Expression of 14-3-3 Sigma Protein in Cholangiocarcinoma Progression

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Background and Objective: The 14-3-3 sigma is found in cytoplasm of all eukaryotic cells and has been most directly links to cancer. The expression of this protein was increased by DNA damage which occurs in many cancers including cholangiocarcinoma. Therefore, this study was to investigate the expression profiles of 14-3-3 sigma protein in human CCA tissues and correlation analysis of clinicopathological parameters.

Method: The human CCA tissue sections were examined the expression of 14-3-3 sigma by immunohistochemistry (IHC) and the stained sections were examined under microscope. Fisher's exact test was used for analyzing of 14-3-3 sigma expression and clinicopathology of CCA patients. Kaplan- Meier, with a Log-Rank test was used for determining the survival analysis.

Result: Thirty-eight CCA tissue samples were studied. Immunohistochemical staining of 14-3-3 sigma overexpression occurred in hyperplastic ducts (68.4%) and tumor tissues (79%) when compared with normal bile ducts. Its high expression was significantly associated with patients' shorter survival and overall metastasis of CCA patients (p=0.041).

Conclusion: The overexpression of 14-3-3 sigma was found in hyperplastic lesion and CCA. Moreover, its overexpression was significantly associated with shorter survival rate and CCA metastasis. These results indicate that the overexpression of 14-3-3 sigma is possibly a prognostic indicator for CCA patients

Keywords: 14-3-3 sigma, Cholangiocarcinoma

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Proceeding

Introductions

The 14-3-3 protein was found in cytoplasm of eukaryotic cells by Moore and Pretz.[1] In mammals, there are seven different isoforms: beta, gamma, epsilon, eta, sigma, theta and zeta encoded by seven different genes. One of all 14-3-3 proteins seven isoforms, 14-3-3 sigma directly links to human cancer. The 14-3-3 sigma expression was increased by DNA damage.[2] Previous reports demonstrated that high expression of 14-3-3 sigma was found in many human cancers such as pancreatic ductal adenocarcinoma and lung cancer, which it correlated with poor prognosis.[3] As indicated, the 14-3-3 sigma overexpression might be a biomarker and a predictor of response to cancer therapy. Therefore, the role of 14-3-3 sigma was elucidated in liver fluke-associated CCA progression.

Objectives

This study aimed to investigate the expression profiles of 14-3-3 sigma protein in human CCA tissues and the correlation with clinicopathological parameters was analyzed.

Materials and Methods

The localization of 14-3-3 sigma protein on human CCA tissue sections was detected by Immunohistochemistry (IHC) using a mouse anti-14-3-3 sigma antibody (Cambridge, England) with a dilution of 1:50 and anti-mouse secondary antibody conjugated peroxidase with a dilution of 1:100. Then, the stained sections were reviewed under a bright- field microscope.

Statistical analysis

The statistics software SPSS for Windows (SPSS version 13.0) was used for the statistical analysis. The correlation of 14-3-3 sigma protein with clinicopathological parameters of CCA patients was analyzed by Fisher's exact test. Patient survival was calculated from the time of surgical resection to death and the survival curves were constructed according to Kaplan- Meier, with a Log-Rank test. The significance of different data was determined by the Student's t-test. A p-value of less than 0.05 was considered statistically significant.

Results

Positive immunohistochemical staining of 14-3-3 sigma was seen in the human CCA tissues. We found that 14-3-3 sigma had no expression in normal bile ducts observed for cadaveric donor (Fig. 1A) whereas low expression was observed for non-tumor adjacent tissues of 68.4% CCA patients (Fig. 1B and 1F). Strong staining of 14-3-3 sigma was found in hyperplastic ducts (Fig. 1C) of 68.4% CCA case (Fig. 1F) and tumor tissues (Fig. 1D) of 79% CCA cases (Fig.1F). The 14-3-3 sigma was also expressed in inflammatory cells (Fig. 1B) and fibroblasts (Fig. 1E) but not in hepatocytes surrounding the tumor area. Our results concluded that the 14-3-3 sigma was significantly overexpressed in hyperplastic lesion and CCA ($p<0.0001$) (Fig.1F) when compared with non-tumor adjacent tissues of CCA.

As shown in Table 1, among the liver sections of 38 patients with intrahepatic CCA examined, 24 (63.2%) cases were male and 14 (36.8%) cases were female. The age of patients ranged from 36 to 74 years old (median age = 59 years). In this study, the CCA histological types were classified as the papillary type of 16 (42.1%) cases and non-papillary type of 22 (57.9%) cases. Fisher's exact test showed a significant positive correlation between high 14-3-3 sigma expression with overall metastasis ($p = 0.041$). Age, sex and histological grade did not show any association with 14-3-3 sigma protein. In addition, cumulative survival of CCA patients with low and high 14-3-3 sigma expression in CCA

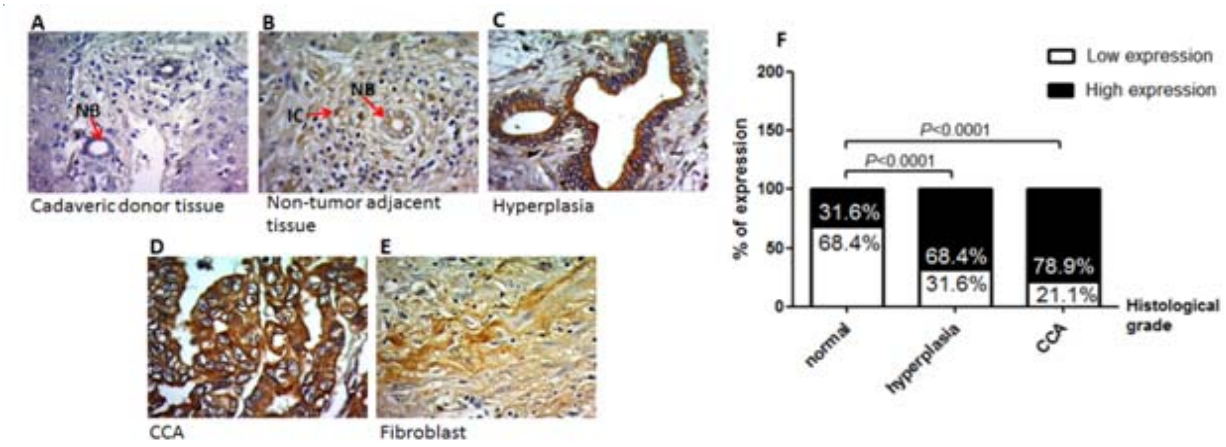

Figure 1 Representative immunohistochemical staining of 14-3-3 sigma expression in human CCA tissues (x400) (**A-E**). **A**, a normal bile duct in cadaveric donor liver tissue; **B,** a normal bile duct in non-tumor adjacent tissue; **C,** hyperplastic biliary cells in hyperplastic lesion; **D**,CCA cells in tumor tissue; **E**, fibroblasts surrounding the tumor area. **F,** a comparison between low and high expressions of 14-3-3 sigma in non-tumor adjacent tissues, hyperplastic lesion and cancerous tissues. NB, normal bile duct; IC, inflammatory cells.

Table 1 Correlation between the expression of 14-3-3 sigma and clinicopathology of CCA patients demonstrated by immunohistochemical staining.

Factors	n	14-3-3 sigma		
		Low	High	p- value
Age (years)				
< 59	16	7	9	0.449
≥ 59	22	8	14	
Gender				
Male	24	10	14	0.495
female	14	5	9	
Overall metastasis				
Non metastasis	20	11	9	0.041*
Metastasis	18	4	14	
Histological grade				
Non-papillary	22	8	14	0.449
Papillary	16	7	9	

* p- value less than 0.05 was considered statistically significant.

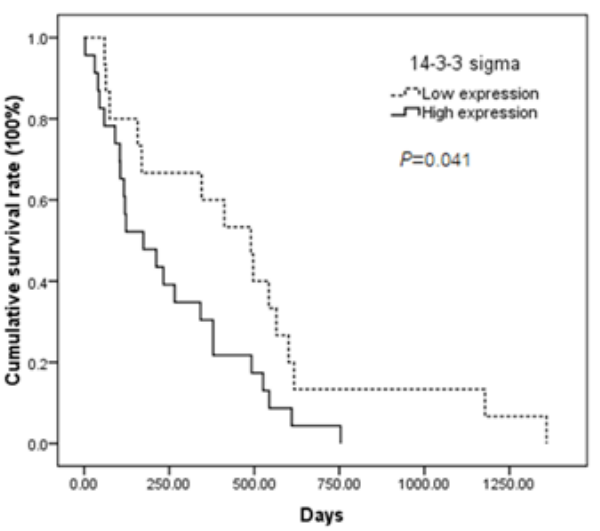

Figure 2 Cumulative survival analysis showed a significantly shorter survival rate of CCA patients with high 14-3-3 sigma expression when compared to those patients who had low 14-3-3 sigma expression (p =0.041).

tissues was analyzed using the Kaplan-Meier method. We found that patients with high 14-3-3 sigma expression had significantly shorter survival rate than those with low 14-3-3 sigma expression (p = 0.041) (Fig. 2).

Conclusion

This study demonstrated that the 14-3-3 sigma was significantly overexpressed in hyperplastic lesion and CCA. Its high expression was significantly associated with shorter survival rate of CCA patients and contributed to CCA metastasis. Our results suggest that 14-3-3 sigma overexpression is possibly a potential prognostic indicator of CCA patients.

Acknowledgements

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