

involved in ATX-mediated tumor cell motility stimulation.

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### **Delayed ERK Activation by Ceramide Reduces Melanin Synthesis in Human Melanocytes**

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We investigated the effects of sphingolipid metabolites and the possible signaling pathways involved in human melanocytes. Our data shows that C2-ceramide inhibits cell growth in a dose-dependent manner, whereas SPP has no effect. Moreover, we observed that the melanin content of the cells was significantly decreased by C2-ceramide. The pigmentation-inhibiting effect of C2-ceramide at 1-10  $\mu$ M was much stronger than that of kojic acid, tested at 1-100  $\mu$ M. The tyrosinase activity of cell extracts was reduced by C2-ceramide treatment. However, in the cell-free system, C2-ceramide could not suppress tyrosinase, whereas kojic acid directly inhibited tyrosinase. These results suggest that C2-ceramide decreases the pigmentation of melanocytes indirectly regulating tyrosinase. Furthermore, we found that C2-ceramide inhibited the production of microphthalmia-associated transcription factor (MITF), which is required for tyrosinase expression. To identify the signaling pathway of ceramide, we studied the ability of C2-ceramide to influence extracellular signal-regulated protein kinase (ERK) and Akt/protein kinase B (PKB) activation. C2-ceramide induced a delayed activation of ERK (>1 h) and a much later activation of Akt/PKB (>3 h) in human melanocytes. In addition, the specific inhibition of the ERK and the Akt signaling pathways by PD98059 and LY294002, respectively, increased melanin synthesis. Thus, it seems that sustained ERK and Akt activation may lead to the suppression of cell growth and melanogenesis.

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### **Intracellular signaling pathway associated with sphingosylphosphorylcholine-induced contraction in feline ileal smooth muscle cells.**

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It is proposed that SPC-induced contraction mechanism involves a certain G protein, phospholipase and kinases-dependent pathway. G protein subtypes of Gi1, Gi3 and Go are immunoblotted in feline ileum. To investigate which subtype mediates the contraction, permeabilized cells were used. Gi3 antibody-treated cells showed the decrease of contraction induced by SPC. In addition, incubation of [<sup>35</sup>S]GTP $\gamma$ S with membrane fraction increased its binding to Gi3 subtype after SPC treatment, suggesting that the signaling pathways invoked by SPC were mediated by Gi3 protein. After treatment of PLC inhibitor neomycin, the contraction was reduced. Of existing PLC subtypes, PLC $\gamma$ 1 antibodies decreased SPC-induced response when treated to permeabilized cells. MEK inhibitor PD98059 and PKC inhibitor chelerythrine blocked the contraction significantly, but p38 MAP kinase inhibitor SB202190 did not. However, co-treatment of PD98059 and chelerythrine showed no significant difference. SPC-induced contraction was inhibited with the incubation of PKC $\epsilon$  antibodies to penetrate permeabilized cells. Phosphorylation and activity of p44/42 MAP kinase and was increased by SPC treatment, which was reversed by pretreatment of inhibitors of signaling molecules that decreased SPC-induced contraction previously. Thus, in feline ileal smooth muscle cells, the mechanism of contraction by SPC involves Gi3 protein, followed by the activation of PLC $\gamma$ 1, PKC $\epsilon$ , and p44/42 MAP kinase.

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### **Autotaxin increase the motility is breast cancer cells**