Beta Catenin Gene Expression in Hepatocellular Carcinoma and Hepatoblastoma

Karrie Lin

ABSTRACT

The Wnt signal transduction pathway is primarily involved in many differentiation events during embryonic development. Cytoplasmic beta catenin, one of the pathway's chief components, serves to transmit wnt signals to the nucleus: in the absence of wnts, beta catenin is degraded in proteasomes, whereas in the presence of wnts, beta catenin is stabilized and associates with TCF transcription factors, leading up to the growth of particular cells. In tumors, beta catenin degradation is inhibited by mutations of beta catenin itself. In order to further study the molecular mechanism of beta catenin mutations in hepatocarcinogenesis, we think it is important to determine the frequency of beta catenin mutations in a larger patient population and correlate the mutations with the clinicopathological features. The present study aims to determine the beta catenin somatic mutation and its protein distribution in hepatocellular carcinoma and hepatoblastoma.

Results showed that beta catenin protein did accumulate in 100% (7/7) hepatoblastoma tumor cells, suggesting its role in tumorigenesis. However, this nuclear accumulation of beta catenin protein did not correlate with the exon 3 mutations suggesting that other mutations or other molecular events are involved in beta catenin protein nuclear translocation. Results did not detect any beta catenin protein accumulation of hepatocellular carcinoma (HCC) nuclei. The conclusions of this study may provide data for further pursuit of the molecular basis for hepatocarcinogenesis.

INTRODUCTION

Beta catenin is a protein involved in the Wingless/Wnt signal transduction pathway. In a normal cell, beta catenin can interact with two proteins: E-cadherins and APC (adenomatous polyposis coli). The beta catenin-cadherin complex plays a role in cell adhesion. The interaction between APC and beta catenin has been considered as an essential step for the beta catenin degradation process, a mechanism yet to be determined. It has been proposed that the mutant APC causing cell transformation may partly be due to the inability of beta catenin degradation. Interestingly, among the 15% of colorectal cancers that do not show APC mutations, beta catenin mutations were identified as a major initiating event for tumorigenesis. Beta catenin mutations have also been identified in melanomas, particularly their recent discovery in mouse and human
hepatocellular carcinomas and hepatoblastomas. All the mutations reported so far are located in the exon 3, which harbors the phosphorylation sites. The mutated amino acids alter the phosphorylation sites for the glycogen synthase kinase-3, an important process for beta catenin degradation. The under-phosphorylated beta catenin will not be degraded through ubiquitin mediated pathway, resulting in the accumulation of this protein in both cytoplasm and nucleus. In the nucleus, beta catenin interacts with transcription factors such as T cell factor to activate target genes involved in cell growth control and apoptosis, presumably initiating the onset of oncogenesis. The molecular details for this process are largely unknown.

Few specific genetic alterations are identified in human hepatocellular carcinomas (HCC); however, p53 mutations have been consistently found in some HCCs. It is considered that the p53 mutation mainly involves later stages of carcinogenesis. On the other hand, APC gene mutation plays a role in early cancer development. But there is no evidence of APC involvement in most hepatocellular carcinomas. There is a possibility that the beta catenin mutation may be important in early carcinogenesis. Recent studies have demonstrated that both mouse hepatocellular carcinomas in H-ras and c-myc transgenic mice and human hepatocellular carcinomas have similar beta catenin mutations. This strongly supports the postulation that beta catenin plays a critical role in hepatocarcinogenesis. A study showed that 50% of mouse HCCs and 26% (8/31) of human HCCs carried beta catenin mutations. It has also been demonstrated that hepatoblastoma has a higher incidence of beta catenin mutations. In order to further study the molecular mechanism of beta catenin mutations in hepatocarcinogenesis, we think it is important to determine the frequency of beta catenin mutations in a larger patient population and correlate the mutations with the clinicopathological features. Our current study proposes to determine the beta catenin somatic mutation and its protein distribution in hepatocellular carcinoma and hepatoblastoma. This study may provide preliminary data for further pursuit of the molecular basis for hepatocarcinogenesis.

MATERIALS AND METHODS

Case Selection

Mutations Seventy (70) archived hepatocellular carcinoma tissue sections and 7 archived hepatoblastoma tissue sections were selected for this study.

Laser Capture Microdissection (LCM) and DNA sequencing

The tumor cells were dissected from the H&E stained uncoverslipped tissue sections. Approximately 1000 laser dissected cells were used for DNA extraction and PCR amplification. The primers used for PCR amplification cover the Exon 3 of beta catenin gene. The 5í end primer is CTG ATT TGA TGG AGT TGG AC, the 3í end primer is TAC CAG CTA CTT GTT CTT GAG. The PCR amplification was performed with the following program, 95ÉC - 30", 60É C - 45", 72 ÉC - 60" with final extension 72É C - 5 minutes. The PCR products, amplified Exon 3 (200-bp) DNA fragments,
were then resolved and purified on an agarose gel. The DNA sequence was determined by an automatic sequencer.

**Immunohistochemistry**

A monoclonal anti-beta catenin antibody was obtained from Transduction Research. Immunohistochemical staining was performed on the tissue sections using this anti-beta catenin antibody (1:250 dilution). The procedure involved the following sequence:

1. **dried tissue sections in 42°C incubator for 20 minutes;**
2. **deparaffinized and rehydrated tissues in xylene (2 times), 100% EtOH, 95% EtOH, and water for 5 minutes each;**
3. **quenched endogenous peroxidase activity with 5-mL 30% hydrogen peroxide and 45-mL methanol solution for 15 minutes each, 2 times**
4. **unmasked antigens with high-temperature procedure: placed tissues in 5-mL 0.1% sodium citrate buffer (pH=6.0) and 45-mL ddH2O solution, and microwaved for 5 minutes;**
5. **applied 1:100 goat serum for 20 minutes;**
6. **applied 1:250 anti-beta catenin antibody for 1 hour in 37°C incubator;**
7. **applied DAKO LSAB2 system;**
8. **counterstained tissues with hemotoxylin, 95%EtOH, 100% EtOH, and xylene (2 times);**
9. **mounted slide cover and allowed to dry.**
RESULTS

1. Beta catenin gene mutation in hepatocellular carcinoma (HCC) and hepatoblastoma:

Among the seven cases of hepatoblastoma, only one case showed the gene mutation at the codon 37, S37Y site. The rest of the hepatoblastoma did not show detectable DNA mutations in the exon 3. Fifteen cases of hepatocellular carcinomas (HCCs) were studied by sequencing analysis. None of the fifteen cases of HCCs showed any detectable DNA mutation in the exon 3.

2. Beta catenin protein accumulates in hepatoblastoma.

Seven hepatoblastoma cases were studied by immunohistochemical staining using a monoclonal anti-beta catenin antibody. It was clearly demonstrated that the beta catenin protein is exclusively located in the nucleus of the tumor cells. As shown in Figure 1A and Figure 1B, both the mesenchymal components and the glandular components show nuclear localization of the beta catenin protein. All of the seven cases displayed similar results.

Figure 1. Beta catenin expression in hepatoblastoma. The protein is clearly expressed in the nucleus of the tumor cells in both glandular area (1A) and mesenchymal area (1B).
3. Beta catenin protein did not accumulate in the nucleus of hepatocellular carcinoma cells (HCC).

Seventy of the HCC tissue sections were studied by immunohistochemical staining using a monoclonal anti-beta catenin antibody. As shown in Figure 2A and Figure 2B, the beta catenin protein is exclusively located on the cell membrane. It appears that the staining intensity is stronger in tumor cells than the staining intensity in the adjacent normal cells. However, there is no nuclear staining in any of the seventy cases of the hepatocellular carcinomas.

Figure 2. Beta catenin protein expression in hepatocellular carcinoma. The protein is exclusively expressed on the cell membrane of the tumor cells in two different tumors.

CONCLUSION

1. The beta catenin protein nuclear accumulation was found in 100%(7/7) of hepatoblasoma tumor cells, suggesting its role in tumorigenesis.

2. The beta catenin protein nuclear accumulation did not correlate with the exon 3 mutations,
suggesting other mutations or other molecular events are involved in beta catenin protein nuclear translocation.

3. Beta catenin protein nuclear accumulation was not identified in hepatocellular carcinomas.

ACKNOWLEDGEMENTS

I would like to thank Dr. Chen Liu for his guidance and continual support as a mentor. I appreciate the opportunities he has provided to encourage learning in the research environment. I would also like to thank Dr. Haizhen Zhu for his assistance.

REFERENCES


