

Chinese herbal medicine *Fructus Ligustri Lucidi* (FLL) promotes cell differentiation in C2C12 myoblasts via increasing vitamin D sensitivity

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Abstract:

Introduction: Aging-related sarcopenia is characterized by the progressive loss of skeletal muscle mass and strength and/or low physical performance (1). Currently, the pharmaceutical remedies for treatment of sarcopenia are unavailable. The relevance of vitamin D to skeletal muscle function has been highlighted, but the benefit of vitamin D supplementation on muscle is also being questioned due to the controversial results of clinical trials (2). Thus, there is a strong demand to develop alternative approach to improve vitamin D status for management of sarcopenia. *Fructus Ligustri Lucidi* (FLL) is a commonly used kidney-tonifying herb that may have anabolic actions in skeletal muscle. In the present study, we hypothesize that FLL promotes cell differentiation in C2C12 myoblasts via modulating vitamin D sensitivity.

Materials and Methods: The C2C12 mouse myoblast cell line (ATCC#: CRL-1772™) was applied for investigating the *in vitro* effects of FLL extract (5, 10 or 20 µg/ml) on cell differentiation. IGF-1 (100 ng/ml) and 1,25(OH)₂D₃ (10 nM) are positive controls. The expression of muscle regulatory factors (MRFs) - myosin heavy chain (MHC), myoblast determination protein 1 (MyoD) and myogenin in differentiating C2C12 myoblasts were determined by using immunocytochemistry and western blotting. The protein expression of vitamin D receptor (VDR) and CYP27B1 were also determined.

Results: FLL could exert stimulatory effects on myogenesis in which FLL (5 and 10 µg/ml) significantly increased the number, diameter and area of myotubes ($p < 0.001$) in differentiating C2C12 myoblasts. FLL was found to significantly upregulate the protein expressions of MRFs as well as the expression of VDR and CYP27B1 in differentiating C2C12 myoblasts ($p < 0.05$).

Conclusions: These results suggest that the stimulatory effects of FLL on cell differentiation in C2C12 myoblasts might be mediated by an improvement of vitamin D sensitivity. This study provides the evidence for supporting the use of FLL for the management of sarcopenia.

References:

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2. Remelli, F., *et al. Nutrients*, 2019. 11(12). doi:10.3390/nu11122861.

Acknowledgement:

This work was supported by the Health and Medical Research Fund of Health Bureau (HKSAR; 19200411), Start-up Fund for RAPs under the Strategic Hiring Scheme (1-BD4V) and Dean's Reserve internal fund (1-ZVXG) from The Hong Kong Polytechnic University. We also thank the University Research Facility in Life Sciences at the Hong Kong Polytechnic University for their technical support.