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CELL-CELL INTERACTIONS: CLUES TO HEPATOCYTE HETEROGENEITY AND BEYOND?

Kuo FC, and Darnell JE Jr. Evidence that interaction of hepatocytes with the collecting (hepatic) veins triggers position-specific transcription of the glutamine synthetase and ornithine aminotransferase genes in the mouse liver. Mol Cell Biol 1991;11:6050-6058.

ABSTRACT

We previously demonstrated that glutamine synthetase (GS) and ornithine aminotransferase (OAT) mRNAs are expressed in the mouse liver acinus preferentially in pericentral hepatocytes, that is, those immediately surrounding terminal central veins (A. L. Bennett, K. E. Paulson, R. E. Miller, and J. E. Darnell, Jr., J. Cell Biol. 105:1073-1085, 1987, and F. C. Kuo, W. L. Hwu, D. Valle, and J. E. Darnell, Jr., Proc. Natl. Acad. Sci. USA, (in press). We now show that hepatocytes surrounding large collecting hepatic veins but not portal veins also express these two mRNAs. The pericentral hepatocytes are the most distal hepatocytes with respect to acinar blood flow, whereas this is not necessarily the case for hepatocytes next to the large collecting hepatic veins. This result implies that it is contact with some hepatic venous element which signals positional expression. In an effort to induce conditions that change relationships between hepatocytes and blood vessels, regenerating liver was studied. After surgical removal of two-thirds or more of the liver, there was no noticeable change in GS or OAT expression in the remaining liver tissue during regeneration. However, treatment with carbon tetrachloride (CCl_4), which specifically kills pericentral hepatocytes, completely removed GS- and OAT-containing cells and promptly halted hepatic transcription of GS. Repair of CCl_4 damage is associated with invasion of inflammatory and scavenging cells, which remove dead hepatocytes to allow regrowth. Only when hepatocytes resumed contact with pericentral veins were the pretreatment levels of OAT and GS mRNA and high levels of GS transcription restored.

COMMENTS

The liver parenchymal cells apparently represent a very flexible cell type. In relation to their position along the porto-central axis, these cells show a variable degree of heterogeneity with respect to enzyme content and activity and thus with respect to function (1). This heterogeneity responds in a dynamic fashion to changes in the physiological state, and the various signals participating in its regulation may involve, among others, the blood concentrations of oxygen, substrates and hormones as well as nervous input (1). A few remarkable exceptions exist. They are the enzymes glutamine synthetase (GS) (2) and ornithine aminotransferase (3), which are localized in about 8% of the most distal perivenous hepatocytes surrounding the terminal hepatic venules. This particular heterogeneity plays an important role in nitrogen detoxification, pH regulation and possibly several other functions (4). The strict limitation of GS expression to some few hepatocytes in a well-defined topographical localization independent of the physiological state has led to the term "positional expression" for this particular phenomenon and has prompted investigators to assume that the liver parenchyma may be subdivided into different "compartments" of gene expression (5). However, the subtle distinction between the generation of such compartments and cell differentiation, if any does exist, has still to be defined.

In the article under discussion, Kuo and Darnell addressed the question of how the positional expression of GS might be explained. The following alternatives were considered by these authors: is the positional expression of these enzymes the result of the location of the respective hepatocytes at the most distal end of the perivenous zone (i.e., in the most downstream area of the lobulus), or is it the result of their contact with the central veins? To approach this question Kuo and Darnell used selective zonal damage by CCl_4 to wipe out hepatocytes in zone 3 and especially to destroy the hepatocytes in contact with the central veins. During regeneration after such a treatment, the reestablishment of the original pattern of GS distribution has been observed with immunologically detectable enzyme protein occurring only in hepatocytes, which resume contact with the central veins (6). Because the induction of the respective messenger RNA (mRNA) precedes detectable levels of enzyme protein, Kuo and Darnell have now strengthened this argument by using *in situ* hybridization to localize the site of the earliest rise in GS and ornithine aminotransferase mRNAs. Their results clearly demonstrate that contact between hepatocytes and pericentral veins triggers the expression of both enzymes. Combining these results, one may conclude that no hepatocyte located further apart from these efferent vessels ever expresses mRNA and protein of these enzymes during the process of regeneration, which takes at least 5 to 7 days.

A second argument for the importance of cell-cell or cell-matrix interactions raised by Kuo and Darnell relies on the observation that a one-cell-thick margin of GS-positive hepatocytes can be found even around large collecting veins where the layer of hepatocytes is no

longer perforated by sinusoids. In this particular case, the adjacent hepatocytes do not *per se* belong to a perivenous zone; rather, they may show varying enzyme patterns sometimes resembling those of midzonal or even periportal hepatocytes because they may occasionally be located quite close to a portal field. The fact that GS is present within all these cells at a level comparable to that in truly perivenous hepatocytes (i.e., those surrounding small terminal hepatic veins) suggests that the location within the acinus is not a major determinant for the expression of this enzyme. Moreover, in accordance with their otherwise periportal phenotype, some of the GS-positive hepatocytes in this location may survive even extended exposure to CCl_4, thus contributing to the residual number of these cells found under such conditions (6).

This argument can even be stressed by looking more carefully at the structure of the liver. The hepatocytes surrounding the large hepatic veins form some kind of a limiting plate that is continuous with the subcapsular limiting plate (7). GS, however, is found only in hepatocytes around the veins and not in the hepatocytes underneath the capsule, indicating that it must be the interaction with the endothelial cells of the hepatic veins or with their basement membrane that contributes to the acquisition of the GS-positive phenotype. However, no simple explanation exists for the fact that the GS-positive area around small branches of the central vein is relatively broad (up to three cells thick), whereas a one-cell layer is always seen around the large vessels, as has been determined directly using image analysis (8).

Although the *in vivo* study by Kuo and Darnell removes the last doubts of the original hypothesis that the heterogeneous distribution of GS may depend on cell-cell or cell-matrix-interactions (2), it still leaves us with the question of what mechanisms might be involved.

In general, such interactions can lead to cell differentiation or contribute to reversible inductive phenomena within already differentiated cells (9). No definitive answer has yet been found for which of these different possibilities is effective in confining GS expression to this small hepatocyte population touching the central veins. To illustrate the complexity of this phenomenon, some of the arguments favoring the one or the other possibility are discussed below.

Direct experimental evidence for the involvement of cell-cell interactions in the regulation of GS expression comes from cocultures between hepatocytes and so-called epitheloid cell lines of thus far unknown origin (10). Under appropriate culture conditions these cells spontaneously induce GS expression in originally enzyme-negative periportal hepatocytes that, in pure culture, would never acquire the ability to express GS. What is still puzzling with these coculture experiments is the fact that despite a more than 10-fold induction of GS in the periportal hepatocytes the level is still relatively low compared with the estimated activity of pure GS-positive hepatocytes. Furthermore, the hormonal response of GS characteristic for originally GS-positive hepatocytes (2) is not established when GS-negative periportal hepatocytes are maintained in coculture. This does not seem to reflect limited performance of the cocultured hepatocytes because perivenous GS-positive hepatocytes maintain this regulation. Rather, it is likely that an important step generating truly GS-positive hepatocytes is missing.

As shown by Schrode, Mecke and Gebhardt (10) the induction in the coculture system seems to be the result of the combined action of several inducing factors produced by the epitheloid cells: a heat and acid labile-soluble polypeptide factor and factors acting only within a limited distance, most likely components of the extracellular matrix. In addition, evidence has been seen for a soluble factor produced by periportal hepatocytes that might inhibit GS expression at least in cultured cells (10).

Certainly, the balance of such positive and negative signals under *in vivo* conditions might ensure that the enzyme distribution would show a very sharp border. However, if such signals do the whole job, what then is the difference between the extremely stable expression of GS and the more flexible expression of α_2-microglobulin (11) or of the brain-type glucose transporter (12), two proteins that normally show a distribution quite similar to GS but that can relatively easily be induced in adjacent, newly recruited hepatocytes? Obviously, the signals regulating these two proteins behave more in a dynamic, gradient-like fashion, although they might be produced by the endothelial cells of the terminal hepatic venules as well (11).

At first glance, these findings seem to support simple enzyme induction as the mechanism, but some evidence also exists for the hypothesis that the massive expression of GS in hepatocytes might be caused by a differentiating event (13). For instance, cell differentiation occurring *in vivo* but not in the cocultures could explain why originally GS-positive hepatocytes remain enzyme positive for 10 days and more during pure cultivation even in the absence of inducing stimuli, whereas the induction of GS in periportal GS-negative hepatocytes during cocultivation that occurs already within 24 hr seems to be reversible (Schrode W, et al., Unpublished observation). Furthermore, the GS-positive hepatocyte population not only shows a distinct pattern of ploidy (6) but is also characterized by an individual proliferative potential *in vitro, in vivo* and in experimental hepatocarcinogenesis (8, 13).

Indeed, other examples exist of induced cell differentiation in the liver. Hepatocyte-mesenchyme interactions in the periportal limiting plate have been described as inducing the differentiation of bile ductular cells and the formation of bile ductules in the embryonic liver (14). Furthermore, Gumucio (15) recently summarized findings indicating that even after this event the hepatocytes of the first layers of periportal cells seem to be distinct from those in more downstream locations,

just as the GS-positive hepatocytes are at the opposite end of the acinus.

In conclusion, short-range cell-cell interactions between endothelial and mesenchymal cells of the portal triad or the central veins and the adjacent hepatocytes seem to play an important role in defining phenotypic characteristics of these hepatocytes. Even the possibility that these interactions lead to higher levels of differentiation remains a serious option. Thus the unequivocal demonstration of the importance of such interactions marks only the beginning of a new challenge for future hepatological studies.

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NUTRITIONAL THERAPY FOR ALCOHOLIC HEPATITIS: ARE WE THERE YET?

Kearns PJ, Young H, Garcia G, Blaschke T, O'Hanlon G, Rinki M, Sucher K, et al. Accelerated improvement of alcoholic liver disease with enteral nutrition. Gastroenterology 1992;102:200-205.

ABSTRACT

This prospective study compared the effects of tube-fed nutrition with those of a regular diet in alcoholic liver disease. The high prevalence of malnutrition in patients with alcoholic liver disease requires clarification of the benefits of aggressive nutritional support. Patients were randomly assigned a regular diet without or with tube-fed supplementation, delivering 1.5 g/kg protein and 167 kJ/kg daily. Comparisons of encephalopathy, antipyrine clearance, metabolic rate, and biochemical parameters were performed weekly for 4 weeks. Sixteen patients receiving enteral supplementation had antipyrine half-life (50% vs. 3% reduction), serum bilirubin (25% vs. 0% reduction), and median encephalopathy scores that improved more rapidly than those of controls. Initially, 15 controls did not consume adequate calories to meet measured resting energy expenditure. Aggressive nutritional intervention accelerated improvement in alcoholic liver disease. Adverse effects did not offset the demonstrated benefits of a 2-cal/mL, casein-based tube-fed supplement. These findings support the use of standard, casein-based solutions in the treatment of alcoholic liver disease and as the control condition for future studies.

Bonkovsky HL, Fiellin DA, Smith GS, Slaker DP, Simon D, Galambos JT. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. I. Short-term effects on liver function. Am J Gastroenterol 1991;86:1200-1208.

ABSTRACT

The present studies were designed to provide careful measures of effects of oxandrolone, an anabolic steroid, intravenous nutritional supplementation, and the combination of these two treatments on liver functions, metabolic balances, nitrogen metabolism, and nutritional status in patients with moderate to severe alcoholic hepatitis. Of 43 patients originally recruited, 39 (19 men, 20 women) with typical clinical and laboratory features of alcoholic hepatitis (11 Child's-Pugh class B; 28 class C) were admitted to a metabolic unit and completed a 35-day three-phase protocol. Phase I was a 10-day baseline period of observation, during which routine and special quantitative tests of liver function (galactose and antipyrine metabolism), a 7-day elemental balance study, and a ^{15}N, ^{13}C-leucine metabolism study were done. Phase II was a 21-day treatment period during which patients were randomly assigned to receive one of four regimens: 1) standard therapy, consisting of abstinence, a balanced, nutritionally adequate diet, and multivitamins; 2) oxandrolone (20 mg orally four times a day) plus standard therapy; 3) nutritional supplementation,