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Chrysin induces G1 phase cell cycle arrest in C6 glioma cells through inducing p21$^{Waf1/Cip1}$ expression: Involvement of p38 mitogen-activated protein kinase

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Abstract

Flavonoids are a broadly distributed class of plant pigments, universally present in plants. They are strong anti-oxidants that can inhibit carcinogenesis in rodents. Chrysin (5,7-dihydroxyflavone) is a natural and biologically active compound extracted from many plants, honey, and propolis. It possesses potent anti-inflammatory, anti-oxidant properties, promotes cell death, and perturbing cell cycle progression. However, the mechanism by which chrysin inhibits cancer cell growth remains poorly understood. Therefore, we developed an interest in the relationship between MAPK signaling pathways and cell growth inhibition after chrysin treatment in rat C6 glioma cells. Cell viability assay and flow cytometric analysis suggested that chrysin exhibited a dose-dependent and time-dependent ability to block rat C6 glioma cell line cell cycle progression at the G1 phase. Western blotting analysis showed that the levels of Rb phosphorylation in C6 glioma cells exposed to 30 μM chrysin for 24 h decreased significantly. We demonstrated the expression of cyclin-dependent kinase inhibitor, p21$^{Waf1/Cip1}$, to be significantly increased, but the p53 protein level did not change in chrysin-treated cells. Both cyclin-dependent kinase 2 (CDK2) and 4 (CDK4) kinase activities were reduced by chrysin in a dose-dependent manner. Furthermore, chrysin also inhibited proteasome activity. We further showed that chrysin induced p38-MAPK activation, and using a specific p38-MAPK inhibitor, SB203580, attenuated chrysin-induced p21$^{Waf1/Cip1}$ expression. These results suggest that chrysin exerts its growth-inhibitory effects either through activating p38-MAPK leading to the accumulation of p21$^{Waf1/Cip1}$ protein or mediating the inhibition of proteasome activity.

Keywords: Chrysin; Cell cycle; p21$^{Waf1/Cip1}$; p38-MAPK; Proteasome activity

1. Introduction

Flavonoids are dietary polyphenolic compounds present in many fruits, vegetables, and beverages [1]. Many experiments have strongly demonstrated flavonoids to be preventive in coronary heart disease [2], stroke [3] and cancers [4]. The most common nonmutagenic flavonoid, apigenin (4′,5,7-trihydroxyflavone), has shown remarkable effects in inhibiting cancer cell growth both in cell culture systems and in vivo tumor models [5,6]. Apigenin also possesses anti-inflammatory and free radical scavenging properties [7,8] and inhibits tumor cell invasion, metastasis [9], and mitogen-activated protein kinases (MAPKs) and downstream oncogenes [10]. These findings suggest that flavonoids possess strong cancer-preventative effects. Chrysin (5,7-dihydroxyflavone) (Fig. 1), an apigenin analog, present at high levels in honey and propolis has been shown recently to be a potent inhibitor of the enzyme aromatase [11], of human immunodeficiency virus activation in models of latent infection [12], and of modulation of $GABA_A$ and $GABA_C$ receptors through binding to the benzodiazepine site located on the $GABA_A$ receptor [13]. Chrysin also has anti-inflammatory [14], and anti-oxidant [15] effects, and it has been found to possess cancer chemopreventive activity through inhibiting malignant cell growth by down-regulated expression of PCNA in HeLa cells [16], and induced apoptosis through caspase activation and Akt inactivation in U937 leukemia cells [17], and induced cell cycle arrest in human colon carcinoma cells [18]. The molecular mechanisms of induced cell cycle arrest, however, remained to be elucidated.

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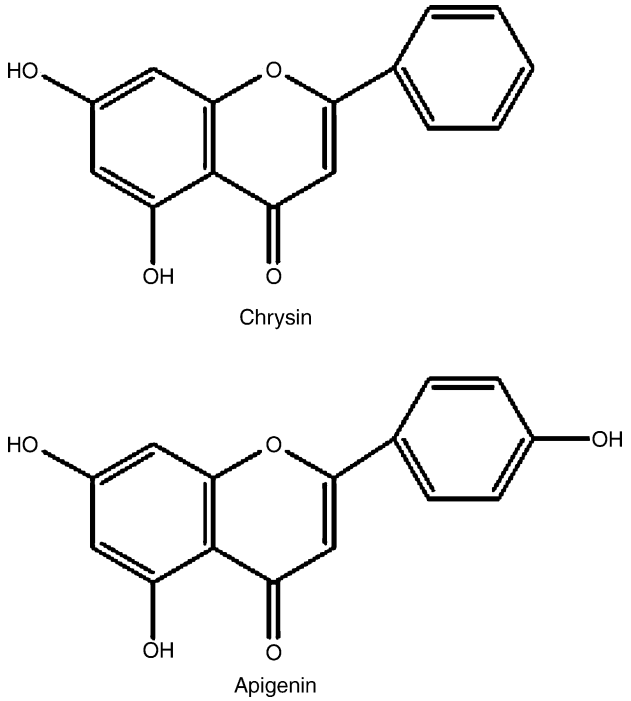

Fig. 1. Chemical structure of the flavonoid chrysin and apigenin.

Cancer cells that grow uncontrollably, such as brain glioma, are largely resistant to chemotherapy. One of the strategies of cancer management is to inhibit cell proliferation [19]. The eukaryotic cell cycle is regulated by activating or deactivating cyclin/cyclin-dependent kinase (CDK) through coordinating internal and external signals at several key checkpoints [20]. CDK activation requires cyclin binding and phosphorylation of conserved threonine residue by CDK-activating kinase (CAK), which leads to the phosphorylation of the Rb protein [21]. This phosphorylation releases a number of factors, including the E2F family of transcription factors. E2F has been shown to activate the transcription of several genes, the products of which are important for entering the S phase and for DNA replication [22]. Otherwise, CDK2, associated with either D- or E-type cyclins, and CDK4 and CDK6, associated with D-type cyclins, regulate G1 progression [23]. The activated CDK/cyclin complexes can be changed to an inactive state by phosphorylation of a conserved threonine–tyrosine pair or binding to CDK inhibitory subunits (CKIs) [24]. The CKIs fall into two classes: (1) p21 (Cip1/Waf1/Cap20/Sdi1/Pic1), p27 (Kip1), and p57 (Kip2), related proteins with a preference for Cdk2 and Cdk4/cyclin complexes; and (2) $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$, closely related CKIs specific for CDK4 and CDK6/cyclin complexes [25]. Most in vivo study suggests the $p21^{Waf1/Cip1}$ is a potent inhibitor protein of CDK/cyclin complexes, which play an important role in G1 and G2 arrest by inhibiting CDK2/cyclin E and CDC2/cyclin B activities, respectively. The expression of $p21^{Waf1/Cip1}$ appears to be regulated by both transcriptional and post-transcriptional mechanisms [26], and by the ubiquitin-proteasome system [27]. At the transcriptional level, $p21^{Waf1/Cip1}$ is induced either dependently or independently by p53 tumor suppressor protein and by the presence of DNA damaging agents [28–30]. Post-transcriptional regulation of $p21^{Waf1/Cip1}$ has been demonstrated during hematopoietic differentiation [31] or TNF-α treatment [32].

The mitogen-activated protein kinase family of serine/threonine protein kinases are involved in a wide range of cellular functions [33]. Upon stimulation, the MAPKs phosphorylate their specific substrates at serine and/or threonine residues. Thus, the MAPK signaling pathways modulate gene expression, mitosis, proliferation, and programmed cell death [34,35]. Three subfamilies of MAPKs have been identified: extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38-MAPKs. The ERK pathway is primarily activated by growth factors and the regulation of Ras-regulated Raf-MEK-MAPK/ERK protein kinase cascade has been linked to cell proliferation, cell growth, and differentiation [36–39]. JNKs are ubiquitously expressed and control a spectrum of cellular processes, including cell growth, differentiation, transformation, or apoptosis [34,39]. Like JNK pathways, p38-MAPK signaling pathways are involved in a variety of cellular responses, including survival enhancement, cell growth, inflammation, and differentiation [40–43]. However, controversial evidence has indicated that more complex roles of these pathways exist to transmit more ultimately distinct cellular effect in different cell lineages. For example, the persistent activation of ERK mediates growth arrest or differentiation signals in muscle cells and leukemia cells [44–46]. In contrast, transient p38 and JNK induction could provide a survival signal, whereas persistent activation induces apoptosis [47,48].

Recent study has demonstrated that chrysin can induce malignant cell apoptosis and cell cycle arrest [17,18]. The mechanism of chrysin-induced apoptosis was through caspase activation and Akt inactivation [17], but the mechanism by which chrysin-induced cell cycle arrest in malignant cells remains poorly understood. Here, we developed an interest in the relationship between MAPK signaling pathways and cell growth inhibition after chrysin treatment. We have found that chrysin induced a dose- and time-dependent G1 cell cycle arrest in C6 glioma cells. Our data demonstrated that chrysin enhanced the protein level of $p21^{Waf1/Cip1}$ and concomitantly inhibited CDK4 and CDK2-mediated phosphorylation of Rb. Besides, p38-MAPK phosphorylation and the ubiquitin-proteasome system are also down-regulated in chrysin-treated cells, which may have resulted in the accumulation of $p21^{Waf1/Cip1}$ protein.

2. Materials and methods

2.1. Cell lines and reagents

The rat C6 glioma cell line was obtained from the American Type Culture Collection (Manassas, VA) and