

Analysis of the mechanism of action of constituents from licorice root using monoclonal antibody against natural compounds

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Licorice (*Glycyrrhiza* spp.) is the most important ingredient for the Japanese Kampo medicine and the traditional Chinese medicine, and has been reported to show various pharmaceutical functions. Liquiritin (LQ) and its aglycon liquiritigenin (LQG) are major licorice flavonoids classified as a phloroglucinol. Our screening of components of licorice extract revealed that LQ and LQG activate melanin synthesis in melanoma cells. In this study, we elucidated the molecular mechanism of melanin synthesis activated by LQ and LQG. In addition, we investigated the intracellular accumulation and target protein of LQ and LQG using anti-LQ/LQG monoclonal antibody (anti-LQ/LQG mAb). LQ and LQG activated melanin synthesis in mouse (B16-F1 and B16-4A5) and human (HMV-II) melanoma cell lines. LQ and LQG enhanced the expression of tyrosinase (Tyr), TRP-1, and TRP-2. Molecular analysis suggests that LQ and LQG activate the p38 and PKA signaling pathways leading to the MITF expression. Enzyme-linked immunosorbent assay (ELISA) using anti-LQ/LQG mAb indicated that LQ and LQG cannot be detected in cellular lysate, and Western blotting using anti-LQ/LQG mAb as the primary antibody demonstrated that LQ binds to an approximately 35-38 kDa protein. These results suggest that LQ and LQG enhance melanin synthesis via the activation of p38 and PKA signaling pathways by binding to an approximately 35-38 kDa protein.