Introduction

Breast cancer is ~~the~~ a common malignant tumor in females. The incidence~~s~~ of breast cancer is progressively on the rise~~increase~~, especially in the urban areas of China. Official data from China predicted that the mortality rates will continue to rise in the ensuing 5 years [1–3]. This presents breast cancer as a major ~~S~~obreast cancer~~is still a~~ major public health ~~problem~~concern. According to the ~~present~~ current research, tumor metastasis remains as the ~~dominate~~ dominant ~~reason~~ cause for cancer-related mortality. In addition, ~~, and~~ metastatic breast cancer is associated with ~~indicated~~ poor~~er~~ outcome~~s~~ in patients. ~~So~~ Therefore, prevention and management of ~~metastasis~~ malignant nature of breast cancer are necessary for cancer therapy.

The characteristic properties of cancer: invasion and metastasis results due to ~~of cancer is the results of~~ the comprehensive action on the host–, tumor cells, and microenvironment. Extracellular matrix (ECM) is ~~the~~ an important microenvironment-, which is composed of ~~complex mixture including~~ collagens, non-collagenous glycoproteins, ~~and~~ proteoglycans, ~~as well as~~ and soluble molecules, such as ~~e.g.~~ growth factors, –chemokines, and cytokines. Extracellular matrix plays an essential role by providing an adhesive structure for cancer cells. These cells adhere to the ECM, which is essential for ~~Moreover,cancer endothelial cells require adhesion to ECM for their~~ their proliferation, migration, morphogenesis, and blood vessel stabilization [4–6]. ~~In~~ During metastasis, the malignant tumor cells ~~devastating~~ adhering to the ECM~~,~~ penetrate the blood vessels ~~walls~~ and ~~then~~ enter the metastatic target tissues. ~~So~~Thus, the ~~structure~~ structural integrity of ~~extracellularmatrix(~~ECM~~)~~ and basement membrane (BM) acts as ~~is a~~ natural barrier ~~for of inhibition of~~ tumor metastasis.

Heparan sulfate proteoglycan (HSPG) ~~are~~ is a complex molecule~~,s~~ which is composed of a core protein ~~with~~ covalently attached to several linear chains of heparan sulfate (HS) ~~chains~~, which is a ubiquitous macromolecule~~s~~ associated with cell surface and ECM. ~~Moreover,~~ ~~HS~~Heparan sulfate mediates the interactions with a variety of extracellular ligands, such as growth factors and adhesion molecules [7,8]. Heparanase ~~(HSPE)~~ is a mammalian endo-D-glucuronidase, which is capable of cleaving heparin and HS. In 1999, 3 different research groups independently reported its ~~It's~~ complementary deoxyribonucleic acid (cDNA) sequence ~~was independently reportedin1999 by 3 research group~~ [9–11]. Cleavage of pro-heparanase yields 8- and 50-kDa subunits that heterodimerize to form the active enzyme~~and exert enzymatic activity~~ [12]. Malignant tumor cells express high levels of HPSE, ~~e.g.~~including U87 glioma [13], HT27 colon carcinoma [14], MCF-7 [15], MDA-MB-231 [16] and MDA-MB-435 [17]. Previous studies have ~~found~~ showed that HPSE can affect the ~~aggressiveness and~~ proliferation of breast cancer cells. The overexpression of HPSE is associated with ~~a~~ metastatic potential and ~~a~~ poor prognosis of the cancer cells [15,18,19]. The ~~released~~ secreted HPSE degrades HS~~,~~ –, thereby destroying the structural integrity of ECM ~~structure~~. ~~Meanwhile~~Consequently, the degradation of HS chains promotes ~~to~~ the release the growth factors, such as *FGF* and *VEGF* from ECM, which, in turn, ~~storage.The released growth factors can further~~regulate ~~regulates~~ the downstream

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signaling pathways, such as PI3K/Akt/mTOR~~–~~, RAS/RAF/MAPK, thereby ~~which stimulate~~ stimulating the proliferation of cancer cells and ~~facilitate~~ facilitating tumor metastasis and angiogenesis.

Several evidences have reported ~~support~~ the application of ~~elemene (ELE)~~ELE as an effective anti-tumor and anti-metastatic agent. However, the underlying cellular mechanisms demonstrating the ~~on~~ the anti-angiogenic and anti-metastatic effects of ELE remain to be ~~further~~ determined. ~~As is known, ELE~~Elemene is a natural plant drug extracted from *Curcuma wenyujin*.[20] Previous study has demonstrated ~~In previous study , ELE has shown~~ an extensive spectrum of anti-tumor effects of ELE, ~~such as~~ including lung cancer, breast cancer, gastric cancer, and brain ~~cancer~~tumor. The anti-tumor activities based on induction apoptosis of cancer cells are attributed to the regulation of *survivin*, *Bcl-XL*, and the activation of Ras/Raf/MEK/ERK or PI3K/Akt/mTOR pathways.[21–24] ~~And these~~ These pathways are regulated by HPSE and growth factors. In addition, ELE also has a strong potency in anti-invasion and anti-angiogenesis via suppressing the *VEGF* ~~vascular endothelial growth factor~~.[25] In light of the previous results, it is intriguing to propose that ELE may down-regulate HPSE expression and ~~then~~ decrease the release of growth factors, ~~such as~~ ~~e.g.~~*FGF* and VEGF. In this study, we evaluated the anti-tumor and anti-metastatic effects of ELE in 4T1 cells. Additionally, ~~At the meanwhile,~~ we ~~detect~~determined the expression levels of HPSE~~,~~ *FGF-2*, and *VEGF*~~,~~S in order to ~~o as to~~ further elucidate the effects and ~~the~~ molecular mechanisms of ELE in breast cancer cells.

Materials and Methods

Chemicals and ~~Reagent~~reagents

The ββ-elemene (98% purity; ~~ELE~~) with molecular formula of C15H24 and molecular weight of 204.35) was obtained from Dalian Jingang Pharmaceuticals ~~,~~Ltd (Liaoning, China). The ~~low~~ low-molecular weight heparin (LMWH) was purchased from Aventis Intercontinental (Paris~~,~~, France).

Primary antibodies against heparanase and *VEGF* were purchased from Abcam Biotechnology (Cambridge, UK). The primary antibodies against *FGF-2* and *β-actin* were purchased from Santa Cruz Biotechnology (CA, USA). The secondary antibodies including *Dylight 800-conjugated goat anti-mouse* and *Dylight 680-conjugated goat anti-rabbit IgG* were purchased from KPL (~~,~~MD, USA).

Cell ~~Culture~~culture

The murine breast cancer ~~cell~~ cell-line 4T1 (Cell Bank of the Chinese Academy of Sciences, ~~shanghai~~Shanghai, China) were cultured in RPMI-1640 (Gibco, Carlsbad, CA, USA)~~.~~, Human breast cancer ~~cell~~ cell-line MCF-7,MDA-MB-231 and MDA-MB-435S were purchased from Cell Center of Medical ~~research~~ Research ~~institute~~ Institute, ~~of~~ Chinese Academy of Medical Sciences (Beijing, China~~–~~). Cell-line MCF-7 ~~were~~ was cultured in DMEM (Gibco, USA~~,~~), whereas MDA-MB-231 and MDA-MB-435S were cultured in Leibovitz's L15 (Hyclone, Logan, UT, USA). ~~All the~~ The media ~~were~~was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were trypsinized with 0.25%

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trypin (Gibco, USA) and seeded to micro-plates for the ~~next~~ subsequent experiment. All the ~~cell~~ cell-lines were cultured at 37°C~~C~~ in a humidified incubator (SANYO, Osaka, ~~JAPAN~~Japan) supplied with 5% carbon dioxide (CO_2).

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