Oncogene expression in the peri-articular osteophytes

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Summary

**Objective:** The aim of this study was to ascertain the proliferative and probably reparative potentials of the peri-articular osteophytes by evaluating the sites of expression of c-myc, c-jun and c-fos oncogenes in this neoplastic repair tissue.

**Materials and Methods:** Sections of osteophytes were obtained from knees of patients undergoing total knee arthroplasty for osteoarthritis. Decalcified sections of osteophytes were stained for c-myc and c-jun oncogenes using the avidin HRP technique. Sections of breast carcinoma were used as positive controls. Undecalcified or frozen sections of osteophytes were stained for c-fos oncogene using the avidin alkaline phosphatase technique. Sections of the human skin were used as positive control. For both techniques, sections of normal articular cartilage were used as negative controls.

**Results:** The chondrocytes of the entire cartilage mantle of the peri-articular osteophyte had positive staining for c-myc oncogene but no staining for c-jun oncogene. The basal chondrocytes of the deep layer of the cartilage mantle of the peri-articular osteophyte had positive staining for c-fos oncogene. The normal articular cartilage sections had no staining for any of the oncogenes evaluated.

**Conclusion:** The expression of c-myc oncogene in the osteoarthritic chondrocytes suggests that these cells are actively proliferating. However, c-fos expression in the basal chondrocytes implies that these cells are capable of transformation. This result confirms the proliferative ability of the peri-articular osteophytes and this may suggest that this osteochondral repair tissue, which is apparently wrongly sited, may be a source of tissue for osteochondral grafting for full thickness articular cartilage defects.

**Keywords:** Osteophyte, Osteoarthritis, Oncogenes, Osteochondral graft.

Résumé

**Objectif:** L'objet de cette étude est de décider des possibilités de réparation probable et prolifération de l'ostéophyte péri-articulaire à travers l'évaluation des sièges de l'expression de l' oncogènes c-myc, c-jun et c-fos dans cette réparation néoplasie d'un tissu.

**Matériaux et Méthodes:** Parties des ostéophytes ont été obtenues à partir des genoux des patients qui suivent un traitement d'arthroplastie complexe du genou pour l'ostéopathie. Les parties décalcifiées d'ostéophytes ont été tachées pour l' oncogènes c-myc et c-jun avec l'utilisation de la technique HRP avidin. Des parties du carcinome du sein ont été utilisés comme des contrôles positifs. Les parties

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Expression of c-myc has been shown to drive quiescent cells into the cell cycle and at the same time block cell differentiation. C-jun and c-fos belong to the bZIP (basic/leucine zipper) super family of transcription factors. C-fos is involved in the determination of cell differentiation along the osteoclast/macroage lineage and it is critical for bone remodelling. However, c-jun on the other hand is a relatively weak inducer of transformation and in general, normal mammalian cells (including humans) are resistant to transformation by c-jun.

The aim of this study was to localise the site(s) of expression of c-myc, c-jun and c-fos oncogenes in periarticular osteophytes with a view to ascertaining the proliferative ability or otherwise of this repair tissue.

Materials and method

Osteophytes were obtained from the margins of the femoral condyles in 6 patients aged 66-88 years undergoing total knee replacement for osteoarthritis (OA). Visually normal articular cartilage was obtained from the compartments not affected by the disease (for example posterior femoral condylar cuts) in another 6 patients undergoing total knee replacement for OA.

Decalcified specimens

One set of specimens was formalin fixed, decalcified, routinely processed and embedded in paraffin. 7μm sections were obtained and stained for c-myc and c-jun oncogenes using the avidin HRP immunohistochemical technique.

Undecalcified or Frozen specimens

Another set of specimens from the same patients were embedded in Cryo-M-Bed (Bright) on a piece of cork and snap frozen in liquid nitrogen. Thereafter, 7μm sections were cut using the Bright cryostat model with a blade specifically designed for skeletal tissue. Sections were stained for c-fos using the avidin alkaline phosphatase immunohistochemical technique.

Staining technique. Avidin HRP technique for c-myc and c-jun.

Sections were de-waxed through graded alcohol and xylene, taken to water and dipped in 6% hydrogen peroxide (to block endogenous alkaline phosphatase) for 10 minutes. Thereafter they were washed in water and PBS (pH 7.6). Sections were now floated with normal goat serum at 1:20 dilution in tris-buffered saline (TBS) for 10 minutes and excess buffer drained but not washed. Primary antibody was added to labelled slides (100μl/slide): mouse anti-myc (Novoceastra Lab. Ltd. Newcastle-upon-Tyne, UK) primary antibody at 1:150 dilution in TBS and mouse anti-jun (Novoceastra Lab. Ltd. Newcastle-upon-Tyne, UK) primary antibody at 1:20 dilution in TBS. The primary antibody was allowed to incubate for 60 minutes before washing the sections in phosphate buffered saline (PBS) for 20 minutes. Biotinylated goat universal anti-mouse secondary antibody (DAKO Ltd. High Wycombe, UK) at 1:200 dilution was then added to the sections and left to incubate for 30 minutes. Thereafter, the sections were washed in PBS for 20 minutes before adding the ABC complex horse radish peroxidase (HRP) (DAKO Ltd. High Wycombe, UK) for 30 minutes. The sections were then washed in PBS for a further 30 minutes before adding 0.05% DAB (BDH) solution (Sigma, Poole, UK) for 5 minutes. Sections were then washed in tap water for 5 minutes and the nuclei were counterstained with haematoxylin for 30 seconds before rinsing again in running tap water. The sections were now dehydrated through graded alcohol and xylene, mounted and examined with the light microscope.

Avidin alkaline phosphatase technique for c-fos.

Sections were brought to room temperature, washed in water for 2 minutes and flooded with TBS buffer for 2 minutes. Excess buffer was wiped from the slides and the sections flooded with normal rabbit serum in TBS at 1:20 dilution (DAKO Ltd. High Wycombe, UK) for 10 minutes. Excess buffer was drained and mouse anti-fos primary antibody (100μl/slide) was added to the sections at 1:20 dilution in TBS (Novoceastra Lab. Ltd. Newcastle-upon-Tyne, UK). The primary antibody was allowed to incubate for 60 minutes and thereafter, the sections were washed in TBS for 20 minutes. Biotinylated rabbit anti-mouse secondary antibody was now added to the sections at 1:400 dilution in TBS (DAKO Ltd. High Wycombe, UK) for 20 minutes after which the sections were washed with TBS for 20 minutes. Avidin conjugated alkaline phosphatase (ACAP) at 1:400 dilution in TBS (DAKO Ltd. High Wycombe, UK) was now added to the sections and allowed to incubate for 30 minutes. The sections were then washed in TBS for a further 20 minutes before immersing the sections in a developer made up of: Levamisole (24mg), Fast Red TR (50mg), Veronal acetate buffer (VAB) pH 9.2 (100ml) and Naphthol as B1 PO4 (50mg) for 30 minutes. Thereafter, the sections were washed in water for 5 minutes, counterstained with haematoxylin for 30 seconds, washed again in running tap water, mounted and examined with a light microscope.

Result

C-myc.

Sections of breast carcinoma (positive control) had brownish cellular staining. Positive staining was observed in the chondrocytes in the entire cartilage mantle of the osteophyte sections (Figure 1). Positive staining was also observed in the vascular endothelium and the endosteal lining of the subchondral bone of the osteophytic sections. There was no staining in the chondrocytes or the subchondral bone of the visually normal articular cartilage (negative controls).

C-jun.

Sections of breast carcinoma were also used as positive controls and these sections had brownish cellular staining (positive control). The chondrocytes of the osteophyte and those of the normal articular cartilage did not stain for c-jun either in the cartilage mantle or the subchondral bone.

C-fos.

Sections of the human skin used as controls for c-fos had a reddish cellular staining (positive control). The chondrocytes at the upper and middle layers of the cartilage mantle of the osteophyte sections had negative staining.
Fig. 1a  C-myc expression in the chondrocytes of the cartilage mantle of osteophytes (superficial and middle zones). ▲ Mag x 100.

Fig. 1b  C-myc expression in the chondrocytes of the cartilage mantle of osteophytes (deep zone including the subchondral bone). ▲ Mag x 100.

Fig. 2  C-fos expression in the chondrocytes at the basal region of the deep layer of the cartilage mantle of osteophyte. ▲ Mag. x 100.

However, the basal chondrocytes of the deep layer of the cartilage mantle of the osteophytic sections had intense positive staining (Figure 2). The vascular endothelial lining and the endosteal lining of the cancellous subchondral bone also had positive staining. The chondrocytes in the entire cartilage mantle of the visually normal articular cartilage had a negative staining for c-fos.

Discussion

From this study, the expression of c-myc oncogenes by the chondrocytes in the entire cartilage mantle of osteophyte suggests that active proliferation is apparent in these cells. The lack of expression of c-myc in the chondrocytes of the visually normal articular cartilage suggests that these cells are propelled towards terminal differentiation. This may suggest that the metabolic activities of osteophytic chondrocytes are higher than those of normal articular cartilage and it might be appropriate to view osteophytes as repair tissues.

The poorly regulated cellular localisation of c-jun compared to c-fos may explain the lack of expression of c-jun oncogene in both the osteophytic tissues and the normal articular cartilage.

The cellular localization of c-fos is well regulated and the expression of this oncogene is found and retained in the nuclei of quiescent or non-active proliferative cells. C-fos has also been shown to aid the transformation of fibroblasts into osteogenic and chondrogenic lineage. The expression of c-fos by the chondrocytes at the basal region of the deep layer of the cartilage mantle of osteophytes where the cartilage is continuous with bone strongly suggests that this oncogene has been switched on in osteophytic tissues and that these cells are capable of transforming into bone.

This result shows that osteophytes are truly reparative tissues and that the chondrocytes in the various layers of the cartilage mantle have different peculiarities similar to what is observed in the normal articular cartilage.
Although the cartilage mantle of osteophytes are thinner than that of the normal articular cartilage, the cartilage mantle of osteophytes may be capable of undergoing adaptive hypertrophy if osteophytes were to be used as osteochondral grafts for full thickness articular cartilage defect. The ability of the c-fos expressing basal osteophytic chondrocyte to transform into bone along with the proliferative ability of the remaining chondrocytes may further aid good integration of the osteophytic osteochondral graft into the recipient bed. The proliferative potential of the chondrocytes may also make osteophytes an alternate source of abundant chondrocyte for autologous chondrocyte implantation when osteophyte is found in conjunction with full thickness articular cartilage defect.

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