EXPRESSION OF CYCLOOXYGENASE-2 IN FELINE CHOLANGIOCARCINOMA, INFLAMMATORY HEPATIC DISEASE, HEPATIC ADENOMA, AND NORMAL LIVER

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Abstract

Cholangiocarcinoma is an aggressive disease in both human and feline patients. In people, chronic inflammatory conditions of the liver involving over-expression of cyclooxygenase-2 (COX-2) appear to predispose patients to the development of cholangiocarcinoma. An association between feline inflammatory liver disease and the development of cholangiocarcinoma has not been established. The purpose of this study was to evaluate COX-2 expression in feline cholangiocarcinoma, biliary adenoma, inflammatory hepatic disease, and normal liver samples. Immunohistochemical staining for COX-2 was performed in 77 formalin-fixed feline liver biopsy samples, including 22 cholangiocarcinoma, 17 biliary adenoma, 19 chronic neutrophilic cholangitis, and 19 normal liver samples. Positive staining was seen in 13/22 (59%) carcinoma samples, 8/17 (47%) adenoma samples, 8/19 (47%) inflammatory samples, and 5/19 (26%) normal liver samples. Cholangiocarcinoma samples had a significantly higher proportion of positive samples ($p < 0.05$) and intensity ($p < 0.05$) and grade ($p < 0.05$) of staining compared to normal liver samples. The finding of COX-2 overexpression in feline cholangiocarcinoma suggests a pathway for malignant transformation and a potential therapeutic target for this disease. Further studies may reveal cats as a model for human cholangiocarcinoma.

Introduction

Cholangiocarcinoma is an aggressive and typically fatal disease in both human and feline patients. In people, it is known that chronic inflammatory conditions of the liver predispose patients to the development of cholangiocarcinoma.[1] Cyclooxygenase-2 (COX-2) is an inducible enzyme involved in inflammation that can also play a role in carcinogenesis (Figure 1). COX-2 has been shown in vitro to cause malignant transformation of biliary cells.[2] The enzyme is over-expressed in human cholangiocarcinoma and chronic hepatitis when compared to normal liver and adenomas and is thought to play a key role in the transition from hepatic inflammation to neoplasia.[2]

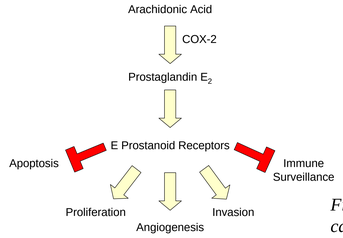


Figure 1: COX-2 and carcinogenesis

Cholangiocarcinoma is the most common non-hematopoietic feline hepatic neoplasm. It is locally aggressive with a high metastatic rate and a prognosis of less than 6 months.[3] An association between feline inflammatory liver disease and the development of cholangiocarcinoma has not been established. The purpose of this study was to evaluate COX-2 expression in feline cholangiocarcinoma, biliary adenoma, inflammatory hepatic disease, and normal liver samples.

Methods

Samples were identified in the pathology database at Tufts Cummings School of Veterinary Medicine. All diagnoses were confirmed by one pathologist (NP). Tissue sections were mounted on positively charged slides. The slides were de-paraffinized and re-hydrated routinely. Antigen retrieval was performed by heating slides in 10mM citrate buffer. After a series of blocks, the sections were incubated with the primary antibody (RM-9121, Lab Vision Corp, Fremont, CA). A streptavidin-alkaline phosphatase system was used for antibody detection. Positive control was feline macula densa cells and other feline tissues served as the negative control. Stain evaluation (blinded) of all samples was performed by one person (JW) according to a previously reported system.[4] Intensity was evaluated as 0, 1+, 2+, 3+, or 4+. Grade was defined as the percentage of positive cells: 0 (negative), 1 (<10% cells positive), 2 (10-30%), 3 (31-60%), or 4 (>60%).

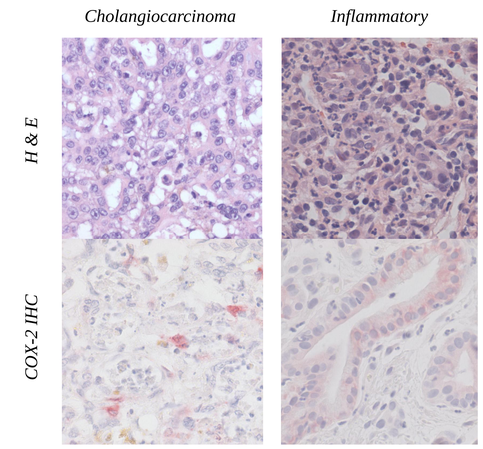
Groups were compared by chi-square analysis for statistically significant differences in the proportion of positively stained samples. Groups were compared for differences in stain intensity or grade using the Mann-Whitney test. Correlation between intensity and grade was measured using the Pearson correlation test.

Results

COX-2 staining was performed in 19 normal, 22 cholangiocarcinoma, 17 adenoma, and 19 inflammatory liver samples. The COX-2 staining results results are outlined in Table 1. Positive staining was seen in 13/22 (59%) carcinoma samples, 8/17 (47%) adenoma samples, 8/19 (42%) inflammatory samples, and 5/19 (26%) normal liver samples. Significant differences in COX-2 expression were detected between the cholangiocarcinoma and normal liver groups ($p < 0.05$). Intensity ($p < 0.05$) and grade ($p < 0.05$) of staining also differed significantly between the carcinoma and normal groups. For all groups, grade and intensity were significantly correlated ($p < 0.01$)

Table 1: COX-2 staining results

	Normal (n = 19)	Carcinoma (n = 22)	Adenoma (n = 17)	Inflammatory (n = 19)
COX-2 Positive	5 (26%)	13 (59%)	8 (47%)	8 (42%)
Intensity:				
1+	5	7	6	5
2+	0	5	2	1
3+	0	1	0	2
4+	0	0	0	0
Grade:				
1	3	7	1	0
2	0	0	0	0
3	0	1	3	5
4	2	5	3	3



Conclusion

These results indicate that feline cholangiocarcinoma over-expresses COX-2, similar to the tumor in humans. COX-2 represents a potential mechanism of carcinogenesis and continued tumor growth and angiogenesis in this aggressive feline neoplasm. It also represents a potential therapeutic target in this tumor that is difficult to treat. Although not significant, the inflammatory and adenoma samples had a higher proportion of positive samples compared to normal liver samples. The power of this study due to the low sample size may have prevented the detection of a significant difference in these conditions with COX-2 expression at levels intermediate to normal liver and cholangiocarcinoma. Inflammatory liver disease may still represent a preneoplastic condition, but further studies are required to establish this connection and the pathways involved in carcinogenesis. Identification of predisposing factors and these pathways will also allow preventative measures and treatments to be undertaken before tumor development.

References

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