

D101 Transforming growth factor- β s differentially regulate chondrogenesis of mesenchymal cells from chick wing and leg bud

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Transforming growth factor- β (TGF- β) can inhibit or stimulate cell proliferation, promote extracellular matrix formation, regulate cell differentiation. In the present study, TGF- β s inhibited and promoted cell proliferation and chondrogenesis of leg and wing bud mesenchymes, respectively. During regulation of chondrogenesis, TGF- β s affected expression and/or phosphorylation of N-cadherin and β -catenin, indicating stabilization of the N-cadherin/ β -catenin cell adhesion complex at the plasma membrane in leg bud mesenchymes. On the other hand, tyrosine phosphorylation and expression of FAK and paxillin were dramatically diminished by treatment TGF- β s, which indicates unstableness of focal adhesion complex in leg bud cells. These alterations in leg bud mesenchymes by TGF- β s treatment were opposite in wing bud mesenchyme cells: decreased N-cadherin/ β -catenin and FAK/paxillin complex. Collectively, these data suggest that TGF- β s can mediate opposite signaling pathways in wing and leg bud mesenchyme cells which helps to distinguish the wing and leg cell fates in chick limb.

D102 High dose of glucose promotes chondrogenic differentiation via PKC- δ signaling pathway in chick limb buds mesenchymes

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Exposure to 25 mM glucose induced an

increase in cellular condensation and nodule size of in vitro micromass cultured limb bud mesenchymal cells, which results in stimulation of chondrogenic differentiation up to 2-2.5 fold. However, cell proliferation of those cells was not changed. Cell condensations are the initial structures in the formation of proper cartilage and skeletal patterning in the developing vertebrate limb. As chondrogenesis proceeded, expression of N-cadherin is reduced whereas expressions of adhesion molecules such as fibronectin and 51 integrin are transiently increased. Exposure to 25 mM glucose increased protein levels of PKC- δ and p70S6K but decreased protein level of N-cadherin, fibronectin and 51 integrin, and phosphorylation of ERK at a later period of chondrogenesis. These alterations in adhesion molecules and PKC- δ were significantly blocked by PKC inhibitor, PMA. Co-treatment of glucose and PMA remarkably decreased cellular condensation and thus significantly inhibited glucose-induced chondrogenesis. Taken together, our data indicate that high dose glucose promotes cellular condensation by modulating expression of adhesion molecules via PKC- δ signaling pathway.

D103 Disruption of Actin Organization During Apoptosis is Coupled to a Caspase-dependent Cleavage of Tensin

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Integrin-associated focal adhesion complexes provide the main adhesive links between the cellular actin cytoskeleton and the surrounding extracellular matrix. Recent studies indicate that members of both calpain and caspase protease families can degrade several components of focal adhesions leading to disassembly of these complexes. In this report, we investigated

the disappearance of tensin from the cell adhesion sites of Chicken embryonic fibroblasts (CEFs) exposed to etoposide, coincident with the disruption of actin cytoskeleton and morphological change during apoptosis. Furthermore, tensin was identified as a new substrate for caspase-3. Tensin cleavage is occurred by caspase-3 at DYPD1226G sequence during etoposide-induced apoptosis in vivo and after caspase-3 treatment in vitro and removes the actin binding domains and SH2 domain of the protein. The resulting tensin product is unable to bind phosphoinositide 3-kinase (PI 3-kinase) that is responsible for cell survival signaling. We also showed that expression of amino-terminal tensin fragment containing actin-binding regions induced disruption of actin cytoskeleton in CEFs. Thus, these results suggest that caspase-mediated cleavage of tensin contributes to the disruption of actin organization and interrupts survival signals through PI 3-kinase from extracellular matrix.

D104 Expression of Epithin in Mouse Preimplantation Development: Functional Role in Compaction

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The present study was performed to examine the expression and function of a mouse type II membrane serine protease, epithin during mouse preimplantation development. RT-PCR analysis showed that epithin mRNA was expressed throughout the cleavage stages from 1 cell zygote to blastocyst. Immunocytochemical study revealed that epithin protein was expressed at the blastomere junction. Inhibition of epithin synthesis by double strand RNA injection into 2 pronucleus stage embryo resulted in the block of the preimplantation development at 8 cell stage just before the compaction. Epithin was colocalized with

E-cadherin at the membrane junction of the compacted morula stage embryo as revealed by double staining immunocytochemistry and confocal microscopy. Taken together, we demonstrate for the first time that epithin is expressed in mouse preimplantation embryo and might play an important role in the compaction process that precede the first differentiation into trophoectoderm and inner cell mass.

D105 Effect of Calcium Ion on mammalian period 1(*mPer1*) mRNA Expression in NIH-3T3 Cell Line

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Circadian rhythms have a periodicity of approximately 24 hours and are entrained by environmental cues among which light is the dominant regulator. In mammals, light/dark information is transmitted to suprachiasmatic nucleus *via* glutamate receptors which mediate Ca^{2+} influx. In the present study, calcium ionophore (A23187) was used as an environmental cue to examine the molecular mechanism of *mammalian period1* (*mPer1*) mRNA induction in NIH-3T3 cells in vitro. A23187 (1M) significantly increased *mPer1* mRNA levels and induction of *mPer1* mRNA expression by A23187 was blocked by actinomycin D, a transcriptional inhibitor, but not by puromycin, a protein synthesis inhibitor. These results suggest that *mPer1* mRNA is synthesized *de novo* and such *de novo* synthesis does not require any newly synthesized proteins. To examine the downstream signal mediators of Ca^{2+} effects, several specific inhibitors of protein kinase A (PKA), protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) were used. However, no notable changes in the A23187-induced *mPer1* mRNA transcription were observed by pretreatment of these inhibitors. These