Hepatocyte growth factor induces *MAT2A* expression and histone acetylation in rat hepatocytes: role in liver regeneration[1]

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SPECIFIC AIMS

We have studied the molecular mechanisms and mediators behind the induction of methionine adenosyltransferase 2 A (*MAT2A*) gene expression in the regenerating rat liver after partial hepatectomy. The involvement of hepatocyte growth factor (HGF) and cellular S-adenosylmethionine (AdoMet) levels in the regulation of *MAT2A* expression are evaluated in a model of cultured rat hepatocytes.

PRINCIPAL FINDINGS

1. Acetylation of histone H4 associated with *MAT2A* promoter is tissue specific and enhanced in the remaining liver after partial hepatectomy (PH)

In mammals, *MAT2A* is expressed in all cells of the organism with the exception of the mature and quiescent hepatocyte. Chromatin immunoprecipitation experiments using an antibody specific to hyperacetylated histone H4 revealed enhanced acetylation of histone H4 associated with *MAT2A* promoter in an expressing tissue such as kidney, whereas the opposite situation was observed in the liver. As previously reported, *MAT2A* expression was induced in the hepatic parenchymal cell shortly after PH. Activation of *MAT2A* transcription was accompanied by time-dependent enhancement in the acetylation status of histone H4 associated with its promoter, as evidenced by chromatin immunoprecipitation assays.

2. HGF induces the hyperacetylation of histone H4 associated with *MAT2A* promoter and *MAT2A* expression in cultured rat hepatocytes

HGF is a key growth factor in the induction of hepatocyte proliferation and one of the main stimuli leading to the rapid changes in gene expression after PH. We studied its effect on *MAT2A* expression in a model of cultured rat hepatocytes. In this experimental system, we have observed that HGF (50 ng/ml, for 1 h) induces the hyperacetylation of histone (H4) associated with *MAT2A* promoter (**Fig. 1**). This effect was blocked by the tyrosine kinase inhibitor genistein (10 μg/ml) (Fig. 1). As would be expected from the effect of HGF on *MAT2A* promoter-associated histones, transcription of *MAT2A* was stimulated by this growth factor in a dose- and time-dependent fashion. The induction of *MAT2A* transcription by HGF was also impaired in the presence of genistein. HGF activated the transcription of a reporter gene (luciferase) under the control of *MAT2A* 5′ region in transient transfection experiments performed in cultured hepatocytes.

3. AdoMet modulates HGF-induced *MAT2A* gene expression and DNA synthesis in cultured rat hepatocytes

In isolated rat hepatocytes, we had previously shown that the expression of *MAT2A* was progressively induced with time in culture, whereas that of the liver-specific gene *MAT1A* was dramatically reduced, probably reflecting the degree of dedifferentiation of cultured hepatocytes. The addition of AdoMet to the culture medium prevented such changes in *MAT1A* and *MAT2A* expression. It was therefore important to know whether *MAT2A* induction by HGF could be modulated by AdoMet. Hepatocytes were preincubated with increasing concentrations of AdoMet for 30 min and treated with HGF (50 ng/ml for 3 h). As shown in

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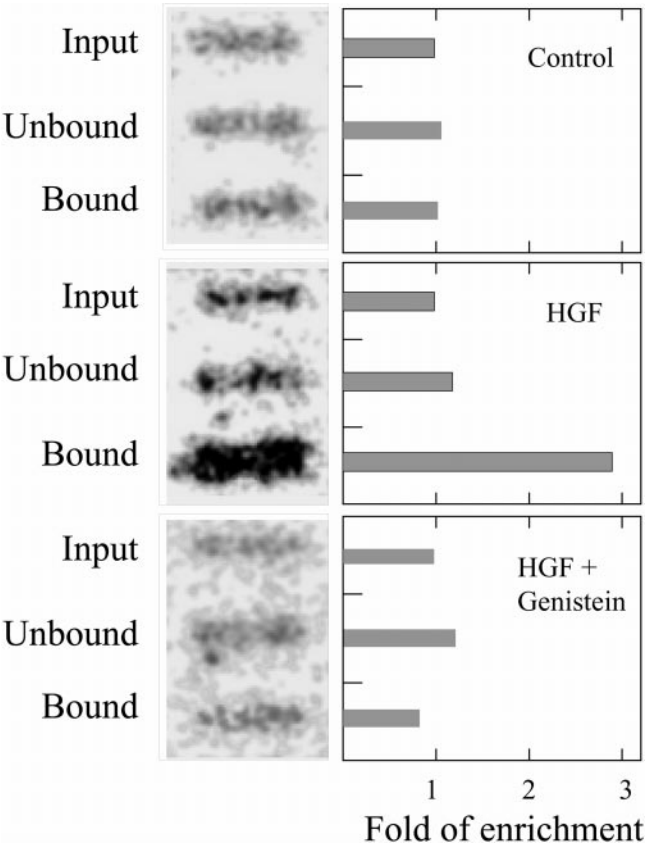

Figure 1. HGF induces the acetylation of histone H4 associated with *MAT2A* promoter in cultured rat hepatocytes. Cells were preincubated or not with genistein (10 μg/ml) for 30 min and then treated for 1 h with 50 ng/ml of HGF. Mononucleosomes were prepared and immunoprecipitated as described in text. DNA was isolated from the different fractions, slot-blotted, and hybridized with a probe derived from *MAT2A* promoter. Quantitation of the radioactivity incorporated in each slot is also shown. Representative autoradiograms are shown.

Fig. 2*A*, AdoMet addition resulted in the dose-dependent inhibition of *MAT2A* expression by HGF. AdoMet effect may be related to its conversion into 5′-methylthio-adenosine (MTA), a metabolite of AdoMet in the polyamine biosynthetic pathway. Pretreatment of hepatocytes with 500 μM of MTA effectively blocked the induction of *MAT2A* expression by HGF, whereas the expression of *MAT1A* was not affected (Fig. 2*B*). It has been reported that AdoMet or MTA administration to rats after PH results in the impairment of DNA synthesis in the liver parenchymal cell. We have now tested the effect of AdoMet on HGF-induced DNA synthesis in cultured rat hepatocytes. In agreement with the in vivo observations in rats after PH, AdoMet was able to partially inhibit HGF-stimulated DNA synthesis (Fig. 2*C*).

CONCLUSIONS

Methionine adenosyltransferase (MAT) catalyzes the formation of AdoMet, a key metabolite central to most cellular transmethylation reactions and a precursor of polyamine biosynthesis. In the adult and quiescent hepatocyte, AdoMet is synthesized by MAT I/III, the product of *MAT1A* gene. When hepatocytes proliferate, however, as occurs during liver regeneration, malignant transformation, or the fetal period, transcription of *MAT2A* is activated resulting in the expression of MAT II, the form of MAT normally expressed outside the liver. Evidence has been reported showing that this switch in MAT gene expression provides the cell with a proliferative advantage, which may stem from the distinct regulatory and kinetic properties of MAT I/III and MAT II that influence intracellular AdoMet levels. However, nothing has been known about the mechanisms that govern *MAT2A* expression during the physiological proliferative response of the hepatocyte.

It is widely accepted that chromatin structure plays a crucial role in the regulation of gene expression in eukaryotes. The interplay of remodeling complexes and covalent histone modifications seems to be essential for the access of DNA binding factors. Histone acetylation is one of such covalent modifications that have been linked to transcriptional activity. We show that histones (H4) associated with *MAT2A* promoter are hyperacetylated in a tissue were the gene is expressed, such as kidney, but are hypoacetylated in the liver. When the hepatocyte proliferates, as in an experimental model of liver regeneration after PH, the acetylation status of histones (H4) associated with *MAT2A* promoter is markedly increased. These in vivo observations suggest that such changes in chromatin at the level of *MAT2A* promoter may play a role in transcriptional activation of this gene.

To identify the factors that could mediate the activation of *MAT2A* expression in the regenerating liver, we turned to an experimental system of isolated rat hepatocytes. HGF is responsible for many of the hepatocellular responses after PH, including the induction of early responsive genes and hepatocyte proliferation. Our observations in primary cultured hepatic cells have identified *MAT2A* as a novel target for HGF. Furthermore, *MAT2A* expression in response to HGF was preceded by the hyperacetylation of histones (H4) associated with its promoter. It has been proposed that the level of selective histone acetylation may depend on signal transduction pathways, but little is known about the possible signal cascades for which histone acetyltransferases (HATs) and/or deacetylases are the end points. It has been shown that steroid hormones and vitamins A and D are able to induce hyperacetylation of histones at promoters of target genes through the recruitment of p300/CBP HAT activity. Together with the recently reported ability of epidermal growth factor to promote the phosphorylation and acetylation of histone H3 associated with c-*fos* promoter, our present observations are the first report of such an effect for a tyrosine kinase-activating growth factor such as HGF. So far, our findings support the more general view that localized changes in chromatin structure induced by

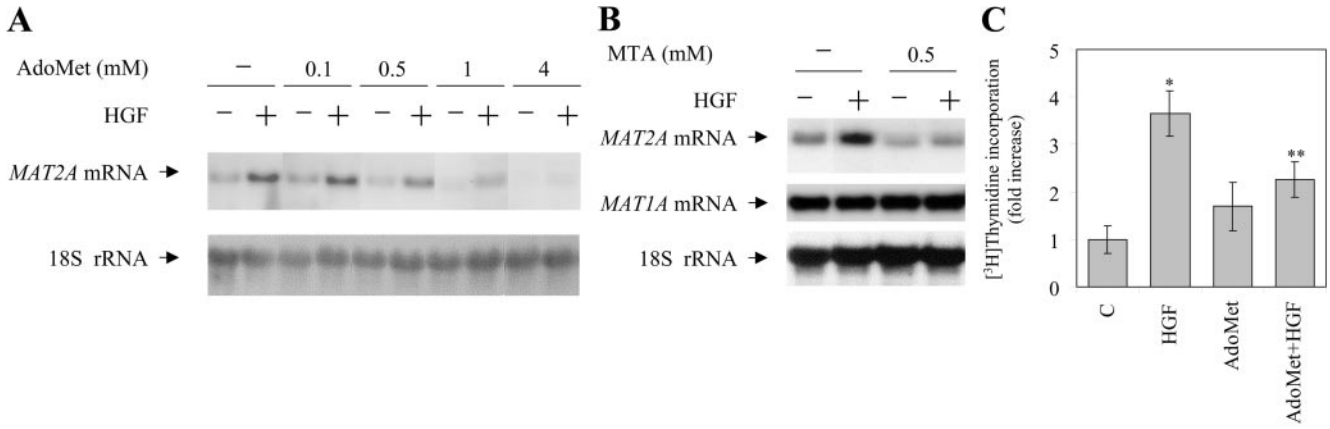

Figure 2. AdoMet and MTA inhibit the induction of *MAT2A* expression by HGF in cultured rat hepatocytes. Cells were pretreated for 30 min with increasing concentrations of AdoMet (*A*) or 500 μM of MTA (*B*), then HGF (50 ng/ml) was added to the cultures and incubation was continued for another 3 h. *MAT2A* and *MAT1A* expression were analyzed by Northern blotting. Hybridization with a probe for 18S rRNA was performed as loading control. Representative blots of three experiments performed in duplicate are shown. *C*) DNA synthesis, measured as [^{3}H]thymidine incorporation, in cultured rat hepatocytes in response to HGF (50 ng/ml) treatment in the presence or absence of AdoMet (4 mM). Data are expressed as fold increase over control and are means ± SE of three experiments performed in triplicate. *$P < 0.05$ respect control (C) value, **$P < 0.05$ respect HGF value.

extracellular signals can be considered a common event in the dynamic regulation of gene expression. Whether HGF also promotes histone H4 phosphorylation or histone H3 modifications (phosphorylation/acetylation) remains to be determined.

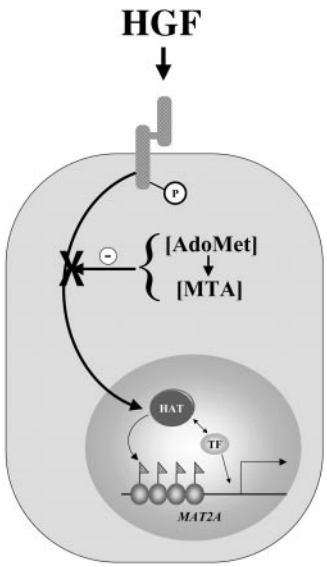

Figure 3. Schematic diagram: model of HGF induction of *MAT2A* gene expression in rat hepatocytes and its modulation of AdoMet/MTA levels. Intracellular signals generated at p190^{MET} receptor after HGF binding result in the recruitment of HAT complexes to *MAT2A* promoter. Such changes in chromatin structure at the level of the target gene would favor the interaction of transcription factors (TF) and promoter transactivation. The induction of *MAT2A* expression in the hepatic parenchymal cell by HGF would be conditioned by AdoMet and MTA contents. AdoMet and MTA levels in the liver are dramatically reduced early after PH, when HGF levels rise and *MAT2A* expression is activated. As intracellular AdoMet and MTA concentrations recover to normal levels, the hepatocyte would subsequently be rendered refractory to HGF, at least regarding the induction of *MAT2A* expression. We would like to propose that fluctuations in the concentrations of these metabolites could be part of the priming events and terminating signals that modulate the liver regenerative process.

The process of liver regeneration involves many complex mechanisms that are not completely understood. A major area of research in this field is the identification of the mechanisms that modulate hepatocyte responsiveness to HGF at the onset of the proliferative response and the signals that determine the termination of liver regeneration. Together with growth factors and cytokines, changes in key metabolite levels may contribute to the orchestration of the regenerative process. We would like to propose that AdoMet and/or MTA could be one of these key metabolites. Hepatic levels of both molecules are dramatically reduced shortly after PH, when HGF levels rise and *MAT2A* expression is activated. As intracellular AdoMet and MTA concentrations subsequently recover to normal levels, the hepatocyte would be rendered refractory to HGF at least regarding the induction of *MAT2A* and DNA synthesis, as we observed in cultured hepatocytes. Although the detailed mechanisms behind AdoMet inhibition of *MAT2A* induction are not completely known, a methylation reaction seems not to be involved, since MTA mimicked this effect and is not a methyl donor compound. Our observations supporting this novel hypothesis are summarized in **Fig. 3** and allow us to suggest that fluctuations in the hepatic concentrations of AdoMet and/or MTA could be part of the priming events and terminating signals that modulate the liver regenerative process. FJ