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Application of metabolomics to investigate the antitumor mechanism of flavopiridol in MCF-7 breast cancer cells

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ABSTRACT

Flavopiridol is reported to have potent antitumor effects by inhibition of cyclin-dependent kinases (CDKs). However, most studies of flavopiridol focus on specific genes and kinases, so the antitumor mechanism needs further elucidation at the metabolic level. In the present study, an UPLC/Q-TOF MS metabolomics approach was used to investigate its antiproliferative effects on MCF-7 breast cancer cells. Comparing flavopiridol-treated MCF-7 cells with vehicle control, 21 potential biomarkers involved in five metabolism pathways were identified. Two pathways involving glutathione metabolism and glycerophospholipid metabolism showed that glutathione (GSH) and phosphatidylcholines (PCs) levels were reduced while their oxidized products oxidized glutathione (GSSG) and lysophosphatidylcholines (LysoPCs) were greatly increased. Further investigation showed an apparent accumulation of reactive oxygen species (ROS) and a decrease in mitochondrial membrane potential (MMP). Thus, we suggest that oxidative stress was provoked in MCF-7 cells to reduce the GSH and PCs levels and cause mitochondria lesions. Moreover, cell cycle analysis showed that flavopiridol blocked cells at G1 stage, which was consistent with the depletion of spermidine and spermine that are believed to promote cancer progression. Taking these together, we concluded that flavopiridol could induce oxidative stress and cell cycle arrest, which finally lead to cell apoptosis in MCF-7 cells. This study provides a new strategy for studying the antitumor mechanism of flavopiridol, which could be used for its further improvement and application.

1. Introduction

Flavonoid derivatives are found as effective components in some plants and spices to exert biological effects including anti-inflammatory, anti-oxidant and anti-cancer activities [1,2]. Some dietary flavonoids have favourable antitumor activities based on properties including kinase activity inhibition, apoptosis induction and suppression of signal transduction pathways [3,4]. Especially, flavopiridol, a synthetic antitumor flavone derivative, is reported to significantly block cell cycle progression at either G1 or G2 stage through inhibition of CDKs (Fig. 1A) [5]. However, previous studies have shown that flavopiridol could not only induce cell cycle arrest in proliferative cells but also cause toxicity in resting cells, and most of the studies concerning its antitumor mechanism were only focused on some specific genes and kinases [6]. Therefore, a comprehensive study of the metabolic alterations after flavopiridol treatment will certainly help reveal the underlying mechanism of drug-cell interactions.

Metabolomics is a quantitative approach to provide a global profile of small-molecule metabolites. It could elucidate the metabolic changes in biological samples subjected to pathophysiological stimuli or genetic modification [7]. Therefore, it has been widely accepted as a powerful tool for disease diagnosis, drug evaluation involving its efficacy and toxicology, biomarker identification and food safety investigation [8]. Currently, there are several analytical approaches and methodologies for metabolomics analysis, which include the liquid chromatography with nuclear magnetic resonance spectroscopy (LC NMR), FT-IR spectroscopy, direct infusion to mass spectrometer (DI-MS) and liquid chromatography-mass

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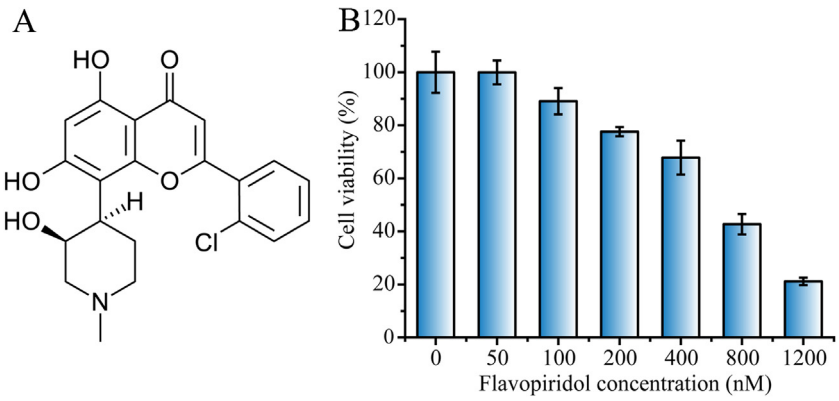

Fig. 1. (A) Chemical structure of flavopiridol; (B) The cytotoxicity of flavopiridol on MCF-7 cells at different concentrations after 48 h treatment (n = 6).

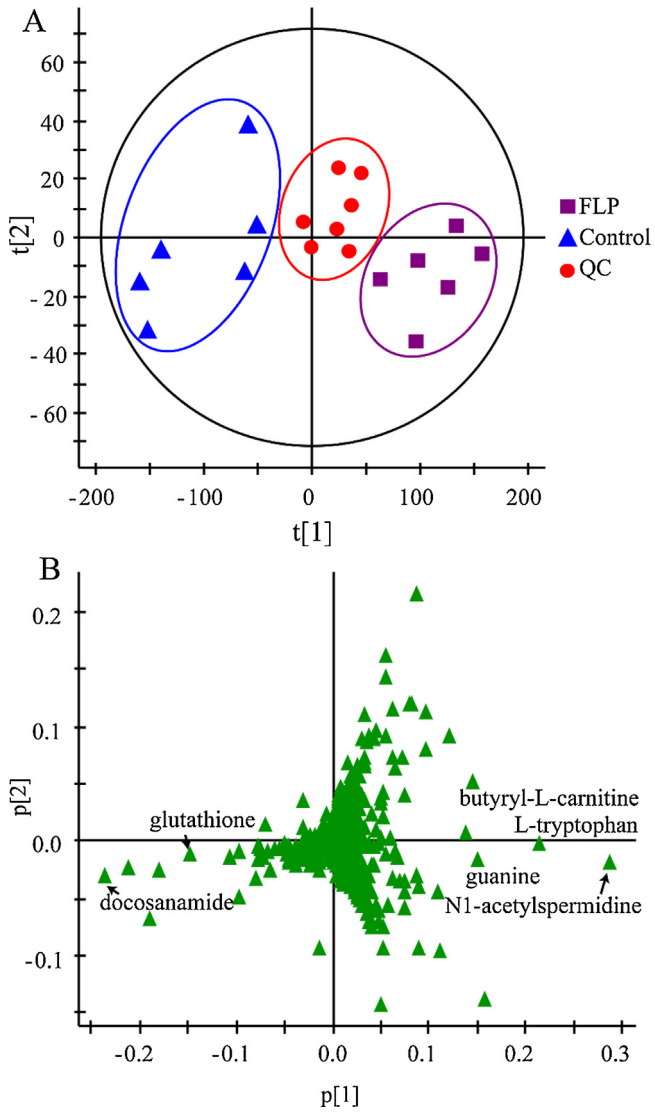

Fig. 2. Statistical analysis of extracted metabolites in MCF-7 cells with or without flavopiridol treatment in positive ion mode. (A) PCA scores plot with solvent control (Control, ▲), quality control (QC, ●) and 400 nM flavopiridol treated group (FLP, ■) included (n = 6). (B) PCA loading plot, including 6 distinct metabolites found in positive mode.

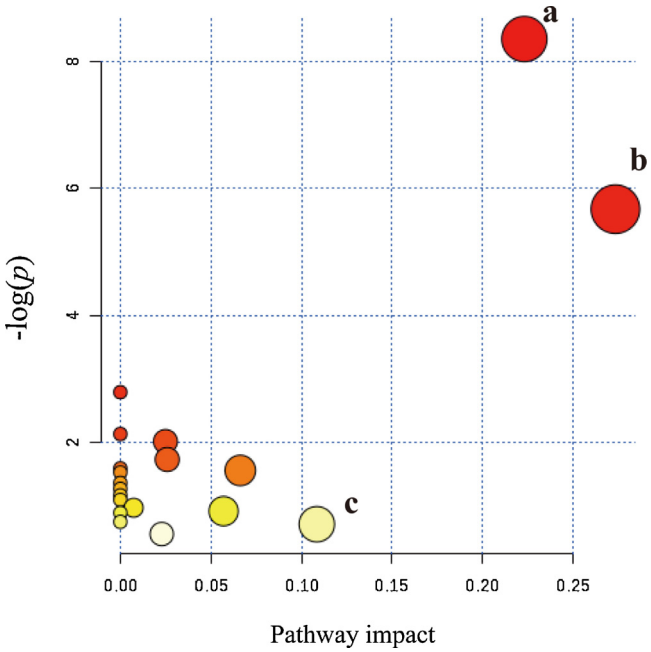

Fig. 3. The summary of metabolic pathways analyzed by MetPA. (a) Glycerophospholipid metabolism; (b) glutathione metabolism; (c) tryptophan metabolism.

spectrometer (LC–MS) [9,10]. In comparison with conventional platforms like LC NMR, UPLC–MS possesses several advantages such as better chromatographic peak resolution, shorter analysis time and higher sensitivity, thus it is more appropriate for metabolomics studies [11,12].

In previous studies, flavopiridol was found to possess potent antitumor activity against acute myeloid leukemia, chronic lymphocytic leukemia and metastatic breast cancer [13–15]. The underlying mechanism has been studied in many *in vitro* experiments with regard to its inhibition of intracellular CDKs and the interaction with intercellular vital signal transducers [16,17]. However, most of the experiments still rely on conventional molecular biological technologies to study specific genes and kinases, and investigation of the metabolic changes is still lacking [18]. The metabolomics information plays a vital role in supporting a thorough understanding of the mechanism of flavopiridol. In this work, a metabolomics study of MCF-7 breast cancer cells treated with flavopiridol was carried out. And the metabolic data were acquired by an ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometer (UPLC/Q-TOF MS). Then the data were analyzed to find out potential biomarkers and related metabolic pathways. Furthermore, the involved