated OPN contributed to more aggressive behaviors of breast cancer cells and can be used as a diagnostic and prognostic marker of breast cancer [25]. In 115 patients suffering from breast cancer, OPN expression was markedly enhanced, and the high expression of OPN was closely related to disease adverse reactions and overall survival rate [26]. In our experiment, it was uncovered that OPN level in MCF-7 and MDA-MB-436 cells was conspicuously elevated. Overexpression of OPN in MDA-MB-436 cells could further increase the proliferative, invasive, migrative, pellet-forming and angiogenesis abilities of MDA-MB-436 cells.

The PI3K/Akt/mTOR pathway acts as a critical player in tumor cell proliferation, vascular growth as well as metastasis, and its downstream mTOR is a vital protein kinase of this pathway, which mainly regulates the biological effects of tumor cell growth, proliferative ability, survival as well as angiogenesis [27]. It showed that the PI3K/Akt/mTOR pathway was triggered in breast cancer, while its inhibitors can inhibit the growth of breast cancer tumors and cause apoptosis of cancer cells [28, 29]. Actin-like protein 8 promotes the proliferative, migrative and invasive abilities of TNBC cells and inhibits apoptosis by triggering PI3K/AKT/mTOR pathway [30]. Elsewhere, OPN can induce proliferation, invasion, migration and angiogenesis in tumor cells by activating the PI3K/AKT/mTOR pathway [11]. In our experiment, it was found that the activation of PI3K/AKT/mTOR was increased in MDA-MB-436 cells compared with normal cells and non-TNBC cells. Inhibition of PI3K/AKT/mTOR expression could significantly reverse the

effects of OPN overexpression on cell malignant progression. These findings implied that OPN in TNBC cells drove the advancement of TNBC by triggering the PI3K/AKT/mTOR signaling pathway.

GPXs are a class of enzymes that play an important antioxidant role in organic cells. By catalyzing the decomposition of various hydroperoxides, GPXs maintains the balance of ROS level in tissues and cells and protects tissues and cells against oxidative stress damage [31]. Among them, GPX4 is an enzyme in the GPXs family with strong lipid peroxidation inhibition, which is located on various membrane structures in the cytoplasm. It can effectively remove various lipid peroxides in cells, protect lipid structures on various membranes in cells from being damaged by oxidative stress, and maintain the integrity of membrane structures and normal organelles [10, 32]. GPX4 has abundant existence in many cancers and acts as a critical oncogenic gene [33–35]. In TNBC, GPX4-mediated lipid peroxidation plays an important role [36, 37]. A case of previous study has found that PI3K/AKT/mTOR is correlated with GPX4 expression. The interaction between mTOR and GPX4 can regulate autophagy-dependent cancer cell death [9]. Autophagy-mediated GPX4 degradation and mTOR inhibition play a synergistic role in killing bladder cancer cells [38]. Therefore, we have reasons to guess that OPN regulates GPX4-mediated lipid peroxidation by regulating PI3K/AKT/mTOR signaling pathway, thus affecting the development of TNBC. Our study uncovered that GPX4 expression was greatly ascended in MDA-MB-436 cells. OPN overexpression can promote GPX4 level in cells, which was subsequently reserved by LY294002 administration, thus inhibiting the lipid peroxidation level of tumor cells.

Conclusion

In conclusion, we found that OPN can promote cell pelletizing and angiogenesis in TNBC by activating the PI3k/Akt/mTOR pathway to regulate GPX4-mediated anti-lipid peroxidation levels.

Declarations

Ethics approval and consent to participate

All animal experiment procedures were operated according to the NIH Guide for the Care and Use of Laboratory Animals approved by the ethical guidelines of Nanyang Central Hospital affiliated to Henan University.

Consent for publication

All the authors agree to be published

Availability of data and materials

The analyzed datasets generated during the present study are available from the corresponding author on reasonable request.

Competing interests

There is no conflict of interest.

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Authors' contributions

Hao Zhang and Man Guo wrote the manuscript and analyzed the data. Weihan Li, Mengyue Liu, Cao Wang and Lu Zhang performed the experiments and supervised the study. Hao Zhang and Man Guo searched the literature and revised the manuscript for important intellectual content. Hao Zhang and Man Guo confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

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Figures

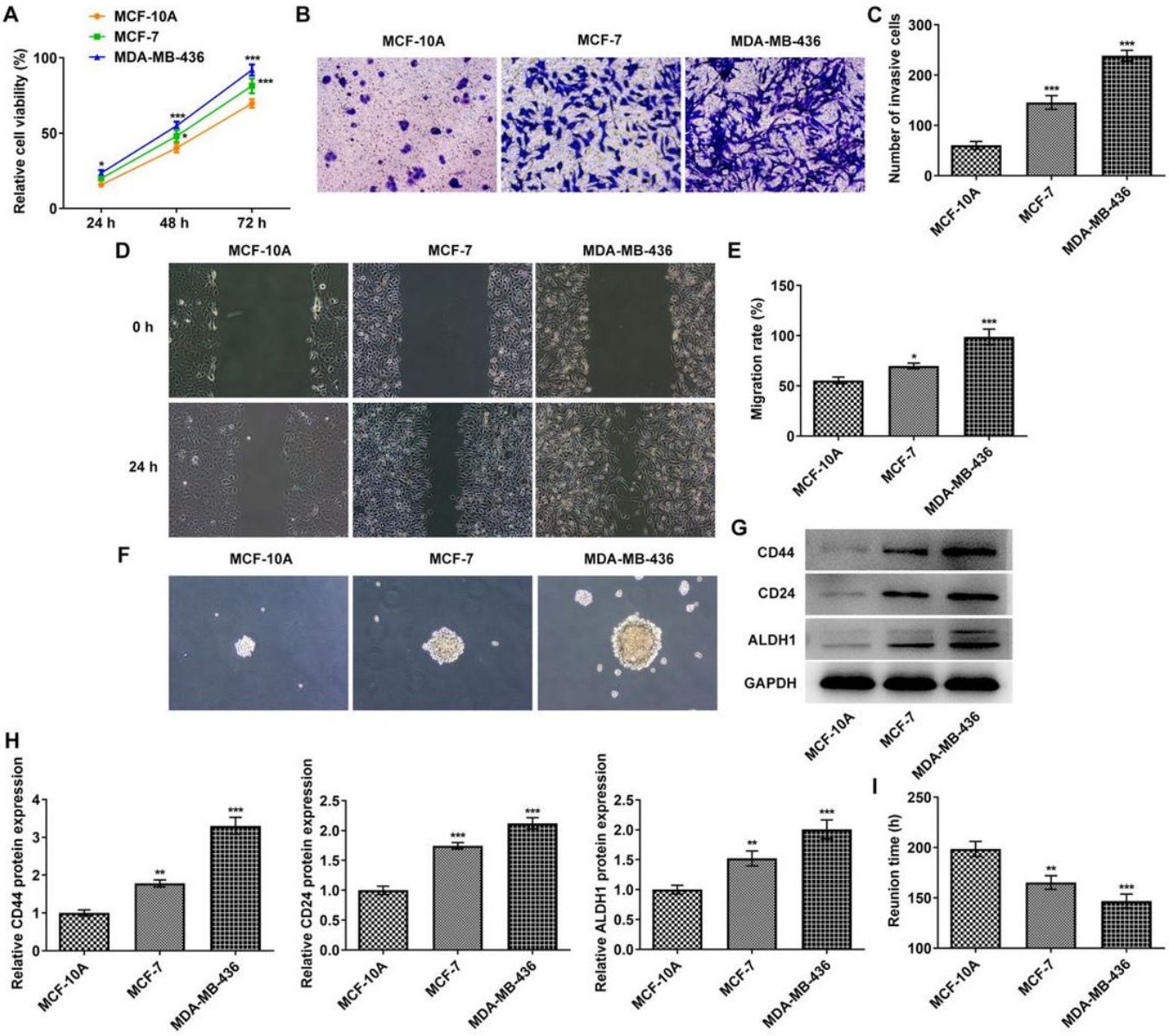

Figure 1

Cell pellet-forming of MCF-10A, MCF-7 and MDA-MB-436 cells. A. CCK8 kit was used to detect the cell proliferative capacity. B. Transwell detected the cell invasion ability. C. Statistical analysis of cell invasion ability. D. Wound healing detected the cell migration ability. E. Statistical analysis of cell migration ability. F. The pellet size of each group was detected by tumor-sphere formation assay. G. Western blot detected the expressions of CD44, CD24 and ALDH1.*p<0.05, **p<0.01, ***p<0.001 vs MCF-10A.

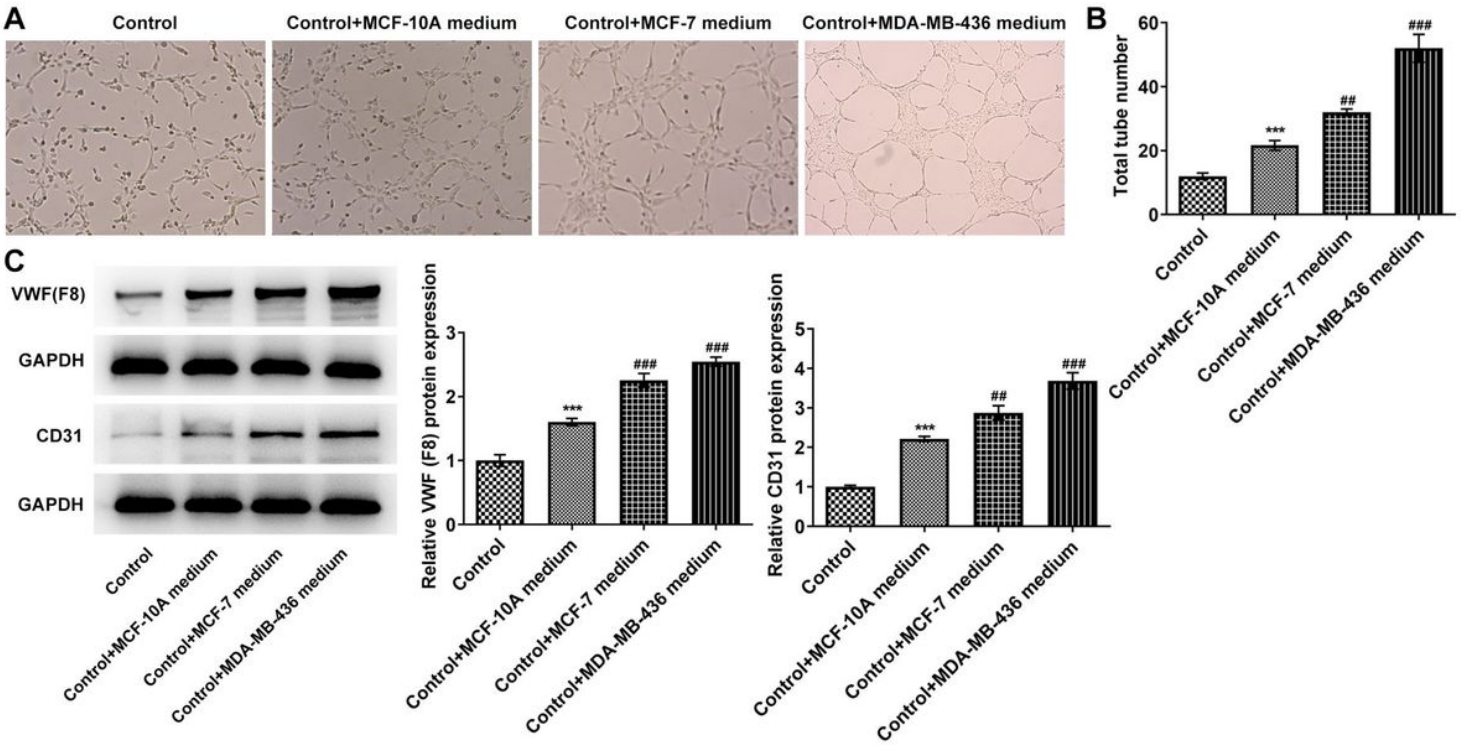

Figure 2

Cell angiogenesis abilities of MCF-10A, MCF-7 and MDA-MB-436 cells. **A.** HUVEC tube formation assay was used to detect the angiogenesis abilities. B. Statistical analysis of tube number. C. Western blot detected the expressions of VWF and CD31. ***p<0.001 vs control; ##p<0.01, ###p<0.001 vs control+MCF-7 medium.

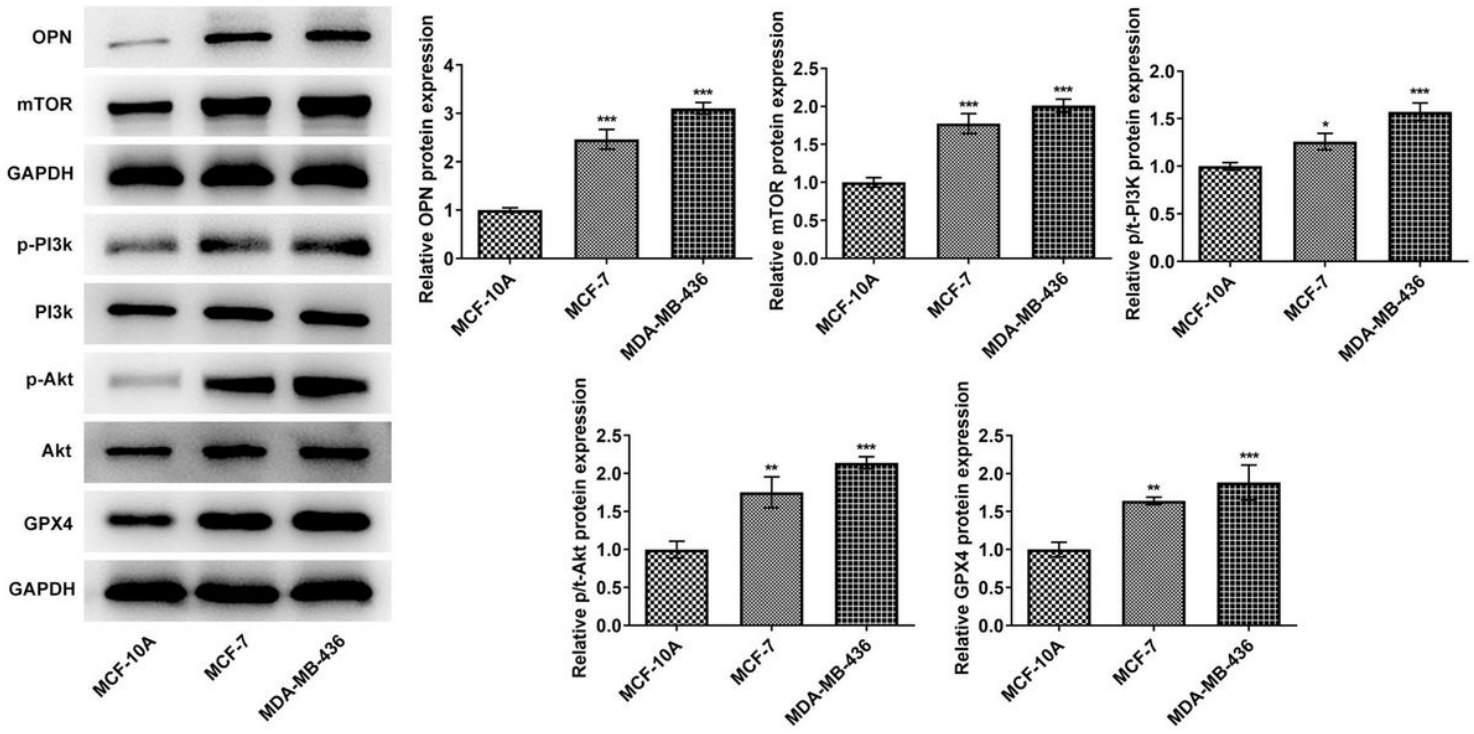

Figure 3

The expressions of OPN and PI3K/AKT/mTOR signaling pathway-related proteins. Western blot detected the expressions of OPN and PI3K/AKT/mTOR signaling pathway-related proteins. *p<0.05, **p<0.01, ***p<0.001 vs MCF-10A.

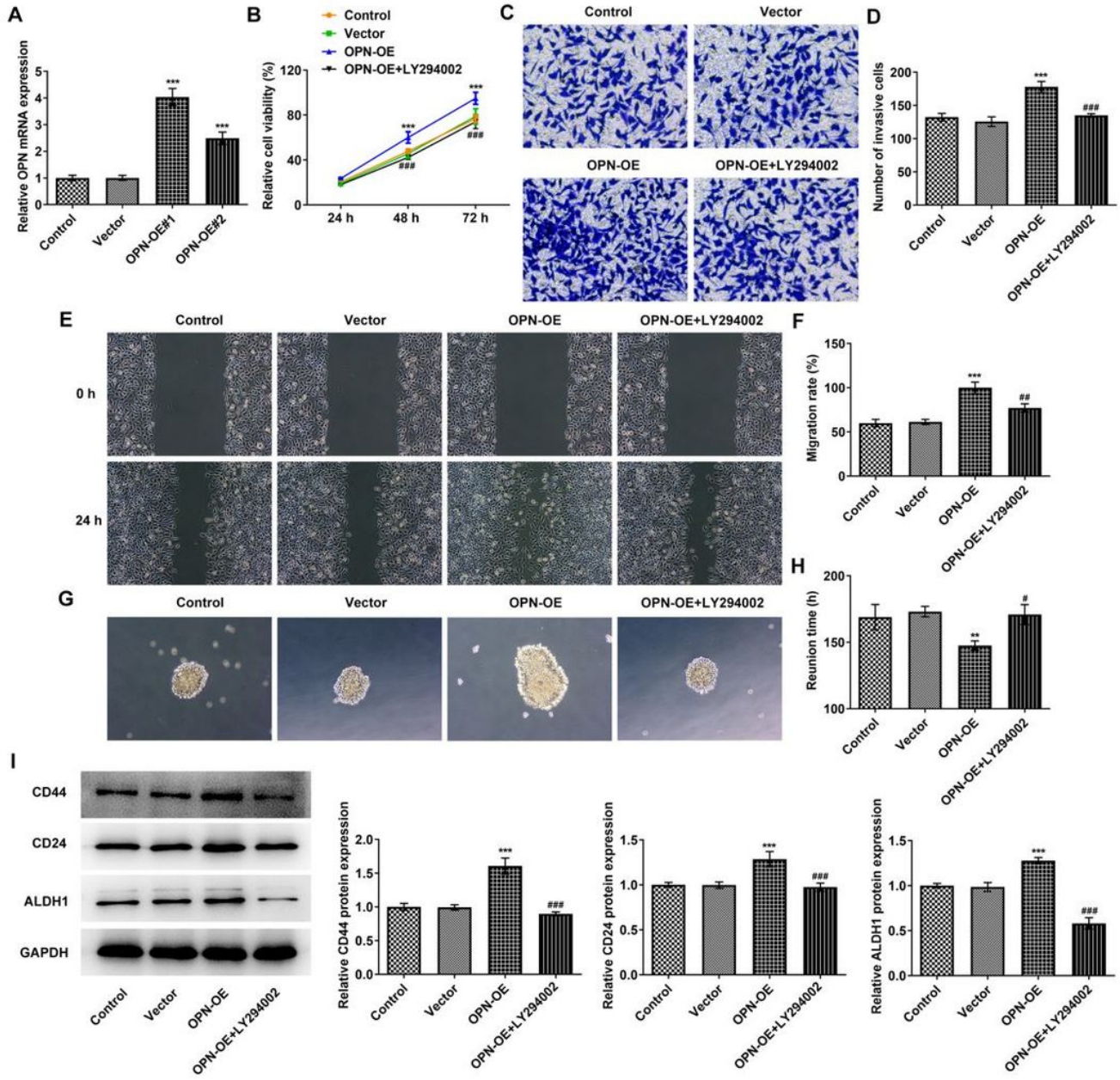

Figure 4

OPN regulates cell pelletizing by activating the PI3K/AKT/mTOR signaling pathway in TNBC. A.RT-qPCR detected the expression of OPN. B. CCK8 kit was used to detect the cell proliferative capacity. C. Transwell detected the cell invasion ability. D. Statistical analysis of cell invasion ability. E. Wound healing detected the cell migration ability. F. Statistical analysis of cell migration ability. G. The pellet size of each group was detected by tumor-sphere formation assay. H. Statistical analysis of the time of tumor-sphere formation. I. Western blot detected the expressions of CD44, CD24 and ALDH1. **p<0.01, ***p<0.001 vs Vector; #p<0.05, ##p<0.01, ###p<0.001 vs OPN-OE.

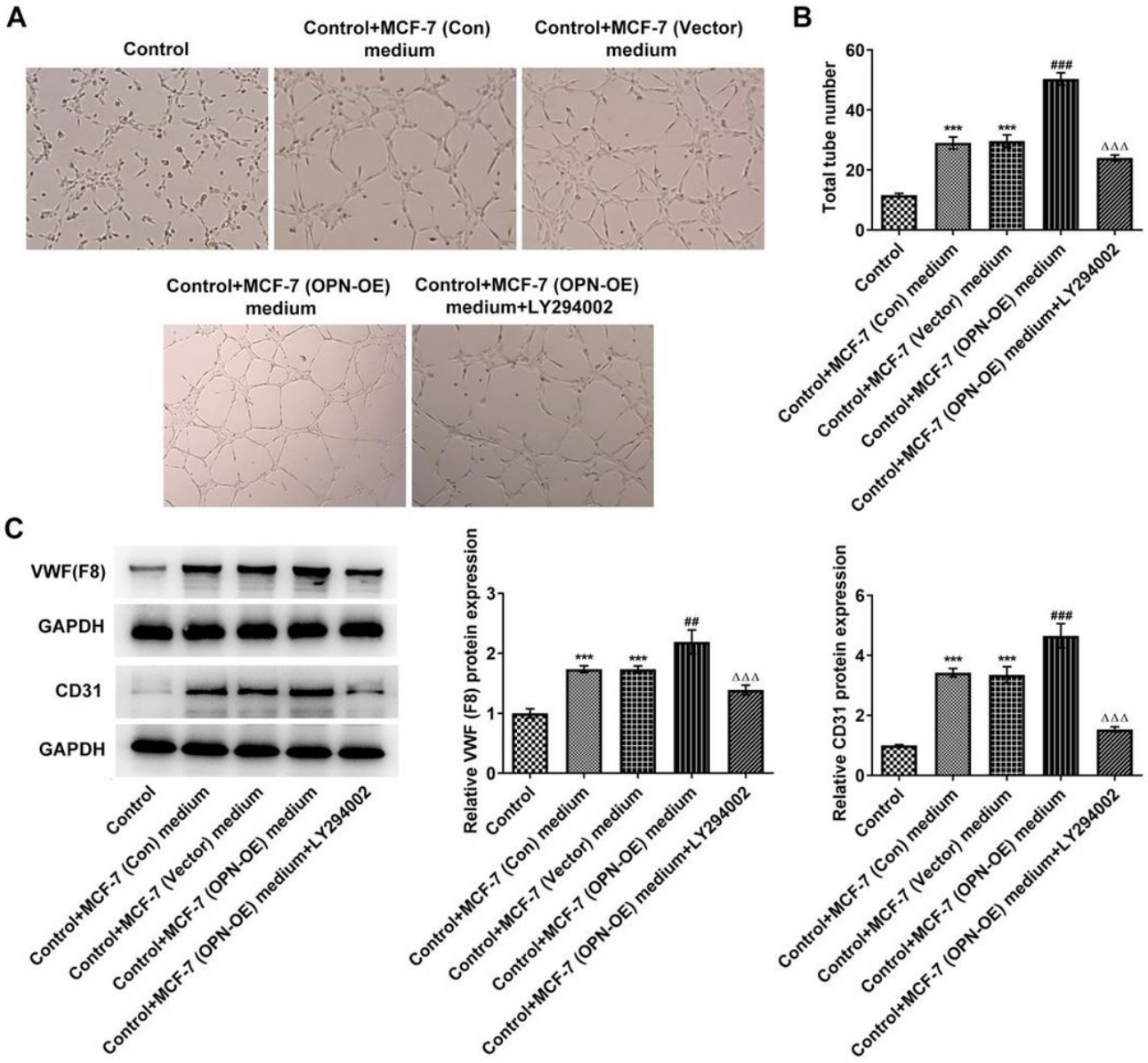

Figure 5

OPN regulates cell vascular formation by activating the PI3K/AKT/mTOR signaling pathway in TNBC. **A.** HUVEC tube formation assay was used to detect the angiogenesis abilities. B. Statistical analysis of tube number. C. Western blot detected the expressions of VWF and CD31. ***p<0.001 vs control; ##p<0.01, ###p<0.001 vs control++MCF-7(Vector) medium; △△△p<0.001 vs control++MCF-7(OPN-OE) medium.

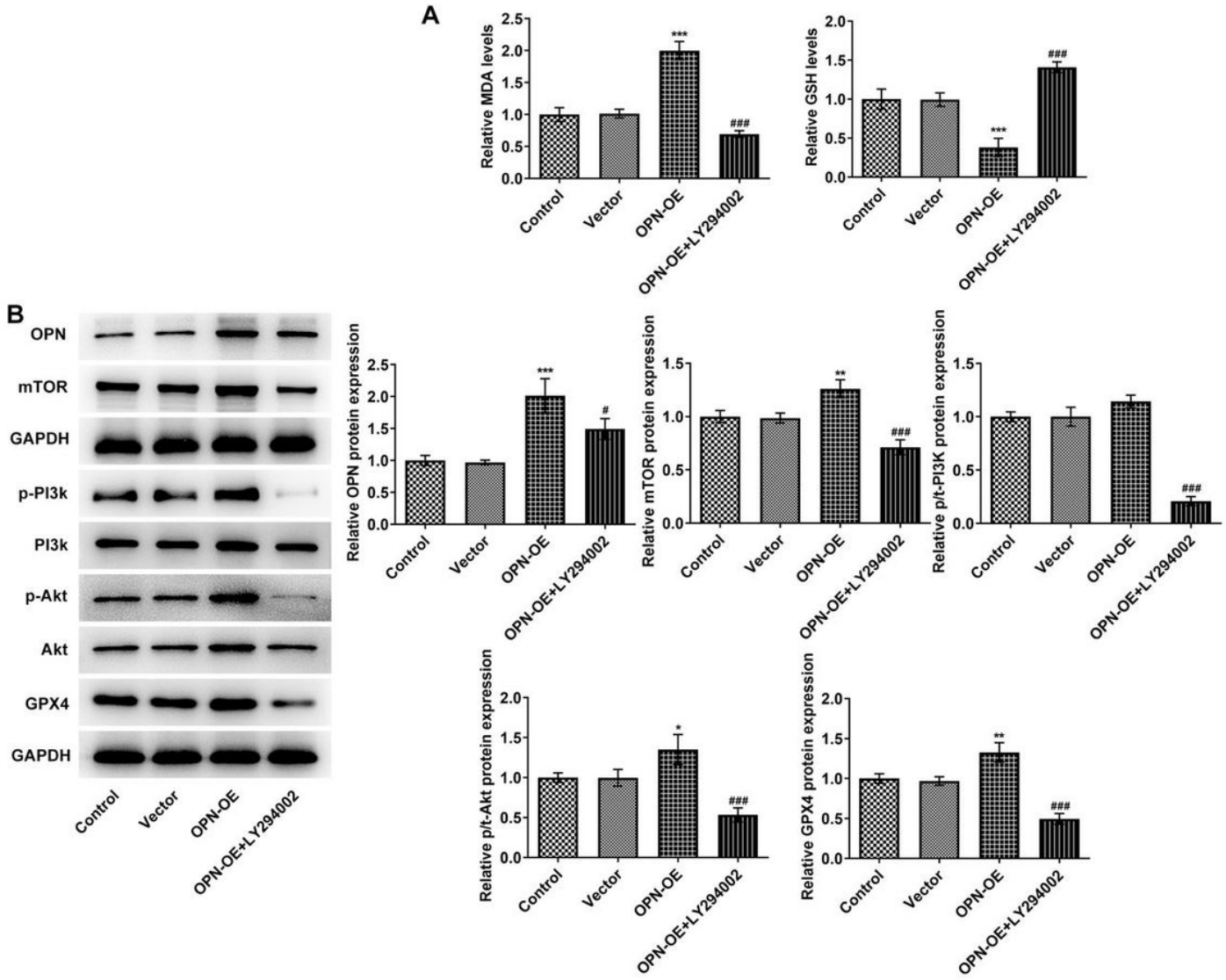

Figure 6

OPN regulates GPX4-mediated anti-lipid peroxidation by activating PI3K/AKT/mTOR signaling pathway to affect cell pelletizing and vascular formation in TNBC. A. The levels of MDA and GSD were detected with corresponding kits. B. Western blot detected the expressions of OPN and PI3K/AKT/mTOR signaling pathway-related proteins. *p<0.05, **p<0.01, ***p<0.001 vs Vector; #p<0.05, ###p<0.001 vs OPN-OE.

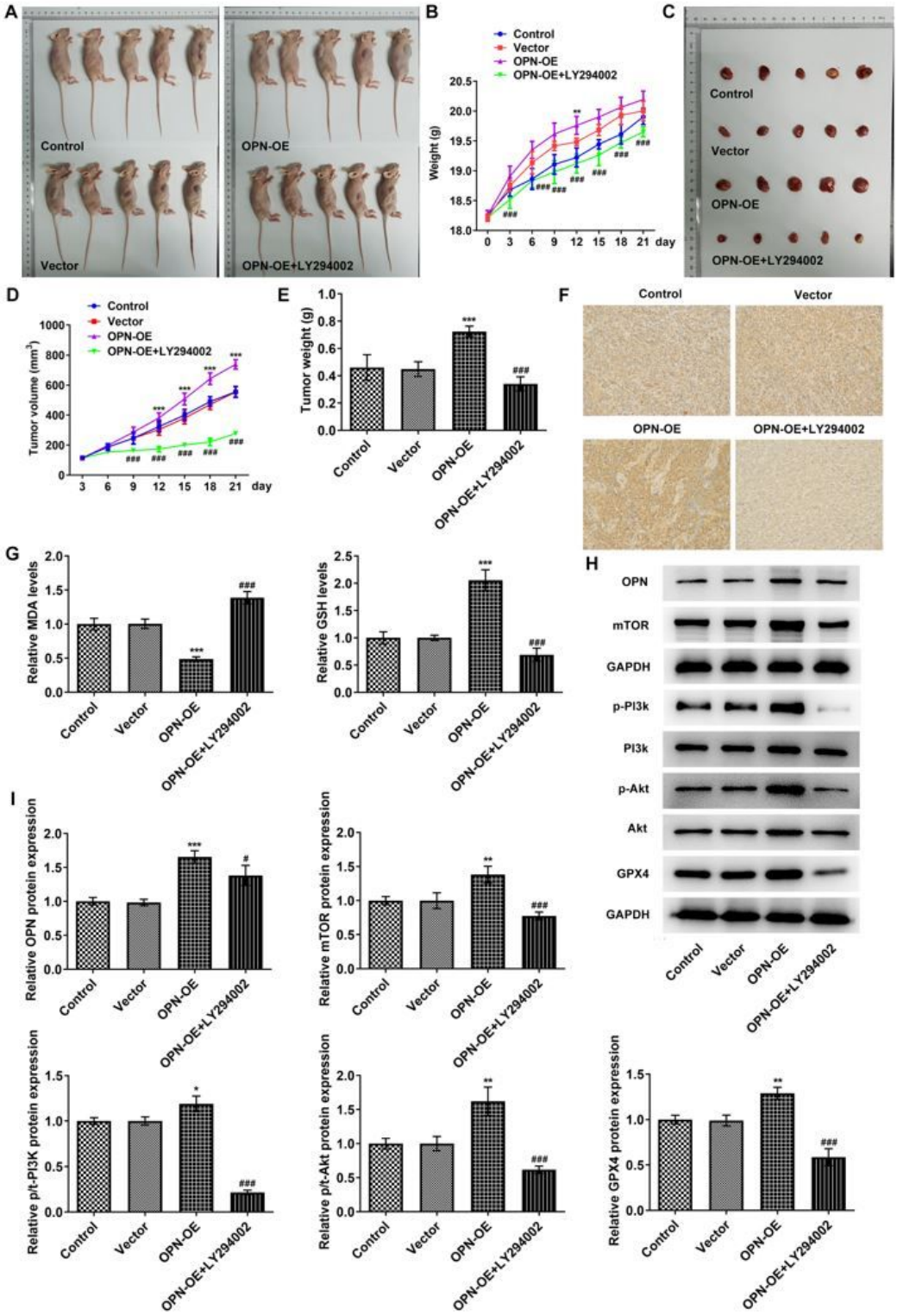

Figure 7

Animal experiments verifies that OPN regulates GPX4-mediated anti-lipid peroxidation by activating PI3K/AKT/mTOR signaling pathway to affect cell pelletogenesis and vascular formation in TNBC. A. The mice were photographed. B. The weight of the mice. C. The tumor of mice was photographed. D. The tumor volume was observed. E. The tumor weight was observed. F. IHC detected the expression of VWF. G. The levels of MDA and GSD were detected with corresponding kits. H and I. Western blot detected the

expressions of OPN and PI3K/AKT/mTOR signaling pathway related proteins. *p<0.05, **p<0.01, ***p<0.001 vs Vector; #p<0.05, ###p<0.001 vs OPN-OE.

Supplementary Files

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