



Expression Patterns of Selenoprotein P in Human Cholangiocarcinoma

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Background and Objective: As a trace element for living organisms, the anti-cancer activity of selenium (Se) attracts thousands of researchers. Selenoproteins are a family of proteins with selenocysteine residuals. Selenoprotein P (SEPP1), the transporter of Se in human plasma, has been reported to have a correlation with various cancers. This study was designed to learn more about the expression pattern of SEPP1 in human CCA.

Method: Forty-nine cases of human cholangiocarcinoma (CCA) tissue sections were stained using immunohistochemistry and then scored in three areas, non-tumor adjacent bile duct, hyperplastic lesion and cancerous tissues. The statistical analysis was executed using two-tailed non-parametric test (Mann-Whitney U-test) to evaluate the categorical data.

Result: The statistical analysis between the expression levels in the 3 areas presented that SEPP1 was frequently expressed in hyperplastic lesion rather than in non-tumor adjacent bile ducts or cancerous tissues. The frequency of SEPP1 expression in cancerous tissues was quite similar to that in adjacent bile ducts.

Conclusion: The inducible expression of SEPP1 in hyperplastic lesions in human CCA was uncovered. Our data suggest that SEPP1, which increased in the early stage, might be an early marker for CCA. SEPP1 in CCA sera will be investigated.

Keywords Selenoprotein P, immunohistochemistry, cholangiocarcinoma

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Introduction

Selenoprotein P (SEPP1), an extracellular heparin-binding glycoprotein, is synthesized in liver and secreted into blood circulation⁴. From accumulated papers of unremitting discoveries, the main function of SEPP1 is considered as the transportation center of selenium (Se) that is an essential element required for the structural assembly of many anti-oxidant enzymes⁶. Its locations in several important organs, such as liver, kidney and brain, can be detected and determined by

immunohistochemistry and microscopy. It has been reported that low level of SEPP1 in plasma are frequently found in cancers and correlated with poor prognosis^{1-3, 5}. Therefore, in this study, the expression pattern of SEPP1 was elucidated in human CCA tissues.

Objective

This study aimed to investigate the expression pattern of SEPP1 in human CCA tissues using immunohistochemistry.

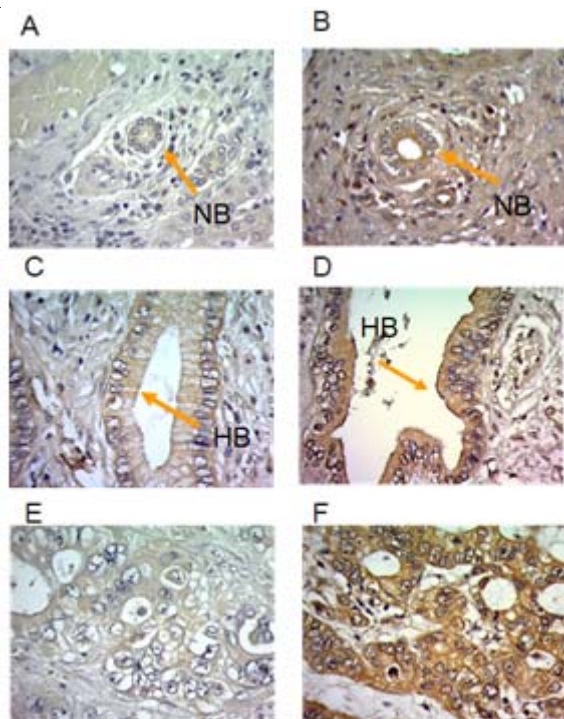


Figure 1: Representative images of SEPP1 expression in; non-tumor adjacent bile ducts (A and B); hyperplastic lesion with apical surface staining (C and D); Cancerous tissues (E and F) in the area of human CCA tissue section; comparison of SEPP1 expression in these three areas. NB, normal bile duct; HB, hyperplastic duct.

Materials and methods

Immunohistochemistry

CCA tissue sections were prepared from CCA specimens that were collected from 49 patients undergoing surgery at Srinagarind hospital. The specimens were fixed by formalin and embedded by paraffin. The liver tissue paraffin blocks were cut into 4 μ m thick sections. The sections were deparaffinized and the antigen was retrieved. The sections were incubated with 1:50 monoclonal anti-SEPP1 antibody (B-9: sc-376858 Santa Cruz Biotechnology, Inc.) and 1:100 rabbit-anti-mouse IgG, horseradish peroxidase conjugate, (R21455, Life Technologies, Inc.) were applied as secondary antibody.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS® version 19.0; SPSS Inc., Chicago, IL, USA). A two-tailed

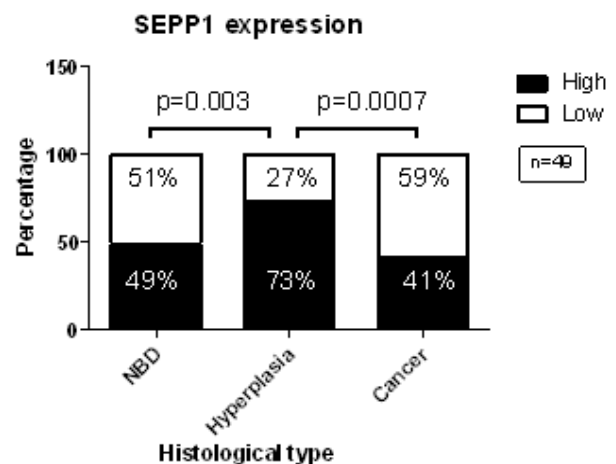


Figure 2 Frequencies of SEPP1 expression in non-tumor adjacent bile ducts, hyperplastic lesions and cancerous tissues. $p < 0.05$ is statistically significant. NBD, normal bile duct.

non-parametric test (Mann–Whitney U-test) was applied to evaluate the categorical data. When p-values were less than 0.05, the two groups of data were considered to be statistically significant.

Results

Of all 49 CCA cases, positive immunohistochemical staining of SEPP1 was seen in the human CCA tissue sections regarding 3 main areas including non-tumor adjacent bile duct, hyperplastic lesion and cancerous tissue. We found that SEPP1 had low (Fig. 1A) and high (Fig. 1B) expression in non-tumor adjacent bile ducts of 51% and 49%, respectively (Fig. 2). In hyperplastic lesion, low and high expressions of SEPP1 were found as 27% and 73%, respectively (Fig. 2). In cancerous tissues, low and high expressions of SEPP1 were found as 59% and 41%, respectively (Fig. 2). Our results concluded that the SEPP1 was significantly overexpressed in hyperplastic lesions when compared with non-tumor adjacent bile ducts ($p < 0.003$) and CCA ($p < 0.0007$) (Fig. 2). It was noted that the apical surfaces of epithelium cells of bile duct were stained (Fig.1D), which suggested that SEPP1 was a secretory protein.



Conclusion

SEPP1 was significantly more likely to be expressed in hyperplastic lesion rather than in non-tumor adjacent bile duct cells or cancerous tissues of CCA. Our finding in overexpression of SEPP1 observed for hyperplastic lesions suggests that SEPP1 might be a potential marker for early stage of CCA genesis. Further investigations of SEPP1 in CCA sera is under-investigated.

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