JOURNAL OF FOOD AND DRUG ANALYSIS XXX (2017) 1–11

Available online at www.sciencedirect.com

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Original Article

The apple polyphenol phloretin inhibits breast cancer cell migration and proliferation via inhibition of signals by type 2 glucose transporter

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ARTICLE INFO

Article history:
Received 6 January 2017
Received in revised form
23 March 2017
Accepted 29 March 2017
Available online xxx

Keywords:
Apple polyphenol
Breast cancer
Glucose transporter 2
Phloretin
Triple-negative breast cancer

ABSTRACT

Human triple-negative breast cancer (TNBC) is the most aggressive and poorly understood subclass of breast cancer. Glucose transporters (GLUTs) are required for glucose uptake in malignant cancer cells and are ideal targets for cancer therapy. To determine whether the inhibition of GLUTs could be used in TNBC cell therapy, the apple polyphenol phloretin (Ph) was used as a specific antagonist of GLUT2 protein function in human TNBC cells. Interestingly, we found that Ph (10–150 μM, for 24 h) inhibited cell growth and arrested the cell cycle in MDA-MB-231 cells in a p53 mutant-dependent manner, which was confirmed by pre-treatment of the cells with a p53-specific dominant-negative expression vector. We also found that Ph treatment (10–150 μM, for 24 h) significantly decreased the migratory activity of the MDA-MB-231 cells through the inhibition of paxillin/FAK, Src, and alpha smooth muscle actin (α-sMA) and through the activation of E-cadherin. Furthermore, the anti-tumorigenic effect of Ph (10, 50 mg/kg or DMSO twice a week for six weeks) was demonstrated *in vivo* using BALB/c nude mice bearing MDA-MB-231 tumor xenografts. A decrease in N-cadherin, vimentin and an increase in p53, p21 and E-cadherin were detected

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http://dx.doi.org/10.1016/j.jfda.2017.03.009

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Please cite this article in press as: Wu K-H, et al., The apple polyphenol phloretin inhibits breast cancer cell migration and proliferation via inhibition of signals by type 2 glucose transporter, Journal of Food and Drug Analysis (2017), http://dx.doi.org/10.1016/j.jfda.2017.03.009

in the tumor tissues. In conclusion, inhibition of GLUT2 by the apple polyphenol Ph could potentially suppress TNBC tumor cell growth and metastasis.

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1. Introduction

Glucose is a major source of energy for cancer cells. In proliferating cancer cells, the transport of glucose across the cell membrane by glucose transporters is the rate-limiting step during metabolism [1]. There are several different subtypes of the mammalian glucose transporter (GLUT1–12, 14) family, which can be identified in various human organs [2]. In this study, we focused on the type 2 glucose transporter (GLUT2), which is detected primarily in the pancreas, intestine, liver and kidney [3]. GLUT2 expression can be regulated by the extracellular glucose concentrations and insulin [4]. Metastasis is the major cause of death in many different types of cancer. Previous studies have demonstrated that the apple polyphenol phloretin (Ph) [5] and an extracted modified Fuji apple polysaccharide [6] could have significant anti-tumorigenic effects on colon cancer cells. Many previous studies have also demonstrated that apple extracts have significant anti-tumorigenic effects in breast cancer cells [7–10]. However, the mechanisms that relate the apple-derived components with potential tumor preventative or therapeutic effects remain unclear.

Ph is detected in apples or apple-derived products and is conjugated to glucosidic to form phloridzin (phloretin 2′-O-glucose) [11]. An *in vitro* study demonstrated that Ph can be produced in *Erwinia herbicola* Y46, which degrades phloridzin to yield Ph [12]. Furthermore, Ph glycosides have been detected at a high level in apple purees and in commercial juices as a consequence of the processing conditions [13]. Apples also contain other phytochemicals or polysaccharides with anti-tumorigenic effects in breast cancer cells [7]. In addition, apple components have chemopreventive activity in breast cancer [14,15]. Increased consumption of apples and their derivatives has been associated with the prevention of breast and colon cancer [14,16]. Most previous reports have focused on apple polysaccharides, which affect breast cancer cell growth or induce apoptosis [17,18]. However, many studies have reported that the phytochemicals produced by apples may function as antioxidants and have anti-proliferative effects in breast cancer cells [19,20]. In our previous studies, it was shown that Ph is a specific inhibitor of GLUT2 and that the significant anti-tumorigenic effects are due to the suppression of trans-membrane glucose transport [5,21,22]. *In vivo* studies also demonstrated that Ph suppresses the growth of xenograft tumors including bladder and liver cancer [21–23]. These findings suggest that Ph has potential anti-tumorigenic activity. However, the mechanism of the effects of Ph in human breast cancer cells is not well known.

In this study, we demonstrate that Ph can significantly inhibit TNBC cancer cell growth in an *in vivo* xenograft mouse model. Our results showed that apple polyphenols inhibit GLUT2 and can be effectively used for breast cancer chemoprevention.

2. Materials and methods

2.1. Cell lines

The human mammary gland epithelial adenocarcinoma cell line MDA-MB-231 (ATCC HTB-26) [24] and the normal human breast epithelial cell line MCF-10A (ATCC CRL-10317) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). MCF-10A cells were maintained in a MCF-10A culture media consisting of DMEM/F12 (Thermo Fisher Scientific, Passau, Germany) supplemented with 20 ng/mL epidermal growth factor, 10 g/mL insulin, 0.5 g/mL hydrocortisone, and 1× non-essential amino acids (Thermo Fisher Scientific). MDA-MB-231 cells were maintained in DMEM (Thermo Fisher Scientific). The cells were cultured according to standard protocols [25].

2.2. Cell proliferation and viability assays

Cell growth and proliferation were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and trypan blue assays [26]. This assay was repeated four times with two technical replicates each time.

2.3. Protein extraction, Western blotting analysis and antibodies

Cells treated with DMSO and Ph were harvested for immunoblotting analysis [25]. Primary antibodies were purchased from multiple sources. Antibodies against WAF1/Cip (p21, #2947), Rb (#9309), cyclin D1 (#2922), cyclin E1 (#4129), phospho-FAK (Tyr397, #3283), FAK (#3285), phospho-Src (Tyr416, #2101) and E-cadherin (#14472) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Anti-Kip1 (p27, # 610241) was purchased from BD Bioscience Pharmingen (San Diego, CA, USA). Anti-GAPDH (ab9485), anti-paxillin (ab32084), anti-alpha smooth muscle actin (α–SMA, ab21027), anti-N-cadherin (ab18203) and anti-Src (ab47405) antibodies were purchased from Abcam (Cambridge, UK). Antibodies against GLUT2 (H-67), p53 (DO-1), and PARP (F2) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

2.4. Flow cytometry analysis

The populations of cells treated with Ph or DMSO were sorted and analyzed based on cell cycle phase using flow cytometry [27]. The population of cells in each of the different phases of

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