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Molecular mechanism of Korean mistletoe lectin-induced up-regulation of IL-3 expression

Joo Young Kim, Sungyoul Hong[1] and Jae Youl Cho*

School of Biotechnology and Bioengeering, Kangwon National University, Chuncheon 200-701, Korea, [1]Department of Genetic Engineering, Sungkyunkwan University, Suwon 440-746 Korea

Lectins (KML) isolated from Korean mistletoe (Viscum album L. coloratum) are known to be a strong anti-cancer protein. In this study, Molecular mechanism of KML-induced up-regulation of IL-3 expression was examined in detail. To do this, murine and human macrophage cell lines (RAW264.7 and U937 cells) were employed and expression of IL-3, a cytokine involved in hematopoiesis, and its signaling mechanism for expression were carefully investigated using RT-PCR and Western blot analyses. KML at low concentrations (10 ng/ml) augmented cytokine (IL-3 and IL-23) expression and the up-regulation of IL-3 expression was clearly affected by inhibitors [U0126 (a ERK inhibitor) and genistein (a protein tyrosine kinase (PTK) inhibitor)]. The involvement of these enzymes was also confirmed by Western blotting analysis. Thus, KML treatment stimulated the tyrosine phosphorylation of p130 and ERK phosphorylation. However, the up-regulation of IL-3 expression seemed to be independent of NF-(kappa)B and AP-1 activation assessed by NF-(kappa)B reporter gene assay system. Therefore, our results suggest that KML may be involved in regulating hematopoiesis and IL-3 mediated cellular responses(*:Corresponding Author). (This work was supported by ARPC.)

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Monitoring altered T cell subpopulation and cytokine production during progression of breast cancer growth in mice

Mi Young Jung and Tae Sung Kim

School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea

Growth, invasion and metastasis of breast carcinomas may be controlled by regulatory factors derived from the malignant cells and/or their neighbors during tumor progression. Such progressive malignancies may also be influenced by tumor-related lymphocytes. However, it remains unclear how tumor progression kinetically affect T cell subpopulations and cytokine production by tumor cells and T lymphocytes. In this study, we assessed the phenotype of T cell subpopulation and kinetics of cytokine profiling during progression of tumor growth in mice subcutaneously challenged with SB5b breast adenocarcinoma. Cytofluorometric analysis showed that the populations of the CD4+CD25+ regulatory T cells as well as activated T cells increased at the late stage of tumor progression. In addition, coculture of tumor cells with T cells resulted in changing cytokine balance, in which TGF-β, IL-10 and IL-6 increased whereas IL-4 and IFN-ɣ decreased. These results indicated that the cell-cell contact between tumor cells and T cells may affect regulatory factors for tumor immunity, which are favorable for tumor progression. Our study suggests that the altered properties of T lymphocytes and the microenvironment of tumor site which is mainly affected by cytokines from tumor infiltrating lymphocytes(TILs) and breast cancer cells may lead to growth and/or metastasis of tumor cells.

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Negative regulation of TGFβ1-induced GLα transcription by Smurf1 and Smad7

Seo-Hyun Choi[1], Goo-Young Seo[1], and Pyeung-Hyeun Kim[1,2]

[1]Department of Molecular Bioscience, School of Bioscience and Biotechnology, [2]Vascular System Research Center, Kangwon National University, Chunchon 200-701, S. Korea

Smurfs (Smurf1 and Smurf2) are E3-ubiquitin ligases that inhibit TGF-β signaling. Recent studies show that Smad7 has a synergistic effect with Smurfs resulting inhibition of TGF-β signaling. Smad7 binds type I TGF-beta receptors and prevents binding and phosphorylation of Smads 2 and 3. Smad7 also facilitates Smurf-induced type I TGF-beta receptor degradation. To determine the effects of Smad7 and Smurfs on IgA class switching, we transfected germ-line α (GLα) promoter reporter to B cell line CH12F3-2A. Either Smurf1 or 2 reduced basal and TGFβ1-induced GLα promoter activity. In contrast, Smurf1 but Smurf2 suppressed the GLα promoter activity enhanced by Smad3/4 in the presence of TGF-β1. Similarly, Smurf1 but Smurf2 further suppressed the GLα promoter activity inhibited by Smad7. These results indicate that Smurf1 is a key molecule that mediates negative regulation of IgA class switching.

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NF-kB dependent expression of MMP-9 gene by CpG-oligodeoxynucleotides in a mouse macrophage cell line RAW 264.7.

Jae-Won Rhee[1], Keun-Wook Lee[2], Hyung-Joo Kwon[2], and Doo-Sik Kim[1]

[1]Dept. of Biochemistry, Yonsei University, Seoul 120-749 [2]Dept of Microbiology, Hallym University College of Medicine, Chunchon, 200-702

Matrix metalloproteinase-9 (MMP-9) is a secreted type IV collagenase that plays an important role in the remodeling of the extracellular matrix (ECM) and the migration of normal and tumor cells. We have shown that CpG-ODN-induced migration of cells is regulated by the inhibition of MMP-9 activity in RAW 264.7 cells treated with tissue inhibitors of MMP-1 (TIMP-1). The MMP-9 gene expression is transcriptionally activated by CpG-ODN in a time-dependent manner. A MMP-9 promoter-reporter was activated by ectopical expression of NF-&;B transcription factor. Inhibition of NF-&;B nuclear localization by co-expression of a mutant I&;Bα protein blocked CpG-ODN-induced transcription from a MMP-9 promoter-reporter construct, showing NF-&;B activation is required for MMP-9 gene expression. In addition to ectopic expression assays, we also confirmed that the NF-&;B contributes to the expression of MMP-9 gene in response to CpG-ODN by chromatin immuno-precipitation using NF-&;B antibody. In summary, our data suggest that NF-&;B-dependent expression of MMP-9 gene in response to CpG-ODN plays an important role in the recruitment of immune cells.