INTRODUCTION
Animal models have substantially contributed to our understanding of the pathogenesis of osteoarthritis (OA). The unilateral canine model is the most commonly used model of experimental OA. In this model, transection of the anterior cruciate ligament (ACL) produces a mechanically unstable knee joint with progressive degeneration in articular cartilage. Similarly to human, dogs can also develop OA spontaneously with old age. The goal of this study was to compare the pathological changes of proteoglycans (PGs) in OA cartilage from dogs that developed OA either spontaneously or by ACL transection. This study may lead to a better understanding of these animal models and may shed light on the underlying pathomechanisms of these respective OA processes.

MATERIALS AND METHODS
Cartilage samples: Cartilage was collected from the operated and the contralateral stifle (knee) joints of 5 dogs (4-6 years old) that developed full-thickness cartilage damage 2 years after ACL transection. Two control (sham-operated) dogs of the same age were used. Samples were also taken from six 10-12 year old beagle dogs that developed OA spontaneously. From additional 3 dogs, cartilage was collected from the area, the lesion surrounding the lesion and the “normal-looking” area of the cartilage. Six age-matched dogs without cartilage damage were used as controls. Samples were extracted with 4M guanidine-HCl in the presence of protease inhibitors and digested with chondroitinase ABC (for biglycan and decorin) or keratanase II (for fibromodulin) [1]. Western blot hybridization: Cartilage extracts were separated by 12% SDS-PAGE followed by transfer to nitrocellulose membranes, and probed with anti-small PG antibodies. Signals were detected by the enhanced chemiluminescence kit and bands were quantified. Antibody production: Canine biglycan and decorin cDNAs were sequenced in our laboratory. Specific peptide sequences were selected from the C-terminal of biglycan and decorin. Synthetic peptides were coupled to ovalbumin and injected to rabbits. Polyclonal antisera were purified on affinity columns on which the corresponding peptides were bound. These antibodies and a cross-reactive anti-human fibromodulin were used for this study. Histology and immunohistochemistry: Frozen cartilage sections (8μm) were stained with Safranin O/Fast Green and anti-small PG antibodies described above.

RESULTS
Cartilage samples from dogs that developed OA spontaneously showed loss of aggrecan staining. Small PG levels, however, were increased in the OA joints compared to those of age-matched normal dogs (Fig. 1). Degradation products of biglycan, and to a lesser extent of decorin, were detected in the OA cartilage except at the lesion site, where, surprisingly, only intact small PGs were found. Decorin was localized mainly in the superficial and upper middle layer of the cartilage along with biglycan, while fibromodulin was found in the full-length of the cartilage. Each of these small PGs showed more intensive staining in OA. In cartilage from dogs that underwent ACL-transsection, aggrecan content was increased slightly in the operated joints compared to the control joints, as detected by Safranin O staining. Western blot analysis showed that the protein level of biglycan increased 1.5-2-fold (Fig. 1) and fibromodulin by 3-4-fold in the operated knee compared to that of the control knee. However, decorin content decreased in the operated knees, having only 50% of the decorin of the control knees. Sham-operated dogs did not exhibit those differences of PG levels between the two legs. Immunohistochemical staining showed similar results to Western blots.

DISCUSSION
In human cartilage, PGs are sensitive to joint load, tissue damage and osteoarthritic changes of articular cartilage [1,2]. While aggrecan content of the cartilage is decreased in human OA cartilage, small PG levels are increased [1]. As described in this study, these phenomena were also detected in cartilage of old dogs that developed OA spontaneously. An interesting aspect of this study is that we could not detect degradation products of small PGs in the lesion areas, however, fragments of biglycan and decorin were found in the areas surrounding the lesion and also in the “normal-looking” areas. This is most likely due to the activation of the repair process at the site of damage, i.e. fragments were most likely removed and replaced by newly synthesized forms of these two PGs. In contrast to spontaneous OA, aggrecan content of the ACL-operated dog cartilage was increased and decorin content was decreased in the operated knees compared to the control knees. We have to consider that the decrease in loading and altered kinematics may be also responsible for the adverse change of decorin and aggrecan. Due to the decrease in the range of extension of the knee joints, ACL transection causes a general unloading and changed kinematics of the knee. This result is consistent with our previous data [2] that biglycan is up-regulated while decorin is down-regulated by lesser stress to the articular cartilage. In summary, the changes in protein levels of PGs in the cartilage of old dogs with spontaneous OA are very similar to those of human OA cartilage. However, when OA develops as a result of ACL-surgery, the changes of PGs, in some respect, are atypical of the slowly developing spontaneous OA. Thus, there are differences between these two animal models of OA, which should be considered in the future.

REFERENCES

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