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ORIGINAL PAPER

# Immunomodulatory effects of phytocompounds characterized by in vivo transgenic human GM-CSF promoter activity in skin tissues

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**Abstract** To investigate the immunomodulatory activities of phytocompounds for potential therapeutics, we devised an in vivo, transgenic, human cytokine gene promoter assay using defined epidermal skin cells as test tissue. Test compounds were topically applied to mouse skin before or after gene gun transfection, using a cytokine gene promoter-driven luciferase reporter. Croton oil, an inflammation inducer, induced transgenic GM-CSF and TNF-$\alpha$ promoter activities in skin epidermis 6-fold and 3.4-fold, respectively; however, it produced a less than 1.5-fold and 1.7-fold change in IL-1$\beta$ and IL-18 promoter activity, respectively. The phytocompound shikonin drastically inhibited inducible GM-CSF promoter activity. However, a fraction of *Dioscorea batatas* extract significantly increased the GM-CSF promoter activity in normal and inflamed skin. Shikonin suppressed the transcriptional activity of GM-CSF promoter by inhibiting the binding of TFIID protein complex (TBP) to TATA box. Our results demonstrate that this in vivo transgenic promoter activity assay system is cytokine gene-specific, and highly responsive to pro-inflammatory or anti-inflammatory stimuli. Currently it is difficult to profile the expression and cross-talk of various types of cytokines in vivo. This investigation has established a bona fide in vivo, in situ, immune tissue system for research into cytokine response to inflammation.

**Keywords** *Dioscorea batatas* · Immune-modulating activities · In vivo assay · Human granulocyte macrophage-colony stimulating factor · Phytocompounds · Shikonin

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## Introduction

Cytokines, the key regulators of the immune system, have been studied intensively as a class of powerful immunomodulators for clinical applications. However, the adverse side effects, high cost, and labile features of cytokine proteins often prohibit their routine use. Efforts are therefore underway to develop alternative immunomodulators as therapies. One source of alternative immunomodulators may be traditional herbs or their derived phytocompounds reputed to confer medicinal efficacy [1]. The historically successful development of medicinal compounds from plants, such as aspirin and taxol, has led to research into the potential of a large range of herbs and their derived phytocompounds as a source of immunomodulators [2, 3].

In drug discovery research, in vitro cell-based screening systems are well established as methods for evaluation of candidate lead compounds. For example, in vitro assays of NF-$\kappa$B [4, 5] and COX-2 [6], two examples of drug targets, are employed to develop therapeutic strategies to counter inflammation. However, it is also known that the regulation of immune-modifiers and their gene expression is highly dependent upon three dimensional microenvironments. Therefore, an in vivo assay that can accurately evaluate the effects of immune modulators/drugs on the expression