kidney, duodenum of non-pregnant female mice. The placental TRPV5, 6, PMCA1 and CaBP-9k were investigated by realtime PCR. And all experimental data was presented mean ± standard error of the mean (S.E.M.); p values were calculated using one-way analysis of variance.

Results: Placental TRPV5, TRPV6 and PMCA1 expressions were down-regulated by COMT inhibitor (ro41-0960). In addition, the reduced PMCA1 expression in the placenta was reversed by calcium supplementation. Duodenal expressions of TRPV5, TRPV6, and PMCA1 were decreased in the COMT-inhibited mice, and slightly recovered after calcium supplementation. Renal expression of TRPV5, TRPV6, and PMCA1 was also decreased by COMT inhibition, while it was reversed by calcium supplementation to the level of control. Duodenal and renal calcium transporting genes, TRPV5, TPRV6, PMCA1 and CaBP-9k, were down-regulated by COMT treatment in female mice.

Conclusions: Taken together, these results indicate that physiological changes observed in COMT inhibition were showed similar symptom to preeclampsia, which may be related with disturbance of calcium metabolism during pregnancy.

References

[1] Yang H, Kim TH, Lee HH, Choi KC, Jeung EB. Distinct expression of the calcium exchangers, NCKX3 nd NCX1, and their regulation by steroid in the human endometrium during the menstrual cycle. ReprodSci 2011; 18: 577-585.

[2] Koo TH, Yang H, An BS, Choi KC, Hyun SH, Jeung EB. Calcium transport genes are differently regulated in maternal and fetal placenta in the knockout mice of calbindin-D(9k) and -D(28k). MolReprod Dev 2012.

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Tumorigenic Characterization of Human Breast Cancer Cell in *in vitro* and Nude Mouse

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Introduction: Human breast tumors are genetically heterogeneous, consisting of phenotypically diverse cells. A small subpopulation of cancer cells, referred to as cancer stem cells (CSC), which have characteristics of tumors initiation with as few as small amount of cells, self-renewal and differentiation. The aim of this study is to characterize tumorigenicity human breast cancer cells (BT-474) in *in vitro* and nude mouse.

Materials and Methods: To select cancer stem cells, BT-474 cells were seeded in 100 mm dish at density of 1.0×10^5 cells/plate with serum free condition, and then, $CD24^{-/low}CD44^+$ cells were purified by MACS using CD24 and CD44 antibody. $CD24^{-/low}CD44^+$ cells and tumorsphere-forming colonies from BT-474 were used to compare the cancer specific gene, protein expression and tumor producing ability in nude mouse. The gene expression of Oct4, Sox2, c-Myc, Klf4 and Nanog were measured by qRT-PCR in $CD24^{-/low}CD44^+$ cells, tumorsphere and BT-474 cells. The protein expression of CD24 and CD44 were determined by immunofluorescence staining. For evaluating tumor formation ability, tumorsphere (1.5×10^4 cells/100㎕) and $CD24^{-/low}CD44^+$ cells (1.7×10^4 cells/100㎕) were injected subcutaneously in mammary fat pad in 7-weeks-old female BALB/c nude mice.

Results: In the culture condition of serum free and suspension, the tumorsphere colonies were formed at 7 days of culture from BT-474 cells. The expression of stem cell marker Oct4 was 1.7 times increased in the tumorspheres. C-Myc and Klf4 were significantly (2.0 and 2.7 times) increased for $CD24^{-/low}CD44^+$ cells, respectively, but Oct4 was decreased (0.2 times) in $CD24^{-/low}CD44^+$ cells, whereas Sox2 was significantly decreased in both $CD24^{-/low}CD44^+$ cells (0.5 times) and the tumorspheres (0.3 times). Regarding to tumor forming capacity of two types of cell in BALB/c nude mice, 3/5 mouse appeared tumor mass at inject site by tumorsphere injection, whereas none of five mice appeared tumor mass in $CD24^{-/low}CD44^+$ cells injected mice, even though all mice did not show any clinical signs, during three weeks of injection.

Conclusions: In the present study, cancer specific gene and protein expressions were different between tumoshpere and $CD24^{-/low}CD44^+$ cells derived from BT-474 cells. Furthermore, tumorshpere derived from cancer cell effectively produced tumor mass in female BALB/c nude mice.

References

[1] Masaki M. et al., characterization of spheres derived from canine mammary gland adenocarcinoma cell lines. Research in Veterinary Science 91 (2011) 254-260.

[2] Gulnur G. et al., Stem cell-related markers in primary breast cancers and associated metastatic lesions. Modern Pathology (2012) 25, 949-955.

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Morphological Study of *Corynosoma strumosum* (Acanthocephala) from Harbour Seal, *Phoca vitulina*, in East Sea

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Introduction: The phylum Acanthocephala is a parasitic worms known as acanthocephalans, thorny-headed worms, or spiny-headed worms, characterized by the presence of an evitable proboscis, armed with spines. Acanthocephalans are common in seal intestines and found occasionally in dolphins and whales. Acanthocephalans have complex life cycles, involving at least two hosts, which may include invertebrates, fishes, amphibians, birds, and mammals. *Corynosoma* from seals, and occasionally dolphins, and *Bolbosoma* from whales are typical examples and, with one exception (*Echinorhynchus*) the only two genera represented in marine mammals. The definitive host of *Corynosoma strumosum* Luhe, 1904 is known to seals and other marine mammals. This acanthocephala can even develop to fully mature adults in warm blooded animals such as rats. Larvae develop in amphipods and many species of fish serve as paratenic hosts. Juveniles are commonly found in erratic hosts such as mammals including birds, human, and other fish eating animals. This paper shows morphological features of *C. strumosum* by scanning electron microscope (SEM) from a harbor seal in East Sea.

Materials and Methods: In this study, over 50 specimens of acanthocephalanwere collected in the intestine of harbour seal, *Phoca vitulina*. The worms were tentatively identified under the light microscope, and then the worms were precisely classified by SEM) For the finding of inner organs, the worms were placed in the lacto-phenol solution (glycerin 20 mL, lactic acid 10 mL, phenol 10 mL, DW 10mL) for 24 hrs. Also, worms were fixed with neutral buffered formalin, serially paraffin sectioned, and H&E stained. For the SEM, parasites were washed 5 times with 0.2 M cacodylate buffer (pH 7.3) and fixed in 2.5% glutaldehyde, post fixed in 1% osmium tetroxide at 4℃. The specimens were dehydrated in a graded ethyl alcohol series, dried by CO_2 critical point, coated osmium and examined by SEM (S-4800, Hitachi) at 10-25kV.

Results: Sexual dimorphism is a little in trunk size. Body lengths are 1.52 –2.12 mm (Mean 1.88 mm). Trunk is small, less than 5.00 mm long, bulboid anteriorly and cylindrical with parallel sides posteriorly. Sexual dimorphism is a little in trunk size. Two types of proboscis (inverted and everted) were found, and the everted proboscis bent ventrally, with 16–18 (usually 16) longitudinal rows of 9–10 (rarely 11) hooks each. Hard thin outer layer of hooks porous enveloping inner medullary core. Dorsal hooks relatively smaller than ventral hooks. Apical end of proboscis bare. Anterior hooks small slender, only slightly longer but more robust posteriorly up to hook no. 5. Hook no. 7-8 invariably longest and most robust. Posterior 4 hooks smallest becoming progressively and relatively smaller and more slender more posteriorly. Hooks 7-8 more widely spaced. Roots of anterior 6 hooks simple, slightly longer than blades. Roots of posterior hooks short, small, stubby. Lemnisci equal, slightly shorter than double walled proboscis receptacle; receptacle longer than proboscis. Gonopore terminal in both sexes.

Conclusions: This is the first report of *Corynosoma strumosum* from the Republic of Korea.

References

[1] Schmidt J.D. 1971. Acanthocephalan infections of man, with two new records. *Journal of Parasitology*, 57, 582–584.

[2] Yamaguti S. 1963. Systema helminthum, vol. 5 Interscience Publishers, Acanthocephala. New York.

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Overexpression of 11β-HSD1 Induces Dysfunction in Energy Balance

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Introduction: **:** 11β-hydroxysteroid dehydrogenase type 1 (11 β-HSD1) effectively amplifies glucocorticoid action in liver, adipose tissue, and brain. This enzyme converts from inactive 11-keto form to glucocorticoid (GC). GCs are lipophilic and readily access their intracellular receptors. GCs regulate carbohydrate, fat and protein metabolism.

Materials and Methods: In a previous study, we constructed vector composed of two parts; the 11β-HSD1 expression cassette and the selection cassette containing EGFP and Neo resistant genes. Based on transgenic fibroblasts overexpressing 11β-HSD1, transgenic piglets came into being without obesity through somatic cell nuclear transfer (SCNT) and re-cloning method, which use the somatic cells derived from stillborn TG piglets.

Results: **:** Six live piglets, one stillborn piglet and three mummies were born. Integration of target gene into the genomic DNA was confirmed from all of them. But, all of six live piglets died within one month, which were showed the hypoglycemia. Increased expression of 11β-HSD1 in metabolic tissues induced up-regulation of gluconeogenesis related genes in liver and kidney, and showed up-regulation of lipogenesis related genes in muscle. Also, it stimulated AMPK and SIRT signaling which controls energy balance and mitochondrial biogenesis.

Conclusions: We propose that overexpression of 11β-HSD1 evokes the excess production and action of glucocorticoid or its receptors, and activates gluconeogenic and lipogenic pathways. For this reason, AMPK and SIRT1 signaling was induced. Also, to compensate energy loss by anabolic process, the expression of mitochondrial biogenesis-related genes was increased.

References

[1] Jung EM, An BS, Kim YK, Jeong YH, Hwang WS, Jeung EB. Generation of porcine fibroblasts overexpressing 11β -HSD1 with adipose tissue-specific aP2 promoter as a porcine model of metabolic syndrome. Molecular medicine reports 2013; 8: 751-756

[2] Sugden MC, Caton PW, Holness MJ. PPAR control: it's SIRTainly as easy as PGC. J Endocrinol. 2010 ; 204(2): 93-104