

## Fermented *Viola mandshurica* inhibits melanogenesis in B16 melanoma cells

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### Objectives

To elucidate the molecular mechanism involved in inhibition of melanogenesis by fermented *Viola mandshurica* extract, we prepared the chloroform extract of fermented *Viola mandshurica* (CEFV) and treated in  $\alpha$ -MSH-induced B16 melanoma cells. In general, ERK signaling pathway is intimately involved in regulation of melanogenesis and activation of ERK pathway induces MITF degradation, which subsequently decreases melanin synthesis. In addition, it has been reported that CREB phosphorylation by cAMP pathway induces MITF expression, and subsequently up-regulates the tyrosinase expression leading to the increased melanin synthesis. the MITF expression is induced through phosphorylation of CREB in  $\alpha$ -MSH-treated B16 cells. Thus we investigate to determine whether CEFV affects MITF expression and ERK activation or CREB phosphorylation in  $\alpha$ -MSH-stimulated B16 melanoma cells.

### Materials and Methods

#### ○ Materials

- Chloroform extract from fermented *Viola patrinii* (CEFV)
- Cell lines : B16F10 murine melanoma cells
- $\alpha$ -Melanocyte stimulating hormone ( $\alpha$ -MSH) : Sigma

#### ○ Methods

- MTT assay, Zymography
- Melanin content and tyrosinase assay
- DOPA staining in the B16 melanoma cells
- Real-time RT-PCR and Western blot analysis

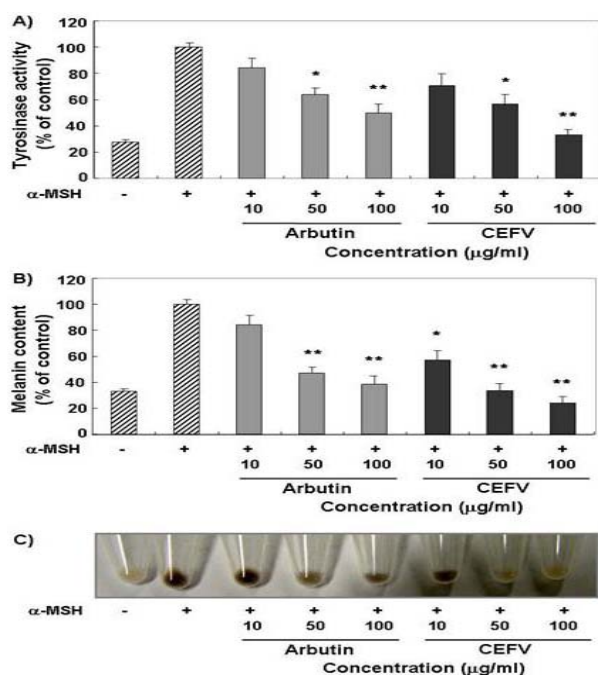
### Results and Discussion

Tyrosinase activity and melanin content were significantly decreased by treatment of CEFV in a dose-dependent manner without cytotoxicity. In the RT-PCR and Western blot analysis experiments, CEFV significantly inhibited both mRNA and protein expressions of tyrosinase and MITF, but had no significant effect on TRP-1 and TRP-2 expressions. In signaling pathway experiment, CEFV significantly

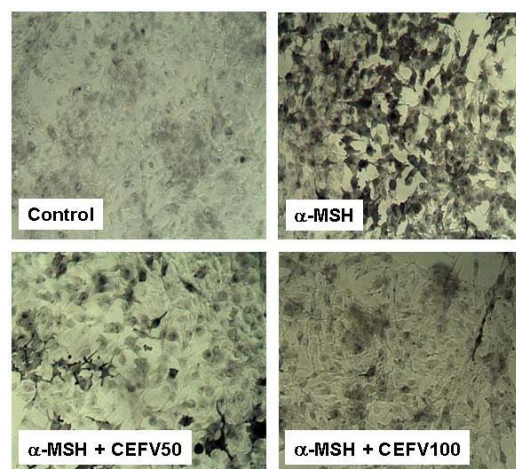
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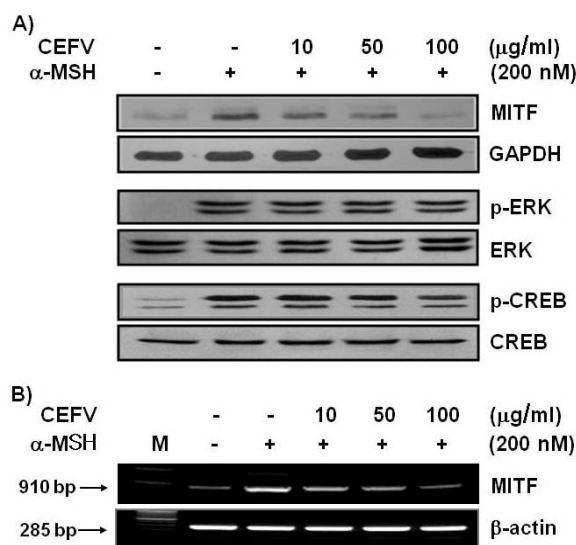
decreased phosphorylation of CREB and induced the duration of ERK activation, leading to the reduction of MITF expression and subsequently tyrosinase. Therefore, we suggest that CEFV induces the down-regulation of melanogenesis through decreased CREB phosphorylation and ERK activation, leading to reduction of MITF expression and consequently decreased tyrosinase expression and melanin production in  $\alpha$ -MSH-stimulated B16 melanoma cells.



**Fig. 1.** Inhibitory effect of CEFV on melanogenesis.



**Fig. 2.** Observation of intracellular tyrosinase activity by DOPA staining.



**Fig. 3.** Effects of CEFV on melanogenesis-related signal targeting genes in  $\alpha$ -MSH-induced B16 melanoma cells.