INTRODUCTION

Analysis of the development of normal synovial joints may lead to a better understanding of joint-related diseases. A major process in the formation of synovial joints is the development of a cell-free space between opposing skeletal elements, termed cavitation. Factors that regulate the development of synovial joints are the subject of much discussion. Skeletal movement has long been known to be an important factor (1), and in the chick limb model studied here it has been demonstrated that immobilization inhibits joint cavity formation (2). Chicks are immobilized by in ovo administration of the neuromuscular blocker, decamethonium bromide (DMB) which induces spastic paralysis. Other factors which appear to be important in joint cavitation include the non-sulfated proteoglycan hyaluronan (HA), which has been localized in elevated levels at the presumptive joint line and appears to be associated with initial tissue separation (3). The activation of uridine diphosphate glucose dehydrogenase (UDPGD, supplies monosaccharides for HA) has been shown to be selectively decreased at the joint line following immobilization-induced inhibition of joint cavitation. The mechanisms by which mechanical strain induced by movement exerts control over the differentiation of cells bordering presumptive joint spaces are unknown.

Mitogen-activated protein (MAP) kinase modules including the extracellular signal-regulated kinase (ERK), p38 and c-Jun NH2-terminal kinase (JNK) are involved in signal transduction of a wide variety of cellular responses including proliferation, differentiation and apoptosis. It is well known that endothelial cells respond to mechanical stimuli by activating the ERK1/2 family of MAPKs (4). The study of the selective activation of ERK1/2, JNK or absence of mechanical stimuli, is the subject of an abstract by K. Lamb et al. and demonstrates sustained joint-line selective expression of phosphorylated ERK1/2 in fully cavitated joints, that is lost in immobilized limbs. This points to an involvement of ERK in the upstream signaling events associated with joint-line related articular surface cell differentiation. Recent evidence supports a significant degree of cross-talk between ERK1/2 and p38 MAPK (5). In this study the involvement of p38 MAPK has been investigated.

METHODS

Embryonic chick limbs develop proximo-distally with cavitation beginning at stages 35, 37 and 38 in knee, tibiotarsal and metatarsophalangeal joints respectively. Immobilization was induced by administration of decamethonium bromide (DMB) encompassing the period before, during and after cavitation of joints. For comparison another group of chicks received 4-AP to induce hypermobility at the same times and a third group of chicks received vehicle alone (Tyrode’s solution) as a control. All animal experiments were performed according to the Home Office Animals Scientific Procedures Act 1986. After the immobilization period, joints were snap frozen in n-hexane at –70°C. Serial cryostat sections (10µm) were cut and immunolabelled with anti-total p38 antibodies (Santa Cruz Biotecnologies) followed by appropriate FITC-conjugated secondary antibody. Antibody binding was visualized using a Zeiss laser scanning confocal microscope. Sections were examined from at least 6 chicks in each treatment group.

Chick articular surface cells dissected from stage 42 tibiotarsi were released by collagenase digestion and grown to confluence on plastic strips in DMEM containing 5% chick serum. Confluent cells were serum-deprived for 24 hours prior to the application of uniaxial dynamic (1Hz) four-point bending with a load of 20 g. Control groups of cells experienced culture medium perturbation alone (flow) or were unperturbed (static). After treatment cells were cultured for 20 mins, 6 h or 24 h prior to cell lysis. Proteins in cell lysates were separated by electrophoresis, transferred to nitrocellulose and p38 detected on western blots using enhanced chemiluminescence.

RESULTS

In agreement with previous in ovo experiments, spastic paralysis induced by DMB results in complete failure of separation of presumptive elements. In control joints prior to cavitation, p38 is localized within fibrocartilaginous articular surface cells of opposing elements; this is particularly apparent in cells at the presumptive joint line. Epiphyseal chondrocytes within the underlying tissue also show p38 expression, as do cells bordering canals (Figure 1A). After cavitation the joint-line related p38 expression rapidly declined. The expression of p38 in cells of all these regions is limited to the cytoplasmic compartment. However, following immobilization of joints by administration of DMB this level of p38 expression is diminished and joint line selective labeling is lost (Figure 1B). Whereas, the administration of AP which causes hypermobility results in an increased expression of p38 in all regions (Figure 1C). A high power image of the joint line prior to cavitation shows increased cytoplasmic localization of p38 within these cells (Figure 1D). This cytoplasmic labeling pattern is confirmed by a lack of co-localization with propidium iodide.

Western blotting for p38 in cultured articular surface cells 20 minutes, 6h or 24h after exposure to load, flow or static treatment revealed no change in the level of p38 expression.

Figure 1. Localization of p38 at the presumptive joint line in control (A), immobilized (B) and hypermobilized (C) joints prior to cavitation. (D) Higher power image showing cytoplasmic localization of p38.

DISCUSSION

This study demonstrates that p38 is found at the presumptive cavitating joint. However, this joint line-selective p38 expression appears only transiently during the cavitation process and is restricted to times when initial separation is evident. In contrast, joint line-selective activated ERK1/2 expression at these sites is sustained. Immobilization of joints results in diminished p38 labelling whereas, hypermobility increases p38 expression at the joint line, demonstrating a role for mechanical stimulation in the regulation of p38 expression. Paradoxically, in vitro studies on isolated articular surface cells show no change in p38 expression in response to the application of mechanical strain or fluid flow, suggesting that the regulation of p38 expression is not strain-related. Studies to detect active phosphorylated p38 are required in order to determine if the activation of p38 co-localizes with active ERK1/2 and whether its activation is induced by strain in vitro. Co-distribution of p38 and phosphorylated ERK1/2 in the cytoplasmic compartment of cells at the developing joint line, contrasts with their distinct locations in chondrocytes within the developing limb and may nonetheless provide scope for their interaction at the site of cavitation.

REFERENCES


48th Annual Meeting of the Orthopaedic Research Society
Poster No: 0345