

## **From atypical nodule to cancer: unravelling the mysteries of hepatocarcinogenesis for the practicing histopathologist**

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### **Hepatocellular carcinoma: a multistep process.**

Human HCC is one of the most common tumor, ranking fifth in incidence and third in mortality worldwide. From epidemiologic studies, it appears that liver carcinogenesis is a multistep process. Although a few HCC arise in normal liver, the vast majority arise from the pathway: normal liver - fibrosis - cirrhosis - HCC. Therefore, cirrhosis is recognized a precancerous lesion. Due to careful follow-up of cirrhotic patients and detailed pathologic analysis of cirrhotic explanted livers, there are now relevant insights into the early pathogenesis of HCC from a pathologist standpoint. Data are less clear when associated molecular events are concerned. There is consensus to support that HCC result from cumulative genetic and epigenetic events. Although recurrent gene abnormalities are reported in fully developed HCC, the early molecular events are far less unknown. The rarer but otherwise interesting mechanisms relevant of HCC in normal liver will not be considered here.

### **From cirrhosis to cancer : the pathologist sight**

The transition from cirrhosis to cancer might progress according two pathways. One goes through an intermediate precancerous lesion that is the development of a large nodule (macronodule), the other proceeds directly from cirrhosis to cancer.

Macronodule is defined as liver cell nodules larger than the background cirrhotic nodules. This is a lesion of significant interest for clinical practice since, when of sufficient size, it can be visualized using ultrasound examination during follow-up of cirrhotic patients. The natural history of macronodules is still debated. Based on ultrasound follow-up, studies have shown that some macronodules might disappear totally or regressed with time whether others are stable, enlarge or evolve to HCC. In order to reflect this diversity, pathologists have proposed to grade macronodules according to architectural and cellular histopathologic criteria. Most of these classifications subdivide macronodules into 3 or 4 grades. Beside benign macroregenerative nodules and early hepatocellular carcinomas, a group of dysplastic nodules (low and high grade) have been isolated. Main criteria evocative of malignancy are small liver cell changes, nuclear crowding, pseudogland formation, clear cell change and steatosis. Intermediate features suggest dysplasia. Although it is suggested that grading of macronodules resume the progressive transformation of a regenerative lesion to a malignant one, this approach has several drawbacks. The first is that there is significant observer variation between pathologists in the grading especially when dysplastic nodules are concerned. The second is that this classification is established on purely descriptive criteria and not supported by any experimental models or longitudinal study demonstrating the validity of this progressive evolution. Finely, grading criteria of macronodules rely on examination of the complete sample obtained after surgery and the relevance of this grading system has not been demonstrated for microfragments obtained with guided fine needle biopsies.

HCC can also arise directly from cirrhotic micronodules in the absence of any detectable macronodules. In this context, two major histopathological features deserve attention: large and small liver cell dysplasia. These features have initially been coined to describe group of liver cells with very characteristic microscopic features but without any macroscopic lesion. Due to the uncertainty in their relationship with carcinogenesis, the term liver cell change has been preferred to liver cell dysplasia. Large liver cell change is defined as group of large irregular liver cells with hyperchromatic and pleomorphic nuclei, multinucleation and often conspicuous nucleoli. Large liver cell change is a common

lesion in cirrhotic liver and may occupy the periphery or the whole cirrhotic nodule. It is more often observed in virus B-induced cirrhosis. Evidences suggest that there is a significant association between large liver cell changes and HCC since foci are observed around HCC or might be associated with an HCC elsewhere in the liver or even predispose to the outcome of an HCC. However, it must be underlined that both positive and negative predictive values of large liver cell change remains low. Furthermore no significant molecular evidence support that LLCC is real preneoplastic or already neoplastic lesions. It is hypothesized that LLCC might reflect liver cell senescence, a condition that most often lead to cell death by apoptosis but that, in some circumstances, might also favor the development of tumor after additional molecular events.

Small liver cell change (SLCC) consists of foci of small atypical liver cells with basophilic cytoplasm and a higher nuclear/cytoplasmic ratio. There is typical nuclear crowding and cells are often organized in thick trabecula. SLCC is a relatively rare lesion whose diagnosis might be difficult and which might be easily confused with regenerative foci of benign liver cells. By contrast to LLCC, most authors agree that SLCC is a precancerous or already in situ cancerous lesion.

#### **From cirrhosis to cancer: the molecular sight**

Several molecular alterations have been described in full-blown HCC. Among the most frequently dysregulated tumor suppressor genes and oncogenes in fully developed HCC are pRb, p53, E-Cadherin, cyclin-D1,  $\beta$ -catenin, axin and c-myc. Data are rarer regarding the early steps of liver carcinogenesis. Several molecular markers have been evaluated in macronodules and none of the up-mentioned oncogenes or tumor suppressor genes have been repeatedly found altered in the precancerous lesions. By contrast, proliferation markers, angiogenesis, telomerase expression, allelic losses, clonality have been studied with more consistent results. It emerges from these studies that dysplastic nodules do not form a homogeneous intermediate group between macroregenerative and malignant nodule but that they cluster either in the group of benign nodules or in the group of early HCC. Dysplastic macronodule is a concept that useful for the pathologist to grade difficult lesions of doubtful evolution but this group does not correspond to a homogeneous entity at the molecular level.

#### **Cirrhosis: a multilineoplastic lesion**

Of significant importance in our understanding of the pathogenesis of precancerous lesions are several studies using different approaches to look for early molecular abnormalities in regular cirrhosis. Interestingly, these studies convincingly demonstrate, using clonal analysis, that among cirrhotic micronodules that looked all similar on light microscope, some are already monoclonal (neoplastic) and other polyclonal (regenerative). Telomerase, an enzyme that allows unrestricted cell proliferation and which is specifically expressed in cancer, can be detected in some but not all of these clonal micronodules without any remarkable histopathologic abnormalities. In the same orientation and using cell cycle markers, several studies have shown that cirrhosis associated with HCC or that will give rise to HCC already expressed increased cell cycle markers. Therefore, it clearly appears that even in regular cirrhosis with a bland histopathologic appearance, foci of neoplastic clonal proliferation of liver cells with a limitless capacity of proliferation are already present.

In conclusion, further studies are needed to draw a parallel between morphological features and molecular markers in the early phases of HCC but studies are concordant to demonstrate that those precancerous lesions are composed of both regenerative innocuous foci and monoclonal foci prone to develop HCC. The transformation from clonal to malignant foci seems a very rare event under the dependence of additional oncogenic stimulation that needs further investigation.