Hepatocellular dysplastic nodules

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The multistep process of hepatic carcinogenesis is mirrored by the morphologic classification of lesions detectable in cirrhosis, that include large regenerative nodules (LRN), low grade dysplastic nodules (LGDN) and high grade dysplastic nodules (HGDN). The latter belong to the "bordeline malignancy" cathegory requiring an accurate distinction from well-differentiated and early hepatocellular carcinoma. Nodules in cirrhosis are usually detected by non-invasive imaging techniques, being the latter unable to discriminate malignant from non-malignant forms, particularly in the 1-2 cm sized group. Liver biopsy is essential in providing practical diagnostic information to hepathologists in the management of cirrhotic patients with US detectable nodules. The histologic diagnosis on liver samples is based on the accurate search of a set of cyto-architectural features (cell atypia, cell crowding, trabecular thickness, microacini etc) and by a supplement of histochemical (Gomori staining) and immunocytochemical stainings. The latter rely upon the search of both well established and novel markers, targeted to evaluate stromal invasion (CK7/19), the vascular pattern (ASMA and CD34) or tumor markers (HSP70 and Glipican 3 among others). Still, the diagnostic sensitivity is limited by the type and size of sampling and by its representativity of the entire lesion. The best diagnostic approach thus requires the integration of clinical, morphological and immunocytochemical information with imaging data (US pattern, perfusional pattern, helical CT/MR pattern). Molecular data are still under evaluation as to their diagnostic efficacy in this controversial field. Discrepancies have emerged recently between Eastern and Western interpretation of these lesions, particularly in the cathegory of "borderline" nodules, that are mostly labelled as early, well differentiated HCC by eastern pathologists and as HGDN by western pathologists. Novel and more objective phenotypical and molecular markers are needed to discriminate within the grey area of borderline lesions that, epidemiologically, are likely distinct between eastern and western geographic areas. These tools might allow a better understanding of the boundaries of the process going from high grade dysplasia to in situ HCC and from the latter to microinvasive HCC and advanced HCC, for a proper clinical management and optimal therapy.

KEY WORDS: Hepatocellular modules

Introduction

Space-occupying sizable nodules arising in cirrhosis are hyperplastic and neoplastic growths (benign, dysplastic and malignant) whose diagnostic criteria and nomenclature require a consensus between western and eastern pathologists [1,2] There is disagreement between pathologists as to the dividing line between dysplasia and well differentiated HCC and this disagreement occurs more frequently as the size of the lesion decreases [3].

A great deal of morphological and molecular information on these lesions has been originally achieved through the systematic macro- and micro-scopical examination of native transplanted end-stage cirrhotic livers; however these livers have a background far advanced as compared that of cirrhotics, routinely followed up by US, for early HCC detection [4-7] (Conversely there is a substantial lack of information on how to recognize lesions on liver biopsies as well as on the phenotypic and genotypic features useful to discriminate among early nodules. Available data on hepatocellular nodules heterogeneity

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and natural history in cirrhosis have ultimately rested upon imaging and the sampling of small <2cm lesions as well as on handful prospective studies carried out by eastern and western groups [8-16].
From these studies emerged that:
– most *de novo* or recurrent hepatocellular nodules >2 cm in cirrhosis are HCC or suspicious lesions proved to be HCC after short-term follow-up;
– imaging alone is not able to discriminate within a consistent fraction of <2cm lesions; 34% are hypovascular and 38% malignant nodules go unrecognized after imaging [8];
– the majority of <2cm hepatocellular nodules detected in cirrhotics are already malignant and their prevalence ranges from 60% (M Borzio, personal communication) to 70% [3] to 75% [3] to 85-90% [10];
– non-malignant, *de novo* or recurrent, <2 cm hepatocellular nodules are a consistent but heterogeneous group of lesions, ranging from 10%-40% in the different series; in *in vivo* series they include both regenerative (60%) and dysplastic nodules (40%) with various malignant potential and with a 1:1 ratio between low grade (LGDN) and high grade (HGDN) dysplastic nodules;
– high-grade dysplastic nodules carry the worse outcome as compared to the other non-malignant nodules, with a four-fold risk developing HCC as compared to non-dysplastic (Borzio et al, 2003); eastern authors tend to interpret these nodules as early, well-differentiated HCC (eWDHCC) [17,18];
– HCC more rarely develops from regenerative and low grade dysplastic nodules (10-25%) [9] while it is hard to estimate how many HCCs will develop from the inconspicuous cirrhotic background;
– all types of non-malignant nodules including HGDN can undergo internal remodeling with 20-73% nodule disappearance at US [9,14-16];
– the most controversial diagnostic issue is between HGDN and eWDHCC. The disagreement between pathologists on the most appropriate diagnostic criteria and related nomenclature [3] likely reflects a *continuum* spectrum of lesions (high grade dysplastic nodules, *in situ* HCC, micro-invasive HCC, overt HCC) whose boundaries are biologically (and consequently morphologically, phenotypically and genotypically) ill-defined [18]. The incidence HGDN in a surveillance setting of cirrhotics is rather low likely accounting for less than 5% <2cm malignant and non-malignant nodules [8-10]. However the number of cases uncertain for malignancy is higher because, in this basket, small eWDHCC are also included;
– eWDHCC due to its lack of vascular invasion, is expected to be clinically less aggressive as compared to overt, potentially angioinvasive, well-differentiated HCC; early, eWDHCC is a heterogeneous entity including *in situ* HCC and micro-invasive HCC [18]. HGDN and *in situ* HCC lie very close in the multistep model [19] of hepatic carcinogenesis and there is the suggestion that they can be the very same entity (M Kojiro, personal communication, 2005).

Liver biopsy is essential for the proper characterization and management of <2cm hepatocellular nodules. Despite a 10% false-negative rate [20], when the imaging profile is not characteristic for malignancy, liver biopsy still remains the diagnostic milestone; morphologic data should always be integrated with imaging that gives information about the whole lesion.
For <2cm nodules a "wait and see" policy has been suggested particularly in patients waiting OLT, limiting an aggressive diagnostic and therapeutic strategy to >2cm nodules [21]. Emerging data indicate that the smaller the lesion the less likely there is to be microscopic vascular invasion and more likely local ablation will be complete[3]. It is therefore important to make the diagnosis of HCC as early as possible. However it is equally important not to apply invasive treatment to lesions that do not have any malignant potential and may still regress. Our opinion is that a distinction of <2cm malignant from non-malignant nodules in cirrhosis and their further morphological sub-categorization is essential for the following reasons:
– to make a correct diagnosis: a) candidating patients with malignant nodule(s) to surgical resection/waiting list for transplantation/ablative therapy; b) decreasing the likelihood of transplanting patients prematurely who don't have HCC and, conversely, avoiding the exclusion of patients with multiple nodules, some of which could be non-malignant;
– to perform the clinical staging of patients with malignant nodules in particular for those amenable to hepatic resection;
– to make a correct prognosis: a) reassuring patients harboring regenerative/LGD nodules of their not aggressive nature and to plan a pertinent recall policy; b) in case of HGDN/eWDHCC discussing with patients the therapeutic options or planning a more aggressive recall policy (enhanced follow up);
– to increase our understanding of the cirrhosis-dysplasia-HCC sequence by studying the natural history and the phenotypic and genotypic profile of the different lesions.
This will permit to find novel markers particularly useful to discriminate low from high malignant potential lesions (including *in situ* HCC) and the latter from well differentiated HCC with stromal and/or vascular invasion.

We retain that an early distinction between malignant and non-malignant nodules, in particular between regenerative/LGDN *versus* HGDN/ eWDHCC is an affordable task for liver-trained pathologists, provided the availability of pertinent clinical and imaging information, adequate sampling and accurate morphologic evaluation.

Integrated clinico-pathological approach

Approaching the biopsy of a focal liver lesion pathologists should be aware of:

– clinical setting: surveillance of patients with cirrhosis or in the liver transplant waiting list or with previous HCC;
– background liver: chronic hepatitis/cirrhosis
– specific serologic markers: viral and oncofetal (AFP)
number, location and size of nodule(s);
– US pattern (and variation during follow up); CT-, US-perfusional-, MRI- patterns when available;
– previous cyto/histopatologic liver sampling and diagnostic report(s);
– previous diagnosis of HCC and type of treatment.

Sampling

An intra- and extra-lesional sampling is highly recommended. Subtle architectural and cytologic abnormalities can be better appreciated through the accurate comparison with extra-lesional referenced liver. When both intra- and extra-lesional samples show overlapping, cirrhosis-like features, without a major nodule standing on the nodular background, the sample should not be considered adequate; as such a new sampling is recommended shortly after. We do not agree with the opinion that only a positive biopsy is helpful and that a negative one can never be taken as conclusive [3]. There are criteria to distinguish unadequate from adequate but negative samples. Sample adequacy is not only linked to the size of biopsy but it is also dependent on the type of lesion, cell differentiation, liver background and on the consistency between morphologic and clinico-radiologic features. An important issue to be taken into account is when the patient harbors more than one small nodule. Given that the diagnostic uncertainty is greater in small lesions, we recommend to sample the smaller one, assuming that the larger is likely to be already malignant.

TABLE I – *H&E features to be looked for in order to preliminary distinguish the different categories of nodules in cirrhosis*

	LRN	LGDN	HGDN	eWDHCC
Clone-like foci	–	±	±	±
Plate thickening	–	±	+	+
Cell crowding	–	–	+	+
Pseudoglands	–	–	+	+
Stromal invasion	–	–	–	±
Unpaired arteries	–	±	±	+
Nuclear Atypia (*)	–	±	+	+

(*) hyperchromasia and nuclear irregularities
Legend: – absent; ±: may be present but not necessarily detectable in biopsy; +: usually present and detectable in biopsy.

Imaging of dysplastic nodules

At conventional US, these lesions are mainly hypoechoic with a well defined rim, but iso-hyperechoic patterns can also be found [14,22]. Consequently US are unable to reliably distinguish benign from malignant or potentially malignant lesions. Recently, different imaging techniques (perfusional-US, helical-CT and MRI) have been introduced in the diagnostic workout, relying on their ability to explore the vascular pattern [8,23-34]. Unfortunately most dysplastic nodules and some well differentiated HCC do not achieve a mature arterial hypervascularisation, so eluding being detected by these techniques. In studies where the gold standard is the explanted liver, CT showed a lower sensitivity (40%) as compared to MRI (96%) in the detection and diagnostic accuracy of <2 cm nodules [28,35]. Perfusional-US do not seem to add more information as compared to helical-CT [36] EASL consensus conference held in Barcellona on 2001 (Bruix et al, 2001) recommended for > 2 cm nodules to explore hypervascularity by at least two techniques, in order to make a reliable diagnosis of malignancy. This policy has been recently revised and restricted to <2 cm lesions, because usually equivocal at imaging [37]. In the current practice, however, small nodules are detected by conventional US, followed by helical-CT, which is available in almost all general hospitals as opposed to perfusional-US and MRI, that are much less diffuse because expensive and experienced-operator dependent. In this setting only the CT showing arterial hypervascularization and venous wash-out is reputed enough for malignancy, not being usually followed by liver biopsy. Unfortunately CT, also in experienced hands, does not detect a consistent fraction (30-50%) of small hepatocellular nodules, because hypovascular or with undefined vascular pattern. In this "real word" scenario we think that the most quick and probably less expensive way to get a conclusive diagnosis is histology.

Dysplastic nodules: general morphologic features on liver biopsy

Dysplastic nodules are classified as LGDN or HGDN on the basis of cytologic and architectural atypia. More specifically, while LGDN only show cytologic atypia and minimal architectural disturbances, HGDN are characterized by both cytologic and architectural atypia but not so overt as to make a diagnosis of malignancy [38] . At low magnification at least one or two unconnected portal tracts should be recognized within the nodules (Figure 1a). Key-morphological features of differential diagnosis are summarized in Table I.

LOW GRADE DYSPLASTIC NODULES

A lesion with low malignant potential. When looking a low grade dysplastic nodule under the microscope the

TABLE II – *Differential diagnosis between HGDN/early well differentiated HCC*

a) Quantitative key-features to be accurately evaluated on liver biopsy

Feature	HGDN	early WD HCC
Thickness of plates	2 or 3	> 3 (*)
Pseudoglands	focal	plurifocal
Cell crowding	x 1,5/2	x >2 (*)
Capillarized vessels (CD34+)	focal	plurifocal

(*) as compared to adjacent cirrhotic nodules.

b) Qualitative key-features diagnostic for malignancy (*)
Decreased/absent reticulin framework (**)
Stromal invasion (**)
Vascular invasion (**)
Nodule in nodule growth (expansive hypercellular nodule)
Diffuse CD34+ capillarized vessels

(*) Each feature diagnostic for malignancy. (**) Each feature discriminating between *in situ* HCC and invasive HCC.

differential diagnosis with a malignant hepatocellular nodule does not come to mind. LGDN are characterized by: 1) preserved hepatic architecture; 2) two cell-thick hepatocyte plates; 3) low grade cytological atypia, usually large cell changes, cytoplasmic eosinophilia, minimal nuclear abnormalities and slightly increased cell density; 4) facultative clone-like foci without compression of the adjacent liver (map-like type), mostly composed by clear cells (or, more rarely, by cells containing fat, haemosiderin, Mallory bodies or iron-free); 5) very few if any, barely visible, unpaired arteries 6) very few CD34 immunoreactive capillarized vessels, mostly peripherally located.

HIGH GRADE DYSPLASTIC NODULES
A lesion with high malignant potential. Under the microscope a differential diagnosis with well differentiated HCC comes to mind; pathologists, at first glance, may be uncertain about malignancy. Both architectural and cytological atypia are detectable but in a focal and uneven distribution, merging within inconspicuous-looking hepatocytes plates. Cytologic atypia include 1) small cell changes (small cells with high N/C ratio) in scattered, irregular foci; 2) cytoplasmic basophilia or clone-like changes (usually clear but also fatty, etc); 3) slight to moderate nuclear abnormalities (hyperchromasia and nuclear irregularities). It is not however indicated to draw any diagnostic conclusion from a mere cytologic approach. Architectural disturbances include 1) increased nuclear crowding (Figure 1b); 2) hepatocyte plates thickening (Figure 1c); 3) a few focally recognizable pseudoglands having small, empty, or barely visible lumina (Figure 1d); 4) a very few unpaired arteries; 5) a few centrally and peripherally located CD34+, capillarized vessels; 5) clone-like foci with (bulging-type) (Figure 1e) or without (map-like type) compression of adjacent hepatocytes, frequently featuring cytologic homogeneous changes (clear, fatty, iron-free). The former type of growth (bulging type) is only encountered in HGDN, where it may determine the appearance of a subnodular growth, pushing apart the surrounding parenchyma [17]. Usually the subnodule shows more atypia and increased cell kinetic [39] as compared to the adjacent liver. The progressive growth of the inner nodule can then originate the so-called *nodule in nodule*, a peculiar radiologic figure with morphologic equivalents (Figures 1f e 1g); the *nodule in nodule* is a sign of malignancy developing within a HGDN (the inner nodule usually being eWDHCC) or within an eWDHCC (the inner nodule being a less well-differentiated HCC) [2]. The prevalence of *nodule in nodule* in Europe is difficult to assess and it has been reported <4% in a series of explanted livers (3 out of 77 carefully sampled lesions), 6 and in a surveillance setting of cirrhotic patients [12]; in Japan it is considered a feature of the multistep hepatocarcinogenesis in cirrhosis [2,17-19,40].

Differential diagnosis

LGDN VERSUS LARGE REGENERATIVE NODULE (LRN)
At high magnification LRN are microscopically undistinguishable from the surroundings. The intralesional parenchyma, when sampled in the middle and near periphery, may even simulate a normal liver, given the presence of portal tracts. Large cell changes, minimal nuclear abnormalities and clone-like changes can be detectable in low grade dysplastic but not large regenerative nodules.

LGDN VERSUS HGDN:
As shown in Table I this distinction is based on qualitative features such as the absence of architectural abnormalities in LGDN while the detection of clone-like changes, when present, should be restricted to minute foci. Overall, at first glance, LGDN do not alert pathologists.

HGDN VERSUS EWDHCC
The most useful morphologic features of differential diagnosis to be always looked for are summarized in Table II a,b. Overtly malignant, even if still well-differentiated HCC, likely corresponding to the so-called "distinctly nodular type" of eastern authors [17], does not enter in the differential diagnosis, being its malignancy easily recognizable at first microscopic glance. The diagnostic issue with eWDHCC can be preliminary addressed by the accurate evaluation of a) hepatocyte plates; b) reticulin framework; c) neoangiogenesis; d) stromal invasion/pseudoinvasion.

Hepatocytes plates. A diagnosis of well differentiated

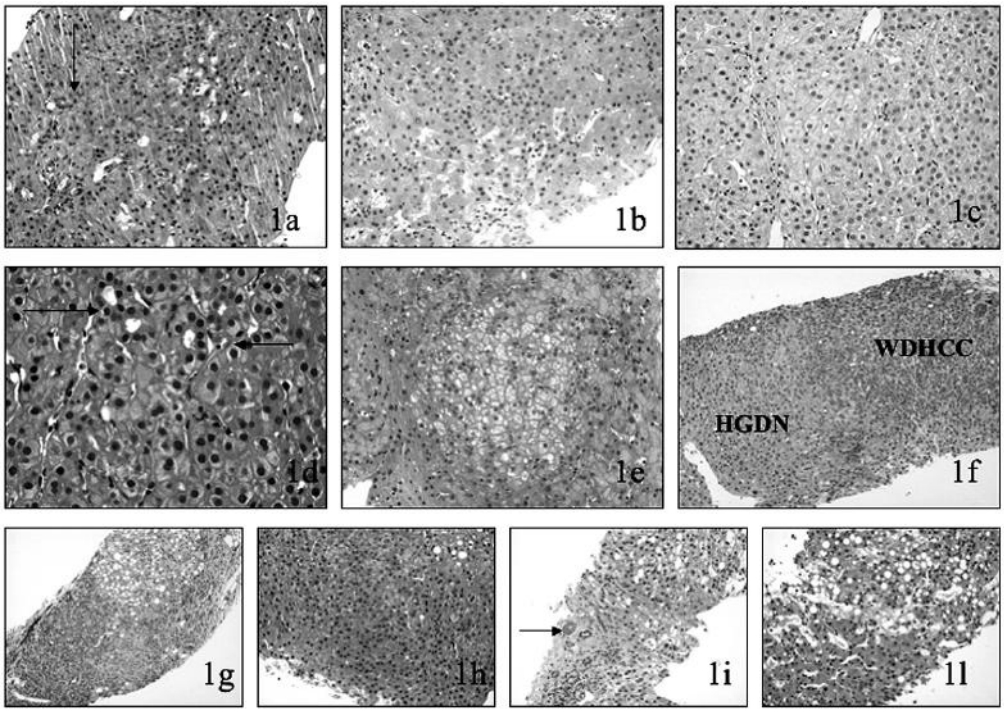

Fig. 1: a) LGDN: arrow points to an unconnected portal tract; b) HGDN: nuclear crowding merging within bland-looking hepatocytes; c) HGDN: focal increased hepatocytes plate thickening; d) HGDN: isolated pseudoglands (arrows); e) HGDN: a focus of clear cell clone-like changes (bulging-type); f) eWDHCC growing within a HGDN; e) G1/G2 HCC growing within an eWDHCC with fatty changes; h) eWDHCC: evenly distributed architectural abnormalities; i) eWDHCC with fatty changes containing a portal tract (arrow); l) eWDHCC with fatty changes at higher magnification.

hepatocellular malignancy should be considered when architectural [3,8,17] for uncomplete, (mainly portal) neovascularization and it is reputed to grow without destruction of the adjacent liver parenchyma, by progressive replacing of cirrhotic nodules. As such it merges with normal hepatic plates, the sinusoids containing both arterial and venous portal flow. Japanese authors consider this eWDHCC to have peculiar macroscopic and microscopic features (average diameter 1,5 cm, unencapsulated, well differentiated histology, fatty changes) [17]. They call it "indistinctly nodular type" as opposed to the "distinctly nodular type", being the latter easily detectable for sharp boundaries at both imaging and on surgical resections. Small HCC of distinctly nodular type is reported to be more aggressive and angioinvasive than eWDHCC (a step further on the road of HCC progression) [17].

The eWDHCC may contain a few portal tracts (less than 1/3 as compared to hepatic surroundings) [17] (Figure 1i). A trabecular arrangement (which would facilitate the diagnosis) is not obviously detectable. It may contain clone-like populations with homogeneous cytoplasmic features (clear, fatty etc). Interestingly a still controversial feature between eastern and western studies is the relative lack of fatty change in western as opposed to eastern countries (30-40% of cases) [17,41]. Indeed fatty changes in HGDN and early HCC are uncommonly reported in European explanted livers (L Libbrecht, personal communication 2004). Recently Rapaccini et al (2004) [12] showed, in a surveillance setting of prospective cirrhosis, that echogenicity of small HCC was unrelated to tumor differentiation, a notable feature given that fatty metamorphosis is the main cause of hyperechogenicity in liver nodules. In our experience, both clear and fatty changes are the main clone-like features detectable in small, eWDHCC (Figure 1l) as well as in HGDN. Further studies are needed comparing the rate of fatty metamorphosis and clear cell changes in western and eastern series in both HGDN and eWDHCC

Reticulin framework. HGDN always display a well preserved reticulin framework (Figure 2a), while obviously invasive HCC do not. Pathologists agree that if the reticulin framework is decreased or lost the tumor is malignant. However, eWDHCC by growing in a replacing pattern, may be associated with a still intact (Figure 2b) or slightly decreased (Figure 2c) reticulin framework. In conclusion a decreased reticulin is very helpful and indicates malignancy but a preserved one does not necessarily exclude malignancy.

6.3.3 Neoangiogenesis. There is a progressive increase in the number of unpaired arteries (barely visible by H&E and better visualized by smooth muscle actin immunostaining) and capillarized sinusoids (documented by CD34 immunostaining) from LGDN to HGDN to eWDHCC to overt HCC) [41,42]. However a threshold of vessels/mm^2 separating pre-malignant from early malignant lesions has not been determined possibly because these lesions form a *continuum* spectrum. As for reticulin framework, an uncomplete sinusoidal capillarization (that is more easily detectable on liver biopsies than unpaired arteries) would still be in keeping with a pre-malignant nodule (HGDN) (Figure 2d) or eWDHCC (Figure 2e), while diffuse CD34 immunoreactivity is, in our experience, a feature of overt malignancy (Figure 2f). However there is a lack of studies on the variation of both reticulin

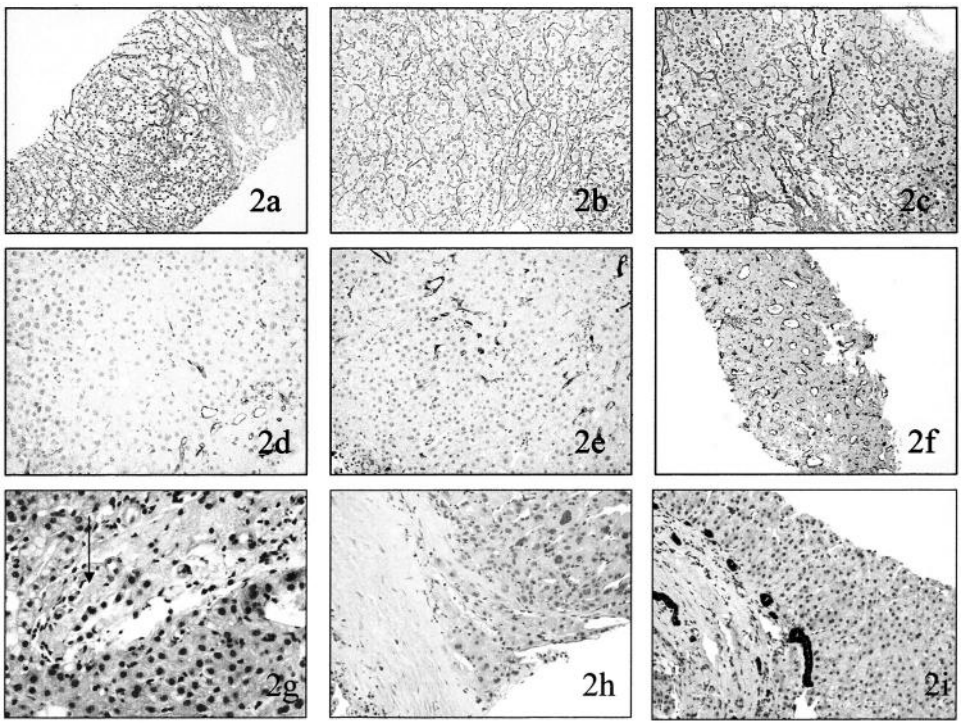

Fig. 2: Preserved reticulin framework in a) HGDN and b) eWDHCC; c) eWDHCC showing slightly decreased reticulin framework. CD34 immunorectivity showing capillarized vessels in: HGDN (d, focal staining); eWDHCC (e, plurifocal staining); overt WDHCC (f, diffuse staining). Stromal invasion detected by g) H&E (arrow); h) lack of CK7 immunoreactivity; i) pseudoinvasion: diffuse ductular reaction bordering a HGDN highlighted by CK7 immunostaining.

framework and vascular capillarization in the sequence HGDN-eWDHCC.

Stromal invasion/pseudoinvasion. Given that HGDN have features partly overlapping those of eWDHCC, the only difference being the less extensive and uneven pattern of growth of the former, there is the suggestion that the most critical feature separating HGDN from eWDHCC is stromal invasion. Stromal invasion may occur in eWDHCC [44] and therefore the infiltration of portal tract/fibrous septa by single hepatocytes is expected to be a cardinal feature of malignancy that should be carefully looked for in intralesional biopsy samples (Figure 2g). It has been preliminary suggested that CK7/19 immunostaining, by highlighting the ductular reaction that takes place only around non-malignant hepatocellular nodules, might also prove useful to distinguish true invasion (lack of staining) (Figure 2h) from pseudoinvasion (positive staining) (Figure 2i) (NY Park, personal communication, 2004).

In conclusion HGDN should be suspected when features of eWDHCC are focally and unevenly distributed, merging with normal-looking hepatocytes, in a setting of preserved reticulin framework, uncomplete sinusoidal capillarization and lack of stromal invasion. Conversely when stromal invasion is the only feature missing in a morphologic scenario of diffuse architectural changes, retained/slightly decreased reticulin framework and discrete sinusoidal capillarization the most likely diagnosis should be eWDHCC (possibly *in situ* HCC). The differential diagnosis between *in situ* HCC and HGDN is obviously very difficult because these two lesions are biologically very close. We can concur with eastern authors that a demarcation line cannot be drawn between HGDN and *in situ* HCC. However, a number of novel phenotypic, genotypic and expression markers are expected to provide information on the putative heterogeneity of this spectrum of lesions.

Novel immunocytochemical markers

Several immunocytochemical markers have been proposed as helpful for a diagnosis of malignancy. Particularly interesting are novel markers of cycling cells such as those of the MCM (minichromosome maintaining proteins) family, (A Quaglia et al, personal communication 2005), or true markers of hepatocellular malignancy such as as glypican- 3 45-47, HSP 70 [48,49] and glutamyne synthetase [50]. However the specificity, sensitivity and heterogeneity of the above markers is largely unknown; a comprehensive study evaluating their expression along the morphologic sequence cirrhosis-dysplasia-HCC is still lacking.

Molecular markers

It has been shown that dysplastic nodules are monoclonal proliferation of hepatocytes [51]; progressive chromosomal instability under the form of allelic imbalance characterizes the sequence LGDN-HGDN-HCC (Maggioni et al, 2000 [52]; reviewed by Libbrecht et al, 2005 [53] and an increased number of chromosomal gains

and loss (reviewed by Moinzadeh et al, 2005) [54] is a feature of the multistep progression towards malignancy. Recently Oh et al. [55]reported telomere shortening involving up regulation of telomere-binding proteins. We have preliminary results confirming that genetic abnormalities affecting key-cancer genes such as p53 and ?-catenin mutations occur in a fraction of overt HCC but not in their precursors; interestingly we are also detecting an increased number of epigenetic changes, such as RASSF1A and p16 genes methylation in dysplastic nodules, likely related to early hepatocarcinogenesis.
A few studies attempted to address the issue of the molecular profile of dysplastic nodules in the cirrhosis-HCC sequence, by gene expression analysis. Anders et al [56] reported that novel genes including caveolin1, semaphorin E and FMS-like TK3 ligand are abnormally regulated in dysplastic nodules. More recently Nam et al [57] were able to identify 240 out of 3084 genes that could accurately classify tumors according to histological grade, especially when attempting to discriminate LGDN, HGDN and grade 1 HCC. Interestingly enough these authors reported that G1 HCC are molecularly heterogeneous, sitting on the border between the transition from pre-malignant lesions to overt malignant carcinoma and that this heterogeneity is distinguishable at the molecular level. It is expected that genomic and proteomic studies, targeted to address gene expression profile in the sequence cirrhosis-dysplasia-HCC, should provide specific markers to distinguish among early lesions and, hopefully, to more accurately predict the risk of malignant transformation of non-malignant nodules.

Natural history of dysplastic nodules

While large regenerative nodules are thought to carry a malignant potential not greater than that of the adjacent cirrhosis, dysplastic nodules are considered precancerous. LGDN have a natural history showing malignant transformation in a few cases [8,9]; as such these lesions can be considered to have a low malignant potential. Conversely, HGDN have been reported to be statistically associated with malignant transformation [9]. These lesions can thus be considered at high malignant potential. Both low and high grade dysplastic nodules can undergo internal remodeling with nodule disappearance [9,14]. In the clinical practice the suggested management of LGDN is surveillance with repeat biopsy when the imaging features of the lesion changes, while HGDN have to be at least strictly monitored or ablated by percutaneous ethanol injection or termal therapy [9,52].

Riassunto

La sequenza di eventi molecolari coinvolti nel processo di epatocarcinogenesi si rispecchia nelle lesioni che possono essere individuate nel fegato cirrotico: noduli rigenerativi (NR), noduli con displasia a basso grado (NDBG), noduli con displasia ad alto grado (NDAG); l'ultima entità si colloca nel contesto delle lesioni a "malignità borderline" e deve essere distinta con attenzione dall'epatocarcinoma in fase iniziale (c.d. early-EC) e dalle forme ben differenziate. La presenza di noduli nel contesto di un fegato cirrotico è in genere appannaggio di metodiche non invasive, tuttavia queste ultime non sono in grado di discriminare tra forme maligne e forme non maligne, specie se la lesione è di dimensioni < 2 cm. In questo contesto diviene essenziale, ai fini di una diagnosi ed un corretto approccio terapeutico, valutare una biopsia della lesione (meglio se unitamente ad una biopsia perilesionale). La diagnosi istologica verte su di una accurata valutazione di caratteri cito-architetturali (atipia citologica, affollamento delle cellule, ispessimento delle trabecole, formazione di microacini, ecc), supportate da indagini istochimiche (colorazione per il reticolo sec. Gomori) ed immunoistochimiche. Queste ultime constano nell'utilizzo di marcatori elettivi, in parte nuovi e in parte di comprovata validità, in grado di evidenziare l'infiltrazione stromale (CK7/19), il pattern vascolare (actina muscolo liscio e CD34) o la trasformazione neoplastica degli epatociti (HSP70 e Glypican-3, fra gli altri). Nonostante ciò la sensitività nella diagnosi è drasticamente limitata dal tipo di prelievo, minimamente rappresentativo della intera lesione. L'approccio migliore, dunque, è rappresentato da una integrazione dei dati clinici, morfologici ed immunoistochimici con le informazioni di imaging (ecografia, pattern di perfusione, RMN/TC). Il ruolo della biologia molecolare è ancora dibattuto. Infatti i dati prodotti hanno dato adito a discrepanze di interpretazione, specie all'interno della categoria delle lesioni a "malignità borderline", fra patologi occidentali ed orientali: i primi a favore della diagnosi di NDAG, i secondi a favore del c.d. early-EC. Si auspica che nuovi, e più obiettivi, marcatori molecolari e fenotipici possano aiutare a discriminare la zona grigia rappresentata da queste le lesioni a "malignità borderline", seppur queste, almeno da un punto di vista epidemiologico, risultano ben separate fra paesi occidentali ed orientali. Questi putativi marcatori dovrebbero anche permettere di meglio comprendere i confini di questo processo che inizia dai NDAG, evolve nell'EC in situ, si modifica ulteriormente assumendo i caratteri dell'EC microinvasivo sino a divenire un EC avanzato, ai fini di ottenere una migliore gestione di questo paziente e un trattamento terapeutico ottimale.

References

1) Roncalli M: *Hepatocellular nodules in cirrhosis:Focus on diagnostic criteria on liver biopsy. A Western experience*. Liver Transpl, 2004; 10: S9-15.

2) Kojiro M: *Focus on dysplastic nodules and early hepatocellular carcinoa:an Eastern point of view*. Liver Transpl, 2004; 10: S3-6

3) Bruix J, Sherman M: *Management of hepatocellular carcinoma.* Hepatology, 2005; 42:1208-36.

4) Hytiroglou P, Yheise ND, Schwartz M, Mor E, Miller C, Thung SN: *Macroregenerative nodules in a series of adult cirrhosis liver explants:Issues of classification and nomenclature.* Hepatology, 1++5; 21: 703-8.

5) Le Bail B, Belleannee G, Bernard PH, Saric J, Balabaud C, Bioulac-Sage P: *Adenomatous hyperplasia in cirrhotic livers:Histological evaluation, cellular density, and proliferative activity of 35 macronodular lesions in the cirrhotic explants of 10 adult French patients.* Hum Pathol, 1995; 26: 897-906

6) Libbrecht L, Desmot V, Roskams T: *Preneoplastic lesions in human hepatocarcinoma.* Liver Int, 2005; 25:16-27

7) Theise ND, Schwartz M, Miller C, Thung SN: *Macroregenerative nodules and hepatocellular carcinoma in forty-four sequantial adult liver explants with cirrhosis.* Hepatology, 1992; 16: 949-55

8) Bolondi L, Gaiani S, Celli N, et al: *Characterization of small nodules in cirrhosis by assessment of vascularity: The problem of hypovascular hepatocellular carcinoma.* Hepatology, 2005; 42: 27-34.

9) Yamamoto K, Takenaka K, Kajiyama K, et al: *Cell proliferarion and cell loss in nosulw.in-nodule hepatocellular carcinoma.* Hepatogastroenterology, 1999; 46: 813-19.

10) Caturelli E, Solmi L, Anti M, et al: *Ultrasound guided fine needle biopsy of early hepatocellular carcinoma complicating liver cirrhosis. A multicentre* study. GUT, 2004; 53: 1356-362.

11) Kaji K, Terada T, Nakanuma Y: *Frequent occurrence of hepatocellular carcinoma in cirrhotic livers after surgical resection of atypical adrnomatous hyperplasia (borderline hepatocellular lesion):A follow-up st*udy. Am J Gastroenterol, 1994; 89: 903-8.

12) Rapaccini GL, Pompili M, Caturelli E, et al: *Hepatocellular carcinomas <2 cm in diameter complicating cirrhosis:Ultrasound and clinical features in 153 consecutive patients.* Liver Int, 2000; 24: 124-30.

13) Sakamoto M, Hirohashi S: *Natural history and prognosis of adenomatous hyperplasia and early hepatocellular carcinoma: Multi-institutional analysis of 53 nodules followed by surgical resection or percutaneous ethanol injection.* Jpn J Clin Oncol, 1998; 28: 604-8.

14) Seki S, Sakaguchi H, Kitada T, et al: *Outcomes of dysplastic nodules in human cirrhotic liver: A clinicopathological study*. Clin Cancer Res, 2000; 6: 3469-473.

15) Takayama T, Makuuchi M, Horphashi S, et al: *Malignant transformation of adenomatous hyperplasia to hepatocellular carcinoma.* Lancet, 1990; 336: 1150-153.

16) Terasaki S, Kaneko S, Kobayashi K, Nonomura A, Nakanuma Y: *Histological features predicting malignant transformation of nonmalignant hepatocellular nodules: A prospective study.* Gastroenterol, 1998; 115: 1216-222

17) Kojiro M: *Pathology of early HCC.* In: Bruis J (ed): *Hepatocellular carcinoma.* Permanyer Publ, 2004 Bruix (Ed); 41-53

18) Kojiro MT: *Early hepatocellular carcinoma and displastic nodules*, Semin Liver Dis, 2005; 25: 133-42.

19) Oikawa T, Ojima H, Yamasaki S, Takayama, Hirohashi S, Sakamoto M: *Multistep and multicentric development of hepatocellular carcinoma: Histological analysis of 980 resected nodules.* J Hepatolo, 2005; 42; 225-29.

20) Denies L, Ernst O, Sergent Baudson G, Duvet S, Lìhermine C: *Contribution and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma.* J Hepatol, 2001; 35:254-53

21) Stigliano R, Burroughs AK,: *Should we biopst each liver mass suspicious for HCC before liver translantation?—No,please don't.* J Kepatol, 2005; 43: 563-68

22) Borzio M, Borzio F, Croce A, et al.: *Ultrasonography-detected macroregenerative nodules in cirrhosis:A prospective study.* Gastroenterology, 1997; 112: 1617-623.

23) de Ledinghrn V, Laharie D, Lecesne R, et al.: *Detection of nodules in liver cirrhosis : Spiral computed tomography or magnetic resonance imaging? A prospective analysis of 88 nodule in 34 patients.* Eur J Gastroenterol Hepatol, 2002; 14: 159-65.

24) Denies L, Ernst O, Sergent.- Baudson G, Duvet S, L'hermine C: *Contribution of spiral CT for the early diagnosis before surgical resection of hepatocellular carcinoma in chirrotic patients.* J Radiol, 2002; 83: 633-40.

25) Gaiani S, Celli N, Piscaglia F, et al: *Usefulness of contrast-enhanced perfusional sonography in the assessment of hepatocellular carcinoma hypervascular at spiral computed tomography,* J Hepatol, 2004; 41: 421-26.

26) Kim CK, Lim JH, Lee WJ: *Detection of hepatocellular carcinomas and dysplastic nodules in cirrhotic liver: accuracy of ultrasonography in transplant patients.* J Ultrasound Med, 2001; 20: 99-104.-

27) Krinsky GA, Lee VS, Theise N., et al: *Hepatocellular carcinoma and dysplastic nodules in patients with cirrhosis:Prospective diagnosis in small carcinoma of humans.* J Hepatol, 2000; 33: 282-89.

28) Lim JH, Kim CK, Lee WJ, et al.: *Detection of hepatocellular carcinoma and dysplastic nodules in cirrhotic livers: Accuracy of helical CT in transplant patients.* AJR Am J Roentgenol, 2000; 175:693-98.

29) Martin J, Puig J, Darnell A, Donoso L: *Magnetic resonance of focal liver lesions in hepatic cirrhosis and chronic hepatitis.* Semin Ultrasound CT MR, 2002; 23:62.78.

30) Miller FH, Butler PS, Hoff FL, Fitzgerald SW, Nemcek AA, Gore RM: *Using triphasic helical CT in detect focal hepatic lesions in patients with neoplasms.* AJR. 1998:171:643-49

31) Nicolau C, Catala V, Vilana R, et al : *Evaluation of hepatocellular carcinoma using SonoVue , a second generation ultrasound contrast agent: Correlation with hepatocellular differentiation.* Eur Radiol, 2004-14:1092-99.

32) Quaia E, Calliada F, Bertolotto M, et al : *Characterization of focal liver lesion with contrast-specific US modes and a sulfur hexafluoride.filled microbubble contrast agent :Diagnostic performance and confidence.* Radiology, 2004; 232:420-30.

33) Rode A. Bancel B, Doucek P, et al : *Small nodule detection in cirrhotic livers: Evaluation with US, spiral CT, and MRI and correlation with oaathologic examination of explanted liver.* L Comput Assist Tomogr, 2001; 25:327-36.

34) Stoker J, Romijn MG, de Man RA, et al: *Malignant transformation of adenomatous hyperplasia to hepatocellular carcinoma.* Lancet, 1990; 336:1150-153.

35) Burrel M, Lovet JM, Ayuso C, et al: Barcelona Liver Cancer Group-. *MRI angiography is superior to helical CT for detection of HGG prior to liver transplantation: An explant correlation.* Hepatology, 2003; 38:1034-42.

36) Fracanzani AL, Burdick L, Borzio M, Roncalli M, Bonelli N, Borzio F, Maraschi A, Fiorelli G, Forgion S: *Contrast-enhanced Doppler ultrasonography in the diagnosis of hepatocellular carcinoma and premalignant lesions in patients with cirrhosis*. Hepatology, 2001; 34:1109-12.

37) Bruix J, Sherman M, Llovet JM, et al.: *EASL Panel of Expert on HCC: Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference*. Hepatol, 2001; 35:421-30.

38) International Working Party: *Terminology of nodular hepatocellular lesions*. Hepatology, 1995; 21:703-8.

39) Yamamoto K, Takenaja K, Kajiyama K, et al.: *Cell proliferation and cell loss in nodule-in-nodule hepatocellular carcinoma.* Hepatogastroenterology, 1999; 46:813-19.

40) Kojiro M: *Nodule-in-nodul 'appearance in hepatocellular carcinoma:Its significant morphologic marker of differentiation*. Intervirology, 2004b: 47:179-83.

41) Kutami R, Nakashima Y, Nakashima O, Shiota K, Kojiro M: *Pathomorphologic study on the mechanism of fatty change in small hepatocellular carcinoma of humans*. J Hepatol, 2100:283-89.

42) Roncalli M, Roz E, Coggi G, et al.: *The vascular profile of regeneration and dysplasia nodules of the cirrhotic liver implications for diagnosis and classification.* Hepatology, 1999; 30:1174-178.

43) Park YN, Yang CP, Fernandez GJ, Cubukcu O,Thung S, Theise N: *Neoangiogenesis and sinusoidal capillarization in fdysplastic nodules of the liver*, Am J Surg Pathol, 1998; 22:656-62.

44) Nakano M, Saito A, Yamamoto M, Doi M, Takasaki E: *Stromal and blood vessel invasion in well differentiated hepatocellular carcinoma*. Liver. 1997; 17:41-46

45) Capurro MI, Wanless IR, Sherman M, et al.: *Glypican-3 :A novel serum and histochemical marker for hepatocellular carcinoma*. Gastroenterology, 2003; 12:89-97.

46) Capurro MI, Xiang XY, Lobe C, Filmus J: *Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling*. Cancer Res, 2005; 65:6245-254.

47) Hippo Y, Watanabe K, Watanabe A, et al: *Identification of soluble NH2-terminal fragment of glycan-3 as a serological marker for early-stage hepatocellular carcinoma.* Cancer Res, 2004; 64:2418-423

48) Chuma M, Sakamoto M, Yamazaki K, et al.: *Expression profiling in multistage hepatocarcinogenesis:Identification of HSP70 as a molecular marker or early hepatocellular carcinoma*. Hepatology, 2003; 37:198-207.

49) Lim SO, Park SG, Yoo JH., et al.: *Expression of heat shock proteins (HSP27, HSP60, HSP70, HSP90,GRP78, GRP94) in hepatitis B virus-related hepatocellular dysplastic nodules.* World J Gastroenterol, 2005; 11:2072-79.

50) Osada T, Nagashima Im Tsuno NH, Kitayama J, Nagawa H: *Prognostic significance of glutamine synthetase expression in unifocal advanced hepatocellular carcinoma.* J Hepatol, 2000; 33:247-53.

51) Paradis V, Laurendeau I, Vidaud M, Bedosan F: *Clonal analysis of macronodules in cirrhosis.* Hepatology, 1998; 28:953-58.

52) Libbrecht L, Desmet V, Roskams T: *Preneoplastic lesions in human hepatocarcinogenesis.* Liver Int, 2005; 25:16-27.

53) Maggioni M, Coggi G, Cassani B, et al.: *Molecular changes in hepatocellular dysplastic nodules on microdissected liver biopsies.* Hepatology, 2000; 32:942-46.

54) Moinzadeh P, Breuhahn K, Stutzer H, Schirmaker P: *Chromosome alterations in human hepatocellular carcinomas correlate with aetiology and histological grade-results of an explorative CGH meta-analysis*. Br J Cancer. 2005; 92:935-41.

55) Oh BK, Kim YJ, Park C, Park YN: *Up-regulation of telomere-binding proteins, TRF1,TRF2, and YIN2 is related to telomere shortening during human multistep hepatocarcinogenesis*. Am J Pathol, 2005; 166:73-80.

56) Anders RA, Yerian LM, Tretiakova M, et al.: *cDNA microarray analysis of macroregenerative and dysplastic nodules in end-stage hepatitis C virus-induced cirrhosis*. Am J Pathol, 2003; 162:991-1000

57) Nam SW, Park YJ, Ramasamy A, et al.: *Molecular changes from dysplastic nodule to hepacellular carcinoma through gene expression. Profiling*. Hepatology, 2005; 42:809-18.