様式（二）

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論文題目　　犬の肝疾患に対する新規診断法に関する前臨床的研究

Although there are various diagnostic examinations in canine liver disease, these have problems with diagnostic accuracy and objectivity.　Especially, not only the subjectivity but also the invasiveness is a major problem of liver biopsy and subsequent histopathological examination, which is the gold standard of most of the liver diseases. In this thesis, I attempted to develop novel diagnostic approach to canine liver disease to conquer these problems.

In this thesis, I attempted novel diagnostic approaches to canine liver disease to improve the usefulness of diagnostic methods.

In chapter 1, I aimed at the application of new ultrasound contrast agent Sonazoid to distinguish malignant from benign tumor in canine liver. First, I studied on the enhancement effect of Sonazoid in healthy canine liver. As in other species, I found significant enhancement effect in hepatic artery, portal

vessels, and liver parenchyma in dogs. In addition, the enhancement effect of liver parenchyma was sustained for more than 30 minutes. Furthermore, no adverse effect was observed in that experiment. All these things lead me to the following clinical study of dogs with neoplastic disease. I performed contrast enhanced ultrasound (CEU) using Sonazoid as the protocol that I developed in the first half of this chapter with a series of clinical cases with liver neoplastic diseases. As a result, enhancement pattern of CEU using Sonazoid in parenchymal phase successfully distinguish malignant from benign lesion (Figure 1) with the sensitivity of 100% and specificity of 88.9%. These figures are as high as these of portal phase in this study. Furthermore, the parenchymal phase in this enhanced ultrasound longed for 30 minutes, which is far longer than other contrast agents previously reported in veterinary medicine. In addition, several characteristics were found especially in hepatocellular carcinoma compared to other malignancies in contrast enhanced ultrasound using Sonazoid, i.e. hyperenhancement in arterial phase and irregular defect in praehchymal phase. Taken together, CEU using Sonazoid was considered to be useful and noninvasive diagnostic method for canine neoplastic liver disease.

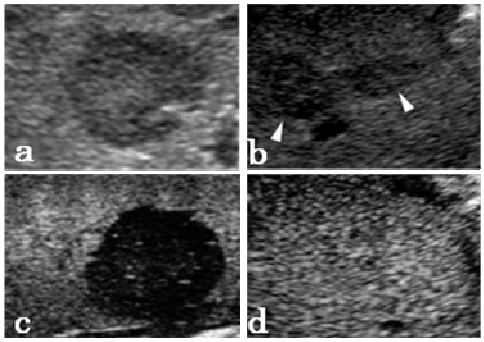

Figure 1 The ultrasonographic and sonazoid–enhanced ultrasonographic findings of malignant and benign lesions. In conventional ultrasound, a malignant tumor (cholangiocellular carcinoma, a) and a benign lesion (nodular hyperplasia, b) is indistinguishable. However, contrast-enhanced ultrasound using Sonazoid showed clear defect in the malignant tumor (c) and sustained enhancement (d) in the benign lesion, which allowed differentiation of these two different types of lesion.

In chapter 2, the histological fibrosis and the blood hyaluronan concentration in dogs with non-cirrhotic liver parenchymal disease and those with cirrhosis were compared, and hyaluronan was stained histochemically in diseased liver sample. As a result, I concluded that hyaluronan can be a new, noninvasive diagnostic tool as a marker for liver cirrhosis in dogs and that it has a possibility to be an aid to estimate the stage of chronic liver disaease. These are supported by following results. First, I confirmed significant blood hyaluronan concentration in dogs with cirrhosis compared to that of healthy dogs, dogs with hepatic disease without cirrhosis, and dogs with non-hepatic disease (Figure 2) although serum total bile acid concentration, one of the frequently used markers for liver dysfunction, was not higher in cirrhotic

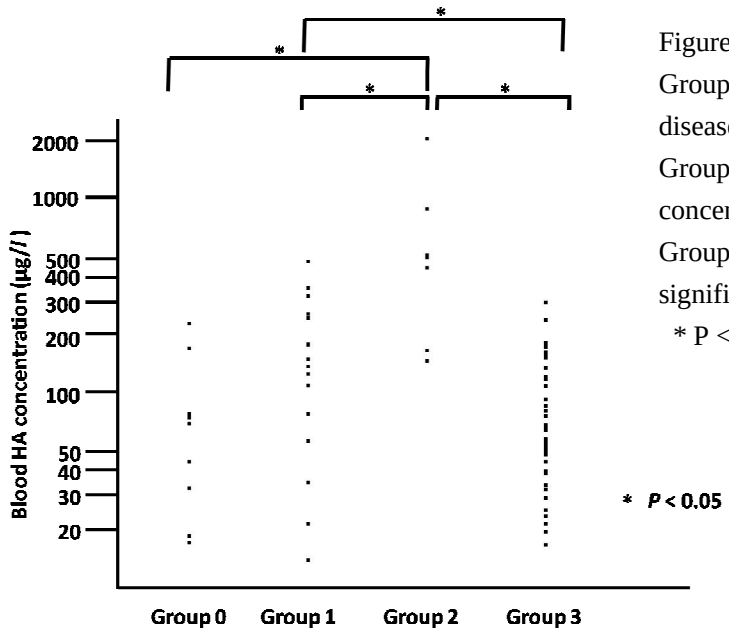

Figure 2 Blood HA concentration in the 4 groups
Group 0: healthy beagles; Group 1: dogs with chronic liver disease without cirrhosis; Group 2: dogs with cirrhosis; Group 3; dogs with extrahepatic disease. The blood HA concentrations were significantly higher in Group 2 than in Group 0, Group 1, and Group 3. Also, Group 2 had significantly higher HA concentration than and Group 3.
* P < 0.05

patients than those with non-cirrhotic liver disease. Second, in dogs with chronic hepatitis and cirrhosis, blood hyaluronan concentration was higher in dogs with clinical signs reported to be negative prognostic factor than dogs without these signs, although blood hyaluronan showed significant but weak correlation to the degree of fibrosis. The usefulness of measurement of hyaluronan in dogs with non-tumor disease was confirmed in this chapter.

In Chapter 3, I attempted to apply gene expression analysis for diagnostic test in canine parenchymal liver disease. First, microarray analysis revealed the difference of the gene expression profile between chronic hepatitis (CH) and American cocker spaniel hepatopathy (ACSH), a newly suggested canine liver disease, supporting the concept that ACSH has different pathophysiological condition from CH (Figure 3).

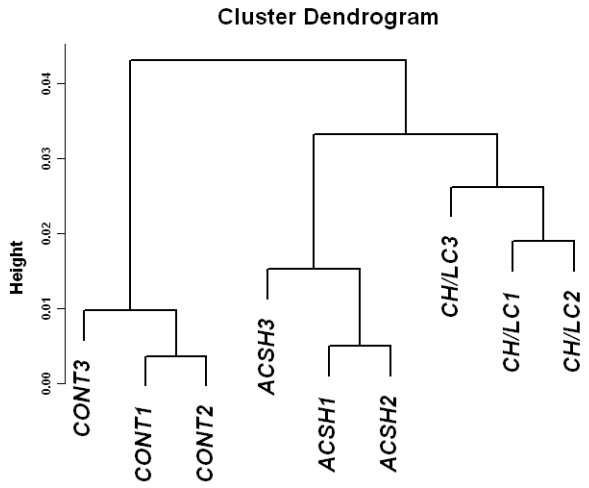

Figure 3 Unsupervised clustering for gene expression profiles of nine samples. The unsupervised clustering distinguished three groups (control group, American cocker spaniel hepatopathy group (ACSH), and chronic hepatitis/cirrhosis group (CH/LC))

The gene expression profile of ACSH determined by reflected the presence of fibrogenic process and lack of severe inflammation. Genes specifically up- or down- regulated in ACSH were also identified, but the evaluation of the importance of each gene was beyond the scope of this thesis. In future, further study is needed to show which genes are important in ACSH is needed. Second, the candidate genes were extracted from the result of microarray experiment and the gene expression levels and the histological degree of fibrosis using CH samples were compared. Expression of all the genes examined was increased in accordance with the histological severity of fibrosis (Figure 4), and multivariate analysis showed that the combination of the expression levels of two genes, namely *PDGFD* and *THBS1*, explained the degree of fibrosis very well, suggesting that the quantification of these genes can be used as an objective scoring of fibrosis in canine chronic hepatitis.

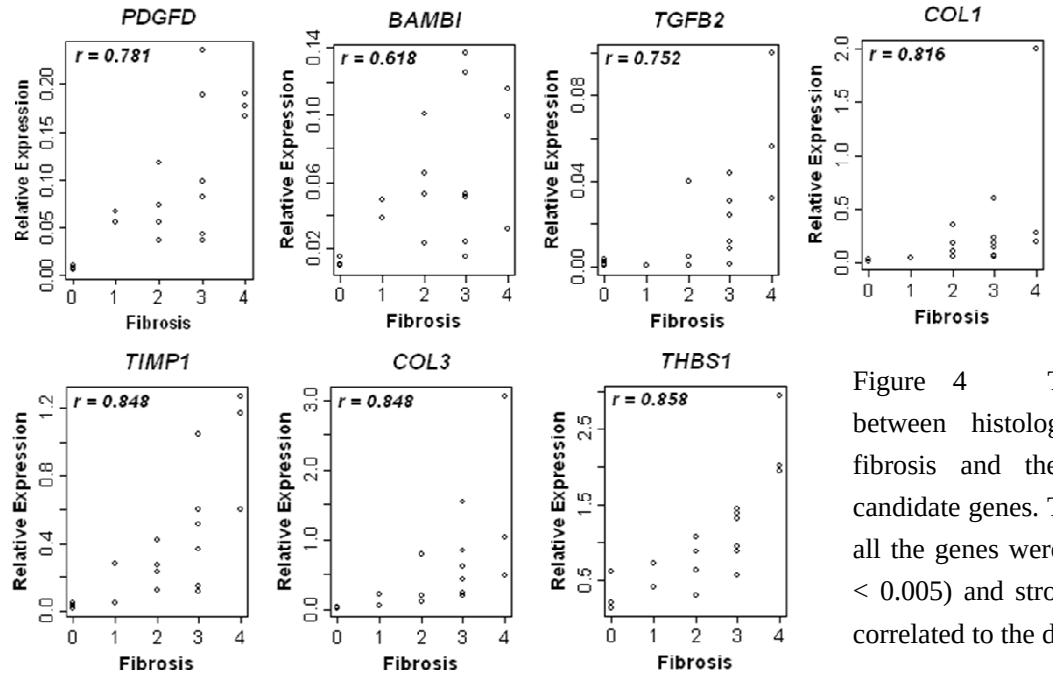

Figure 4 The relationship between histological degree of fibrosis and the expression of candidate genes. The expression of all the genes were significantly ($P < 0.005$) and strongly ($r > 0.6$) correlated to the degree of fibrosis

In conclusion, I suggested a series of clinical examination methods, i.e. contrast-enhanced ultrasound using Sonazoid for neoplastic disorder, determining plasma hyaluronate concentration as a marker of cirrhosis, and gene expression analysis for chronic parenchymal liver disorders in dogs. Using the new diagnostic methods developed here, I hope that the owner of the dogs with liver disease and clinical hepatologists can obtain better and more objective information than previous methods, leading to better diagnosis and treatment.