Molecular Biology of Hepatocellular Carcinoma

Dr Taher AL-Tweigeri MD, HBIM, FRCPC
King Faisal Specialist Hospital and Research Centre
Riyadh, KSA

ISSN: 2070-254X

Hepatocellular carcinoma (HCC) is the fifth most frequent tumor and the third cause of tumor-related death in the world, and is now a major health problem worldwide. In the United States, the incidence increased by 80% between 1975 and 1998, whereas the incidence of all other malignancies declined. Based on mathematical models, its frequency has been estimated likely to further increase in the upcoming years because of the spread of the hepatitis C virus, and the longer survival of patients with liver cirrhosis.

In fact, in Western European as well as North American countries, HCC usually develops in cirrhotic patients, and cirrhosis is the most important risk factor. So far, therapy has been mainly ablative, including embolization, alcohol or acid instillation, radiofrequency ablation and radical surgery, and although some improvements prognosis and survival are still unsatisfactory, even for those patients undergoing liver transplantation. On the other hand, we cannot rule out the possibility that an advanced stage of the underlying liver cirrhosis can be the cause of death in patients with HCC. Tumor recurrence, together with metastasis formation, represents the most common reasons for the unfavorable prognosis, and no therapies are currently available to block or reduce tumor growth and/or metastasis dissemination. This is mainly because the molecular and biologic mechanisms regulating cancer growth and spread are still poorly understood. For instance, the underlying chronic liver disease, as well as the pathologic characteristics of the tumor, does not explain why the natural history of HCC is so different in each patient. In fact, growing evidence suggests that intrinsic biologic characteristics of the tumor in terms of proliferation, survival, and invasiveness are probably due to different composition and activity of the microenvironment, this leading to very different clinical outcome. For example, only a few of the questions that are still seeking an answer includes why and how HCC develops and can recur, why it develops metastases, why the sites of metastasis are so different, and how the malignant phenotype changes, often becoming very aggressive and invasive during the natural history. In this review I will try to point out what we know and what we need to know to be better able to face the challenges posed by HCC. Moreover, I will deal with the capacity of invasion and spread of HCC in the liver or in other organs, devoting a particular interest to the interactions between the surrounding microenvironment and the cancer cells.

The tissue microenvironment is a kind of biologic ''soup'' made up of different cell types (hepatic satellite cells, Kupfer cells, macrophages, endothelial cells); extracellular matrix (ECM) proteins such as laminins (Lns), collagens, fi-bronectin, vitronectin, fi-brinogen; proteolytic enzymes: matrix metalloproteases (MMPs), serin proteases; growth factors: transforming growth factor (TGF)-b1, hepatocyte growth factor etc. To invade, HCC cells need to penetrate through the tissues, negotiating this progression with the ECM proteins. However, this does not happen as a result of a breakdown of the molecular tissue boundaries but rather due to the unlocking of a dynamic gate. Proteolytic enzymes such as MMPs and serin proteases act as molecular scissors, that can cleave different components including ECM molecules, growth factors etc. changing their biologic properties, with a consequent effect on the HCC malignant Phenotype.

In fact, in the last few years, the ''seed and soil'' theory of Paget formulated in 1889 has been revisited in light of the growing evidence suggesting that metastases occur as a consequence of modifications of the microenvironment (the soil), rather than of the ''primary metastatic genes'' we have focused on for many unsuccessful years.

Metastatic dissemination or multifocal tumor?

The possibility that more than one nodule may be detected in patients with HCC has been known since 1957, when this was pointed out by Plopper and Schsffiner, but this manifestation has been most commonly considered as a multifocal carcinoma rather than a metastatic cancer, although such an assumption would be quite unusual for a malignant type of cancer like HCC. More recently, improvements in diagnostic techniques together with the availability of larger portions of tissue obtained from radical surgery or from liver transplantation, have made more systematic studies possible, thereby showing the presence of satellite micronodules around the main lesion, and so suggesting that HCC effectively is an invasive cancer. This is consistent with the recurrence of HCC in transplanted liver, which would otherwise be difficult to explain. The distinction between multifocal or metastatic nodules is not merely academic as the occurrence of metastasis is correlated with worst prognosis.

Among the different sites of metastasis, the liver represents the most frequent target, whereas the blood vessels are the most dramatic, being associated with a particularly unfavorable prognosis. ''Metastatic dissemination or multifocal tumor?'' is not a Hamletic question, because the 2 possibilities are not mutually exclusive as cirrhotic liver can generate more than one cancer nodule with the

same, still unknown mechanisms, during the history of the disease, as observed for other tumors, that is, urinary bladder. However, the identification of these 2 distinct hypothesis seems to be an underestimated problem by clinicians although a patient with a multifocal tumor has a better prognosis than a patient with a metastatic cancer. The main problem is still how to differentiate between the two, as the currently available morphologic criteria are definitely obsolete. Molecular genetic approaches seem to be the most appropriate to investigate the clonality of the different nodules: monoclonal multiple nodules stem from a common malignant precursor, as in metastatic lesions, whereas different clonalities are an expression of distinct tumors, as in multifocal HCC.

A more sophisticated approach to investigating a common pattern of chromosomal alterations has documented karyotypic alterations serving to differentiate between multifocal tumor and metastatic HCC.Similar results have been obtained by different investigators, showing that multiple HCC nodules are an expression of metastasis rather than of multifocal cancer in more than 60% of cases. However, in both studies the techniques used could not be immediately introduced into clinical care, as it is not reasonable to perform multiple biopsies in patients to assess the correct diagnosis based on chromosomal or DNA alterations. These types of studies are very sophisticated and would need more clinical validation, also in view of the possibility that some alterations might not be the rule for HCC of multifocal origin. In conclusion, it is important to consider HCC as a highly metastatic cancer with a particular tropism for blood vessels, in which other tests are needed to recognize a metastatic from a multiple HCC.

Is HCC invasiveness a tumoral or a peritumoral problem?

In HCC tissue, the microenvironment seems to play a key role because the malignant cells grow embedded in a microenvironment enriched with ECM proteins, deposited as a consequence of the underlying cirrhosis. HCC cells, like other epithelial cells, cross talk with the surrounding ECM proteins thanks to several integrins, which act as transmembrane receptors.
In the last few years many proposed a model whereby α3b1 integrin, the main receptor for Ln-5, is present on the cellular surface of invasive but not of noninvasive HCC cells in vitro; consistently, α3b1 integrin was detected only in the tissue specimens obtained from aggressive and invasive HCC. Ln-5 is a member of the Ln family that has been reported to be involved in the metastatic spread of several malignancies after the proteolytic cleavage of its γ2 chain. In the liver, Ln-5 is expressed "de novo" in HCC but is absent in the peritumoral tissues; furthermore, the γ2 chain has been found to be expressed along the invasive edge, thus correlating with the occurrence of metastasis and with a worse survival and prognosis. Which cell type secretes Ln-5 in the liver is not yet known, but HCC cells that express α3b1 integrand can use Ln-5 as a preferential route to invade surrounding tissues, whereas HCC cells that do not express α3b1 do not migrate and do not invade. However, these cells can acquire migratory and invasive abilities through TGF-b1 that stimulates the expression of the integrand α3b1 at a translational level, on the cellular surface of the "noninvasive" HCC cells, that thus acquire migratory and invasive properties.TGF-b1 has been reported to be increased in the serum of HCC patients, stored in an inactive form in the microenvironment, where it can be activated after proteolytic cleavage by MMP-2 or MMP-9. Consistently, in HCC a decreased expression of the tissue inhibitor of MMP-2, responsible for a proteolytic imbalance, has been reported.

This induces the activation of TGF-b1 and the metastasization of the HCC. Whether the proteolytic imbalance is brought about by the HCC cells or by other surrounding cells such as myoepithelial or inflammatory cells, as reported in breast and colon cancer, respectively, is not yet known. Another possibility is that inflammatory cells are commonly present in the tissue surrounding the cancer, and may represent an important source of proteolytic enzymes that can activate TGF-b1, inflammatory cytokines, growth factors, etc. This is the rationale behind the proposed use of anti–COX-2 as a potential anticancer drug. In any case, it is very difficult to define each component of the microenvironment, also because up to now studies have usually been focused on investigating the biologic functions of just one or few components present in the microenvironment. Several ongoing studies are investigating the "fingerprint" of HCC compared to the peritumoral tissues, using highly technologic approaches based on microarray techniques, proteomics, etc., and different hierarchical genes have been reported, but the interpretation of the results is strongly limited until the biologic role of those genes has been explained in an experimental model.

Molecular Mechanisms of HCC

An extensive study of HCC resulting from three of the main etiological factors HCV infection, HBV infection, and chronic alcohol intake indicates common molecular/genetic changes, with Rb1, p53, and Wnt the main pathways affected. Typically, tumors associated with alcoholism have more frequent alterations of Rb1 and p53 pathways than those caused by HCV infection.
The most common alterations were p16INK4A methylation, loss of Rb1 expression through promoter methylation, and Cyclin D1 amplification. Analysis of HCC from human and animal models demonstrates up-regulation of the MAPK pathway as well as genes associated with an activated cell cycle. In addition the down-regulated genes mainly encode hepatocyte specific gene products and detoxification enzymes producing a less differentiated phenotype.

Later in hepatocarcinogenesis tumor cells undergo increasing levels of chromosomal aberrations including loss of gene heterozygosity. P16INK4A normally inhibits cyclin dependent kinases (cdk) 4 and 6 which block G1 phase progression via dephosphorylation of Rb, the latter of which binds to and inactivates E2F1. Germ line mutations of this tumor suppressor protein have been identified in adult cases of HCC in Switzerland suggesting familial HCC however, the concept of inheritable HCC is relatively new and requires further confirmation and analysis. Diethylnitrosamine-thioacetamide treatment of Fischer rats, which induces HCC, also causes hypermethylation of the p16INK4A exon 1 in the later stages of carcinogenesis, further highlighting its importance in this process. Mutations of β-Catenin are commonly observed in the early development of HCC and disruption of this Wnt signaling protein affects the expression of its target genes including c-myc, c-jun, cyclin D1 and fi-bronectin β-Catenin is an important submembranous protein that functions in cell– cell adhesion. Its mutation disrupts normal cell– cell interactions and strongly stimulates hepatocellular growth. P53 is the most common molecular target in human carcinogenesis.

However, a study of primary HCCs from many different origins shows that frequency of mutation of this tumor suppressor was low. Usually mutations of p53 are recognized only in advanced stages of HCC and it is not a prerequisite for hepatocarcinogenesis.

The most common mutations of p53 in HCC are the G to T transversions in codon 249 caused by aflatoxin exposure. Recently, work to begin to define the complex signaling networks involved in the development of HCC has been undertaken using DNA–microarray-based gene expression profiling of stored human HCC.

Analyses of gene expression arrays have identified two distinct subtypes of HCC based upon relative gene expression patterns, termed subclass A and B. These two distinct subgroups carry dramatically different survival curves and patterns of gene expression. Subclass A that carries a poor prognosis has high levels of expression of genes associated with proliferation and ubiquitination. Expression of apoptotic proteins in subclass B HCC is low. Conversely, subclass B tumors express lower relative up-regulation of growth pathways and ubiquitination proteins but express high levels of antiapoptotic proteins. Subclass A cancers may involve a greater degree of c-myc dysregulation or that associated with transforming-growth factor alpha expression while providing little evidence of β-catenin expression. Conversely, subclass B HCC appears associated with variable expression of B-catenin and appear to have a larger expression of the transcription factor E2f1. Work is ongoing to further identify differences in patients with HCC. These differences may have profound effects in the future diagnosis as well as in choosing molecular pharmacologic targets to improve outcomes associated with chemotherapy.

Conclusions

In conclusion, what we know best is that our knowledge of HCC biology is definitely still poor. The importance of gaining a deep understanding of the biologic and molecular mechanisms of HCC growth and metastasis seems to be underestimated by clinicians.

Nevertheless, there is a strong demand for new therapies, although their development is hampered because the potential therapeutic target is still unclear. Furthermore, HCC is a cancer with peculiar characteristics, due to the underlying cirrhosis that could limit drug administration because of potential hepatoxicity. In addition, the altered tissue remodelling commonly occurring in liver cirrhosis could trigger cancer aggressiveness because of activated signal pathways. As consequence of this impediment, and based on other cancer models, biologic therapy targeting microenvironment components seems to be a potentially interesting strategy. Pharmaceutical research in this sense would be greatly improved by a greater insight into the identification of the molecular mechanisms responsible for the different biologic and clinical behavior of HCC.