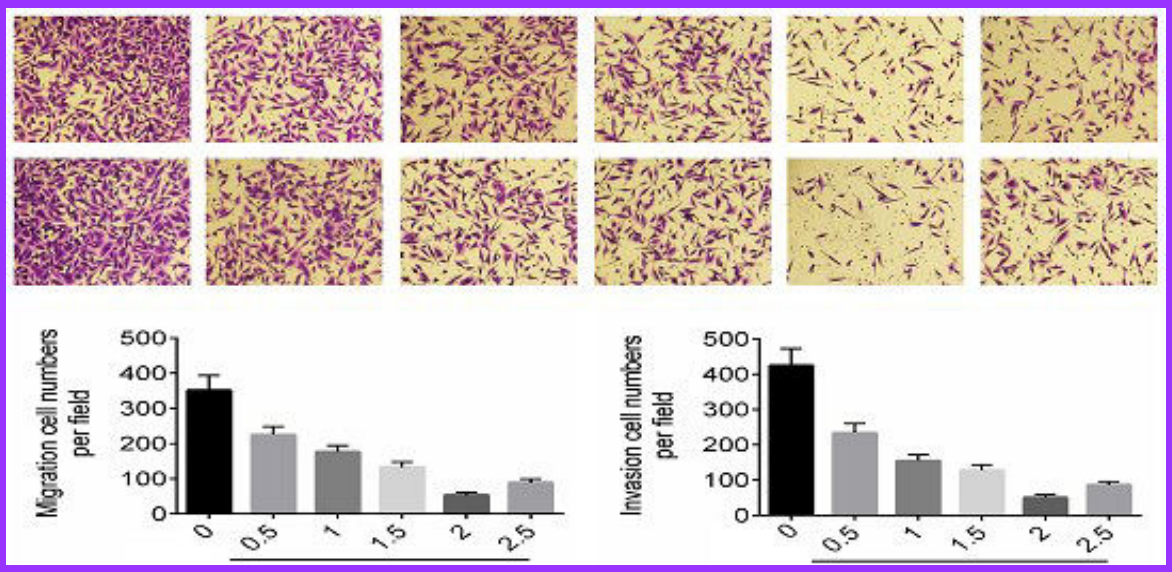

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Lycorine inhibits MDA-MB-231 breast cancer cell proliferation, migration and invasion that are associated with Wnt/β-catenin signaling

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Abstract

This study was aimed to determine the effects of lycorine, a toxic crystalline alkaloid, on MDA-MB-231 breast cancer cells proliferation, migration and invasion, and to investigate the mechanism involved. The cells were cultured with different concentrations of lycorine *in vitro*. MTT assays were performed to determine the proliferation of cells. Transwell assays were performed to measure the migration and invasion of cells. The activation of Wnt/β-catenin signaling pathway and expression were assayed by Western blot. This study showed that proliferation, migration and invasion of MDA-MB-231 breast cancer cells could be inhibited by lycorine. Furthermore, we found that Wnt/β-catenin signaling was markedly blocked in MDA-MB-321 cells treated with lycorine. In conclusion, lycorine inhibits the proliferation, migration and invasion of MDA-MB-231 breast cancer cells that is associated with the suppression of Wnt/β-catenin signaling.

Introduction

Breast cancer is the second leading cause of cancer death among women after lung cancer in the United States (DeSantis et al., 2014). In the treatment for breast cancer, some adverse effects could cause serious complications and affect the health and quality of life among survivors (Odle, 2014). Therefore, it is necessary to develop novel anti-cancer candidates for breast cancer.

In the past several decades, natural compounds such as resveratrol (Sinha et al., 2016), epigallocatechin-3-gallate, oldenlandia diffusa and *Ziziphus jujube* have been demonstrated that possessed potential anti-breast cancer activity (Bonofiglio et al., 2016).

Lycorine, known as a multifunctional compound, is extensively studied for its pharmacological activities, such as anti-tumor activity (Lamoral-Theys et al., 2010). Previous studies have shown that lycorine presented a potential anti-cancer activity in breast cancer via the inhibition of tumor growth and metastasis (Wang et al., 2017; Ying et al., 2017). Src/FAK (focal adhesion kinase) -involved pathway and STAT3 (signal transducer and activator of transcription 3) signaling pathway were involved in growth, invasion and metastasis of breast cancer.

However, Wnt/β-catenin signaling, a multifunctional pathway that has not been studied to be involved in proliferation, invasion and migration of breast cancer. This study is meant to confirm the inhibitory effects of lycorine on the proliferation, migration and invasion of MDA-MB-231 breast cancer cells and investigate the activation of Wnt/β-catenin signaling pathway involved.

Materials and Methods

Cell lines and reagents

Human MDA-MB-231 breast cancer cells were obtained

from American Type Culture Collection (ATCC). MDA-MB-231 cells were cultured in Dulbecco's modified Eagle's (DMEM) medium (ThermoFisher, USA) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher, USA) and incubated in 5% humidified CO_2 at 37°C. Primary antibodies for Western blot including β-catenin, cyclin D1 and c-Myc were purchased from Santa Cruz Biotechnology (USA). β-actin antibody, mouse and rabbit IgG-horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from the Santa Cruz Biotechnology (USA).

MTT proliferation assay

Cell proliferation was determined using 3-(4.5-methylthiozol-2yl)-2.5-diphenyltetrazolium bromide (MTT) assay (Beyotime, China) according to manufacturer's instruction. MDA-MB-231 cells (1 x 10^4 cells/well) were seeded into 96-well plate and cultured in 150 µL of DMEM medium supplemented with 10% FBS for 24 hours. At the indicated time, various concentrations of lycorine were added into the culture medium. Following incubation at 37°C for 24 hours, the culture medium was replaced with 100 µL of DMEM medium and 10 µL MTT reagent, and MDA-MB-231 cells were cultured with another 4 hours at 37°C. After plates were agitated to dissolve the dark blue crystals, the optical density was measured using an enzyme-linked immunosorbent assay plate reader (Roche Diagnostics GmbH, Germany) at 490 nm.

Transwell migration and invasion assay

Cell migration and invasion were determined using a transwell chamber (Corning incorporated, USA). MDA-MB-231 cells (1×10^5 cells/well) were added into the upper chambers with 200 µL DMEM medium. DMEM media (600 µL) containing 10 FBS was added to lower chambers. Different concentrations of lycorine were seeded in both chambers. For the invasion assay, MDA-MB-231 cells (1 x 10^6 cells/well) were placed in the upper chamber coated with 30 µL matrigel (BD, USA). After culturing for 24 hours in 5% CO_2 at 37°C, 4% paraformaldehyde was used to fix the cells in the upper chambers for H and E staining. Migrated MDA-MB-231 cells were counted in five randomly picked fields under microscope.

Western blot analysis

MDA-MB-231 cells were added into 6-well plate. Various concentrations of lycorine were then added into each well and incubated for 24 hours. Whole cell lysates were prepared in RIPA buffer containing protease inhibitor cocktail (ThermoFisher, USA). Total protein was quantified with Coomassie brilliant blue method. Equal amounts of protein were run on 8-12% SDS-PAGE gels and transferred onto nitrocellulose membranes. The membranes were blocked in 5% skim milk and stored overnight at 4°C with specific primary antibodies. The immunoblots were washed with TBST (TBS/0.1% Tween 20) and incubated with relevant IgG-horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 hour at room temperature. After blots were washed again with TBST, the chemiluminescent signals were captured using Odyssey Fluorescence Scanner (LI-COR Bioscience Inc., USA).

Statistical analysis

Quantitative results were expressed as mean ± standard deviation (SD). The statistical analysis were conducted using SPSS 19.0 statistical software (USA). The one-way analysis of variance (ANOVA) for independent analysis was applied to evaluated statistical comparison of more than two groups. The significant differences of means were determined at the level of $p<0.05$.

Results

Proliferation of MDA-MB-231 cells

Results of MTT assays showed that lycorine suppressed MB-231 breast cancer cells proliferation in a dose-dependent manner (Figure 1).

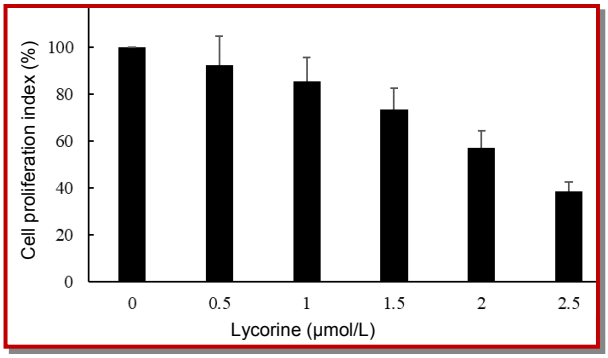

Figure 1: Lycorine suppresses proliferation of MDA-MB-231 breast cancer cells in a dose-dependent manner

Migration and invasion of MDA-MB-231 cells

Lycorine decreased the MB-231 breast cancer cells migration and invasion in a dose-dependent manner (Figure 2). The chemotactic motility and metastasis of breast cancer cells were suppressed by lycorine.

Wnt/β-catenin signaling pathway in cells

To further illuminate potential mechanism by which lycorine suppress the proliferation, migration and invasion in MDA-MB-231 cells, we investigated the effects of lycorine on the down-stream target proteins of Wnt/β-catenin signaling pathway. The protein expression levels of β-catenin, cyclin D1 and c-Myc were significantly decreased in MDA-MB-231 cells treated with different concentrations of lycorine in a dose-dependent manner (Figure 3). The data suggested that the inhibitory effects of lycorine on the proliferation, migration and invasion of MDA-MB-231 breast cancer cells is

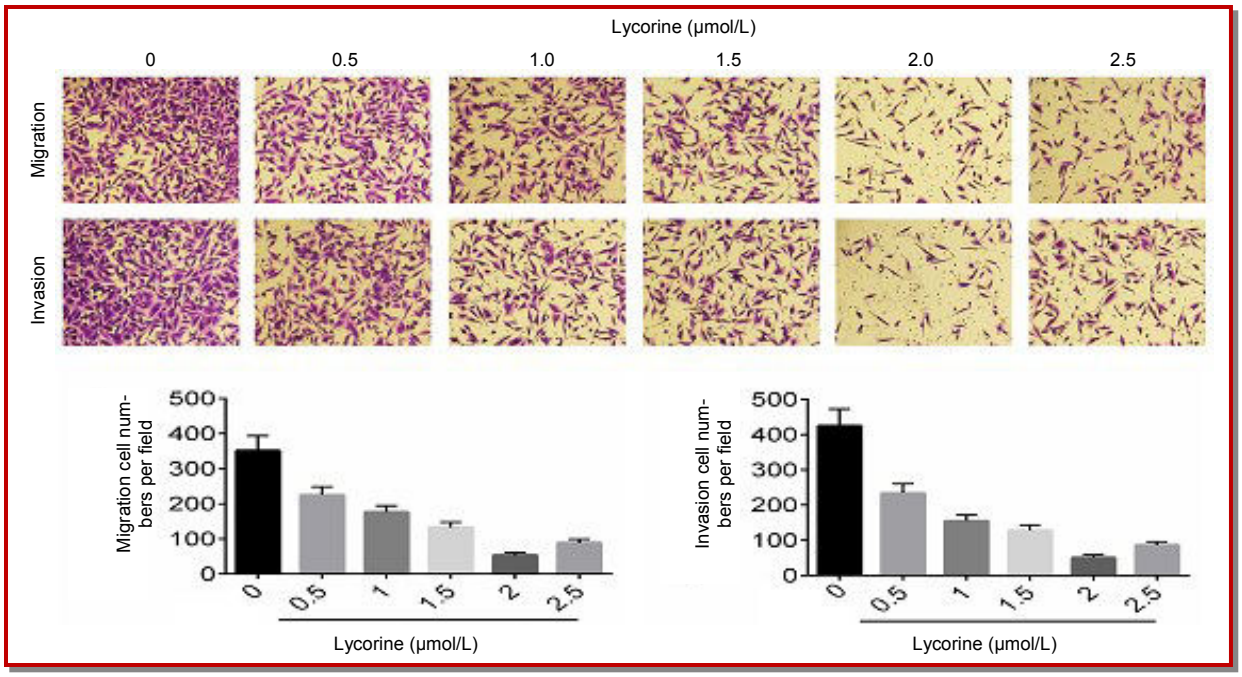

Figure 2: Lycorine suppresses migration and invasion of MDA-MB-231 breast cancer cells in a dose-dependent manner

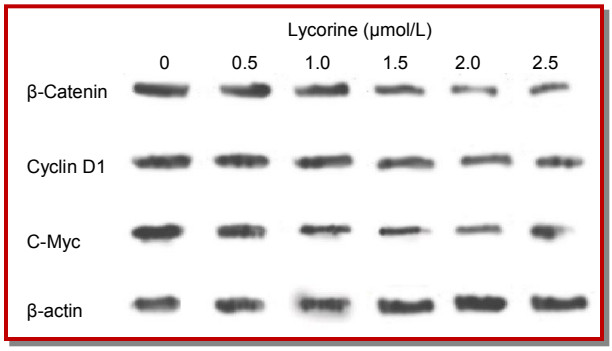

Figure 3: Lycorine blocks Wnt/β-catenin signaling pathway in MDA-MB-231 breast cancer cells in a dose-dependent manner

associated with suppression of Wnt/β-catenin signaling.

Discussion

Lycorine is a multifunctional compound isolated from the Amaryllidaceae genera. The dichloromethane extracts, three alkaloids (lycorine, vittatine and montanine) exhibited potential antiproliferation activity in MCF7 breast cancer cells (Silva et al., 2008). Furthermore, it could induce the apoptosis of MCF7 (Ji et al., 2017).

Wnt/β-catenin signaling pathway were involved in a wide range of disease in humans (Clevers and Nusse, 2012). Especially, Wnt/β-catenin signaling pathway is aberrantly activated in many carcinoma types. Moreover, Rosenbluh et al. (2014) reviewed new insights into the biology of Wnt/β-catenin signaling pathway and provided new components of Wnt/β-catenin signaling pathway (Rosenbluh et al., 2014). The Wnt receptor frizzled-7 (FZD7) and co-receptor the low-density lipoprotein receptor-related protein-6 (LRP6) expression increased significantly in triple-negative breast cancer. Moreover, the transcriptional knockdown of FZD_7 or LRP6 in triple-negative breast cancer cells represses tumor growth (Jamdade et al., 2015). Accumulating evidence demonstrated that activated Wnt/β-catenin signaling participates in the pathological process of breast cancer (King et al., 2012; Lu and Li, 2014; Ma et al., 2014).

Resveratrol is a non-flavonoid polyphenol present in grape, berry, peanut, pomegranate and soy bean. Resveratrol significantly inhibits the proliferation of breast cancer stem-like cells isolated from MCF-7 and SUM159, and decreased the percentage of breast cancer stem-like cells population, consequently reduced the size and number of mammospheres in non-adherent spherical clusters. Like lycorine, resveratrol suppresses the Wnt/β-catenin signaling pathway (Fu et al., 2014).

Epicatechin-3-gallate is flavonoid present in green tea. Anti-breast cancer effect of green tea may be due to inhibition of vascular endothelial growth factor (VEGF) expression and tumor angiogenesis via inhibiting hypoxia-inducible factor 1α and nuclear factor κB activation (Li et al., 2014).

The extract of *Oldenlandia diffusa* showed antiproliferative and apoptotic effects on human breast cancer cells through ERα/Sp1-mediated p53 activation (Gu et al., 2012).

Ziziphus jujube shows anti-proliferative and apoptotic effects in breast MCF-7 cancer cells which is associated with enhanced expression of Bax and decreased Bcl2 gene leading to time-dependent increase in the Bax/Bcl -2 ratio (Abedini et al., 2016).

The cytotoxic effect of *Orthosiphon stamineus* extract in MCF-7 cells is due to suppression of nucleolin and Bcl2 (Saravanan et al., 2017).

Conclusion

Lycorine inhibits the proliferation, migration and invasion of MDA-MB-231 cells. These effects are associated with the suppression of Wnt/β-catenin signaling.

Conflict of Interest

The author declared no conflict of interest.

Acknowledgement

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