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## ACCEPTED MANUSCRIPT

## Transient Expression of CCL21as recombinant protein in tomato

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Highlight

- Use plants to produce recombinant protein and oral vaccine
- The present study is the first to report gene expression of *ccl21* construct in tomato via agro infiltration to use this plant as oral vaccine
- To investigate the expression of *ccl21* in tomato leaves via agroinfiltration
- To investigate the effect of CCl21 that expressed in tomato leaves on the proliferation of human cancer cell line and also their migration capacity in a cultured monolayer, serving as in vitro wound model.

#### Abstract

The main goal of this study was to investigate the possibility of expressing recombinant protein of C-C chemokine ligand 21 (CCL21) in *Solanum lycopersicum* via agroinfiltration. CCL21 is a chemokine can be used for anti-metastatic of cancer cell lines. To examine the expression of CCL21 protein in *S. lycopersicum*, the construct of *ccl21* was synthesized. This construct was cloned into pBI121 and the resulting CCL21 plasmid was agro-infiltrated into *S. lycopersicum* leaves. Within three days after infiltration, Expression of the foreign gene was confirmed by quantitative Real-time PCR. A recombinant CCL21 protein was immunogenically detected by western blot, dot blot and ELISA assay. And results showed that the foreign gene was expressed in the transformed leaves in high level. Also scratch assay was used to investigate the role of this protein in anti-metastatic function. The results demonstrated anti-metastatic of cancer cells in the presence of this protein.

keywords: Recombinant vaccine; Agroinfiltration; Tomato; Transient gene expression; Migration; scratch assay.

#### 1. Introduction

Chemokines and their receptors play essential roles in leukocyte recruitment, tumor cell growth and metastasis (Cheng, Guo, Yang, & Yang, 2015; Zhao et al., 2014). Among them, C-C chemokine ligand 21, Secondary lymphoid tissue chemokine (CCL21), exerts antitumor immunity by co-localizing dendritic cells and T cells at the tumor sites (Sohi, Jourabchi, & Khodabandeh, 2005a; Zhao et al., 2014). Application of plants as bioreactors for production of recombinant proteins has emerged as a molecular farming over the past two decades. (Sohi et al., 2005a). Many strategies have been proposed for the enhancement of recombinant protein expression including; use of strong promoters, chloroplast transformation (Sohi et al., 2005a), signal peptide codon optimization and untranslated leader sequences (Singer & Clark, 1999). The Long time required for the generation of transformed plants expressing foreign antigens is another limitation for the production of recombinant proteins (Simmons, VanderGheynst, & Upadhyaya, 2009). Transient gene expression methods are appropriate alternatives to stable transformation because they allow an inexpensive and rapid method for

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