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EFFECT OF MNU ON THE METHYLATION PATTERN OF HEPATIC DNA DURING COMPENSATORY CELL PROLIFERATION

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We have used the initiaton-promotion model of MNU-induced hepatocarcinogenesis to test the hypothesis that alteration of the methylation status of DNA cytosines could be involved in the initiation of carcinogenesis. In fact cell proliferation plays a fundamental role in the initiation of liver carcinogenesis and hepatocytes in the S phase are more sensitive towards MNU initiation than at other times in the cycle. The molecular mechanisms involved in these processes, however, are still poorly understood and it seemed of value to monitor the DNA methylation status in this system. The results obtained indicate that MNU hepatocarcinogenic action might consist also of the inhibition of DNA hypomethylation biologically associated with cell proliferation.

Changes in the DNA methylation status have been proposed as one of the mechanisms involved in the perturbed gene expression of cancer cells (1,2) and, more specifically, hypomethylation has been related to the initial step of carcinogenesis (3,4). The ability of chemical carcinogens to interfere with the DNA methylation process (3,5,6) and the fact that 5-azacytidine, an inhibitor of DNA methylation, potentiates the initiation process induced by chemical carcinogens strongly support this hypothesis (7). On the other hand, the molecular mechanisms by which DNA hypomethylation would affect transcription and cause aberrant

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Abbreviations: PH, partial hepatectomy; MNU, N-methyl-N-nitrosourea.

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genic expression remain largely speculative (8-10). In testing the validity of the hypothesis that site-specific hypomethylation may play an important role in the initial phases of chemical carcinogenesis (11), we used the MNU-induced hepatocarcinogenesis in partially hepatectomized rats as a model. This model is a particularly intriguing example of chemically induced liver carcinogenesis since MNU is not hepatocarcinogenic, but becomes such when given after partial hepatectomy (12). It has been suggested that cell proliferation is necessary "to fix" the carcinogen-induced damage into DNA, but the molecular mechanisms underlying the MNU-induced hepatocarcinogenesis are still unclear. Consequently, given also the well known hypomethylating action of MNU (5,13,14), it seemed of value to study the DNA methylation patterns under these conditions.

Materials and Methods

Wistar male albino rats weighing 180-200 g were given MNU (60 mg/kg; i.p.) and killed 4 or 8 h later. When the effect of cell proliferation was investigated, rats were submitted to 2/3 partial hepatectomy and killed at 20 and 24 h. The effect of carcinogen on the DNA methylation status of partially-hepatectomized rats was explored by giving MNU 16 or 20h after PH, and sacrificing the animals 4 or 8 hours later. The animals had free access to water and food.

DNA was isolated and purified from control, partially hepatectomized and/or carcinogen-treated animals as already described in detail (15,16). Purified high-mol wt. hepatic DNA was digested by using the restriction endonucleases HpaII/MspI/HaeIII, and resolved on 1% agarose gel according to described procedures (16).

Results and Discussion

Figure 1 shows that: (i) after partial hepatectomy, the degree of digestibility of genomic DNA in the S phase (lane B and C) was higher than in control DNA (lane A); (ii) DNAs from normal non-treated rats exposed to the carcinogen for 4 and 8 h (lanes D and E) exhibited a slight degree of hypomethylation; (iii) hepatic DNA from partially hepatectomized rats which had been exposed to the

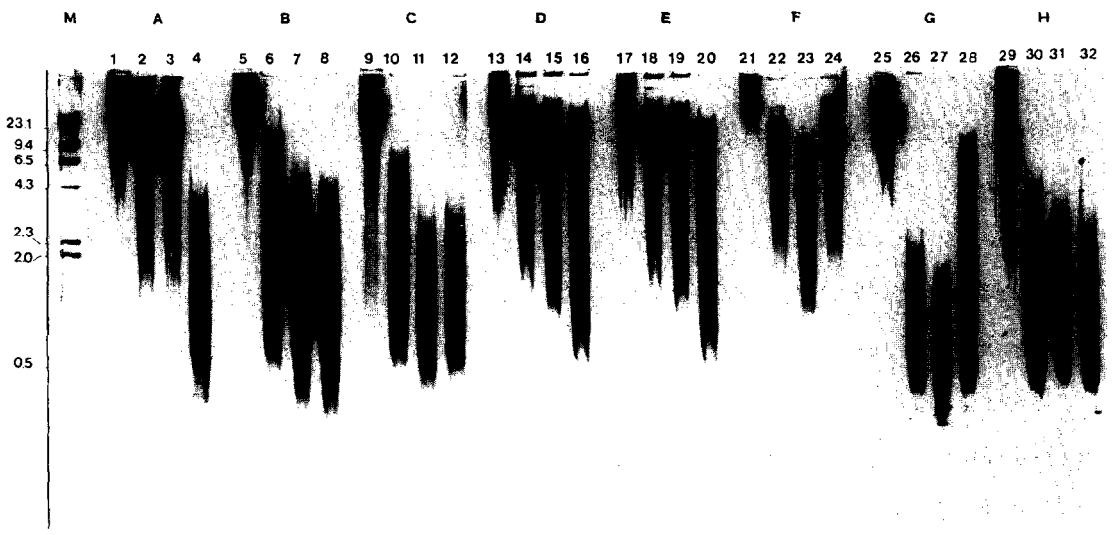

Fig. 1. Effect of N-methyl-N-nitrosourea on hepatic DNA hypomethylation induced by partial hepatectomy.

Purified high-mol wt. hepatic DNA was digested with restriction enzymes and resolved on 1% agarose gel. The photograph illustrates the analysis of DNA isolated from rats: non-treated (A), partially hepatectomized, and sacrificed at 20 (B) or 24 h (C) after PH; exposed for 4 (D), and 8 h (E) to MNU; partially hepatectomized, injected with MNU at 20 h after PH and killed 4 h later (F); partially hepatectomized, injected with MNU at 16 h after PH and killed 4 h (G) and 8 (H) h later. Lanes 1, 5, 9, 13, 17, 21, 25, 29 are undigested DNAs; lanes 2, 6, 10, 14, 18, 22, 26, 30 correspond to HpaII enzymic digests; lanes 3, 7, 11, 15, 19, 23, 27, and 31 are MspI digests; lanes 4, 8, 12, 16, 20, 24, 28, and 32 are HaeIII digests. M is the gel pattern of the molecular weight standard lambda-DNA cut with HindIII.

Further experimental details have been described in our earlier publication (16). The experiments were repeated at least four times with similar patterns of results.

carcinogen for 4 hours, starting 20 hours after PH (lane F), exhibited an intermediate degree of hypomethylation between that caused by PH and MNU given separately; (iiii) hepatic DNAs from partially hepatectomized rats which had been exposed to the carcinogen for 4 or 8 hours starting 16 hours after PH, were heavily hypometylated (lanes G and H).

The results presented in this communication show that differential alterations in the DNA methylation status are produced at genomic level by MNU given after PH, the type of alteration depending on the administration time of the N-nitroso compound. When given 16 hours after PH, the hypomethylating effect of the carcinogen appears to add to the hypomethylation caused by

the compensatory cell proliferation. On the contrary, when given at 20-h after PH, a time critical for the carcinogenic attack (12), MNU inhibits PH-induced hypomethylation. The present findings thus suggest that one of the earliest genomic responses to MNU administration at a time after PH when the hepatocytes are actively engaged in DNA synthesis, is the partial block of the DNA hypomethylation biologically associated with cell proliferation (15,16). This result is consistent with the fact that chemical carcinogens are strong mitoinhibitors and therefore are likely to block DNA hypomethylation by blocking cell proliferation. Thus the present findings might indicate that a prevented hypomethylation, rather than a normal hypomethylation, could be an aspect of the operative mechanism of the hepatocarcinogenicity of the nitroso compound under these conditions. Indeed it is possible that this hampered hypomethylation might prevent from expression some part of the genome possibly involved in the regulation and control of proliferative processes. Thus the comparative analysis of the non-expressed genes in this model could be a promising approach to singling out critical genes targeted by MNU, such as tumor suppressor genes.

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