THE REACTION OF CHONDROCYTES IN EXPLANT CULTURE TO EXPERIMENTAL WOUNDING

Introdution

It is well known that articular cartilage lesions that fail to penetrate the subchondral bone do not heal. The standard method for eliciting a repair response is to penetrate the vascularised subchondral bone to produce fibrocartilaginous repair tissue. Many studies have endeavoured to improve the quality of the cartilage produced through the introduction of synthetic matrices, allogenic tissue grafts, tissue culture expanded cells or growth factors [1]. Results have remained largely equivocal possibly due to the poor integration between host and repair tissues and this seems to be related to the response of the chondrocytes at the lesion edge. Histological examination of the edge of wound sites has revealed a band of cell death along the lesion edge, with an area displaying low levels of cellular proliferation adjacent to it [2]. To date, detailed information about aeteges at the edges of articular lesions is scarce.

Results

Much work examining cartilage wounding has been conducted in vivo. Since examining the reactions of chondrocytes at early stages in this way would limit the possibility of manipulating experimental conditions, we have developed an in vitro cartilage explant culture model to investigate the processes occurring after cartilage injury. Wound sites were assessed for signs of programmed cell death and cellular proliferation and also the effect of sealing the wound. Cartilage morphology changes greatly during skeletal maturity and we have used both mature and immature tissues to compare their respective reactions to wounding.

Methods

Cartilage explants (approx. 1cm x 2cm) were excised from both mature and immature bovine metacarpal- and metatarsal- phalangeal joints and wounded using a 1.2mm trephine so that a core of tissue was removed from the centre of the explant. The explants were cultured in DMEM/HAMS-F12 + 10% fetal calf serum for 1, 2, 5 and 10 days before fixation in 10% formal saline. Twenty Four hours before fixation, 20µCi/ml 3H-thymidine was added to the culture media of each explant.

In addition to examining simple open wounds the effect of sealing the wound was investigated. This was achieved by replacing the tissue plug immediately after removal. Other explants were wounded and sealed using the culture media of each explant.

Intriguingly, in samples where the excised plug had been replaced or where agarose had been used to fill the lesion, the numbers of positive cells were confined to immature tissue although the overall trend of the progression of cell death was still visible at days 5 and 10. At the surface compared with mid- and basal areas of immature tissue there was a significantly greater number of TUNEL positive cells and the cell death extended further into the tissue. Interestingly, in samples where the excised plug had been replaced or where agarose had been used to fill the lesion, the numbers of positive cells and the width of the band was markedly reduced suggesting a possible role for a diffusion dependent factor in this process. Autoradiographic analysis of 7H-thymidine incorporation showed occasional labelled cells at days 1 and 2 along the lesion in the immature and mature samples. By day 5 incorporation of the label was elevated in tissues of both ages and at day 10 a number of cells were still incorporating label.

Ultrastructural studies of the cells at the wound edge showed cells displaying characteristics of apoptosis including condensed chromatin within an intact plasma membrane 1 day after wounding. By day 5 all the cells along the wound edge appeared necrotic with no discernible plasma membrane.

Interestingly, inhibition of protein synthesis had no effect on TUNEL labelling.

Discussion

It seems likely that cell death at the edge of articular cartilage wounds combined with only a modest proliferative response plays a major role in the failure of repair tissues to fully integrate with existing tissue. Furthermore, the mode of cell death seems to be apoptosis although the cells quickly degenerate, possibly undergoing secondary necrosis 5 days after wounding. Differences are also evident between tissue ages as it appears immature tissues react far more quickly with surface cells showing preferential cell death.

The absence of healthy, active cells to remodel the matrix in the vicinity of cartilage lesions must severely hamper the repair process. We have shown evidence that sealing a cartilage wound can help to reduce the extent of the apoptosis at the cut edge and this suggests that one or more diffusible factors are involved in the apoptotic response. Treatments that could reduce or even halt this degeneration would contribute greatly to a successful cartilage repair. Inhibition of protein synthesis does not prevent apoptosis occurring and this probably allows commitment to cell suicide to be a rapid process for chondrocytes within the vicinity of a cartilage lesion.

References


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