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DISSERTATION SUMMARY

Changes of chondrocyte gene expression under inflammatory conditions

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Arthritis is a degenerative arthropathy that frequently leads to chronic pain and disability. With the aging of our population, this condition is becoming more prevalent and its treatment increasingly financially burdensome. Finding better treatments for arthritis is a major focus of medically oriented research. This disease is characterized by joint swelling and degradation of the articular cartilage matrix. If we could understand and repress these processes we will be able to give back not only the freedom of moving, but to repress the amount of the analgesic drugs.

Our aims were to map in chondrocytes the inflammatory signal transduction pathways, to compare that with another cell's analogous pathways, to determine possible targets of antirheumatic drugs. To accomplish our goals we needed a test system, which can reflect the behavior of the chondrocytes during (in vivo) inflammation, and will be sufficient to test anti-inflammatory drugs.

The commercially available SW1353 human chondrosarcoma cell line and a rat chondrosarcoma cell line (RCS, Mukhopadhyay et al. 1995) were included in our studies.

In the SW1353 human chondrosarcoma cell line gene expression has been studied by RNA-blot hybridization and RT-PCR. We used GAPD and rRNA as standards. Proinflammatory cytokines induced characteristic changes in the gene expression pattern. After 24 hr induction with interleukin-1 (IL-1) elevated level of MMP13 and MT1-MMP mRNAs was seen. These enzymes are responsible in large part for the degradation of extracellular matrix in arthritic cartilage. At the same time significant decrease of the RNAs encoding the chondrogenic master transcription factor Sox9, and the cartilage specific matrix molecule collagen type II was also observed. Transient increase of the Egr1 mRNA level was also monitored. RCS cells responded well to the inflammatory induction, too.

We followed the nuclear factor κB (NFkB) mediated changes by using SW1353 clones stably transfected with plasmids encoding NFkB-driven luciferase marker gene. We confirmed with the behavior of the transfectants, that the inflammatory symptoms inducible with IL-1, tumor necrosis factor α (TNFα), phytohemagglutinin (PHA), and phorbol 12-myristate 13-acetate (PMA) were mediated at least partly by NFkB.

Several anti-inflammatory drugs were tested in the model cell culture system. PDTC was effective only at high concentrations detrimental to the cells. In some other cases the compounds were effective only at special conditions. Some of the observed discrepancies can be attributed to the difference of the cell line, culture conditions and the proinflammatory agents used in our laboratory and published in the literature. Diacerhein and CTP-N-TPC (Sullivan et al. 1998) in our hands were able to inhibit the effect of PMA and PHA in serum free conditions, but had no effect in case of the induction with IL-1 in SW1353 clones, in contrast to the RCS clones where diacerhein reduced the effect of IL-1 in the presence of serum.

We produced primary cultures of articular chondrocytes and synovial fibroblasts to compare those with the permanent cell lines.

In conclusion the proinflammatory drugs we tested on these two cell lines were able to induce the inflammatory pathways, the luciferase assay supported that NFkB-mediated signal transduction takes part in this induction, and we could make appropriate circumstances in which the anti-inflammatory drugs are effective and are useable to further tests.

References

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