J-29

Evidence of BNIP3 involvement in TNF-α-induced cell death pathway

Su-Mi Kim, Jeong-Hun Ko, Ji-Hye Yim, Jin-Hae Park, Jae-Hoon Park

Department of Pathology and Medical Science and Engineering Research Center for Reactive Oxygen Species, College of Medicine, Kyung Hee University, Seoul 130-701, Korea

BNip3 is a pro-apoptotic molecule that belongs to BH3-only Bcl-2 family proteins. When overexpressed, BNip3 induces apoptotic, necrotic, or autophagic cell death, depending on types of cells or injuries. Given that the suppression of apoptotic cell death by viral protein is an early and essential step for efficient viral replication, we have hypothesized that BNip3 may function as a cell death machinery that has to be blocked by early viral proteins. On this hypothesis, we speculated the involvement of BNip3 in cell death signals provoked by cellular defense mechanisms against viral infection. In this study, we show that BNip3 inserted into the mitochondrial membrane, which is the requisite for BNip3-induced cell death, when cell death is triggered by TNF-α. In this process, BNip3 activation is facilitated by cathepsin B-dependent, but caspase/Bid/Bax- or C-Jun NH2-terminal kinase (JNK)-independent pathway. Furthermore, our results show that BNip3-induced necrotic cell death is an alternative route selected when main Bid pathway was blocked. In conclusion, our data suggest that BNip3 is an effecter molecule responsible for TNF-α-induced cathepsin B-associated mitochondrial damage.

J-30

Ganglioside GM3 modulates cell cycle progression by inducing PTEN expression in colon cancer cells

Tae-Wook Chung[1,4], Hee-Jung Choi[1], Young-Choon Lee[2], Jeong-Heon Ko[3], Jong-Guk Kim[4] and Cheorl-Ho Kim[1]

[1]Molecular and Cellular Glycobiology Unit, Department of Biological Science, SungKyunKwan University. [2]Faculty of Biotechnology, Dong-A University. [3]Systemic Proteomics Research Center, KRIBB. [4]Department of Microbiology, Kyungpook National University

The simple ganglioside GM3 has been shown to have antiproliferative effects in several in vitro and in vivo cancer models. Although the exogenous ganglioside GM3 has an inhibitory effect on cancer cell proliferation, the exact mechanism by which it prevents cell proliferation remains unclear. Previous studies showed that MDM2 is an oncoprotein that controls tumorigenesis through both p53-dependent and -independent mechanisms, and tumor suppressor PTEN, a dual specificity phosphatase that antagonizes phosphatidylinositol 3-kinase (PI-3K)/AKT signaling, is capable of blocking MDM2 nuclear translocation and destabilizing the MDM2 protein. Results from our current study show that GM3 treatment dramatically increases cyclin-dependent kinase (CDK) inhibitor (CKI) p21WAF1 expression through the accumulation of p53 protein by the PTEN-mediated inhibition of the PI-3K/AKT/MDM2 survival signaling in HCT116 colon cancer cells. Moreover, the data herein clearly show that ganglioside GM3 induces p53-dependent transcriptional activity of p21WAF1, as evidenced by the p21WAF1 promoter-driven luciferase reporter plasmid (full-length p21WAF1 promoter and a construct lacking the p53 binding sites). Additionally, ganglioside GM3 enhances expression of CKI p27kip1 through the PTEN-mediated inhibition of the PI-3K/AKT signaling. Furthermore, the down-regulation of the cyclin E and CDK2 was clearly observed in GM3-treated HCT116 cells, but the down-regulation of cyclin D1 and CDK4 was not. Whereas, suppression of PTEN levels by RNA interference restores the enhanced expression of p53-dependent p21WAF1 and p53-independent p27 kip1 through inactivating the effect of PTEN on PI-3K/AKT signaling modulated by ganglioside GM3. These results suggest that ganglioside GM3-stimulated PTEN expression modulates cell cycle regulatory proteins, thus inhibiting cell growth. We conclude that ganglioside GM3 represents a modulator of cancer cell proliferation and may have potential for use in colorectal cancer therapy.

J-31

Geum japonicum Thunberg extracts exhibit anti-metastatic activites via Rho family small GTPases-indenpent pathway in B16 melanoma cancer cells

Hyo-Sook Kim[1], Ja-Young Park[1], Jin-Chul Heo[1], Sang-Mi An[1], Sang-Uk Woo[1], Kang-Jin Cho[2], Jong-Guk Kim[3], and Sang-Han Lee[1]

Departments of [1]Food Science & Techonology, and [2]Microbiology, Kyungpook National University Daegu 702-701, Korea [3]National Institute of Agricultural Biotechnology (NIAB), Rural Development Adminstration, Suwon 441-707, Korea

Geum japonicum Thunberg (GjT) belonged to Rosaceae has been used for a treatment with herbal medicines in Oriental countries for centuries. In order to confirm the biological activities of GjT, we carried out various in vitro assays. In present study, GjT was used for the observance of cell migration in B16 (melanoma cells) during wound healing assay and tube formation in HUVEC cells. We found here that GjT inhibits both cell migration and capillary tube formation. Cell migration is a critical step in tumor invasion and metastasis, and regulation of this process will lead to appropriate therapies for treating cancer. During cell migration, Rho family small GTPases play pivotal roles in reorganization of the actin cytoskeleton. We, therefore, examined possible involvement of Rho family small GTPases activation when NIH-3T3 cells are stimulated by GjT. Examination of four molecules which is known for Rho family small GTPases such as CDC42, Ras, Rac1 and Rho, revealed that the expression of GTP-bound proteins did not change by GjT treatment compared to that of control. Consequently, our data suggest that GjT inhibits cell migration and tube formation via Rho family small GTPases-independent pathway.

J-32

GSK-3β mediated NF-κB transcriptional activation by HBX

Kyoung A Kim, Osong Kwon, Sun Ok Kim, Long Ha, Jong Seog Ahn, Bo Yeon Kim

Metabolic functional research center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Yuseong, Daejeon, 305-333, Korea

HBX is a multifunction protein that can trans activate some transcription factors,including AP-1,NF-κB,CREB and TBP and is essential for HBV infection and may play an important role development of hepatocellular carcinoma.Although the NF-κB activation is extensively exploited,the role of GSK-3β in this process has not yet been clearly defined. We examined the cellular effect of HBX in normal liver cells and constitutive HBX-expressing (chang/HBX) cells.We have explored which HBX was found to activate the NF-κB transcriptional activity by GSK-3β phosphorylation.To further investigate the role of GSK-3β in modulating NF-κB transcription,chang/HBX cells were transiently transfected with either wild type GSK-3β or mutant GSK-3β in which the regulatory ser9 residue is changed to alanine.Mutant GSK-3β abolished NF-κB transcriptional activity but not influenced nuclear translocation and DNA-binding activity by HBX.Inhibition of Akt1 and MAPKs did not affect IkBα degradation or NF-κB DNA binding activity.HBX induced a sustained NFκB activation but NFκB inhibition did not alter the HBX-induced caspase3 activity.In conclusion,we show that HBX-induced GSK-3β phosphorylation activates NF-κB transcription activity independently ofIκBα degradation,nuclear translocation of NF-κB