Telomerase catalyzes the elongation of telomeric repeat DNA to maintain telomere length and structure (1). Although telomerase is a complex composed of a catalytic subunit (hTERT) and an RNA component (hTER), it is the expression of hTERT (2,3) that is the major determinant of telomerase activity in human cells (1,4). Maintaining telomere length by telomerase is associated with human cell immortalization and oncogenesis (1); however, no mutations in the hTERT or hTER telomerase genes have been found in human cancers. Hepatocellular carcinoma is often associated with both telomerase activation and viral infection. Here, we show the cis-activation of the hTERT gene by an insertion of viral DNA into the hTERT promoter region.

A 66-base-pair (bp) sequence within the promoter region of hTERT gene (positions −372 to −307) (Fig. 1, A) is identical to a cellular sequence at the integration site of the hepatitis B virus (HBV) genome in a telomerase-positive (Fig. 1, B) hepatocarcinoma cell line, huH-4 (GenBank X51995) (5,6). This sequence corresponds to the upstream junction of the HBV integration (Fig. 1, A). Southern blot analysis confirmed an huH-4-specific rearrangement within the hTERT promoter and suggested an intact hTERT coding region in this cell line. Genomic polymerase chain reaction (PCR) by use of the primers from HBV (near the enhancer region or at the preS1 region) and hTERT sequences (in exon 1 or intron 2) generated huH-4-specific products (Fig. 1, C) that allowed identification of the downstream integration site of HBV (Fig. 1, A and E). The HBV genome was disrupted within its preS1 region and inserted at position −257 of the hTERT promoter, with an associated 49-bp deletion (positions −306 to −258) of the hTERT promoter. The integrated HBV genome was rearranged so that a HBV enhancer sequence (7) was placed about 1.6 kilobase upstream of the junction. The presence of a full-length hTERT messenger RNA starting from the endogenous transcription initiation site in huH-4 cells has been shown by the ribonuclease protection assay [see (5)]

Fig. 1. Expression of hTERT/telomerase in the hepatocellular carcinoma cell line huH-4 and characterization of the hepatitis B virus (HBV) genome integrated into the hTERT promoter region. A) Upper diagram is a schematic representation of the HBV genome integrated into the hTERT promoter. Numbered boxes indicate hTERT exons. Thick and thin lines are hTERT promoter and intron regions, respectively. The upstream junction location (GenBank X51995) and the size of the integrated HBV genome are indicated on the basis of Kekule et al. (6). Open arrowheads indicate the locations of polymerase chain reaction (PCR) primers to detect genomic DNA and a fusion transcript shown in Fig. 1, C and D, respectively. The transcription start sites from the HBV large S (14) and hTERT promoters are indicated by the filled arrows and are shown in greater detail in the lower diagram. The middle diagram (continued on next page)
HBV–hTERT fusion transcript. Position 1629 of the hTERT complementary DNA (GenBank
integration site. The nucleotide location is shown in
parentheses. F) A PCR product generated from the HBV preS1 and hTERT exon 4 primers in Fig. 1, D, was sequenced to determine the identity of the HBV–hTERT fusion mRNA transcript. Position 1629 of the hTERT complementary DNA (GenBank AF015950) corresponds to the 5’ end of exon 3.

and confirmed by reverse transcription–PCR analyses (Fig. 1, D; data not shown). In addition, the cells express an HBV–hTERT fusion transcript in which a cryptic splice-donor site within the preS1 region was joined out of frame to exon 3 of hTERT (Fig. 1, A, D, and F).

Transcriptional activity of wild-type hTERT and fused HBV–hTERT promoters in huH-4 cells was tested in luciferase assays (Fig. 2, left). Two wild-type fragments (positions −256 to +40 and positions −305 to +40) showed similar activities, excluding a role of the integration-associated, 49-bp deletion (positions −306 to −258) in hTERT activation. Transcriptional activity of a fusion promoter containing the HBV large S-promoter region was not increased compared with the wild-type promoters. In contrast, transcriptional activity of a fusion fragment extending over the HBV enhancer region was approximately sixfold higher than that of the wild-type promoters. This increase was attributed to an orientation-independent activity of the HBV enhancer, which increased the transcriptional activity from both wild-type hTERT and HBV–hTERT fusion promoters. These findings suggest that the integration of HBV enhancer upstream of the hTERT promoter cis-activated the hTERT gene transcription in huH-4 cells. There might also be some trans-activation by cellular and/or viral transcription factors associated with carcinogenic processes, because the wild-type promoters with no HBV sequence showed considerably more activity than the empty-vector pGL3–Basic. Given that HBV infection is an early event in human liver carcinogenesis (8), it is likely that cis-activation by the integrated HBV genome was a primary mechanism for hTERT and telomerase activation in huH-4 cells. In another hepatocellular carcinoma cell line, HepG2, which is HBV negative, similar results were obtained (Fig. 2, right), suggesting that HBV-encoded proteins are dispensable for activity of the fused HBV–hTERT promoter. Transcriptional activity stimulated by the HBV enhancer was slight in HepG2 cells (1.7- to 2.2-fold), consistent with the notion that changes in cellular trans-acting factors resulted in full transcriptional activation from the wild-type promoter in this cell line.

The numeric changes (i.e., increased copy number) of the hTERT and hTERT
genes were found in a fraction of human cancers (9,10). To our knowledge, this study is the first report of a structural alteration in the telomerase genes, which led to the activation of telomerase. Telomerase activity is associated with cell proliferation and differentiation (11,12) and may be affected by multiple factors associated with carcinogenesis (i.e., oncogene and tumor suppressor gene products) (4). However, whether telomerase activation plays a causative role in carcinogenesis is still controversial. Our study provides an example of a primary genetic alteration in hTERT that is similar to the classic examples of oncogene activation (13). This observation adds the hTERT gene to the list of cellular targets that are activated in cis by oncogenic viruses and validates a causative role of telomerase in human liver carcinogenesis.

REFERENCES


NOTES

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