O-03 SIGNAL PATHWAY IN MEDIATOR RELEASES EVOKED BY ACTIVATION OF MAST CELLS CO-CULTURED WITH ASTROCYTES

Dae Yong Kim[1,2], Yun Song Lee[1,2], Jai Youl Ro[1,2]

[1]Department of Pharmacology, [2]Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Korea

It has been reported that functional communication between mast cells and nervous cells occurred in the physiological and pathologic situations. This study aimed to examine the signal pathway of mast cells activated in situation co-cultured with astrocytes (ATs). We co-cultured human mast cell-1 (HMC-1) and human U87 glioblastoma cell lines (U87), and co-cultured mouse bone marrow-derived mast cells (BMMCs) and mice cerebral cortices-derived ATs as a ratio 3:1 for periods indicated, and then mast cells were harvested. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) level was measured by confocal microscope, histamine by automated fluometric analysis, leukotrienes (LTs) by RIA method, surface molecules of mast cells and ATs, MAP kinases, small GTP molecules, and PKCβ by western blot, activity of NF-κB by EMSA, and mRNA of cytokines by microarray and RT-PCR. HMC-1 cells or BMMCs co-cultured with each ATs increased $[Ca^{2+}]_i$ level, histamine, LTs, and cytokine mRNA, and in co-culture time-dependent manner. The expressions of surface molecules of mast cells (CD40L) and ATs (CD40) increased during co-culture. Mast cells co-cultured with ATs also increased MAP kinases, small GTP molecules (Ras, Rac, Rho, cdc42), activity of NF-κB, and cytokine mRNA. These results were inhibited by CD40 antibody pretreatment during co-culture. These data suggest that activation of mast cells caused by CD40/CD40L ligation is induced by activation of small GTP molecules/PKCβ via Ca^{2+} influx, and that mediators released and synthesized from mast cells are related with chronic neurodegenerative and inflammatory diseases of the nervous system.

Key Words: Human mast cell-1 (HMC-1), Human U87 glioblastoma cell lines (U87), Ca^{2+}, PKC, Small GTP molecules

O-04 TGFβ1 GENE REPRESSION BY PPARγ AND RXR RESULTS FROM PTEN-MEDIATED S6K1 INHIBITION

SJ Lee, EK Yang, SG Kim

College of Pharmacy, Seoul National University, Sillim-dong, Kwanak-gu, Seoul 151-742, Korea

PPARγ and RXR heterodimer regulates cell growth and differentiation. Zf9, whose phosphorylation promotes target genes, is a transcription factor essential for transactivation of the TGFβ1 gene. This study investigated whether activation of PPARγ-RXR heterodimer inhibits TGFβ1 gene transcription and Zf9 phosphorylation and, if so, what signaling pathway regulates it. Either 15-deoxy-(12, 14)-prostaglandin J_2 (PGJ_2) or 9-cis-retinoic acid (RA) treatment decreased the TGFβ1 mRNA level in L929 fibroblasts. PGJ_2+RA, compared with individual treatment alone, synergistically inhibited the TGFβ1gene expression, which was abrogated by PPARγ antagonists. Likewise, PGJ_2+RA decreased luciferase expression from the TGFβ1 gene promoter. Promoter deletion analysis of the TGFβ1gene revealed that pGL3-323 making up to −323 bp region, but lacking PPAR-responsive elements, responded to PGJ_2+RA. PGJ_2+RA treatment inhibited the activity of p70 ribosomal S6 kinase-1 (S6K1), abolishing Zf9 phosphorylation as did rapamycin. Zf9 dephosphorylation and TGFβ1 gene repression by PGJ_2+RA were reversed by transfection of cells with the plasmid encoding constitutively active (CA)-S6K1. Ectopic expression of PPARγ1 and RXRα repressed pGL3-323 transactivation with S6K1 inhibition, which was abrogated by CA-S6K1. PGJ_2+RA induced PTEN, whose overexpression repressed the TGFβ1gene through S6K1 inhibition, decreasing ERK1/2-RSK1 and Akt-mTOR phosphorylations. In summary, TGFβ1 gene repression by PPARγ and RXR results from PTEN-mediated S6K1 inhibition, providing insight into pharmacological manipulation of the TGFβ1 gene regulation.

Key Words: TGFβ1, PPARγ, RXR, P70 ribosomal S6 kinase-1, PTEN