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Metformin inhibits hepatic gluconeogenesis by AMP-activated protein kinase dependent regulation of the orphan nuclear receptor SHP

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Metformin is a commonly used antidiabetic drug for the treatment of type 2 diabetes. It plays an important role on glucose metabolism in the liver by activation of AMP-activated protein kinase (AMPK). However, its detail molecular mechanism for the inhibition of hepatic gluconeogeneis has not been fully elucidated yet. Here, we report that metformin-induced small heterodimer partner (SHP; NR0B2), an atypical orphan nuclear receptor, regulates hepatic gluconeogenic gene expression. Hepatic SHP gene expression was induced by metformin, AICAR and adenovirus-containing AMPK (Ad-AMPK). Metformin-induced SHP gene expression was abolished by adenovirus-containing dominant negative AMPK (Ad-DN AMPK) as well as compound C. Metformin down-regulates HNF3β or HNF4α-activated G6Pase and PEPCK promoter activity and these activations were blocked in the presence of siRNA SHP. Additionally, knockdown of SHP by adenovirus siRNA inhibits metformin mediated repression of the cAMP/Dex-induced PEPCK and G6Pase gene expression. Furthermore, oral administration of metformin induces SHP mRNA levels in the liver of ob/ob mice. Hepatic overexpression of SHP by tail vein injection of adenovisus-containing SHP (Ad-SHP) decreased G6Pase and PEPCK gene expression and decreased blood glucose levels. Taken together, this study demonstrates that metformin-induced inhibition of hepatic gluconeogenic gene expression is mediated by SHP. These results provide the new insight that ligands or reagents which are specifically able to up-regulate hepatic SHP expression or activity are promising therapeutic agent in the treatment of type 2 diabetes.

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Liver X receptor alpha enhances the stability and transcriptional activity of HIF-1α

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The Liver X receptor α (LXRα) is a nuclear receptor that functions as a regulator of the cholesterol homeostasis in macrophages and plays a role in development of cardiovascular diseases such as atherosclerosis. Inflammatory responses are associated with significant changes in cholesterol metabolism, which leads to the local hypoxia that promote blood flow and inflammation in the lesion. In the present investigation, we studied the possible cross-talk between LXRα and hypoxia-inducible factor (HIF-1α), a key regulator of hypoxic responses. We first observed that expression of LXRα was induced time-dependently at both transcript- and protein-levels under hypoxic conditions in the macrophage cell line Raw 264.7. The hypoxia-induced LXRα expression was repressed by transfection of anti-sense LXRα and HIF-1α induction under hypoxia was inhibited by siLXRα. TO901317, a specific ligand of LXRα, enhanced the expression of HIF-1α under normoxia. These results suggest an existence of a positive regulatory loop for induction of HIF-1α and LXRα under hypoxia. We also found that stability as well as transcriptional activity of HIF-1α protein was increased in the Raw264.7 cells when LXRα was overexpressed or treated with TO901317. Taken together, our results demonstrate a potential cross-talk between hypoxia and lipid metabolism that is mediated by LXRα.

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Ligand-dependent transcriptional activation of RORα increases protein level as well as activity of HIF-1α

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Hypoxia-inducible factor-1α (HIF-1α) is primarily involved in the sensing and adapting of cells to changes of oxygen level, which is essential for vascular function. Recently, physiological roles of retinoic acid-related orphan receptor α (RORα) has implicated in cardiovascular diseases such as atherosclerosis. When HepG2 cells were exposed to hypoxia, RORα was induced at protein- and mRNA-level after 0.5 h and the induction was continued up to 24 h. When RORα was overexpressed, transcriptional activity of HIF-1α was enhanced up to 8-fold when measured by reporter containing hypoxia-response element (HRE). Knock-down of RORα using siRNA strongly suppressed the increases of HIF-1α protein and HRE activity, indicating that RORα enhances transcriptional activity of HIF-1α. We showed that putative ligands such as melatonin, 22(R)-hydroxycholesterol, stearic acid, cholesterol sulfate, and 7-dehydrocholesterol induces the expression and transcriptional activity of RORα. Ligands of RORα enhanced the expression of HIF-1α as well as vascular endothelial growth factor. The mRNA-level of VEGF was increased however, that of HIF-1α was unchanged in the presence of ligands. Expression of HIF-1α protein was repressed when the expression of RORα was suppressed by RNA interference. Together our results may suggest that ligands of RORα that stabilize HIF-1α could be used for treatment of hypoxia-induced human vascular diseases.

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Kaempferol modulates age-related NF-κB activation via kaempferol modulates age-related NF-κB activation via RAGE-PKCβII signaling pathway

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Advanced Glycation End products (AGEs) result from the reaction between carbohydrates and the free amino group of proteins. AGEs induce the activation of NF- κ B and expression of NF- κ B-dependent pro-inflammatory genes and adhesion molecules. The formation and accumulation of AGE adducts in various tissues are known to be associated with altered protein structure and function, and are able to generate reactive oxygen species (ROS) and induce activation of protein kinase C (PKC) pathway. The binding of AGE to the receptor for AGE (RAGE) is known to show various cell dysfunctions and is implicated in pathogenesis of diabetic and age-related diseases. In this study, it was investigated whether kaempferol modulates AGEs formation, expression of RAGE, and AGEs-induced NF-κB signaling during aging. AGEs and RAGE increased during aging and the increases were reduced by kaempferol. NF-κB activity increased with aging, but kaemferol suppressed it. Kaempferol also suppressed expression of NF- κ B-regulated pro-inflammatory genes and adhesion molecules. Kaempferol modulated age-related RAGE signaling via PKCβII pathway. Furthermore, kaempferol inhibited the formation of AGEs induced by amino group with α-dicarbonyl compound. Besides, Kaempferol inhibited AGEs-induced NF-κB activation via NADPH oxidase. Furthermore, kaempferol modulated RAGE and PKCβII expression via NADPH oxidase. Taken together, the results in this study indicate that kaempferol caused the decrease of the enhanced AGEs with age through RAGE signaling via PKCβII pathway and kaempferol also directly suppressed non-enzymatic glycation.

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Inner ear hair cell generation via stepwise differentiation of mesenchymal stem cell isolated from the human mastoid process bone marrow

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Cochlear sensory epithelia and sprial ganglion neurons in the adult mammals inner ear do not regenerate following severe injury. To replace the degenerated sensory hair cells and spiral ganglion neurons, we used bone marrow-derived mesenchymal stem cells (MSCs) for cell therapy. MSCs isolated from human mastoid process bone marrow expressed cell type specific markers for MSCs including CD29, CD44, CD90 and CD166. When grown in differentiation media, MSCs expressed high levels of neural markers, such as genes TUJ1, NF-L, NF-M. Some of them showed voltage-dependent sodium currents and delayed rectifier potassium currents. Transdifferentiated MSCs were transplanted into guinea-pig inner ear after cisplatin-induced injury. Histological finding demonstrated that numbers of cell body in the organ of Corti and spiral ganglia were increased significantly. Human specific phenotype markers including Math1, P27kip1, BMP4, BMP7, characteristic of inner ear sensory hair cells or supporting cells, were detected in transplanted inner ear. Furthermore guinea-pigs which were taken MSCs transplantation showed a significant recovery in auditory brain stem response test. These results suggest that human bone marrow-derived MSCs have the potential to restore inner ear hair cells (This work was supported by the Korea Reseach Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-311-E00379).

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Inhibitory effects of development of white adipose tissues by OB1, herbal medicine, in Wistar rats fed on a cafeteria diet.

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The object of this study was to find out whether the expression of the markers of adipose tissue and adipocyte were affected by OB-1, which was composed of 6 herbal medicines and had the strong anti-oxidant effects. For this purpose, male Wistar rats were fed with standard laboratory diet or cafeteria diets during 4 weeks and both, cafeteria and standard diet fed groups, were divided into two groups respectively, one was injected with 40mg/100g body weights of OB1 or normal saline daily for 5 weeks. After treated OB1 and saline, there were not remarkable differences of body weight gain between two groups but they showed down-regulated mRNA level of leptin, adiponectin in white adipose tissue. In these results, we could know that OB1 had anti-obesity with no toxicity. And with the difference in adipocyte markers, we studied the open field activities of rats and we have known there were significant differences. In conclusion, OB1 has inhibitory effect of adipocyte marker in diet-induced obesity rats but have no toxity in Wistar rats and we assumed that the effects were related with the energy expenditure and, maybe, anti-oxidation. The authors, in part, of this study were supported by the second stage of BK21 project from the Ministry of Education and Human Resources Development of South Korea and and This study was supported by the Seoul Research and Business Development Program (10524)

Poster Session