Perlecan Expression is Strongly Reduced in Aging Cartilage but Increased by Physiological Loading

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Introduction

Perlecan is a large multi-domain heparan sulfate proteoglycan (HSPG) with a core protein of ~460kDa widely distributed throughout the body in basement membranes. Perlecan is involved in development of the musculoskeletal system and is found in articular cartilage. Perlecan has a multi-faceted molecular composition and, through its multiple domains, can exert a number of biological functions, including cell adhesion, growth factor delivery and sequestration. This latter function is one of the mechanisms by which perlecan influences the pericellular microenvironment. In addition, HS bearing perlecan domain I binds several growth factors important in cartilage growth, including BMP-2; therefore, it can serve as an effective growth factor delivery system for chondrogenic cells.[1] It is not known what changes occur for perlecan expression in aging cartilage, and our hypothesis is that if reduced, loss of perlecan would substantially impact the biosynthetic activity of aging chondrocytes to maintain proper cellular function. In this study we explored the changes of perlecan expression in a large number of bovine cartilages at various ages and tested methods to increase perlecan expression via hydrostatic loading, a component of the physiological loading environment.

Methods

For the study of perlecan expression as a function of donor age, full thickness cartilage explants were harvested (<3 hours post mortem) from bovine metacarpophalangeal joints. Macroscopically, all animals were negative for any indications of osteoarthritis. mRNA was subsequently isolated using TRIzol (Life Technologies) and extracted via the RNeasy mini kit (Qiagen). cDNA was prepared and gene expression measured by SYBR-green qPCR using perlecan domain I specific primers 5'-GTGACCCATGGGCTGAGGGCATA-3' and 5'-GGGCACTGTGCCCAGGCGT-3'. Using the ΔCT method, the expression of perlecan was compared to the average of four housekeeping genes; GAPDH, RPS14, RPL22 and Gusb. For loading studies, fresh hyaline cartilage was harvested from the condyles and the trochlear groove of juvenile bovine knees (3-6 months old). The cartilage was washed in 2X PSF PBS for 30 minutes, minced and digested overnight in DMEM with 10% FBS, 1X PSF and 1mg/ml collagenase type II (Worthington). The suspension was filtered through a 70μm mesh filter and chondrocytes were isolated by centrifugation. The isolated chondrocytes were then aggregated in poly 2-hydroxyethyl methacrylate 96-well plates (Corning) creating cartilage tissue analogs (CTA) at 1 x 10^6 cells/CTA. CTAs for 1 and 2 loading sessions were matured for 5.5 weeks and CTAs for 3 loading sessions were matured for 10 days prior to loading.[2,3] A custom bioreactor was utilized to apply dynamic loading (750 psi, 0.1 Hz, 3 hours) to the CTAs. Loading was applied 1, 2 and 3 times a week for 1 week (n=3). CTAs were harvested 72 hours after the final loading session. Harvested CTAs were crushed in liquid nitrogen and isolated as above. cDNA was synthesized with iScript and qPCR performed using iTaq (Bio-Rad) on a StepOnePlus Real-Time PCR System (Life Technologies) with bovine specific GAPDH primers. Results were analyzed using the $2^{-\Delta Ct}$ method for relative gene expression to GAPDH.

Results

Perlecan expression in cartilage was determined in 41 bovine donors from 0-12 years of age. An inverse correlation exists between donor age and perlecan expression, as the amount of perlecan mRNA decreases with donor age (Figure 1, 2). This is more evident when the donors are grouped in 2 year age brackets (Figure 2). We see the most substantial downregulation of perlecan after 2, 4 and 10 years (40.98%, 56.39% and 67.25%, respectively) when compared to the previous age group. By 10-12 years of age, a drastic >90% downregulation of perlecan expression was observed compared to the 0-2 year age group. Applying a physiologic, cyclic hydrostatic pressure load using a custom designed real-time bioreactor, perlecan expression was quantified under multiple dynamic loading regimens using our CTA model.[2,3] Perlecan expression was not altered by 1 loading session over 1 week (5.9%). However, specific loading regimens of 2

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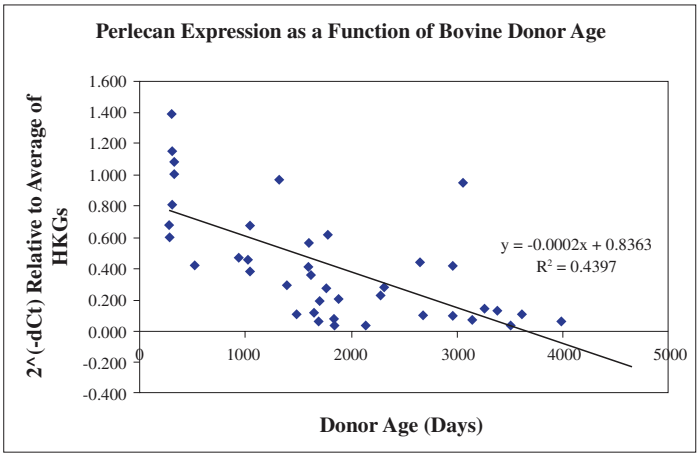

Figure 1. Perlecan expression as a function of bovine donor age.

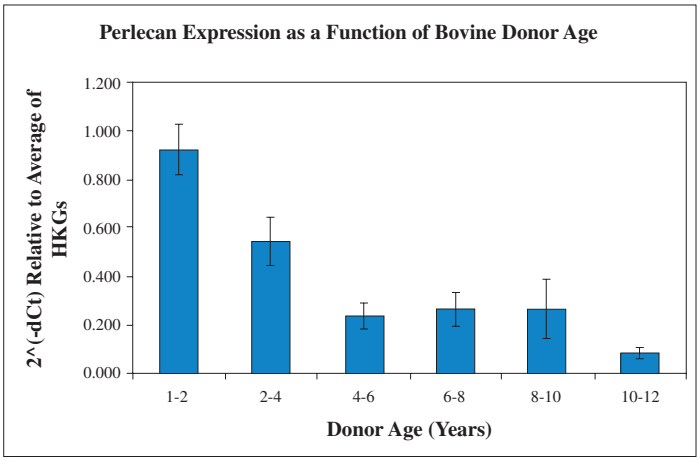

Figure 2. Perlecan expression as a function of bovine donor age in 2-year age groups.

and 3 loading cycles increased perlecan expression in CTA compared to their unloaded counterparts (198.8% and 77.3%, respectively, Figure 3). While result values are sticking and consistent through multiple experiments, they only approach statistical significance.

Discussion

In this study, we identified a significant negative association between age and perlecan mRNA expression via analysis of a large cohort of fresh cartilage specimens (that were not subjected to culture conditions or removal from native tissue that would alter expression levels). Given the increased predilection for developing osteoarthritis with aging, this reduction in perlecan expression as a function of age suggests that perlecan might play a critical role in protecting against the degradation of cartilage and in maintaining proper

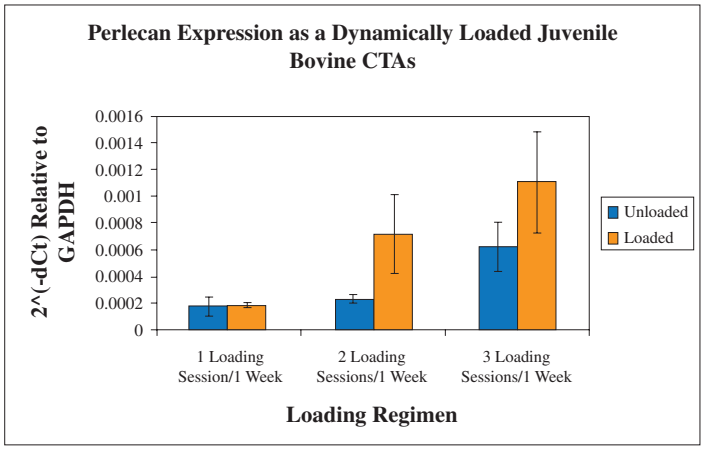

Figure 3. Perlecan expression in dynamically loaded juvenile bovine CTAs.

chondrocyte function. Interestingly, increased perlecan expression was observed when engineered CTAs were exposed to physiologically relevant dynamic hydrostatic loading. It is known that dynamic loading both improves cartilage homeostasis and maintains the chondrogenic phenotype in cartilage. Therefore, it can be suggested that perlecan expression in loaded CTAs is a parallel to normal joint function and in vivo pressure.

Significance

Aging is the main risk factor for OA. We show that perlecan expression is strongly reduced by aging. In contrast, physiological loading increases perlecan expression. Our data suggest that physiological loading might play a role in maintenance of perlecan expression in adult cartilage and in this way contributes to cartilage homeostasis.

Acknowledgments

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