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EXPRESSION OF C-MYC GENE IN HUMAN HEPATOMA

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SUMMARY The level of c-myc transcript was examined in liver samples from seven hepatoma patients. Transcripts were detected in all the normal liver parts examined; in contrast, in two hepatoma parts, there was a dramatic reduction in c-myc transcripts. The restriction enzyme pattern of c-myc gene appeared the same among samples. The data suggest that c-myc gene expression might not be required for the maintenance of the tumor state in human liver carcinogenesis.

Activation of the c-myc gene has been implied in a variety of hematopoietic malignancies (1). It has been postulated that amplification of the myc gene and/or enhanced transcription of proto-myc gene by downstream promotion or by chromosome translocation might play an important role in the cause of chicken B cell lymphoma, human Burkitt's lymphomas and mouse plasmacytomas (2,3,4). The increase of c-myc gene transcripts has also been demonstrated during rat liver regeneration and hepatocarcinogenesis (5,6). In a search for possible known oncogenes involved in the development of human hepatocellular carcinoma, the expression of c-myc RNA and its gene structure were examined in liver samples from seven hepatoma patients.

MATERIALS AND METHODS

Human liver samples --- Human hepatomas and their adjacent normal liver tissues were obtained according to the regulations of Veterans General Hospital. The surgically removed liver samples from hepatoma patients were then carefully dissected into normal

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and hepatoma parts and were immediately frozen and stored in liquid nitrogen until use. The dissected normal liver and hepatoma tissues were confirmed by pathological examination.

Isolation of DNA and RNA from liver samples --- High molecular weight DNA was prepared by the method of Blin and Stafford (7). The total liver RNA was extracted by the guanidinium/cesium chloride method (8).

Electrophoresis and detection of c-myc DNA and RNA --- DNA was digested to completion by restriction enzyme in the condition suggested by the manufacturer's specification (New England Biolabs, Inc.). Electrophoresis, transfer to nitrocellulose paper and detection of DNA were performed as described by Southern (9). RNA was denatured by glyoxal, electrophoresed, transferred to nitrocellulose paper and detected with [^{32}P] labeled nick-translated c-myc probe as described (10,11). The human c-myc DNA probe used for nick translation was kindly contributed by Dr. G. Klein (12).

RESULTS

C-myc RNA of about 2.5 Kb in size was detected in all six of the normal liver tissue specimens and in five out of seven hepatoma tissues examined (Fig.1). Some differences were observed in quantity of c-myc RNA in each sample. In samples 1

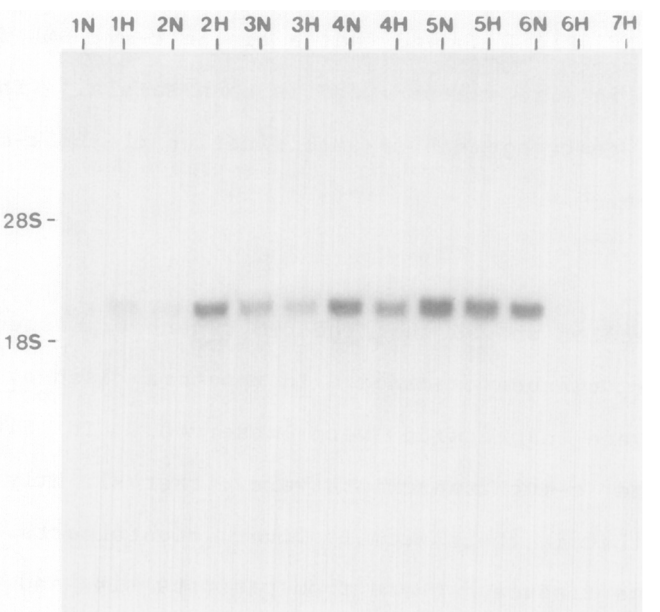

Figure 1. Northern blot hybridization of total RNA from normal liver (N) and hepatoma (H) with c-myc probe. Fifteen ug of total RNA were denatured with glyoxal and applied to a 1.2% agarose gel for electrophoresis. The RNA was transferred to nitrocellulose paper and hybridized with nick-translated [^{32}P] c-myc probe. The amount of probe was 1 x 10^7 cpm/filter and specific activity was 2 x 10^8 cpm/ug. Patient numbers appear at the top.

and 2, the hepatoma tissues showed higher c-myc transcripts than that in normal tissues; in contrast, in samples 3,4,5, there were slightly higher c-myc transcripts in normal tissues. The possibility that these differences might be due to the variation in amount of RNA loaded was ruled out by the similar intensity of ribosomal RNA bands on agarose gel by ethidium bromide staining (data not shown). The most striking observation on the level of c-myc RNA, as shown in Fig.1, was that in hepatoma samples from patients six and seven there were barely detectable c-myc transcripts. The lack of hybridizable signals was not caused by the degradation of RNA, as shown by the intensity of ribosomal RNA bands on the gel and by probing the same RNA with human albumin cDNA probe (data not shown).

To explore the possibility that the low expression of the c-myc gene in hepatoma samples 6 and 7 resulted from an alteration in gene structure, the Eco Rl restriction pattern of c-myc gene was examined. Fig 2 showed that a single c-myc DNA fragment of about 15 kb in size was detected in each sample. There was no significant rearrangement or amplification of the c-myc gene in these tissues.

DISCUSSION

The results showed that the c-myc gene expressed in all six normal liver tissues examined. In hepatoma tissues, variations in c-myc gene expression were observed. In five hepatoma samples, the c-myc transcripts were either slightly higher or lower than that in their normal liver counterparts. In another two hepatoma tissues, those from patients six and seven, the expression of c-myc gene was rather low. We have examined α-fetoprotein expression in patient six in order to confirm the status of normal liver and hepatoma tissues defined by pathological examinations. No detectable α-fetoprotein RNA

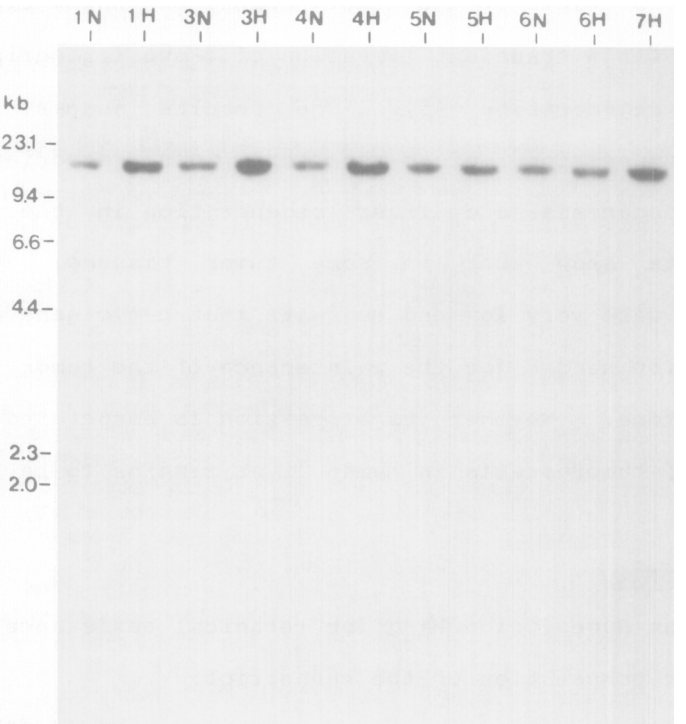

Figure 2. Restriction pattern of genomic DNA using c-myc clone as probe. Fifteen ug of genomic DNA from normal liver (N) and from hepatoma (H) were digested with restriction enzyme Eco Rl. Fragments were separated by electrophoresis in a 0.8% agarose gel and transferred to nitrocellulose paper. The paper was hybridized with nick-translated [^{32}P] c-myc probe. The amount of probe was 2 x 10^7 cpm/filter and specific activity was 2 x 10^8 cpm/ug. Patient numbers appear at the top.

was found in a normal liver sample, and a high expression was noted in the hepatoma sample (Su et al. unpublished result). Southern blot analysis of the c-myc gene demonstrated that the low expression was not associated with significant change in gene structure. The data strongly suggests that c-myc gene expression might not be necessary for the maintenance of the tumor state in human hepatoma. Yaswen et al. examined the c-myc gene expression in rat liver during the course of hepatocarcinogenesis induced by a choline-deficient diet containing ethionine (6). They reported the c-myc transcripts increased by 2 weeks after the start of the carcinogenic diet, and the c-myc expression remained elevated

during the 35 weeks of the diet. Fausto and Shank also showed that there was a transient elevation of c-myc transcripts during rat liver regeneration (5). The results suggest that the elevated expression of c-myc gene was associated with hepatocarcinogenesis and liver regeneration in the rat. The present data show that in some tumor tissues, the c-myc transcripts were very low and suggests that c-myc gene expression might not be needed for the maintenance of the tumor state in human hepatoma. Whether its expression is associated with the promotion of tumorgenesis in human liver remains to be studied.

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