P29-211 Expression of Recombinant Endochitinase of Antarctic *Sanguibacter sp.* KOPRI21702 in *Pichia pastoris*

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Chitin composes the cell walls of some animals and microbes, including insects, crustaceans, and fungi. Chitinases break down glycosidic bonds in chitin. In combination of exochitinases, endochitinases are considered as important enzymes in biomedical industry for producing particularly N-acetylglucosamine (NAG) and others. Endochitinase chi21702 was isolated from Antarctic *Sanguibacter sp.* KOPRI21702 and well characterized in our lab previously. The gene for this enzyme was obtained from the genomic DNA and the sequence was determined successfully. The methylotrophic Yeast *Pichia pastoris* expression system was applied to develop the production process of the enzyme since this system is known to facilitate the purification of the recombinant enzymes secreted to the culture media. The *Pichia* system expressed the recombinant Antarctic endochitinase successfully and revealed enzymatic activity using colloidal chitin as a substrate. The expressed protein showed higher molecular weight than theoretical one due to maybe post-translational modification, presumably glycosylation. This presentation introduces unique characteristics of chitinases from Antarctic bacteria and suggests a potential for the development of biomedical applications.

P29-212 17β-estradiol Attenuates Vascular Contraction by Inhibiting RhoA/Rho Kinase Pathway

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We hypothesized that 17β-estradiol attenuates vascular contraction by inhibiting RhoA/Rho kinase signaling pathway in rat aorta. Rat aortic rings were denuded of endothelium, mounted in organ baths, and contracted with U46619, a thromboxane A2 analogue, after pretreatment with or without 17β-estradiol (30 and 100 μM) for 30 min. We measured the amount of GTP RhoA as a marker for RhoA activation. We also determined the phosphorylation level of the myosin light chain (MLC_{20}), myosin phosphatase targeting subunit 1 (MYPT1) and PKC-potentiated inhibitory protein for heterotrimeric MLCP of 17 kDa (CPI17) by Western blot. Pretreatment with 17β-estradiol significantly inhibited the 30 nM U46619 -induced vasocontraction, RhoA activity and MLC_{20} phosphorylation. 17β-Estradiol not only inhibited vascular contractions induced by U46619, but also decreased the level of phosphorylation of $MYPT1_{Thr855}$ and $CPI17_{Thr38}$, downstream effectors of Rho-kinase. In conclusion, 17β-estradiol attenuates vascular contraction at least in part by inhibiting RhoA/Rho kinase signaling pathway.

P29-213 The Anti-apoptotic Effects of H0181 Extracts Increase Proliferation of Cyclophosphamide-induced Apoptotic Human Dermal Papilla Cells and Stimulate Hair Growth of C57B/6 Mice

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Numerous studies have shown that H0181 has anti-inflammatory, anti-allergic, anti-cancer, and antioxidant properties. Hair loss is a result of premature catagen cycle induced by apoptotic signaling. Here, we investigated the effect of H0181 extracts on the proliferation of a human hair dermal papilla cell (HHDPC) line and the ability to stimulate hair growth on C57 B/6 mice. According to the MTT assays, the proliferation of HHDPCs was barely increased in both a dose and time dependent manner with addition of H0181 extracts, while cell growth decreased when cyclophosphamide was added to the culture medium. If H0181 extract was added to the media with cyclophosphamide, the decreased proliferation was reversed. Regarding the effects of H0181 extracts on Bcl-2, Bax, and p53, the expression of Bax and p53 was decreased and the ratio of Bcl-2/Bax was increased. A depilated C57B/6 mouse model exhibited stimulated production of hair growth when treated with H0181 extracts. Together, our results suggest that H0181 extracts contain compound that stimulate hair growth in mice, possibly by suppressing the apoptotic- mediated induction of the catagen cycle.

P29-214 Morphological Characterization and *in vitro* Eva Two Porous Nano-β-Tricalcium Phosphate/Collagen/Chond phate Composites

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Collagen-β-tricalcium phosphate scaffolds were used in regenerati due to their similarity to the inorganic component of bone. In order to new composite scaffolds, a solution of collagen type I was mixed wi of chondroitin sulphate and nano-β-tricalcium phosphate (β-nTCP) weight ratios of 1:0.5:1 and 1:0.5:2. Porous composite scaffolds quently seeded with osteoblasts and cultured 72 hours *in vitro*. In ord tiate the micro-structure of the two porous composites we used method, immunofluorescence microscopy and polarization micro colonization of two porous composites by the osteoblasts has been histology, fluorescence microscopy after DAPI staining, osteocalcin chemistry and alkaline phospatase histochemistry. Our results sug two composite scaffolds tested are well tolerated by osteoblasts in The results from the *in vitro* study indicate a good compatibility for prepared by mixing collagen with chondroitin sulphate and β-nTC was supported by Project BIOSTEM, No. 61-012/2007

P29-215 Quercetin Enhances TRAIL-induced Apoptotic volvement of the ERK Signal Transduction Pathway

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Combined treatment with quercetin and TRAIL induced cytotoxi hanced annexin V staining and poly (ADP-ribose) polymerase (PAR in human prostate cancer cell lines DU-145 and PC-3. These indica tosis resulted from the activation of caspase-8, -9, and -3. Although sion levels of FLIPs, cIAP1, cIAP2, and the Bcl-2 family were not quercetin-treated cells, significant downregulation of survivin occu down survivin by siRNA significantly increased TRAIL-induced ap data demonstrated that inhibitor of ERK (PD98059), but not (SB203580) or JNK (SP600125), significantly maintained the intrace survivin during treatment with quercetin. Interestingly, PD98059 al quercetin-induced deacetylation of histone H3. Data from surviv activity assay suggest that the Sp1 transcription factor binds to the moter region and quercetin inhibits its binding activity through dea histone H3. Taken together, our findings suggest that quercetin enh induced apoptosis by inhibition of survivin expression, through mediated deacetylation of H3.

P29-216 A Novel Function of Karyopherin β3 assoc Apolipoprotein A-I Secretion

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Human karyopherin β3, highly homologous to a yeast protein secreti (PSE1), has often been reported to be associated with a mediator o toplasmic transport pathway. Previously, we showed that karyoph plemented the PSE1 and KAP123 double mutant. Our research su karyopherin β3 has an evolutionary function similar to that of yeast KAP 123. In this study, we performed yeast two-hybrid screening fo which would interact with karyopherin β3 and identified apolipoprotei I), a secretion protein with a primary function in cholesterol transport *vitro* binding assay, co-immunoprecipitation, and colocalization stu fined an interaction between karyopherin β3 and apo A-I. In addi pression of karyopherin β3 significantly increased apo A-I secretio sults suggest that karyopherin β3 plays a crucial role in apo A-I sec findings may be relevant to the study of a novel function of karyop coronary artery diseases associated with apo A

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212 17β-estradiol Attenuates Vascular Contraction by Inhibiting Rho Kinase Pathway

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P29-214 Morphological Characterization and *in vitro* Evaluation of Two Porous Nano-β-Tricalcium Phosphate/Collagen/Chondroitin Sulphate Composites

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Collagen-β-tricalcium phosphate scaffolds were used in regenerative medicine due to their similarity to the inorganic component of bone. In order to fabricate the new composite scaffolds, a solution of collagen type I was mixed with a solution of chondroitin sulphate and nano-β-tricalcium phosphate (β-nTCP) powder, in weight ratios of 1:0.5:1 and 1:0.5:2. Porous composite scaffolds were subsequently seeded with osteoblasts and cultured 72 hours *in vitro*. In order to evidentiate the micro-structure of the two porous composites we used von Kossa method, immunofluorescence microscopy and polarization microscopy. The colonization of two porous composites by the osteoblasts has been evaluated by histology, fluorescence microscopy after DAPI staining, osteocalcin immunocytochemistry and alkaline phospatase histochemistry. Our results suggest that the two composite scaffolds tested are well tolerated by osteoblasts in cell culture. The results from the *in vitro* study indicate a good compatibility for biomaterials prepared by mixing collagen with chondroitin sulphate and β-nTCP. This work was supported by Project BIOSTEM, No. 61-012/2007

P29-215 Quercetin Enhances TRAIL-induced Apoptotic Death: Involvement of the ERK Signal Transduction Pathway

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Combined treatment with quercetin and TRAIL induced cytotoxicity and enhanced annexin V staining and poly (ADP-ribose) polymerase (PARP) cleavage in human prostate cancer cell lines DU-145 and PC-3. These indicators of apoptosis resulted from the activation of caspase-8, -9, and -3. Although the expression levels of FLIPs, cIAP1, cIAP2, and the Bcl-2 family were not changed in quercetin-treated cells, significant downregulation of survivin occurred. Knockdown survivin by siRNA significantly increased TRAIL-induced apoptosis. Our data demonstrated that inhibitor of ERK (PD98059), but not p38 MAPK (SB203580) or JNK (SP600125), significantly maintained the intracellular level of survivin during treatment with quercetin. Interestingly, PD98059 also prevented quercetin-induced deacetylation of histone H3. Data from survivin promoter activity assay suggest that the Sp1 transcription factor binds to the survivin promoter region and quercetin inhibits its binding activity through deacetylation of histone H3. Taken together, our findings suggest that quercetin enhances TRAIL induced apoptosis by inhibition of survivin expression, through ERK-MSK1-mediated deacetylation of H3.

P29-216 A Novel Function of Karyopherin β3 associated with Apolipoprotein A-I Secretion

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Human karyopherin β3, highly homologous to a yeast protein secretion enhancer (PSE1), has often been reported to be associated with a mediator of a nucleocytoplasmic transport pathway. Previously, we showed that karyopherin β3 complemented the PSE1 and KAP123 double mutant. Our research suggested that karyopherin β3 has an evolutionary function similar to that of yeast PSE1 and/or KAP 123. In this study, we performed yeast two-hybrid screening to find a protein which would interact with karyopherin β3 and identified apolipoprotein A-I (apo A-I), a secretion protein with a primary function in cholesterol transport. By using *in vitro* binding assay, co-immunoprecipitation, and colocalization studies, we defined an interaction between karyopherin β3 and apo A-I. In addition, overexpression of karyopherin β3 significantly increased apo A-I secretion. These results suggest that karyopherin β3 plays a crucial role in apo A-I secretion. These findings may be relevant to the study of a novel function of karyopherin β3 and coronary artery diseases associated with apo A