中文題目：桑葉提取物和 5-咖啡酰奎寧酸可抑制模擬糖尿病培養的血管平滑肌細胞 Ras 和 FAK 相關之遷移和增殖

英文題目：Mulberry leaf extract and 5-Caffeoylquinic acid inhibit Ras and FAK related migration and proliferation in mimic diabetic cultured vascular smooth muscle cells

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Background: Mulberry leaf (*Morus alba L.*) has been used as a health food and in traditional medicine to treat several metabolic diseases, including diabetes, hypertension and hyperlipidemia. However, the mechanism by which mulberry leaf and its functional components mediate atherosclerosis remains unclear. This study aimed to evaluate the effect of mulberry leaf extract (MLE) and its major component, 5-Caffeoylquinic acid (nCGA), on the migration and proliferation of rat aortic vascular smooth muscle cells (VSMCs, A7r5 cell line) under diabetic conditions.

Materials: Fresh mulberry leaves were immediately dried and stored at room temperature. For MLE preparation, 100 g of mulberry leaves in dried powder form was mixed with 300 mL methanol and heated at 95°C for 3 h. The extract was filtered and concentrated through evaporation under reduced pressure at room temperature. The extract was then resuspended with 500 mL distilled water and extracted with ethyl acetate. Then, the ethyl acetate extract was concentrated through evaporation and lyophilized. The MLE was filtered using a 0.22 μm filter for further use in cell culture.

Method: A7r5 cells were seeded in 12-well plates at a density of 2×10^5 cells per well. After incubation overnight with medium, the cells were treated with various concentrations of MLE and nCGA for 24 h. The cell lysates were centrifuged at 10,000 rpm for 3-5 min and the supernatant was collected. The supernatant was thoroughly mixed with 1ml PBS. A 100 mL sample of each cell suspension with an equal amount of 100 mL trypan blue (Invitrogen) was assessed under a microscope to evaluate the live (unstained) and dead (blue) cells using counting chambers (Paul Marienfeld GmbH & Co. KG).

Result: MLE and nCGA significantly inhibited proliferation and cell migration in A7r5 cells as determined by a scratch wound assay and Transwell assay. Protein expression was measured by Western blot analysis and it was revealed that MLE and nCGA inhibited the phosphorylation of focal adhesion kinase (FAK) and phosphoinositide 3-kinases (PI3K)/protein kinase B (Akt), Ras signals, and small GTPase proteins, to attenuate cell migration and proliferation.

Conclusion: we confirmed the anti-atherosclerotic effects of MLE and nCGA in reducing VSMC migration and proliferation under high glucose and oleic acid (OH) cultured conditions, via inhibition of FAK/small GTPase proteins, PI3K/Akt, and Ras-related signaling.