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A CHARACTERISTIC ELECTROPHORETIC PATTERN OF CYTOSOLIC POLYPEPTIDES FROM HEPATOCYTE NODULES GENERATED DURING LIVER CARCINOGENESIS IN SEVERAL MODELS

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The cytosolic polypeptides of hepatocyte nodules in six models of liver carcinogenesis were analysed by SDS-polyacrylamide gel electrophoresis and their patterns campared with these of control and variously treated livers. The amount of a polypeptide of M_r 21,000 was about tenfold elevated in the cytosol of five of the six types of nodules and moderately elevated in the sixth. Certain other polypeptides, particularly one of M_r 26,000, also varied in amount, so that all of the nodules analysed could be distinguished from liver by their electrophoretic patterns. Some possible identities of the two polypeptides are discussed. Their study may have mechanistic as well as diagnostic importance.

Focal proliferations of hepatocytes, hepatocyte nodules, are virtually constant accompaniments of hepatic carcinogenesis during the precancerous period (1-4). These nodules, one site of origin for liver cancer (see 7), have many biochemical properties in common as well as a characteristic arrangement of hepatocytes, blood supply and other structural and physiological features (5,6).

This study was prompted by several recent reports indicating that some liver cytosolic proteins, such as a carcinogen-binding protein (9), some glutathione-S-transferases (9-11), DT-diaphorase (10,12) and aldehyde-NAD(P) oxidoreductase (13), show alterations during liver carcinogenesis (9-13). A comparison was made between the electrophoretic patterns of cytosolic polypeptides from hepatocyte nodules induced in six different models of liver

Abbreviations: CEI, chronic enzyme induction; CMD, choline-methionine deficient; 2-AAF, 2-acetylaminofluorene; PMSF, phenylmethyl-sulfonylfluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; GSH, glutathione; DEN, diethylnitrosamine; PB, phenobarbital; 3-MC, 3-methylcholanthrene; 3'-Me-DAB, 3'-methyl-4-dimethylaminoazobenzene; DMH, 1,2-dimethylhydrazine; GGT, γ-glutamyl transpeptidase.

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carcinogenesis, from adult, fetal, neonatal and regenerating livers and from livers from animals treated with some enzyme inducers including carcinogens. Characteristic differences were found, two polypeptide bands of M_r 21,000 and 26,000 being of special interest.

MATERIALS AND METHODS. Male Fischer 344 and Wistar rats were used for the production of nodules; all other experiments involved the use of the former strain. Resistant hepatocyte-nodules (9 batches) were produced by the method of Solt and Farber (14). CEI-nodules were produced by the methods of Peraino et al (15) (3 batches) and of Pitot et al (16) (2 batches). CMD-nodules (5 batches) were generated by the lipotrope deficiency model of Sells et al (17). AAF-nodules (3 batches) were produced by cyclic treatment with 2-AAF (18). Orotic acid-nodules (4 batches) were generated by the method of Columbano et al (19). All of the batches of nodules were analysed. Two hepatomas produced by the method of Solt and Farber (14) and one by orotic acid (19) were also analysed. Some animals were injected with various xenobiotics (DEN, 2-AAF, PB, DMH, 2-MC and 3'-Me-DAB). The dosages of these agents were as described previously (20), except for DMH which was administered i.p. at 100 mg per kg, the rats being killed 4 days later. Prior to harvesting nodules, all livers were perfused with ice-cold 0.25 mM sucrose containing 1 mM PMSF (Sigma) as protease inhibitor and 1 mM EDTA. Cytosol fractions were prepared by homogenizing tissue (1 g per 3 ml of medium) in 0.25 M sucrose containing PMSF and EDTA as above; the homogenates were centrifuged at 105,000 x g_{av} for 2 h, the clear supernatants collected and then frozen at -70°C until used. Estimations of protein were by the method of Lowry et al (21). SDS-PAGE was performed as described previously (20,22) except that 15% polyacrylamide gels were used routinely. Samples for analysis were reduced and alkylated unless otherwise indicated. The gels were stained with Coomassie Blue B and certain of them were scanned at 540 mM. A sample of purified GSH S-transferase B (ligandin) was generously provided by Dr. G. Litwack. A mixture of GSH S-transferase, partially purified from rat liver, was purchased from Sigma.

RESULTS

Fig. 1A shows a typical separation of the polypeptides of liver, nodules and a hepatoma. At least 35 polypeptides were resolved from liver cytosol (track 1). The nodules (AAF-, resistant hepatocyte-, CMD- and CEI (Peraino)-, tracks 2-5 respectively) and hepatoma (track 6) displayed a pattern that was generally similar to that of liver. A prominent difference in nodules and in the hepatomas was a marked increase in the amount of a polypeptide of M_r 21,000 (P-21). Scanning indicated that P-21 was increased approximately tenfold. A second consistent difference was a marked reduction in the amount of a polypeptide of M_r 26,000 (P-26) in all nodules and hepatomas. The amount of a polypeptide of M_r 12,000 (P-12) was also reduced in the AAF-, the resistant hepatocyte- and CMD-nodules, but not in the CEI (Peraino)-

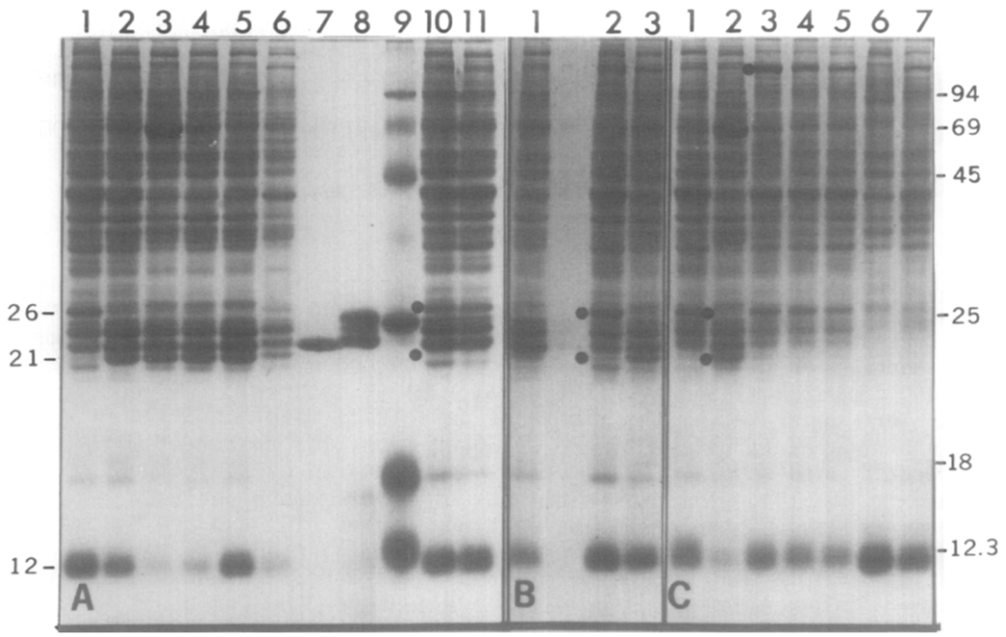

Fig. 1. Analyses by SDS-PAGE (15% polyacrylamide) of the cytosolic polypeptides of livers and of hepatocyte nodules.
A. 1, Control liver; 2, AAF-nodules; 3, resistant hepatocyte-nodules; 4, CMD-nodules; 5, CEI (Peraino)-nodules; 6, hepatoma; 7, ligandin; 8, partially purified preparation (Sigma) of the GSH S-transferases of rat liver; 9, marker proteins; 10, liver from an animal treated with PB; 11, control liver.
B. 1, CEI (Pitot)-nodules; 2, control liver; 3, orotic acid-nodules.
C. 1, Control liver; 2, resistant hepatocyte-nodules; 3,4 and 5, cytosols from livers of rats obtained at 12, 24 and 48 h after partial hepatectomy; 6, pooled livers of fetuses of 15 days of age; 7, pooled livers of newborn animals.

Approximately 30 μg of protein of each cytosolic fraction analysed was subjected to electrophoresis, except in the case of the hepatoma where the amount was approximately 50 μg. The gels were stained with Coomassie Blue B. The markers for estimation of M_r were phosphorylase a (94,000), bovine serum albumin (69,000), ovalbumin (45,000), chymotrypsinogen A (25,000), myoglobin (18,000) and cytochrome c (12,300).

P-26, P-21 and P-12 are indicated in the left hand margin of the figure. A polypeptide of approximately M_r 160,000 whose amount increased after partial hepatectomy is indicated in the left hand margin of track 3 of Fig. 1C by a black dot. The number of batches of each type of nodule analysed is given in the text. The migration of P-21 was not affected by omission of reduction of samples prior to analysis.

nodules. The migrations of the two identical subunits of ligandin and of the subunits of a mixture of partially purified GSH S-transferases of rat liver are shown in tracks 7 and 8 respectively. The former migrated just behind P-21, exhibiting an apparent M_r 22,000, whereas the two other polypeptides (track 8) exhibited values for M_r of approximately 24,000 and 25,000. These values for M_r of the subunits of GSH S-transferases compare well with those reported (cf 23). The amounts of ligandin and of the polypeptide of M_r

24,000 are seen to be moderately elevated (approximately twofold) in the cytosols of the AAF- and CEI (Peraino)-nodules (tracks 2 and 5).

The effects of administration of various xenobiotics on the electrophoretic pattern of the cytosolic polypeptides of liver were investigated. Typical results obtained with PB are shown in track 10. The amounts of ligandin and of the polypeptide of M_r 24,000 are seen to be moderately elevated by this procedure; however, the amount of P-21 was not affected. It was also found that no other xenobiotic administered (DEN, 2-AAF, 3-MC, 3'-Me-DAB or DMH) affected the amount of P-21 in liver. The polypeptides of two batches of CEI (Pitot)-nodules and of four batches of orotic acid-nodules were also analysed. Tracks 1 and 3 of Fig. 1B show typical patterns obtained. The amount of P-21 was moderately (approximately two- to threefold) elevated in both batches of the former and markedly elevated in all batches of the latter type. The amount of P-26 was markedly reduced in all batches of these two types of nodules.

The effects of partial hepatectomy on the polypeptides of liver were also examined (Fig. 1C). This did not appreciably affect the amounts of P-21 or P-26 at 12, 24, 48 (tracks 3-5), 72 or 130 hours (latter two results not shown) after its performance. The amount of a polypeptide of M_r approximately 160,000 was prominently increased at the first three of these time-points. P-21 was not prominent in samples of livers from 15 day old fetuses or newborn animals (tracks 6 and 7) or in samples from 3 day or 10 day old animals (results not shown). The amount of P-26 in these samples was low relative to its amount in control liver (track 1).

DISCUSSION

These results have revealed a number of differences between the patterns of the cytosolic polypeptides of liver and hepatocyte nodules. With regard to P-21, a marked or moderate elevation of its amount was found in every sample of hepatocyte nodules and hepatomas but not under any of the other conditions examined. This apparent high specificity encourages us to think that it may have a close association with hepatocarcinogenesis. The presence of a small

amount of a polypeptide of corresponding migration (Fig. 1A) suggests that a similar protein might be present in the control livers. The estimation of the M_r of P-21 by SDS-PAGE does not exclude the possibility that it may be a subunit of a homo- or hetero- polymer. The finding that omission of the thiol group reduction prior to electrophoresis (see legend to Fig. 1) did not appreciably affect its migration indicates that, if P-21 is present in a polymeric form, the polymer is not linked by disulfide bonds.

A number of other polypeptides were also noted to be altered in amount in the nodules. Every sample of nodules analyzed showed a decrease in the amounts of P-26 and most showed a decrease in P-12. The latter may correspond to the polypeptide of M_r approximately 14,000 studied by Sorof and coworkers (8), since we have observed that it binds metabolites of 2-AAF *in vivo*.

P-21 could be the protein or subunit of an enzyme with elevated activity in the nodules. Our results (Fig 1A) tend to exlude the probability of its being a subunit of one of the major GSH-S-transferases but do not rule out the relationship to a minor species of these enzymes. The activity of GGT is markedly increased in nodules (24), but this appears to be a membrane-bound enzyme (23). GSH peroxidase is a cytosolic enzyme that appears to be closely related to the GSH S-transferases (23); one purified form of this enzyme is comprised of subunits of M_r 21,000 (25).

P-21 and P-26 may be positive and negative growth regulatory proteins respectively. McMahon *et al* (26) have purified a protein of M_r 26,000 from rat liver that exerts an inhibitory effect on the proliferation of normal liver cells. P-21 may be a product of a cellular oncogene. The transforming factors of certain murine sarcoma viruses appear to be proteins of M_r 21,000, designated P-21 (27). It is possible that analogous proteins that are products of cellular oncogenes are to be found in rat preneoplastic and neoplastic liver.

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