artificial insemination and specific sequences of SRY (sex-determining region Y) gene were amplified by PCR to determine the sex of fetal fibroblast. Adult fibroblasts were derived from a male10-year-old beagle. Fibroblasts were cultured with Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum and 1% non-essential aminoacids. Population doubling time, viability, and size of the cells were analyzed during passage 3 to 6. Real-time pcr was used to analyze mRNA expression of bax1, bcl2, Dnmt1, HDAC1, and Oct 4. One-way ANOVA and t-test were performed using Graph Pad Prism 5.0.

Results: Average viability between fetal and adult fibroblasts was not different (95.7 ± 0.6 and 96.8 ± 0.8). While cell size was similar in fetal fibroblasts from passage 3 to 6, it was significantly increased in adult fibroblasts in passage 6 (14.9 ± 0.5 um) compare to passage 3(12.8 ± 0.1um). All of the transcript expressions including ax1,bcl2, Dnmt1, HDAC1, and Oct 4 between fetal and adult fibroblasts were not significantly different.

Conclusions: Similar characteristics between fetal and adult fibroblasts in our study showed that adult fibroblasts can be used to produce atransgenic cell line as well as fetal fibroblasts. Especially, we recommend that transfection up to passage 5 will effectively maintain cell size of adult fibroblasts similar with fetal fibroblast.

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Ginsenoside Rp3, a Novel Ginsenoside Derivative, Inhibits ADP-induced Platelet Aggregation

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Introduction: Korean red ginseng has been used as a traditional oriental medicine to treat illness and promote health. Korean red ginseng extracts and some ginsenosides are known to have anti-platelet activities. Ginsenosides are the main constituents for the pharmacological effects of Panax ginseng. The ginsenosides Rp3 (G-Rp3), a derivative of ginsenoside Re, is isolated from Panax ginseng. Platelet aggregation is an essential part of the haemostatic process when blood vessels are injured. We investigated the ability of G-Rp3 to modulate ADP-induced platelet activation.

Materials and Methods: Whole blood from the rats was collected using a 23G needle which was inserted into the cardiac vascular. Aggregation of washed platelet was monitored by measuring light transmission in an aggregometer. Intracellular Ca^{2+} was measured using fluorescent Ca^{2+} indicator fura 2-AM. The granule secretion of ADP-induced platelet was screened by measuring the release of ATP and P-selectin expression was determined using anti-CD62P. In addition, to analyze the activation of integrin αIIbβ3 and phosphorylation of signaling molecules, we carried out immunoblotting assay and Alexa Fluor488-fibrinogen binding assay using washed platelet.

Results: G-Rp3 inhibited ADP (10 μM)-stimulated platelet aggregation in a dose-dependent manner. We found that G-Rp3 decreased calcium mobilization, ATP release and P-selection expression. Moreover, fibrinogen binding to integrin by ADP was attenuated in G-Rp3 treated platelets. G-Rp3 significantly attenuated phosphorylation of ERK, JNK, as well as PI3K/AKT, PLCγ and Src family kinase phosphorylation.

Conclusions: These results indicate that the inhibitory effect of G-Rp3 on platelet aggregation and granule secretion is mediated by suppression of calcium mobilization. And it is suggested the G-Rp3 mediates its anti-platelet activity by phosphorylation of ERK, JNK, PI3K/AKT, PLCγ and SFK through signaling of GPV binding to ADP. In conclusion, these effects support that G-Rp3 could be potent candidate of therapeutic agent against platelet-related cardiovascular diseases.

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Flavonoid Extract Isolated from Korean *Scutellaria baicalensis* Georgi Induces Apoptosis in AGS Human Gastric Cancer Cells

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Introduction: Gastric cancer is the second most common cancer in the world and the mortality is still high in Korea. *Scutellaria bicalensis* has been used in traditional herbal medicine to treat fever,, jaundice, abdominal pain and diarrhea. *Scutellaria bicalensis* has numerous bioactive flavonoids such as carthamin, and isocarthamin. Also,

Scutellaria bicalensis's roots have flavonoids such as baicalin, wogonin-n-glucuronide, oroxylin-A-glucuronide, β-sitostero, stigmasterol. *Scutellaria bicalensis* has an effect on diseases such as cleanses heat, dries, moisture, purges fire, removes toxin and spontaneous abortion.

Materials and Methods: The flavonoid compounds were extracted with 70% methanol from radix of Korean *Scutellaria bicalensis* Georgi (Jinju, Korea). We got the AGS cells from the Korea Cell Line Bank (Seoul, Korea). All experiments used that AGS cells were seeded into 6-well plates and stabilized for 24h. The cells were then treated with or without *Scutellaria bicalensis*. Cells were cultured inRPMI1640 medium supplemented with 10% FBS, and 1% penicillin, streptomycin in ahumidified atmosphere of 5% CO_2 at 37℃. Cell viability was determined using MTT assay. Apoptotic cells were detected using aFITC annexin-V apoptosis detection kit 1 (BD Pharmingen, San Diego, CA, USA). And the levels of the apoptosis related proteins expression were analyzed by Western blot.

Results: We tested the cytotoxicity of various concentrations of polyphenols for 24 using the MTT assay. MTT results showed flavonoids inhibited cell viability in adose- dependent manner. AGS cells were untreated or treated with plant extract for24 h and analyzed using fluorescence- activated cell sorting. The plant extract significantly increased the sub-G1 phase. In Annexin-V and PI double staining, we checked that flavonoid compounds induce apoptosis in AGS cells. Western blot results showed that expression level of Bax/Bcl-xL ratio was increased in adose-dependent manner. Presently, the levels of caspase-3, -6, -8 and −9 were diminished in a dose dependent manner. Cleavage of PARP is the main hallmark for caspase- dependent apoptosis cell death. Cleaved PARP also significantly increased in a dose-dependent manner. The collective data favored the suggestion that flavonoids induced apoptosis through a shift in Bax/Bcl-xL ratio and the activation of caspase cascades.

Conclusions: Caspases play a pivotal role in apoptosis; their over expression and cleavage is a precursor of apoptosis in mammalian cells. Also, the Blc-2 families of proteins, whose activities are directed to act at the mitochondrial outer membrane, are major regulators of apoptosis. Treatment with the plantextract of *Scutellaria bicalensis* significantly activated Cleaved Caspase-3, Cleaved PARP and Bax/Bcl-xl ratio in AGS human gastric cancer cell.

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Complete Genome of A Novel Phage KBNP 1315 that Infects Avian Pathogenic *Escherichia coli* and Functional Characterization of Its Lysis-cassette

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Introduction: Avian colibacillosis, an infectious disease of birds caused by *Escherichia coli*, is one of the principal causes for heavy economic losses in the poultry industry[1]. With the increasing prevalence of multidrug-resistant bacteria, there are urgent needs to formulate a treatment for bacterial pathogen; phage therapy, the useof bacteriophage itself, and/or recombinant phage-harbored lysin have been generally recognized as a promising alternative of antibacterial against infectious diseases. Here, we studied a coliphage, KBNP 1315, specific for avian pathogenic *E. coli* strain, with a focus on genomic and functional characteristics of phage and its lysis-cassette, respectively.

Materials and Methods: 1) Bacterial strain and its bacteriophage: Bacterial strain, *Escherichia coli* 1315 for phage isolation was kindly provided by KBNP Technology Institute, KBNP, Inc. 2) Transmission electron microscopy (TEM): A solution (8 μl) of purified bacteriophage stained with 8 μl of 1% aqueous uranyl acetate (Merck, Darmstadt, Germany) was examined with Philips TECNAI F12 FEI transmission electron microscope (FEI, Hillsboro, OR) at an accelerating voltage of 120 kV. 3) Genome annotation: Open reading frames (ORFs) were identified using RAST server and annotated by BLASTp, PSI-BLAST and HHpred. 4) Construction of phylogenomic network: Based on the number of shared protein families between phage genomes, we calculated phage-phage similarity using the hypergeometric formula[2]. The significance (Sig) value was obtained as described by Lima-Mendez *et al*[2]. Afterwards, network was visualized with the Cytoscape software (version 3.0.2; http://cytoscape.org/). 5) Lytic proteins expression: Putative holin and endolysin gene were cloned to pDEST24 expression vector and this plasmid was transformed into E. coli DH5α. For lysis test, optical density measurements at 600nm were measured at1h intervals. The samples were induced with 0.5 mM IPTG.

Results: We were able to isolate the phage (KBNP 1315)from an *E.coli* strain that causes avian colibacillosis. The KBNP 1315 genome is 45,403 bp in length and is flanked by 208 bp short direct terminal repeat. Based on annotation tools, only 39 ORF swere annotated and predicted to be functional. The annotated ORFs can be categorized into several functional groups: Transcription/Translation, DNA replication/ modification, Phage structure, and Host lysis. To better understand its evolutionary relationships, we built a similarity network through ACLAME database, which indicates that KBNP1315 belongs to the SP6-like genus. With respect to bactericidal candidate, holin and endolysin were cloned and expressed as lysis-cassette of KBNP 1315. From *in vitro* experiment, we demonstrated that endolysin of this