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Delphinidin의 Raf/MEK/ERK 신호전달 경로 저해를 통한 발암억제 효과

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Delphinidin Attenuates Neoplastic Transformation in JB6 Cl41 Mouse Epidermal Cells by Blocking Raf/MEK/ERK Signaling

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Objectives

Epidemiological studies suggest that a high consumption of fruits and vegetables can reduce the risk of cancer. Anthocyanins are naturally occurring polyphenolic compounds that provide intense color to fruits and vegetables such as berries, red grapes, purple sweet potato and red cabbages. Previous studies showed that anthocyanidins scavenge reactive oxygen species and suppress cell proliferation and migration, tumor cell invasion, and angiogenesis. Delphinidin, a representative dietary anthocyanidin, was shown to exert the strongest antitumor-promoting effects among the anthocyanidins tested, including delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin, in TPA-promoted cell transformation of JB6 promotion-sensitive mou se skin epidermal (JB6 P+) cells. However, the molecularmechanisms and specific targets of the antitumorigenic effects of delphinidin remain unknown.

Materials and Methods

○ Materials

Delphinidin was purchased from Indofine Chemical and EGF, TPA, Sigma/Aldrich.

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○ Methods

MTT assay, Anchorage-independent cell transformation assay, PGE2 assay, Reverse transcription-PCR, Luciferase assays for COX-2 promoter activity and AP-1 and NF-κB transcriptional activities, Reporter gene assay for c-fos promoter activity, Western blot analysis, In vitro MEK1, Raf1, ERK2, and JNK1 kinase assays, Ex vivo Raf1 and MEK1 immunoprecipitation and kinase assays, In vitro and ex vivo pull-down assays, ATP and delphinidin competition assay, Molecular modeling

Results

Neoplastic transformation of cells and inflammation are considered to be major ev ents contributing to carcinogenesis. Here we report that delphinidin, a major dietary a nthocyanidin, inhibits tumor-promoter-induced transformation and cyclooxygenase-2 (COX-2) expression in JB6 promotion-sensitive mouse skin epidermal (JB6 P+) cells by directly targeting Raf and MEK. Delphinidin inhibited 12-O-tetradecanoylphorbol-1 3-acetate (TPA)-induced neoplastic transformation and COX-2 expression at both the protein and transcriptional levels. The activation of activator protein-1 and nuclear fac tor-kB induced by TPA was dose-dependently inhibited by delphinidin treatment. Del phinidin strongly suppressed Raf1 and MEK1 kinase activities and subsequently atten uated TPA-induced phosphorylation of MEK, ERK, p90RSK, and MSK. Although delp hinidin suppressed ERK and JNK activities, it was more effective at inhibiting Raf1 o r MEK1 activities. Pull-down and competition assays revealed that delphinidin binds with Raf1 or MEK1 noncompetitively with ATP. Delphinidin also dose-dependently su ppressed JB6 P+ cell transformation induced by epidermal growth factor and H-Ras, both of which are known to be involved in the activation of Raf/MEK/ERK signaling. Together these findings suggested that the targeted inhibition of Raf1 and MEK activ ities and COX-2 expression by delphinidin contribute to the chemopreventive potential of fruits and vegetables.

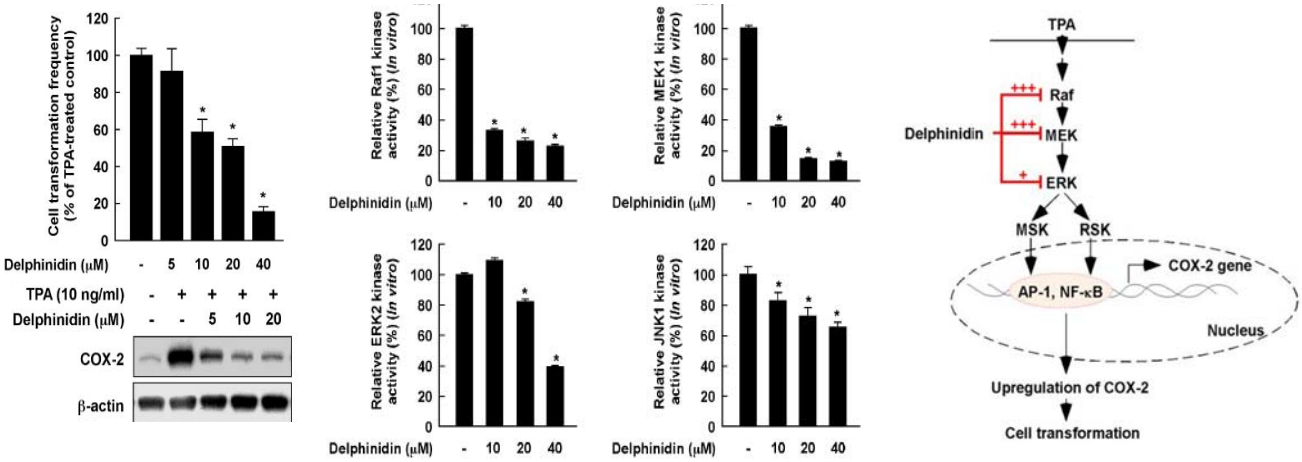

Figure 1. Delphinidin Attenuates Neoplastic Transformation in JB6 Cl41 Mouse Epidermal Cells by Blocking Raf/MEK/ERK Signaling