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Differential proteomic analysis on the effects of 2-methoxy-1,4-naphthoquinone towards MDA-MB-231 cell line

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ABSTRACT

Background: We have previously reported the anti-metastatic effects of 2-methoxy-1,4-naphthoquinone (MNQ) against MDA-MB-231 cell line.
Purpose: To investigate the molecular mechanism underlying the anti-metastatic effects of MNQ towards MDA-MB-231 cell line via the comparative proteomic approach.
Study design/methods: Differentially expressed proteins in MNQ-treated MDA-MB-231 cells were identified by using two-dimensional gel electrophoresis coupled with tandem mass spectrometry. Proteins and signalling pathways associated with the identified MNQ-altered proteins were studied by using Western blotting.
Results: Significant modulation of MDA-MB-231 cell proteome was observed upon treatment with MNQ in which the expressions of 19 proteins were found to be downregulated whereas another eight were upregulated (>1.5 fold, $p < 0.05$). The altered proteins were mainly related to cytoskeletal functions and regulations, mRNA processing, protein modifications and oxidative stress response. Notably, two of the downregulated proteins, protein S100-A4 (S100A4) and laminin-binding protein (RPSA) are known to play key roles in driving metastasis and were verified using Western blotting. Further investigation using Western blotting also revealed that MNQ decreased the activations of pro-metastatic ERK1/2 and NF-κB signalling pathways. Moreover, MNQ was shown to stimulate the expression of the metastatic suppressor, E-cadherin.
Conclusion: This study reports a proposed mechanism by which MNQ exerts its anti-metastatic effects against MDA-MB-231 cell line. The findings from this study offer new insights on the potential of MNQ to be developed as a novel anti-metastatic agent.

Introduction

Metastasis accounts for more than 90% of cancer-related deaths globally (Sethi and Kang 2011). Metastatic disease is characterised by the acquisition of invasive behaviour by cancer cells in order to migrate to distant organ sites via the vasculature and to subsequently develop into secondary tumours (Sethi and Kang 2011). Thus far, the lack of progress in preventing metastasis can be attributed to the scarcity of anticancer drugs that can successfully combat metastatic progression (Weber 2013). Plant natural products have and will continue to be an important source of novel and effective anticancer drugs. Numerous phytochemicals have shown promising anti-metastatic activities in recent years as they are increasingly reported to modulate proteins and intracellular signalling pathways that govern metastasis (Shu et al. 2010). 1,4-Naphthoquinone derivatives such as plumbagin and shikonin are one such group of phytochemicals that have strong anticancer and anti-metastatic activities (Lu et al. 2013).

2-Methoxy-1,4-naphthoquinone (MNQ) (Fig. 1) is a naturally occurring phytochemical in the plant *Impatiens balsamina* L. Previous studies on the anticancer activities of this 1,4-naphthoquinone derivative are mostly focused on its cytotoxic properties via the

Abbreviations: 2DGE, two-dimensional gel electrophoresis; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; AP-1, activator protein-1; DTT, dithiothreitol; DMSO, dimethyl sulphoxide; EMT, epithelial-to-mesenchymal transition; ERK1/2, extracellular signal-regulated kinase 1/2; FAK, focal adhesion kinase; FBS, fetal bovine serum; IPG, immobilised pH gradient; MALDI-TOF/TOF-MS, matrix-assisted laser desorption ionisation tandem time-of-flight mass spectrometry; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MNQ, 2-methoxy-1,4-naphthoquinone; NF-κB, nuclear factor-κB; NMIIA, nonmuscle myosin-IIA; ROS, reactive oxygen species; RPSA, laminin-binding protein; S100A4, protein S100-A4; uPa, urokinase plasminogen activator.

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