Conclusions: We conclude that nucleostemin is expressed within several localised to the nucleoli. Immunohistochemistry showed evidence of nucleostemin expression in articular cartilage. Analysis of fresh and cultured articular chondrocytes by western blotting showed that nucleostemin protein was also detected in fresh extracts of cultured chondrocytes in OA chondrocytes as compared with normal chondrocytes. Although we found that levels of protein expression were slightly raised in osteoarthritic chondrocytes as compared with normal cartilage (n=4) (0.90±0.12 vs. 0.59±0.11) although the differences were not significant (p=0.12). Nucleostemin protein was also detected by western blotting in fresh extracts of articular cartilage. Analysis of fresh and cultured articular chondrocytes by immunohistochemistry showed evidence of nucleostemin expression localised to the nucleoli.

Conclusions: We conclude that nucleostemin is expressed within several articular tissues including chondrocytes where it is localised within the nucleoli. Although we found that levels of protein expression were slightly raised in osteoarthritic chondrocytes as compared with normal chondrocytes, the number of samples studied was small and this will have to be confirmed by further research.

287 DELPHINIDIN BLOCKS IL-1β-INDUCED ACTIVATION OF NF-κB BY MODULATING THE IKKβ GENE EXPRESSION AND THE ACTIVATION OF NF-κB IN HUMAN CHONDROCYTES

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Purpose: Osteoarthritis (OA) is a highly prevalent disease that affects the quality of life of its victims. Interleukin-1β (IL-1β) plays a major role in the pathogenesis of OA and is present in the arthritic joints at high levels. IL-1β has been shown to cause increased production of catabolic enzymes such as MMPs, and inflammatory mediators such as prostaglandins and nitric oxide through the activation of several signaling pathways including NF-κB. Novel approaches using plant derived polyphenols to inhibit signal transduction pathways have shown promise and could overcome some of the toxicity issues related to current therapies. One key signaling mechanism implicated in OA could be that of a target of orally bioavailable inhibitors is the NF-κB pathway. Delphinidin (2-(3,4,5-trihydroxophenyl) chromenium-3,5,7-triol) is an anthocyanidin that is widely distributed in pigmented fruits and flowers and has been shown to possess anti-inflammatory and anti-cancerous properties. The present study was undertaken to determine the effect of Delphinidin on OA chondrocytes.

Methods: Chondrocytes were derived by enzymatic digestion of human articular cartilage (OA chondrocytes) from patients undergoing total knee arthroplasty. OA chondrocytes were treated with Delphinidin for different time points and were then stimulated with IL-1β (10ng/ml) in vitro and total RNA or cell lysate was prepared following our standard protocols. To determine the gene expression, single stranded cDNA was synthesized and the expression of target mRNAs (COX-2, MMP-13, iNOS) was quantified using TaqMan Assays. Hela cells were transfected with promoter reporter vectors and negative control vectors using the Fugene-6 reagent and the effect on promoter activity was verified by Luciferase assay. Activation of NF-κB p65 was determined by ELISA based assay.

Results: Pretreatment of chondrocytes with Delphinidin (50 μg/ml) for 2 hrs significantly inhibited the IL-1β induced expression of COX-2, iNOS and MMP-13 and the production of nitric oxide. Delphinidin also inhibited the phosphorylation of IKKβ and was also a potent suppressor of IKKβ gene and protein expression in OA chondrocytes. IKK promoter had a strong activity in HeLa cells and this activity and production of the Luciferase reporter enzyme was blocked by pre-treatment with Delphinidin. Functional pathway analyses showed that IL-1β-induced phosphorylation of NF-κB-inducing Kinase (NIK), which interacts with and activates IKKα and IKKβ which then phosphorylate IkB, was inhibited by pretreatment of OA chondrocytes with Delphinidin. In agreement with these findings, pre-treatment with Delphinidin inhibited the phosphorylation and degradation of IkB, as determined by Western immunoblotting, and the activation and the DNA binding activity of NF-κB p65 determined by a highly specific ELISA based assay.

Conclusions: Taken together, the data presented here identifies a novel mechanism of NF-κB inhibition by Delphinidin in inhibiting NIK, a kinase upstream of IKK complex in the NF-κB activation pathway. Given the important role played by IL-1β and NF-κB in OA, these results may provide important clues to develop useful pharmacological inhibitors of NF-κB for the prevention and/or treatment of OA.

288 REDIFFERENTIATION OF HUMAN ARTICULAR CHONDROCYTES IN 2D VERSUS 3D CULTURE


Purpose: For cartilage regenerative techniques human articular chondrocytes (HACs) are cultured in vitro. Unfortunately, long expansion time and multiple passaging lead to ‘dedifferentiation’. To overcome dedifferentiation, chondrocytes are ‘redifferentiated’ in 3D-culturemodels (pellets, alginate cultures etc.). However, 2D (monolayer) culture methods are less laborious and have better reproducibility. We therefore set out to compare redifferentiation characteristics of HACs in 2D and 3D systems.

Methods: HACs were expanded in monolayer from 15 independent donors. Redifferentiation in monolayer was performed at subconfluence. Identical medium conditions were used to redifferentiate cells in pellets or alginate beads. After 7 days chondrogenic marker-expression was analyzed.

Results: Monolayer redifferentiation did not support expression of Col2a1 and Sox9 mRNAs, whereas chondrocyte hypertrophy markers Col10A1, Runx2 and ALP were significantly increased. Col2a1 and Sox9 mRNA expression in HACs redifferentiated in pellets or alginate beads increased significantly, as did Col10A1 and Runx2. Suggesting that redifferentiation in monolayer does not fully support a chondrogenic phenotype. However, when corresponding protein samples were analyzed we detected a profound upregulation of Col2A1 and Sox9 protein expression in monolayer samples which was quantitatively equal to samples from 3D cultures. Upregulation of Runx2 and Col10A1 protein expression was also found in both culture systems, with the monolayer cultures inducing these markers to higher levels than 3D cultures.

Conclusions: For the first time redifferentiation capacity of HACs was compared between 2D and 3D culture techniques by gene expression as well as by protein expression. Although gene expression analyses indicate that monolayer does not support chondrogenic redifferentiation, protein expression analysis proved the opposite. Besides a fundamentally interesting contradictory observation, our data show that redifferentiation of HACs is possible in monolayer but behaves differently on the gene expression as compared to 3D culture systems. Monolayer provides a reproducible and low cell amount-demanding culturesystem better suited for molecular and genetic interference studies into chondrocyte biology.

289 IMPAIRMENT OF MITOCHONDRIAL 8-OXOGUANINE DNA GLYCOSONYLYASE (OGG1) AGAINST ACCUMULATION OF 8-OXOGUANINE IN OSTEARTHRITIC CHONDROCYTES.

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Purpose: It is well known that chondrocytes produce excess amounts of reactive oxygen species (ROS) as well as proinflammatory cytokines and chemokines in response to mechanical and chemical stresses. An oxidized form of guanine, 8-oxo-7,8-dihydroxyguanine (8-oxoguanine) is a major causative lesion for mutagenesis by ROS, since it can form a stable base pair with adenine as well as with cytosine during DNA replication.