CATHEPSIN K EXPRESSION BY ACTIVATED FIBROBLASTS AT THE BONE INTERFACE OF THE PSEUDOSYNOVIUM IN ASEPTIC PROSTHESIS LOOSENING

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Introduction The potential role of fibroblasts in aseptic loosening of total hip arthroplasties has only recently been studied, and is a controversial topic. The popular view of osteolytic destruction is that macrophages become activated through phagocytosis of particles and release cytokines that cause bone resorption. Fibroblasts, which compose the majority of cells in the pseudosynovial membrane[1], also have the potential to be both phagocytotic and destructive *in vitro*. However, their possible role in loosening of total hip arthroplasty is often overlooked. Recently we demonstrated that fibroblasts with a "transformed like" appearance and VCAM-1 positive labeling are present at the bone interface of failed cemented prostheses[2]. These fibroblasts are similar to those seen at the destructive interface of the rheumatoid synovium[3]. In rheumatoid arthritis, these fibroblasts have been observed producing cathepsin K[4] and are considered activated in view of their morphology and expression of VCAM-1 and several oncogenes[5]. This study investigated the potential for activated fibroblasts at the bone pseudosynovium interface to produce matrix-degrading enzymes.

Methods Specimens of pseudosynovial membrane were collected from 8 patients undergoing revision surgery for aseptically loosened, cemented prostheses with osteolysis. Pieces of the bone/membrane interface from each patient were snap frozen in isopentane cooled with liquid nitrogen. Serial 6µm sections were cut on a cryostat and labeled with antibodies specific for macrophages (CD68 (EBM11 clone)) and fibroblasts (prolyl-4 hydroxylase, 5B5 clone). Cathepsin K expression was investigated using a polyclonal antibody. Labeling was performed on serial sections using standard PAP for cathepsin K and indirect fluorescent (FITC) immunohistochemistry for CD68 and 5B5. Substitution of the monoclonal antibodies with 0.1% BSA in PBS and isotype immunoglobulins served as negative controls. *In situ* hybridisation was performed using an anti-digoxigenin technique as described previously using sense and anti-sense probes against cathepsin K (position 589 - 1014 of published sequence, GenBank accession-number S79895).

Results The immunohistochemistry results concurred with our earlier results[2] i.e. macrophages were found away from the bone pseudosynovium interface (Figure 1-D), with the predominant cell type being 5B5 positive fibroblasts (Figure 1-A). Cathepsin K expression was evident in the all samples examined by immunohistochemistry and *in situ* hybridisation. The signal visualised by *in situ* hybridisation was present in cells throughout the cryostat sections, with an apparent increase in signal towards the bone pseudosynovium interface (Figure 1-E & 1-F). The cathepsin K immunohistochemical labeling indicated that the only cells producing the protease were 5B5 positive cells at the interface (Figure 1-A & 1-B).

Discussion Earlier studies have demonstrated cathepsin K expression by macrophages[4] and osteoclasts[6]. Previously, we demonstrated the similarity of the fibroblasts seen at the bone interface of the pseudosynovium to the destructive interface in the rheumatoid synovium, i.e. the activated phenotype with an enlarged nucleus with multiple nucleoli and increased cytoplasmic area[2,3]. These activated fibroblasts have been shown, in rheumatoid synovium, to play an active role in matrix degredation[5]. The expression of cathepsin K, a major osteoclastic protease, by fibroblasts at the bone interface of failed cemented prostheses, further demonstrates the similarities between the activated fibroblasts seen in the two pathological situations. In addition, it demonstrates the potential these fibroblasts have to produce proteases that can degrade the extracellular matrix. This observation indicates that tissue fibroblasts seen in the pseudosynovium of failed cemented prostheses may play a more important role in the osteolytic process than previously thought. Whilst other cell types such as macrophages and giant cells produce matrix degrading enzymes, it is likely that activated fibroblasts will also contribute to the bone loss seen in prosthesis loosening.

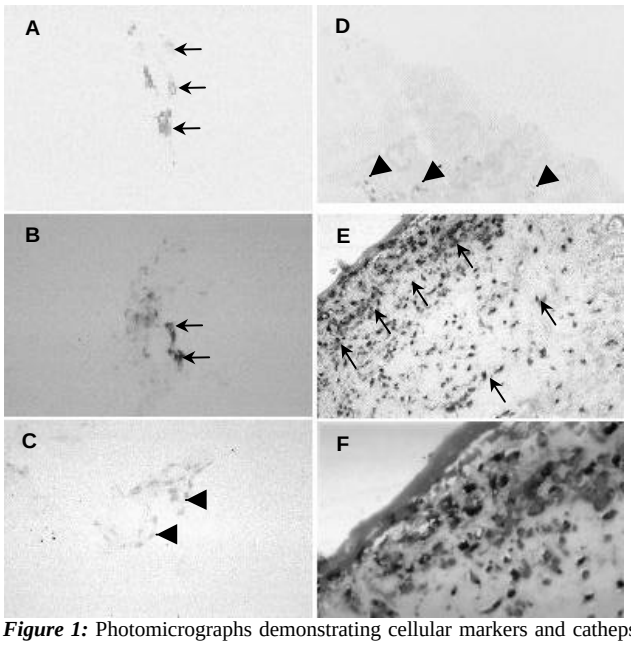

Figure 1: Photomicrographs demonstrating cellular markers and cathepsin K labelling. A) Positive labeling of fibroblasts with 5B5 at the interface (arrows), negative image; B) Positive labeling of cathepsin K at the interface (arrows); C) Isotype negative control for cathepsin K showing haematoxylin staining of the nuclei (arrowheads); D) Labeling of CD68 positive macrophages (arrowheads) away from the interface, negative image; E) *in situ* hybridisation for cathepsin K mRNA signal (arrows) at the interface; F) enlargement of the *in situ* hybridisation in (E)

References

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