bring us closer to solving the puzzle of the pathogenesis of gastric cancer.

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Cirrhosis and Hepatocellular Neoplasia: More Like Cousins Than Like Parent and Child

See article on page 455.

Crucial to the understanding of how carcinoma arises in any organ and crucial to the ability to intervene therapeutically in the malignant process is the identification of the earliest detectable premalignant lesion. It has long been thought and often stated that cirrhosis is such a lesion in the liver. However, whereas most hepatocellular carcinomas (HCCs) arise in the context of cirrhosis, not all types of cirrhosis have the same likelihood of giving rise to carcinoma and not all carcinomas in the setting of chronic liver disease arise after cirrhosis has been fully established. Thus, consideration of cirrhosis itself as the premalignant lesion in the liver gives an incomplete picture, both conceptually and practically.

In the last decade, Japanese clinicians, while screening for the smallest detectable HCC nodules in patients with cirrhosis, found some cirrhotic nodules that were larger than others but were not actually carcinoma. However, many of these nodules contained foci of cytological or architectural atypia, suggesting that these lesions were actually premalignant. Moreover, some nodules were found to contain microscopic foci of HCC, further supporting the background nodule as a premalignant lesion.[1] These nodules have been referred to variously as adenomatous hyperplasia, macroregenerative nodules, or dysplastic nodules (the last term is now preferred).[2] Because cirrhosis was held to be the earliest premalignant lesion, it was thought that development of carcinoma went something like this: small regenerative nodules with a higher rate of proliferation than surrounding nodules became larger—thereby standing out from the surrounding parenchyma allowing their detection—and by virtue of the increased proliferation, became more susceptible to carcinogenic hits.[3]

However, three observations suggested that this hypothesis was untenable. First, nearly all dysplastic nodules contain intact portal triads, often in a virtually normal distribution, implying that as the nodule expanded

new portal tracts might also be forming.[4] However, regrowth of normal portal tracts has not been observed in human tissues or in experimental animal models. Second, dysplastic nodules, including one containing multiple microscopic foci of HCC, have been found in livers from patients with chronic hepatitis but without cirrhosis. Therefore, they could not have begun as smaller cirrhotic nodules.[5] Finally, one study of hepatitis B genome integration, using restriction fragment length polymorphism techniques, showed clonality in two dysplastic nodules from a patient with chronic hepatitis B infection, implying that these nodules were not hyperplastic but already neoplastic.[6] Putting these observations together we suggested an alternate hypothesis of dysplastic nodule development.[1,5] This hypothesis suggested that a clonal, neoplastic expansion of a single hepatocyte or progenitor cell, in the context of increased hepatocyte turnover in chronic liver disease, would spread in an infiltrating fashion around nearby portal and vascular structures. The infiltrating growth replacing nonneoplastic hepatocytes could be accomplished either by increased proliferation or by a decrease in cell death, giving the neoplastic cells a survival advantage. If the population of cells was also resistant to scarring leading to cirrhosis in the surrounding tissue, then it would take on the gross appearance of a large cirrhotic nodule although it would be actually forming an island of relatively preserved hepatic parenchyma in an otherwise cirrhotic liver. Thus, the development of HCC would not follow the development of cirrhosis but would be an independent, although often parallel, process.

Preliminary support for this hypothesis came from two immunohistochemical studies of dysplastic nodules. The first study looked at the expression of proliferating cell nuclear antigen as a marker for proliferative activity and determined that dysplastic nodules were in fact relatively low proliferative lesions compared with the surrounding cirrhotic nodules.[7] The second study used smooth muscle actin as a marker for activated stellate cells and showed dysplastic nodules had fewer of these cells than either cirrhotic nodules or HCC, suggesting that they were in fact undergoing diminished or delayed scarring compared with surrounding tissues.[8] The cornerstone of this hypothesis, however, was the clonality study of Tsuda et al.[6] This study, relying on restriction fragment length polymorphism analysis of hepatitis B genome integration, suggested that dysplastic nodules were clonal, although the technique actually only shows that at least a subpopulation of cells are clonal. Moreover, they did not look at other cirrhotic nodules to determine if some of these nodules were also clonal. Thus, more definitive clonality studies of both cirrhotic and dysplastic nodules, using a technique that assesses the entire population of cells in a sampled nodule, were necessary.

These studies have been performed by Aihara et al.[9,10] The authors based their analysis on restriction fragment length polymorphism of the X chromosome–linked phosphoglycerokinase gene and on random inactivation of the gene by methylation. Thus, in women who are heterozygous for different forms of the gene, polyclonal nodules will produce two recognized bands after restriction enzyme digestion and Southern blotting. On the other hand, entirely monoclonal lesions will produce only one band. The first study by Aihara et al. looked at cirrhotic nodules in patients with hepatitis C–related cirrhosis.[9] They found that 43% of the nodules examined (33 of 76) were clonal. Interestingly, these clonal nodules always clustered together. Because allelic inactivation is random, it would therefore be unlikely that each nodule happens to inactivate the same allele. The authors suggest that "monoclonal cell expansion is initiated before the nodule is established by septum formation." In other words, they postulate a clonal expansion of hepatocytes before the development of cirrhosis. Their second study addresses the clonality of dysplastic nodules in hepatitis C–related cirrhosis.[10] Here they confirm that dysplastic nodules are indeed clonal and therefore already neoplastic.

The difference between clustered, small neoplastic nodules in cirrhosis and dysplastic nodules is the degree to which they have been susceptible to scarring, as suggested earlier. We thus find that the premalignant lesion of the liver is in fact a monoclonal proliferation of cells without cytological features to suggest neoplasia. Depending on differences in stellate cell recruitment and activation or in the timing of the proliferation compared with the surrounding scarring of the liver, these lesions will sometimes, although not always, have a distinctive gross appearance when compared with surrounding parenchyma. However, if they can undergo scarring, they will be indistinguishable from neighboring hyperplastic nodules until foci of atypia or HCC actually arise.

The implications for detection and early treatment are clear. HCC may arise in simple cirrhotic nodules or in dysplastic ones. Neoplastic hepatocyte populations that do not undergo scarring to the same degree as the surrounding parenchyma will have the potential for earlier detection as dysplastic nodules. Those that are able to scar into separate cirrhotic nodules will be undetectable until an HCC arises in one of them, which then overgrows surrounding nodules to become distinct itself. Thus, as Japanese investigators have discovered, a distinct nodule in imaging studies may or may not be cancer. However, because studies of proliferation in dysplastic

nodules suggest that rapid proliferation does not take place until HCC actually develops, follow-up imaging within a shortened time period should serve to distinguish dysplastic nodules from HCC without the need for biopsy; a lesion that expands within a 4-month period or so can be assumed to be HCC and must therefore be resected or ablated.[1] Without rapid expansion, the lesion is believed to be a dysplastic nodule and therefore may be followed up not only for the risk of developing HCC within the nodule itself but because neoplastic proliferations have probably occurred throughout the liver, most as clustered small nodules and some as large dysplastic nodules. Thus the identification of a dysplastic nodule serves as a marker for increased risk in the liver as a whole, warranting more frequent imaging to identify the emergence of new HCCs.

Additionally, with confirmation that the earliest premalignant change is a clonal expansion of cytologically normal hepatocytes, we can perhaps begin to ascertain what genetic abnormalities may be commonly associated with these changes. Aihara et al. have begun this work with further evaluation in the current report of clonal somatic mutations and loss of heterozygosity in dysplastic nodules and HCC. The era for elucidating the early genetic events leading to the development of carcinoma in human livers would appear to be at hand.

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