**Immuno-expression of p53 and cyclin D1 in adenomas of the gallbladder**

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**RESUMEN**

**Introducción:** el adenoma de vesícula biliar es una neoplasia infrecuente, cuya relación con el adenocarcinoma es poco conocida, aunque algunos autores han propuesto que la mayoría de adenomas no degeneran en adenocarcinomas, debido a que ambas lesiones presentan vías moleculares diferentes.

**Material y métodos:** el presente trabajo es un estudio transversal que compara las características moleculares del adenoma y adenocarcinoma de vesícula biliar, mediante la medición inmunohistoquímica de la expresión de las proteínas p53 y ciclina D1 (ambas reguladoras del ciclo celular) en 12 enfermos de cada grupo.

**Resultados:** encontramos una mayor expresión de p53 en los adenocarcinomas (83.3%) que en los adenomas (16.6%) siendo esta diferencia estadísticamente significativa usando el test de chi cuadrado (p = 0.003), mientras que la expresión de ciclina D1 en ambos grupos fue similar.

**Conclusión:** consideramos que nuestros resultados indican que la alteración en el p53 es un paso importante en el desarrollo de los adenocarcinomas de vesícula biliar, mientras que en el desarrollo de los adenomas, la alteración del p53 sería poco trascendente. Por otro lado, la sobreexpresión de ciclina D1 sería un mecanismo molecular común a ambas lesiones.

**Palabras clave:** Adenoma de vesícula biliar. Adenocarcinoma de vesícula biliar. p53. Ciclina D1.

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**ABSTRACT**

**Introduction:** gallbladder adenomas are infrequent neoplasms whose relation to adenocarcinoma is not well understood. It has been suggested that adenomas and adenocarcinomas follow different molecular pathways.

**Material and methods:** this is a comparative, cross-sectional study in which we compared p53 and D1 cyclin expression in adenomas and adenocarcinomas of the gallbladder.

**Results:** we included 12 cases in each group. Expression of p53 occurred in 83.3% of adenocarcinomas and in 16.6% of adenomas (p = 0.003). D1 cyclin was expressed in a similar number of adenomas and adenocarcinomas.

**Conclusion:** our results support the hypothesis that p53 is an important step in the pathogenesis of adenocarcinomas but not of adenomas of the gallbladder. D1 cyclin is apparently a common pathway involved in the genesis of both tumors.

**Key words:** Gallbladder adenoma. Gallbladder adenocarcinoma. p53. Cyclin D1.

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**INTRODUCTION**

Adenomas of the gallbladder are infrequent neoplasias, found in less than 1% of cholecystectomies. Due to this low frequency there are few studies about adenomas. Even so, most authors believe that only a small percentage of adenomas degenerate in adenocarcinomas (1). p53 protein is a tumor onco-suppressor, that is altered in about half of gallbladder cancers, while D1 cyclin is a protein in charge of controlling cellular cycles and has been reported overexpressed more commonly in adenomas than in adenocarcinomas of the gallbladder (2).

We consider that if adenomas and adenocarcinomas of the gallbladder were part of an oncogenic sequence, they should share similar molecular characteristics.
The aim of the present study is to evaluate whether there are differences in the immune expression of p53 and D1 cyclin in adenomas and adenocarcinomas of the gallbladder.

MATERIAL AND METHODS

All gallbladders received at our institution during 2005 and 2006 were reviewed and all cases of adenomas were selected (12 cases). However, from all the adenocarcinomas we found, we could only work with 12 because of the remaining tumors we lacked material enough. Therefore, an equal number of adenocarcinomas was selected from the same study period. A gallbladder adenoma was defined as any outgrowth that histologically showed gland proliferation and some grade of dysplasia. Gallbladder adenocarcinoma was defined as that outgrowth with cells showing atypicality, forming glands and originating on a superficial epithelium that can be observed microscopically.

Immune expression of p53 and D1 cyclin were measured in each specimen and age, gender, and tumor size were also considered.

p53 and cyclin D1 were measured using immuneroxidase technique. For p53, we used the mouse monoclonal antibodies clone D0-7 (DAKO) and for D1 cyclin, we used the rabbit monoclonal antibodies, clone RBT-14 (CELLMARK).

A cell was considered positive for p53 or cyclin D1, if it showed a golden staining in its nucleus, and a case was considered positive for these two proteins if more than 15% of its cells showed a positive staining. Percentage of positivity was measured counting 3 fields of 40X, using a Leica-Olympus light microscopes.

Data was processed using Excel and SPSS 11.0 software.

RESULTS

During the study period a total of 1,798 gallbladder specimens were received at our institution; 91.6% of these gallbladders came from female with an average age of 43.2 years and with an average size of 1.01 cm for adenomas.

As table I shows, there were 12 adenomas and 16 gallbladder adenocarcinomas. Tables II and III show the positivity for D1 cyclin and p53 proteins in both groups of lesions. There were no differences in the grade of expression of cyclin D1 between both groups of lesions, but p53 was more frequently expressed in adenocarcinomas (Figs. 1-5).

DISCUSSION

Cyclin D1 is a protein that controls the cellular cycle, allowing progression from G1 to S phases in the cell cycle. So, it is natural that its overexpression leads to cell proliferation. Overexpression of cyclin D1 has been reported to occur in a number of neoplasias as lymphomas, colon carcinomas, and breast cancer, as well as in some pre-neoplastic conditions such as adenomatous polyps of the colon and ductal hyperplasia of the breast (2).

Regarding the gallbladder, there are few reports on cyclin D1 expression on adenomas and adenocarcinomas. A Japanese report (2) showed a higher expression of this cyclin (61%) in adenomas than in adenocarcinomas (41%), while a Chinese study (3) reported the contrary finding; thus, expression of cyclin D1 was more marked in adenocarcinomas (68%) than in adenomas (57%). Our study found a similar rate of cyclin D1 expression in both adenomas (66.6%) and adenocarcinomas (58.3%). Our results, as well as these of others, suggest that cyclin D1 expression is a common molecular feature of both adenomas and adenocarcinomas of the gallbladder.

Protein p53 is a regulator of cell cycle and one of the most frequently altered targets in the majority of human neoplasias. Its function consists in maintaining the integrity of DNA and inducing apoptosis of those cells with an abnormal DNA impossible to be repaired (4).

In the digestive system, p53 changes have been commonly reported in colon cancer (60-70%), gastric carci-
nomas (20-50%) and in lesser proportions in esophageal carcinomas (5). In the gallbladder, immune-expression of p53 has been reported mainly in adenocarcinomas (35-65%) (4), but it has also been found in adenomas (0-17%) (6-9). Our results agree with those reported in the literature, since we found p53 overexpression in 83.3% of the adenocarcinomas and only in 16.6% of the adenomas.
We believe that the different expression of p53 between adenomas and adenocarcinomas may have two possible explanations.

First, it might be well possible that adenomas express p53 very late in their evolution to adenocarcinoma; in such a way that it may be difficult to detect initially. However, absence of p53 protein expression in those adenomas with foci of adenocarcinoma contradicts this first hypothesis. Second, it is also possible that changes in p53 are molecular events characteristic of adenocarcinomas, which very rarely occur in gallbladder adenomas, possibly due to different molecular origins. This idea has also been postulated by other authors. Thus, Witsuba et al. (10), studying p53, K-ras, and N-ras, as well as loss of heterogeneity, conclude that adenomas show different molecular abnormalities when compared with adenocarcinomas. Lack of changes in p53 expression in the development of gallbladder adenomas would imply the existence of alternative molecular pathways as yet unknown to us. This alternative pathway would have a lesser oncogenic potential than p53 pathway considering the low incidence of adenomatous remnants in the neighboring mucosa to early carcinomas (11).

We consider that our results suggest that changes in p53 are an important step in the genesis of adenocarcinomas, but they have little importance in the development of adenomas of the gallbladder. On the other hand, cyclin D1 would be a common pathway in the development of both type of tumors.

REFERENCES