Gallbladder carcinomas: An immunoprostnostic evaluation of P53, Bcl-2, CEA and alpha-fetoprotein

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The *p53* gene is the most frequently mutated gene in many human cancers, including those of the colon, breast, lung, esophagus, liver and brain. Such genetically mutated tumours are generally associated with progression of the disease and poor clinical outcome. One hundred cases of documented gallbladder carcinomas were reviewed. Twenty-eight cases were randomly selected to evaluate the expression of P53, Bcl-2, carcinoembryonic antigen (CEA) and alpha-fetoprotein, in both the *in situ* (19 cases) and invasive components (28 cases) of the tumour by the avidin-biotin complex method of immunohistochemistry. These results were correlated with the mean survival intervals in an effort to clarify the progression of the disease and evaluate their role as prognostic markers. Staining to alpha-fetoprotein and Bcl-2 remained consistently negative to weak insignificant staining in both the *in situ* and invasive components of the tumour in all cases. P53 staining of the invasive part of the tumour was seen in 24 (86%) of the cases and in 17 (89%) of the *in situ* component. The *in situ* staining patterns of P53 were not statistically significant in relation to the mean survival. However, in the invasive component, moderate to strong staining tumours, as seen in 15 (54%) cases, were associated with a mean survival of 8.8 months. A similar trend was also observed with staining patterns to CEA. Eighty-nine per cent of the invasive and 84% of the *in situ* components of the tumour stained positive to CEA. Moderate to strong staining of both the *in situ* and the invasive components of the tumours was associated with a mean survival of 10.6 months in 76% of cases.

This study shows that altered expressions of P53 and CEA are detectable by immunohistochemistry in gallbladder carcinomas. Tumours with increased expression of P53 and CEA of a strong to moderate staining were associated with poor clinical outcomes as

evidenced by their mean survival. A stepwise progression of altered CEA and P53 expression may reflect ongoing progression of the disease from the *in situ* to the invasive phase. However, such trends need to be evaluated in larger numbers and are thus not considered to be true independent prognostic markers.

Key Words: *Alpha-fetoprotein; Bcl-2; Carcinoembryonic antigen; Gallbladder carcinoma; P53*

Cancers de la vésicule biliaire : Évaluation immunoprostnostique du P53, du Bcl-2, de l'ACE et de l'alpha-fétoprotéine

RÉSUMÉ : Le gène *p53* est le gène le plus muté dans beaucoup de cancers chez l'être humain, y compris le cancer du côlon, du sein, du poumon, de l'œsophage, du foie et du cerveau. Ces tumeurs dérivant d'une mutation génétique sont en général associées à la progression de la maladie et à un pronostic sombre. Cent cas de cancer de la vésicule biliaire documentés ont été passés en revue. Vingt-huit cas ont été sélectionnés aléatoirement pour qu'on y évalue l'expression du P53, du Bcl-2, de l'antigène carcinoembryonnaire (ACE) et de l'alpha-fétoprotéine, dans les composantes *in situ* (19 cas) et envahissantes (28 cas) de la tumeur, par la méthode immunohistochimique du complexe avidine-biotine. Ces résultats ont été mis en corrélation avec les intervalles moyens de survie pour tenter de clarifier la progression de la maladie et d'évaluer leur rôle comme marqueurs pronostiques. La coloration de l'alpha-fétoprotéine et du Bcl-2 est restée négative, ou très faible, tant dans les composantes *in situ* qu'envahissantes de la tumeur dans tous les cas. La coloration du P53 de la portion envahissante de la tumeur a été observée dans 24 (86 %) des cas et dans 17 (89 %) de la composante *in situ*. La coloration *in situ* de 53 cas ne s'est pas révélée en rapport statistiquement significatif avec la survie moyenne. Par contre, dans

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les composantes envahissantes, la coloration de modérée à forte des tumeurs observée dans 15 (54 %) cas a été associée à une survie moyenne de 8,8 mois. Une tendance similaire a aussi été observée dans le mode de coloration de l'ACE. Quatre-vingt-neuf pour cent des composantes envahissantes et 84 % des composantes *in situ* de la tumeur se sont révélés positives avec l'ACE. Une coloration de modérée à forte des composantes *in situ* et envahissantes des tumeurs a été associée à une survie moyenne de 10,6 mois dans 76 % des cas.

L'étude démontre que les expressions altérées du P53 et de l'ACE sont dé-

celables par immunohistochimie dans les cancers de la vésicule biliaire. Les tumeurs présentant une expression accrue du P53 et de l'ACE manifestée par une coloration de forte à modérée ont été associées à un pronostic sombre, comme en témoigne la survie moyenne. Une régression graduelle de l'expression altérée de l'ACE et du P53 pourrait être le reflet de l'évolution de la maladie de la phase *in situ* à la phase envahissante. Par contre, ces tendances devront être évaluées auprès d'échantillons de population plus grands et ne peuvent être considérées comme de réels marqueurs pronostiques indépendants.

Cancer of the gallbladder is the fifth most common cancer of the digestive tract. The mean five-year survival rate has remained at 1% despite surgical intervention; this is mainly attributed to its late presentation, with extensive local spread and concomitant invasion of the liver. It is now accepted that most cases of invasive gallbladder cancers arise from precursor lesions of intestinal metaplasia, dysplasia and carcinoma in situ lesions. The adenoma-cancer sequence is recognized in the minority of cases. In this study, the expression of P53, Bcl-2, carcinoembryonic antigen (CEA) and alpha-fetoprotein was evaluated in both the *in situ* and invasive components of the tumour, and the results were correlated with their mean survival.

MATERIALS AND METHODS

One hundred clinical cases of documented gallbladder carcinomas were reviewed. Twenty-eight *de novo* carcinomas were randomly selected for the study (Figures 1A,B). All clinical details regarding outcomes were obtained from their chart reviews. The mean survival intervals were assessed for each case. Tissue blocks pertaining to these cases were obtained from the files of the Department of Pathology, Royal University Hospital, Saskatoon, Saskatchewan. All of the tissue had been fixed in 10% buffered formalin and embedded in paraffin. Five micron-thick sections were stained with hematoxylin-eosin and assessed by two pathologists independently. Representative blocks were selected for immunohistochemistry. This was performed using a standard streptavidin-biotin method after antigen retrieval. The antibodies used, their source and their dilutions are listed in Table 1.

In each case, the entire section was systematically examined for immunoreactivity to the various antibodies in both the *in situ* and invasive components of the tumour. Only immunoreactive nuclear-stained cells were recorded as being P53 positive (Figures 2A,B). By using a combination of the percentage of positive cells and their intensity of staining, the stained sections were assessed as negative, weak (+), moderate (++) and strong (+++) by semiquantitative analysis. The staining patterns of the *in situ* and the invasive components were correlated to their mean survival.

RESULTS

The staining to alpha-fetoprotein and Bcl-2 antibodies remained consistently negative to very weak in all parts of the tumour in all cases. Moderate to strong P53 staining was observed in 17 (89%) of the *in situ* component and 24 (86%) of

Figure 1) **A** Hematoxylin and eosin staining demonstrating the carcinoma *in situ* component in the gallbladder. Marked cytological atypia limited to the epithelial cells of the mucosa is apparent. **B** Hematoxylin and eosin staining elucidating the invasive component of gallbladder carcinoma. Marked architectural distortion with invasive malignant glands are clearly identified

TABLE 1
Antibodies used

Antibody	Type	Dilution	Source
P53 (DO7)	Mouse	1/50	DAKO(M7001)
Carcinoembryonic antigen	Rabbit	1/5000	DAKO(AO115)
Alpha-fetoprotein	Rabbit	1/6000	DAKO(A0008)
Bcl-2	Mouse	1/100	DAKO(M0887)

Figure 2) A P53 staining of the carcinoma in situ component is strong and intranuclear. B P53 positive immunoreactivity of the invasive component is also strong and intranuclear

TABLE 2
P53 staining profile

P53 staining	In situ component (n=19)		Invasive component (n=28)	
	Number alive (months)	Number dead (months)	Number alive (months)	Number dead (months)
Strong (+++)	2 (58.5)	4 (24.5)	2 (58.5)	8 (9.4)
Moderate (++)	N/A	5 (3.2)	1 (64)	7 (8.3)
Weak (+)	N/A	6 (13.5)	2 (13.5)	4 (13.3)
Negative	2 (60)	N/A	1 (56)	3 (10)

N/A Not applicable

the invasive components (Table 2). CEA staining was observed in both the in situ (84%) and invasive (89%) components (Table 3). The immunoreaction was strong and present along the cell surface, within the cytoplasm and in the gland secretions (Figures 3A,B).

The in situ staining patterns of P53 were statistically insignificant to their mean survival. However, moderate to strong staining of the invasive components, as seen in 15 cases (54%), was associated with a mean survival of 8.8 months (Table 2).

TABLE 3
Carcinoembryonic antigen (CEA) profile

CEA staining	In situ component (n=19)		Invasive component (n=28)	
	Number alive (months)	Number dead (months)	Number alive (months)	Number dead (months)
Strong (+++)	1 (19)	7 (11.4)	4 (59.3)	11 (8.8)
Moderate (++)	1 (98)	5 (15.4)	2 (13.5)	8 (12.5)
Weak (+)	N/A	2 (16)	N/A	N/A
Negative	2 (60 m)	1 (6 m)	N/A	3 (6.3)

Figure 3) A Carcinoembryonic antigen staining is restricted to the apical cytoplasm in the carcinoma in situ component. B Carcinoembryonic antigen staining is stronger and can be seen within the cytoplasm and even within the gland secretions in the invasive components of gallbladder carcinoma

CEA staining of both the in situ and invasive components were associated with a mean survival of 10.6 months in 76% of the cases (Table 3).

DISCUSSION

The precursor lesions of invasive adenocarcinoma of the gallbladder have recently been recognized as those of intestinal metaplasia, dysplasia and carcinoma in situ (1,2). The adenoma-carcinoma sequence occurs only in a minority of cases. Recognition of these lesions is, therefore, important

when removing gallbladders for cholecystitis or cholelithiasis.

Some gallbladder adenocarcinomas are immunoreactive for alpha-fetoprotein (3,4). No such case occurred in our series.

The *bcl-2* gene product is an integral membrane protein located in the inner mitochondrial membrane and principally exerts its effect on the cell cycle as a suppressor of apoptosis. Bcl-2 protein expression is linked to long term survival in female breast carcinomas. In our series, no such linkage could be established because all cases were immunonegative to Bcl-2 staining.

Our study showed moderate to strong staining patterns of CEA in both the in situ and invasive components. Such patterns were also associated with low mean survival rates. The pattern of CEA distribution in the gallbladder is similar to that of the rest of the digestive tract. Normal epithelial cells contain small quantities of CEA that are always distributed along the apical cytoplasm. At least three CEA-related macromolecules, glycoprotein types I, II and III, are found in normal bile. Type I occurs in normal bile and is similar to CEA but lacks the tumour-associated antigenic determinants. In inflammatory and tumorous conditions, type I is replaced with types II and III, the latter being immunologically similar to CEA. The amount of CEA progressively increases in dysplasia, in situ lesions and invasive carcinomas. In addition to a stronger staining, CEA can also be seen within the cytoplasm and even within the gland secretions in the invasive components (5,6). It is possible that serological assessment of CEA may be of value in the close follow-up of such precursor lesions. Such altered CEA staining pattern seems to reflect an early event in the progression of the disease as reflected by increased staining in both in situ and invasive components of the tumour in the majority of cases. Cases with negative staining in the frankly invasive components further demonstrate that cellular antigens are both developed and lost during the process of neoplastic transformation of the gallbladder.

P53 expression was different from that of the CEA, with a dominant staining pattern being confined to the invasive component of the tumour. Mutations in the *p53* gene (tumour suppressor oncogene) are the most common genetic lesion found in human cancers. These mutations play a key role in the multistep process that leads to carcinogenesis. These mutations are generally thought to alter the functional capabilities of the molecule, rendering the cell devoid of the restraint engendered by normal *p53*. Most *p53* mutations described are missense mutations resulting in an abnormal protein that accumulates in cells by virtue of an increased half-life. Such intranuclear accumulations are easily detected by immunohistochemical methods. Overexpression of *p53* is found in gallbladder dysplasias and invasive carcinomas (7-13). The mean survival of 8.8 months in *p53* immunopositivity of the invasive components suggests that *p53* positivity is associated with a worse clinical outcome.

The strong to moderate staining seen in the in situ component in 11 cases, however, clearly suggests that *p53* gene mutations may play a key role in the multiple step evolution of disease progression in gallbladder carcinoma. It probably exerts its major effect via its antiapoptosis effect, thereby promoting cellular immortalization and ongoing genomic instability with resultant acquisition of secondary chromosomal aberrations.

CONCLUSIONS

Our study shows that altered expressions of *p53* and CEA are detectable by immunohistochemical methods in de novo gallbladder carcinomas. Tumours with increased expression of *p53* and CEA of a strong to moderate staining were associated with poor clinical outcomes as evidenced by their short mean survival. Trends of strong to moderate staining in both the in situ and the invasive components suggest a pathogenetic basis for ongoing disease progression. However, that *p53*-negative tumours were not associated with statistically longer survival indicates that *p53* immunoreactivity alone is not an independent prognostic marker. These observations are based on a small number of cases, and larger series of cases with complete follow-up are required.

REFERENCES

- Albores-Saavedra J, Alcantra-Vazquez A, Curz-Ortiz H, Herrera-Goeft R. The precursor lesions of invasive gallbladder cancer: Hyperplasia, atypical hyperplasia and carcinoma in-situ. *Cancer* 1980;45:919-27.
- Duarte I, Llonos O, Domke H, Hartz C, Valdivieso V. Metaplasia and precursor lesions of gallbladder carcinoma. Frequency, distribution and probability of detection in routine histologic samples. *Cancer* 1993;72:1878-84.
- Watanabe M, Hori Y, Nojima T, et al. Alpha-fetoprotein-producing carcinoma of the gall bladder. *Dig Dis Sci* 1993;38:561-4.
- Ng WK, Ng WF. Elevated serum alpha-fetoprotein in a patient with undifferentiated carcinoma of the gall bladder. *J Clin Pathol* 1995;48:1061-3.
- Albores-Saavedra J, Nadji M, Morales AR, Henson DE. Carcinoembryonic antigen in normal, preneoplastic and neoplastic gallbladder epithelium. *Cancer* 1983;52:1069-72.
- Roa I, Araya JC, Wistuba I, et al. [Immunohistochemical expression of the carcinoembryonic antigen in gallbladder cancer]. *Rev Med Chil* 1991;119:129-36.
- Oshashi Y, Watanabe H, Ajioka Y, Hatakeyama K. P53 immunostaining distinguishes malignant from benign lesions of the gallbladder. *Pathol Int* 1995;45:58-65.
- Wee A, Teh M, Raju GC. Clinical importance of p53 protein in gallbladder carcinoma and its precursor lesions. *J Clin Pathol* 1994;47:453-6.
- Teh M, Wee A, Raju GC. An immunohistochemical study of P53 protein in gallbladder and extrahepatic bile duct/ampullary carcinomas. *Cancer* 1994;74:1542-5.
- Kamel D, Paakko P, Nuorva K, Vahakangas K, Soini Y. p53 and c-erb-B2 protein expression in adenocarcinomas and epithelial dysplasia of the gall bladder. *J Pathol* 1993;170:67-72.
- Diamantis I, Karamitopoulou E, Perentes E, Zimmermann A. p53 protein immunoreactivity in extrahepatic bile duct and gallbladder cancer: correlation with tumor grade and survival. *Hepatology* 1995;22:774-9.
- Fujii K, Yokozaki H, Yasui W, et al. High frequency of p53 gene mutation in adenocarcinomas of the gallbladder. *Cancer Epidemiol Biomarkers Prev* 1996;5:461-6.
- Itoi T, Watanabe H, Yoshida M, Ajioka Y, Nishikura K, Saito T. Correlation of p53 protein expression with gene mutation in gall bladder carcinomas. *Pathol Int* 1997;47:525-30.