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EARLY GROWTH RESPONSE 1 COORDINATES EPIGENOMIC REMODELLING AND SOX9 EXPRESSION IN ATDC5 CHONDROGENESIS

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Purpose: Cells use cues from their direct environment to adjust their physiological state. Differentiation is a process in which such environmental cues induce spatio-temporal changes in gene expression profiles; transcriptomic remodeling accompanies acquisition of a terminally differentiated, functional phenotype. Coding and non-coding genes are packed into chromatin. Chromatin structure, which is at least in part determined by epigenomic covalent modifications 'written in' histones, determines whether genes can be accessed or not. The molecular pathways that connect these environmental cues to chromatin remodelling are currently unclear. What are the signaling factors involved in the very early moments of epigenomic remodeling and, hence, differentiation?
We study chondrogenesis as a function of environmental changes and focus on epigenomic remodeling. Early growth response 1 (Egr1) is an "immediate early gene" based on its rapid induction kinetics in response to e.g. proliferative stimuli. Depending on the cellular context Egr1 promotes or inhibits proliferation. Its function in chondrogenesis is currently unknown. We hypothesize that Egr1 is a crucial regulator of early steps in chondrogenic commitment.
Methods: We use an established in vitro model for chondrogenesis, the murine mesenchymal progenitor cell line ATDC5, to study epigenetic remodeling. Using short hairpin RNAs we developed stable knockdown cell lines for proteins of interest. Expression is evaluated by qRT-PCR and immunoblotting. Protein-DNA interactions were tested by chromatin immuno precipitation (ChIP).
Results: We found that Egr1 is rapidly induced upon chondrogenic differentiation and Egr1 appears to initiate chondrogenesis through Sox9 expression. Loss of Egr1 results in strongly impaired epigenetic remodeling and chondrogenic differentiation. We also observed binding of Egr1 on many epigenomically 'open' promoters. Egr1 binding, however, did not necessarily correspond to regulation of gene expression.
Conclusions: Our data suggest a central role for Egr1 in cell fate decision: loss of Egr1 results in strongly impaired chondrogenic differentiation. The observation that Egr1 promoter binding does not always lead to transcriptional induction suggests a requirement for additional factors to induce local changes in gene expression. We speculate that Egr1 fulfills a global role e.g. in marking open chromatin, for processes that require stringent regulation during differentiation (transcriptional regulation, DNA replication and repair).

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ASSOCIATION OF METALLOPEPTIDASE DOMAIN 12 (ADAM12) GENE POLYMORPHISMS AND ADAM12 PROTEIN WITH THE DEVELOPMENT OF KNEE OSTEOARTHRITIS

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Aims: ADAM12 (disintegrin and metalloprotease) is one of the candidate genes demonstrating susceptibility to osteoarthritis. The peptide product of ADAM12 gene, the ADAM12 protein is a multidomain glycoprotein with metalloprotease, cell adhesion and signalling activities. The purpose of this study was to investigate:
i) the potential role of four single nucleotide polymorphisms (SNP) of the ADAM12 gene in susceptibility to radiographic knee osteoarthritis (KOA)
ii) the relationship between ADAM12 protein and KOA
iii) the association between different genotypes of investigated SNP's in ADAM12 gene and concentration of ADAM12.
Methods: The study group of genetic investigation consists of a total of 506 subjects (aged 32-60, average age 47.4 y.o., 66.5% females), including 357 individuals with and 149 without radiographic KOA features. The radiographs were obtained in all participants from both tibiofemoral (TF) and patellofemoral (PF) joints.
Selected SNPs of ADAM12 gene - rs3740199 (c.142G>C, p.G48R), rs1871054 (c.1154+145C>T) rs1278279 (c.1515C>T, p.N505N) and rs1044122 (c.2475T>C, p.A825A) were genotyped using TaqMan allelic discrimination technology.
The ADAM12 protein was measured in 181 subjects with radiographic KOA traits and 95 controls selected from main study group. The DELFIA1/AutoDELFIA research kit was used to measure the ADAM12 protein in serum samples.
Results: *ADAM12 gene SNP's in KOA'* We found remarkable association between an intronic variant rs1871054 and presence of osteophytes of advanced stages (stage II-III) in male patients (OR=2.51, 95%CI 1.23-5.15), the genetic risk was related to CC homozygosity (OR=4.91, 95%CI 1.30-18.53). The same SNP was also related to advanced grades of KOA in PF joint (OR=2.73, 95%CI 1.18-6.33). Near to signifficant association was found between rs1871054 and advanced KOA in TF joint (OR 2.12, 95%CI 0.99-4.54). No significant associations were observed between rs1871054 SNP and the KOA traits in females of pooled group.
SNP's rs3740199, rs1278279 and rs1044122 were not related to KOA radiographic features in investigated groups.
ADAM12 protein in KOA. In our study, the ADAM12 protein in the whole group was related to radiographic KOA grades in TF (p=0.004) as well in PF joint (p=0.003). We also found a correlation between ADAM12 protein and osteophytes in TF and/or PF joints (p=0.003). The level of ADAM12 protein was higher in the serum of patients with advanced stage of osteophytes (p = 0.0008), when compared with the controls (grade 0). Similar difference was also observed between patients with advanced grade of KOA in PF (p = 0.0004) or TF joints (p = 0.0015) and controls.
ADAM12 gene SNP's and ADAM12 protein. No relation between levels of ADAM12 protein and genotypes of investigated SNP was observed.
Conclusions: – The intronic variant rs 1871054 of ADAM12 gene seems to be associated with susceptibility to radiographic KOA in gender-dependent manner, mainly due to development of osteophytes.
– Additionally, the elavated serum level of ADAM12 protein correlates positively with the grades of the radiograhic KOA.
– Functional activity of ADAM12 protein is not determinate by investigated genetic variants (selected SNP's). Hovewer, the involvment of ADAM12 gene and it's protein in pathogenesis of osteoarthritis needs further investigation.

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GENETIC POLYMORPHISMS OF *ADAMTS14* IN SUSCEPTIBILITY TO KNEE OSTEOARTHRITIS IN A CHINESE HAN POPULATION

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Purpose: Osteoarthritis (OA) is the most common form of arthritis and the precise etiology of this disease remains unclear. Genetic factors play a considerable role in pathogenesis of OA. ADAMTS14 is a novel member of the ADAMTS (a disintegrin-like and metalloproteinase domain with thrombospondin type 1 modules) metalloproteinase family which processes extracellular matrix proteins. An nsSNP in *ADAMTS14* (rs4747096) to some osteoarthritis phenotypes was reported associated with OA susceptibility in Caucasians. In the present study we performed a comprehensive investigation of the *ADAMTS14* as a candidate gene for susceptibility to knee OA in a Chinese Han population.
Methods: A case-control association study was conducted. The five SNPs were genotyped in patients who had primary symptomatic knee OA with radiographic confirmation or received total knee replacement surgery and in matched controls. Allelic and genotypic frequencies were compared between them.
Results: A total of 751 OA patients and 766 controls were genotyped in the SNP of rs4747096, while a total of 365 OA patients and 368 controls were genotyped in other four SNPs. Two SNPs (rs4747096 and rs6480463) were significantly associated with knee OA (all *P*[[Unsupported Character - ﹤]]0.05). Significant difference was detected in male samples when stratified by gender (*P*=0.0027; OR=0.68; 95% CI=0.53-0.88). No association between the SNPs genotype and the clinical variables age, sex, BMI (body mass index) and K/L (Kellgren/Lawrence) score was observed in OA patients.
Conclusions: This study is the [[Unsupported Character - ﬁ]]rst attempt to evaluate the role of the polymorphism of OA in the Asia population. These findings suggest a potentially important role for the ADAMTS14 gene in predisposition to OA. Further studies are needed to give a global view of this polymorphism in pathogenesis of OA.