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생쥐 정소에서 Cannabinoid 수용체의 발현

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Cannabinoid 수용체는 뇌에서 주로 발현되는 CB1 receptor와 면역계에서 주로 발현되는 CB2 receptor 2가지가 있으며 7개의 막관통부위를 갖는 전형적인 GPCR이다. CB1 receptor는 G(i/o)-proteins과 결합하여 G(i/o)-coupled receptor를 통한 신호전달을 억제할 뿐만 아니라 Na+/H+ exchanger를 활성화시키며, N-type Ca++ channel을 조절한다. CB1을 경유한 만성적인 agonist 처리 시adenylate cyclase (AC)활성이 증가하는 AC superactivation이 관찰되며 마약중독에 관련된다. 한편 생체 내에 존재하는 anandamide는 CB1 수용체와 결합하여 남성생식기능에 조절 작용을 한다. 본 연구에서는 생쥐에서 출생 후 성체에 이르는 동안 정소 내 CB1 발현의 변동을 조사하였다. 1, 2, 4, 8주령의 정소로부터 RT-PCR 및 real time PCR 법으로 CB1 mRNA의 발현을 분석한 결과 1주령에서는 발현되지 않았고 2주령에서는 미량이 발현되기 시작하였고, 4주령부터 발현량이 급격히 증가하였고 성체에서는 다량으로 발현되었다. Semi-quantitative RT-PCR 법과 real time PCR 법을 비교할 때 주령별 발현 양상은 유사하였으나 4주령과 성체에서는 두 시험법 사이에 양적 차이가 있었다. 면역조직화학염색 결과 Leydig cell에서 강한 발현이 확인되었고 Sertoli cell 및 germ cell에서도 미약한 신호를 검출하였다. Western blot 상에서 분자량 60 및 53 kDa의 항원이 검출되었으며 발현 양상은 mRNA와 유사하였다. 따라서 정소에서 발현되는 CB1의 경우 post-translation 수준의 변형이 수반되는 것으로 사료되며 이러한 생화학적 변화와 CB1의 기능과의 연관성은 추후 연구되어야 할 것이다.

E201

Isolation of Callus Specific mRNAs from Differentiating Embryogenic Somatic Calli in *Pimpinella brachycarpa* by cDNA-AFLP

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cDNA-AFLP was carried out using toisolate transcribed-derived transcripts from genes that express differentially in three different developmental stages (callus, somatic embryo, plant) during somatic embryogenesis in *Pimpinella brachycarpa*. High polymorphism was observed in the AFLP patterns and the fragments in callus stage specific were isolated from the gel to check the stage specificity. Of the 478 callus specific fragments, 71 fragments showed hybridization signals with the callus cDNA probe and the nucleotide sequences of these 71 fragments were determined and searched DNA homology in the GenBank database entries. Thirty-three fragments showed significant homology (BLAST expectation value [E]of 10-5) with the database entries. Twelve of the 33 showed sequence showed homology with the database entries having known functions as ATPase, tubulin like proteins, kinase like proteins, transcription factors, ubiquitin-specific protease, ankyrin like protein. In the RT-PCR analysis of these fragments, they were expressed highly abundantly in callus stage. However, the expression level in other stages were no in somatic embryo and very low in plant stage. The expression profiles and significance of the expression are presented in the presentation.

E701

Double Mutations for Polycomb Group Genes *XYY1* and *Xbmi-1* by RNAi Reveal Dual Control in Early Development of Nervous System in *Xenopus laevis*.

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The *Polycomb group* (PcG) genes have been identified that are necessary for transcriptional repression of *Hox* (homeobox) expression in *Drosophila* and in vertebrate. Also several studies suggested that the Polycomb group proteins probably operate as multimeric complexes that bind to chromatin. Human PcG protein complexes were identified two complexes, the EED-EZH protein complex and the HPC-HPH PcG complex. The EED-EZH protein complex contains EED protein which interacts specificailly with with YY1. The HPC-HPH PcG complex contains the HPC, HPH, BMI1, and RING1 PcG proteins. To understand molecular mechanisms of stable repression of gene activity in vertebrates we have studied *Polycomb group* (PcG) genes in *Xenopus laevis*. A vertebrate DNA binding protein and transcriptional repressor, *XYY1*, shows sequence homology with a Drosophila PcG protein, *Pleiohomeotic (PHO)*. And *Xbmi-1* is a proto-oncogene which has sequence homology with *Posterior Sex Combs* in *Drosophila*. The expression patterns of both *XYY1* and *Xbmi-1* during early development of *Xenopus laevis*were partially ovelapped in hind brain and spinal cord. These results have raised strong possibility that *XYY1* and *Xbmi-1* dually control not only A/P axis patterning but also in development of anterior nervous system. We will show double *loss-of-function*mutations that were produced by RNAi (RNA interference) microinjection for *XYY1* and *Xbmi-1* .

E702

Xhex* Regulates Hepatic Bud Outgrowth and Development in *Xenopus laevis

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The liver has been considered a 'mystery organ' because of the paucity of genetic data regarding its development. *Hex* is one of the important genes in liver development. In Xenopus *Hex* (*Xhex*) encodes a homeodomain transcription factor. *Xhex*is beginning to be expressed in the endomesoderm of the prospective dorsal sideduring gastrulation (stage 10.5), and then specifically expressed in the anterior endoderm and liver diverticulum after early neurulation (stage 16). In the gut of tadpole, strong *Xhex*expression is maintained in the liver and gall bladder. To determine whether *Xhex*is required for Xenopus liver development, a knockout experiment was carried out in the embryo via the injection of *Xhex* morpholino antisense oligonucleotides (Xhex-MO). Xhex-MO was injected into both sides of dorsal-vegetal blastomeres at 16-cell stage embryos. *FOR*[Farnesoid X receptor (*FXR*) like Orphan Receptor] was used as a molecular marker for embryonic liver formation. The results showed that *FOR* expression decreased in Xhex-MO (20ng or 40ng) injected embryos at stage 35, indicating that Xhex-MO inhibited liver formation. Furthermore, cross sections of Xhex-MO-injected embryos revealed that gut coiling and liver formation were severely inhibited. These results suggest that *Xhex* plays an important role in the liver development, especially in the liver-bud outgrowth.