P012 Localisation of β1 integrins on the chondrocyte primary cilium

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The chondrocyte primary cilium is hypothesised to act as a cellular transducer of mechanical and physicochemical forces experienced by hyaline cartilage. We have shown that the primary cilium is directly anchored to tensioned collagen fibres and hydrated proteoglycans of the extracellular matrix. These interactions result in a range of ciliary bending moments that conform to the 'heavy elastica' model of passive ciliary deflection. Transduction of mechanical forces may occur through membrane receptors that interact with pericellularly localised matrix molecules and are linked intracellularly to the cytoskeleton. Using immunofluorescence and confocal microscopy, we investigated whether the chondrocyte primary cilium expresses receptors for matrix molecules. Embryonic chick sternal chondrocytes were labelled *in situ* using antibodies specific for NG2, annexin-V (anchorin-CII) and β1-integrins. These are putative receptors for collagen type VI, type II and/or fibronectin. NG2 and β1-integrins showed a punctate distribution on the plasma membrane while annexin-V was present as plaques on the membrane up to 1μm in diameter and occasionally in the extracellular matrix. Dual immunofluorescence with a cilia–specific antibody to acetylated α-tubulin was used to investigate the presence of the three receptors on the primary ciliary membrane. Although annexin-V and NG2 were clearly absent from the cilium, β1-integrins co-localised with the cilium and these results were confirmed using immunogold electron microscopy. At least three receptors for collagen and/or fibronectin were found in the plasma membrane of chick chondrocytes. We conclude that the primary cilium membrane, although continuous with the plasma membrane, is a separate domain that selectively expresses some proteins (i.e. β1-integrins), but not others (i.e. annexin-V). Therefore, β1-integrins may be one of several mechanosensitive proteins that the primary cilium specifically utilises to interact with the extracellular matrix thus leading to downstream signalling involved in skeletogenesis.