and osteoarthritic cartilage samples (total knee replacement surgery) were
Material and methods: RNA isolation: Normal human knee cartilage
tissue was collected from healthy donors during total knee replacement
surgery. After harvesting, samples were immediately frozen and kept at
cerebral level. All measurements were done in duplicate, and the results
were compared to control group. Each experiment included negative and
positive controls. Matrix Metalloproteinases (MMPs): The by far strongest
were found for MMP-3 and it appears to be upregulated very early in the
degeneration process (p<0.05), whereas it was very significantly down-
regulated in the late stage specimens (p<0.01). This indicates that in late
stages of cartilage degeneration, other degradation pathways might be more
prominent involving other enzymes such as MMP 2 and MMP11. Both are
upregulated in late stage disease (MMP-2: p<0.001, MMP-11: p<0.002).
MT1-MMP (MMP-14) displayed rather constant expression levels.
Collagenases: No MMP-1 (collagenase) was expressed in cartilage RNA,
although signals could be detected in cultured chondrocytes in vitro (own
unpublished results). MMP-13 showed no detectable expression levels in
normal articular chondrocytes in line with the virtually non-existent collagen
turnover in normal articular cartilage. In late stage OA cartilage, a significant
increase of MMP-13 expression was detectable (p<0.05), suggesting that this
element might be involved in terminal breakdown of collagen fibres in the
late stages of OA. In contrast, early stages appear to be more characterised by
degradation of other matrix components such as aggrecan and other non-
collagenous matrix components. MMP-2, an enzyme known to be able to
degradation denatured collagens (=gelatins), is significantly upregulated in late
stage OA cartilage (p<0.001). It is hardly or not detectable in normal and early
degenerated tissue in line with the lack of significant amounts of its substrate,
wheras in late OA stages a significant proportion of all collagen shows various
extents of denaturation after having been cleaved by enzymes like MMP-13,
also predominantly expressed under this condition.
Other Genes: Another interesting gene was chitinase, which showed a high
and significant increase in early stage degenerative lesions (15x; p<0.02).
Thus chitinase might be a sensitive marker of very early degenerative
lesions, although this requires further investigation.
Discussion: This is the first report on the use of cDNA technology in order to identify
differential gene expression patterns in osteoarthritic (late and early stages)
compared to normal articular cartilage. A high number of spotted genes were
negative, which can be taken as good evidence for the specificity of the
revealed signals. On the other side many of the genes known to be expressed
in normal articular cartilage showed significant expression levels on the arrays
confirming the power of the method to pick up at least reasonably expressed
genes. Some of the genes which we expected to detect did not give significant
signals indicating some lack of sensitivity of cDNA-technology compared e.g. to
PCR-based techniques.
Overall, cDNA technology offers for cartilage research a powerful tool in
order to investigate gene expression pattern based on a high number of
different genes simultaneously.