Commentary

Differential Growth: From Carcinogenesis to Liver Repopulation

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The liver, like most organs in adult healthy animals, tends to maintain a steady mass, with a balance between cell gain and cell loss.^{1,2} This apparently simple phenomenon, which is indeed at the heart of the complexity of higher organisms, must be regulated by highly integrated mechanisms, of which there is rather little understanding so far. In fact, neither the sensors nor the exact reference parameters (organ mass, DNA content, number of cells) that maintain such homeostatic balance have been exactly identified.^{3,4} One of the best examples illustrating this concept is the vigorous process of regeneration that follows partial removal of liver tissue.⁵⁻⁷ This process is rapid and stops only when the mass of the organ is largely recovered, indicating that very tight and effective control mechanisms are involved.⁵⁻¹³ Under normal conditions the large majority of pre-existing hepatocytes is able to contribute to the regenerative process, undergoing one or more cell division cycles.³⁻⁷ However, when the proliferative capacity of differentiated hepatocytes is severely impaired, an alternative response pattern is often observed, namely the emergence of a cell population referred to as oval cells that appears to originate from pre-existing bile ductular epithelium.¹⁴⁻²⁰ The biological significance of such response pattern is far from being fully elucidated. Because there is evidence indicating that these cells can differentiate into hepatocytes,^{16,21-24} they are often interpreted as representing an adaptive response of the liver to the cell cycle block imposed on resident hepatocytes, aiming for the recovery of the original hepatocytic mass. However, it is important to recognize that any quantitative contribution of oval cells to the process of liver regeneration, under various experimental conditions of hepatocytic cell cycle block, has not yet been assessed.

In the paper by Gordon et al²⁵ in this issue of *The American Journal of Pathology*, a novel possible pathway is proposed whereby liver mass can be recovered when the majority of pre-existing hepatocytes are inhibited from responding to growth stimuli. The model described by

the authors is based on the use of retrorsine, a pyrrolizidine alkaloid that exerts a long-lasting block on hepatocyte cell cycle.^{26,27} When rats exposed to retrorsine undergo 2/3 partial hepatectomy (PH), clusters of small hepatocytes rapidly emerge among surrounding megakaryocytic cells. These cells are still actively proliferating at 2 weeks after PH, and by 1 month they occupy the bulk of the liver and have virtually completed the regeneration process, apparently replacing the original parenchymal cells.²⁵ Although the cell of origin of proliferating hepatocyte clusters was not exactly identified, the process described represents a clear example of the existence of alternative pathways of regeneration in the liver, which can be activated when the majority of the original hepatocytes are unable to divide. These cells must have escaped the mitotic block imposed by retrorsine, thereby acquiring a selective growth advantage over surrounding hepatocytes. Technically it is a process of liver repopulation by endogenous cells, similar, in principle, to that achieved through the use of transplanted normal hepatocytes in rats given retrorsine.²⁸

The concept that a selective growth advantage could drive focal proliferation of rare resistant cells when the response capacity of surrounding tissue is inhibited was first derived from studies on chemical carcinogenesis.²⁹ In fact, those studies formed the conceptual framework on which the retrorsine model of liver repopulation was initially proposed.³⁰

Over two decades ago Farber and colleagues²⁹ developed a model of cancer induction in rat liver wherein the rapid expansion of initiated cells into hepatocyte nodules was achieved via selective inhibition of surrounding liver. When carcinogen-treated rats received a brief exposure to an agent able to block normal hepatocyte cell division (eg, 2-acetylaminofluorene), a rare population of carcinogen-altered cells (initiated cells) could withstand such growth inhibitory effect and proliferate rapidly on appropriate stimulation.^{29,31} These findings established a new principle in the analysis of the stepwise process of cancer development; ie, a mechanism of differential

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growth inhibition of surrounding cells could form the basis for the emergence of focal proliferative lesions,²⁹ an established precursor of neoplasia. Three pathogenetic components were involved: the induction of a rare population of initiated cells with a resistant phenotype toward cytotoxicity; the imposition of a growth constrained environment for the majority of cells in the surrounding tissue; and a strong selective pressure in the form of an appropriate growth stimulus (eg, 2/3 PH).²⁹ In the absence of any of the above components, no effective focal growth of altered hepatocytes was observed.^{29,31}

Later analyses of other experimental systems have been consistent with this hypothesis, suggesting that the selective expansion of initiated cells in the context of a constrained growth environment may represent one general mechanism for the emergence of focal proliferative areas.³²⁻³⁶ For example, orotic acid, a normal cellular metabolite found to enhance carcinogenesis in rat liver, is also able to inhibit normal hepatocyte proliferation, whereas liver nodules were resistant to such an inhibitory effect.^{33,34} Recent data indicate that TPA (12-O-tetradecanoylphorbol-13-acetate), the first tumor promoter to be identified and one of the most extensively investigated, may also act through a mechanism consistent with the above interpretation.³⁵ Furthermore, in a transgenic mouse model, coexpression of TGF- α and *c-myc*, which is associated with high incidence of liver tumors, promotes early replicative senescence of differentiated hepatocytes, which in turn may provide a driving force for the selective growth of initiated cells in that model.³⁶ In these different model systems of carcinogenesis, a common denominator appears to be the emergence of a resistant cell population in a background of chronic toxicity and/or impaired growth capacity in the target organ, which represents a critical component for the selective expansion of initiated cells.^{37,38}

Within this conceptual framework, it became of interest to ask a basic question: could normal cells also expand selectively when transplanted into a recipient organ whose growth capacity had been previously impaired? And, if so, would they grow in a nodular pattern, similar to that of initiated cells, or would they rather integrate in the recipient parenchyma and behave much like normal endogenous cells? Answers to these questions could provide important insights into the pathogenesis of focal proliferation during carcinogenesis and help designing novel strategies for organ repopulation with transplanted normal cells.

To address some of these questions, an experimental model associated with strong and persistent inhibition of endogenous cells was selected, to be used as a background for the subsequent transplantation of normal cells. Pyrrolizidine alkaloids (PAs), a class of naturally occurring compounds present in several plant species, were known for their ability to impose a long-lasting block on hepatocyte cell cycle even after one or a few exposures,²⁶ and appeared therefore to be ideal for this type of transplantation experiment. In the initial studies, rats were treated with lasiocarpine, a PA, followed by injection (through the portal vein) of 1 million normal hepatocytes isolated from a syngeneic donor.³⁰ When animals were

killed 3 months later, classical chronic lesions associated with PA exposure¹⁵ were found in the liver of rats receiving no cell transplantation, including extensive megalocytosis (enlarged hepatocytes; close to 90% of the liver was megalocytic) and proliferation of oval cells.³⁰ However, the livers of PA-treated and transplanted animals appeared histologically normal, with normal sized hepatocytes and only about 2% residual megalocytes. Because in that study no markers were used to distinguish host from transplanted hepatocytes, no definitive conclusions could be drawn as to the basis for the observed phenomenon. However, the data were consistent with the postulation that transplanted normal cells could expand selectively in the recipient liver inhibited by lasiocarpine.³⁰ Moreover, if such selective growth did in fact occur, it appeared to be well integrated into the host liver, as no discrete nodular lesions were observed in transplanted animals.

A direct testing of the hypothesis came with studies involving the use of a marker enzyme to follow the fate of transplanted cells in the recipient liver. In collaboration with the group of Dr. Shafritz at the Albert Einstein College of Medicine (Bronx, NY), we were able to show that donor-derived normal cells could indeed proliferate extensively in the PA-treated recipient liver, replacing over 90% of the original mass within 2 to 3 months.²⁸ (In these studies retrorsine, a commercially available PA related to lasiocarpine, was used.) It was also confirmed that transplanted cells were integrated in host parenchyma and there was no evidence of nodular type of growth in animals followed for up to 1 year.²⁸ Donor-derived hepatocytes maintained a normal differentiated phenotype and were negative for the expression of glutathione-S-transferase 7-7 (Laconi, Pillai, and Pani, unpublished observation). No proliferation of donor-derived cells occurred in transplanted-untreated animals, indicating that exposure to retrorsine was essential for the phenomenon to occur.²⁸

The development of this model clearly indicated that normal transplanted cells could selectively grow in the liver of animals with a normal genetic background when the proliferative potential of resident cells had been reduced via exogenous treatment. This was similar, at least in principle, to the selective expansion of initiated cells early in carcinogenesis, when the surrounding tissue is inhibited by growth-suppressive agents and carcinogen-altered cells are able to emerge because of their phenotypic resistance to the effect of these agents.²⁹ The apparent analogy between these two biological systems is intriguing and is supported by additional evidence. Partial surgical hepatectomy (2/3 PH), which is known to stimulate the growth of early lesions during carcinogenesis,³¹ is also very effective in accelerating the process of liver repopulation by transplanted hepatocytes in the retrorsine model (>90% replacement within 2 to 3 months, compared to a maximum of 10 to 20% in the absence of PH).²⁹ Conversely, acute administration of a direct liver mitogen such as lead nitrate, which is unable to promote the selective expansion of initiated cells in rat liver,³⁹ was also ineffective toward enhancing repopulation in the retrorsine model of hepatocyte transplantation.⁴⁰ This

type of evidence suggests the existence of basic common mechanisms sustaining proliferation of normal transplanted cells and initiated cells when the bulk of the tissue is inhibited in its capacity to respond to growth stimuli. More specifically, both cell types appear to be capable to undergo compensatory proliferation when the growth potential of the target organ has been impaired by earlier treatments. This in turn raises the intriguing possibility that normal cells could compete for growth with initiated cells when transplanted during carcinogenesis.

Relevant to this point, it is also critical to highlight the existence of at least two fundamental differences in the biological behavior of transplanted normal cells *versus* endogenous initiated cells, ie, growth pattern and extent of proliferation. In rat liver, the growth pattern of initiated cells is typically focal/nodular, ie, they grow into discrete lesions that are sharply demarcated from surrounding tissue; the latter is usually compressed as the size of these lesions increases; invasion is not observed until very late during progression and is in fact a clear sign of overt neoplasia.⁴¹ This is in sharp contrast to what one sees during repopulation of rat liver by normal cells. Transplanted hepatocytes do not form nodules but rather integrate into the recipient parenchyma, forming hybrid canaliculi with adjacent endogenous cells; at no time in the process of liver repopulation was there any sign of compression of surrounding tissue.^{28,30} Paradoxically, the behavior of normal cells appears in this sense more invasive than that of initiated cells, at least at early stages. However, it should be noted that no evidence of neoplastic transformation has been observed so far in donor-derived cells after more than 16 months of follow up (Laconi et al, unpublished observation).

Secondly, the kinetics of liver repopulation by transplanted normal hepatocytes (>90% within 2–3 months) appears to be faster than the growth rate of nodules in most of the available models of chemically induced liver carcinogenesis.^{31,42} This observation suggests that the proliferative potential of transplanted normal cells may be higher than that of endogenous initiated cells and supports the possibility that normal cell transplantation could have the ability to modulate the growth of initiated cells early in carcinogenesis.

In summary, the development of the retrorsine model of massive liver repopulation indicates that normal transplanted cells can also undergo a process of selective clonal expansion when the growth response capacity of endogenous hepatocytes is inhibited via external treatment. This is similar, in principle, to the emergence of focal proliferative lesions in the context of a constrained growth environment during carcinogenesis. However, intriguing analogies and important differences are apparent between liver repopulation by normal cells and selective focal growth of initiated cells during early phases of cancer development. The report by Gordon et al²⁵ in this issue of the *Journal* describes yet another suggestive example of selective clonal growth originating from a rare cell population in the liver. A common denominator that appears to be relevant to all of these processes is the inability of resident, differentiated hepatocytes to respond effectively to normal homeostatic mechanisms,

which tend to maintain the size of the organ within relatively constant limits. When this occurs, alternative response patterns emerge that are strictly dependent on the overall status of the organ. A better understanding of the above mechanisms will provide further insights into the biological significance of each of these processes and may help in the design of novel strategies for their control in a clinical setting.

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References

1. Wright N, Alison MR: The liver. The Biology of Epithelial Cell Populations. Vol. 2, ch. 25. Oxford, Clarendon Press, 1984, pp 880–980
2. Schulte-Hermann R: Reactions of the liver to injury: adaptation. Toxic Injury of the Liver. Edited by Farber E, Fisher MM. New York, Marcel Dekker, 1979, pp 385–444
3. Moolten FL, Bucher NLR: Regeneration of rat liver: transfer of humoral agents by cross circulation. *Science* 1967, 158:272–273
4. Alison MR: Regulation of hepatic growth. *Physiol Rev* 1986, 66:499–541
5. Higgins GM, Anderson RM: Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. *Arch Pathol* 1931, 12:186–202
6. Grisham JW: A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver; autoradiography with thymidine-H-3. *Cancer Res* 1962, 22:842–848
7. Michalopoulos GK, DeFrances MC: Liver regeneration. *Science* 1997, 276:60–66
8. Braun L, Mead JE, Panzica M, Mikumo R, Bell GI, Fausto N: Transforming growth factor β mRNA increases during liver regeneration: a possible paracrine mechanism of growth regulation. *Proc Natl Acad Sci USA* 1988, 85:1539–1543
9. Bissell DM, Wang S-S, Jarnagin WR, Roll FJ: Cell-specific upregulation of transforming growth factor- β in rat liver: evidence for autocrine regulation of hepatocyte proliferation. *J Clin Invest* 1995, 96:447–455
10. Diehl AM, Rai RM: Regulation of signal transduction during liver regeneration. *FASEB J* 1996, 10:215–227
11. Boulton R, Woodman A, Calnan D, Selden C, Tam F, Hodgson H: Nonparenchymal cells from regenerating rat liver generate interleukin-1 α and -1 β : a mechanism of negative regulation of hepatocyte proliferation. *Hepatology* 1997, 26:49–58
12. Fausto N: Knocking out genes to study liver regeneration: present and future. *Am J Physiol* 1999, 277:G917–G921
13. Bucher NLR, Swaffield MN: The rate of incorporation of labelled thymidine into the deoxyribonucleic acid of regenerating rat liver in relation to the amount of liver excised. *Cancer Res* 1964, 24:1611–1625
14. Farber E: Similarities in the sequence of early histological changes induced in the liver of the rat by ethionine, 2-acetylaminofluorene, and 3'-methyl-4-dimethylaminoazobenzene. *Cancer Res* 1956, 16:142–148
15. Schoental R, Magee PN: Chronic liver changes in rats after a single dose of lasiocarpine, a pyrrolizidine (senecio) alkaloid. *J Pathol Bacteriol* 1957, 54:305–319
16. Lemire JM, Shiojiri N, Fausto N: Oval cell proliferation and the origin of small hepatocytes in liver injury induced by D-galactosamine. *Am J Pathol* 1991, 139:535–552
17. Sarraf C, Lalani E-N, Golding M, Anilkumar TV, Poulosom R, Alison M: Cell behavior in the acetylaminofluorene-treated regenerating rat

- liver: light and electron microscopic observations. *Am J Pathol* 1994, 145:1114–1126
18. Petersen BE, Zajac VF, Michalopoulos GK: Hepatic oval cell activation in response to injury following chemically induced periportal or pericentral damage in rats. *Hepatology* 1998, 27:1030–1038
19. Yin L, Lynch D, Sell S: Participation of different cell types in the restitutive response of rat liver to periportal injury induced by allyl alcohol. *J Hepatol* 1999, 31:497–507
20. Demetris AJ, Seaberg EC, Wennerberg A, Ionellie J, Michalopoulos GK: Ductular reaction after submassive necrosis in humans. *Am J Pathol* 1996, 149:439–448
21. Evarts RP, Nagy P, Nakatsukasa H, Marsden E, Thorgeirsson SS: In vivo differentiation of rat liver oval cells into hepatocytes. *Cancer Res* 1989, 49:1541–1547
22. Dabeva MD, Shafritz DA: Activation, proliferation, and differentiation of progenitor cells into hepatocytes in the D-galactosamine model of liver regeneration. *Am J Pathol* 1993, 143:1606–1620
23. Alison MA, Golding M, Lalani E-N, Nagy P, Thorgeirsson SS, Sarraf C: Wholesale hepatocytic differentiation in the rat from ductular oval cells, the progeny of biliary stem cells. *J Hepatol* 1997, 26:343–352
24. Lazaro CA, RhimJA, Yamada Y, Fausto N: Generation of hepatocytes from oval cell precursors in culture. *Cancer Res* 1998, 58:5514–5522
25. Gordon GJ, Coleman WB, Hixson DC, Grisham JW: Liver regeneration in rats with retrorsine-induced hepatocellular injury proceeds through a novel cellular response. *Am J Pathol* 2000, 156:607–619
26. Peterson JE: Effects of the pyrrolizidine alkaloid, lasiocarpine N-oxide, on nuclear and cell division in the liver of rats. *J Pathol Bacteriol* 1965, 89:153–171
27. Laconi S, Curreli F, Diana S, Pasciu D, De Filippo G, Sarma DSR, Pani P, Laconi E: Liver regeneration in response to partial hepatectomy in rats treated with retrorsine: a kinetic study. *J Hepatol* 1999, 31:1069–1074
28. Laconi E, Oren R, Mukhopadhyaya D, Hurston, E, Laconi S, Pani P, Dabeva M, Shafritz A: Long-term, near-complete liver replacement by transplantation of isolated hepatocytes in rats treated with retrorsine. *Am J Pathol* 1998, 153:319–329
29. Solt DB, Farber E: A new principle for the analysis of chemical carcinogenesis. *Nature* 1976, 263:702–703
30. Laconi E, Sarma DSR, Pani P: Transplantation of normal hepatocytes modulates the development of chronic liver lesions induced by a pyrrolizidine alkaloid, lasiocarpine. *Carcinogenesis* 1995, 16:139–142
31. Solt DB, Medline A, Farber E: Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. *Am J Pathol* 1977, 88:595–618
32. Barbason H, Rasenfosse C, Betz EH: Promotion mechanism of phenobarbital and partial hepatectomy in DENA hepatocarcinogenesis: cell kinetics effect. *Br J Cancer* 1983, 47:517–525
33. Laconi E, Li F, Semple E, Rao PM, Rajalakshmi S, Sarma DSR: Inhibition of DNA synthesis in primary cultures of hepatocytes by orotic acid. *Carcinogenesis* 1988, 9:675–677
34. Sheikh A, Yusuf A, Laconi E, Rao PM, Rajalakshmi S, Sarma DSR: Effect of orotic acid on in vivo DNA synthesis in hepatocytes of normal rat liver and in hepatic foci/nodules. *Carcinogenesis* 1993, 14:907–912
35. Karen J, Wang Y, Javaherian A, Vaccariello M, Fusenig NE, Garlick JA: 12-O-Tetradecanoylphorbol-13-acetate induces clonal expansion of potentially malignant keratinocytes in a tissue model of early neoplastic progression. *Cancer Res* 1999, 59:474–481
36. Factor VM, Kao C-Y, Santoni-Rugiu E, Woitach JT, Jensen MR, Thorgeirsson SS: Coexpression of c-myc and transforming growth factor α in the liver promotes early replicative senescence and diminishes regenerative capacity after partial hepatectomy in transgenic mice. *Hepatology* 1997, 26:1434–1443
37. Farber E, Rubin H: Cellular adaptation in the origin and development of cancer. *Cancer Res* 1991, 51:2751–2761
38. Prehn RT: On the prevention and therapy of prostate cancer by androgen administration. *Cancer Res* 1999, 59:4161–4164
39. Coni P, Pichiri-Coni G, Curto M, Simbula S, Giacomini L, Sarma DSR, Ledda-Columbano GM, Columbano A: Different effects of regenerative and direct mitogenic stimuli on the growth of initiated cells in the resistant hepatocyte model. *Jpn J Cancer Res* 1993, 84:501–507
40. Pani P, Laconi S, Pillai S, Scintu F, Curreli F, Shafritz DA, Laconi E: Direct hyperplasia does not enhance the kinetics of liver repopulation in a new model of hepatocyte transplantation in the rat. *J Hepatol* 1999, 31:354–359
41. Farber E, Sarma DSR: Biology of disease: hepatocarcinogenesis: a dynamic cellular perspective. *Lab Invest* 1987, 56:4–22
42. Hayes MA, Roberts E, Farber E: Initiation and selection of resistant hepatocyte nodules in rats given the pyrrolizidine alkaloids lasiocarpine and senecionine. *Cancer Res* 1985, 45:3726–3734