

Chapter 6

Hepatocarcinogenesis

Primary Author

Claude B. Sirlin UC San Diego

Contributing Authors

Victoria Chernyak	Montefiore Medical Center
Jennifer Cui	UC San Diego
Kazim H. Narsinh	Hospital of the University of Pennsylvania
Demetri Papadatos	University of Ottawa
Cynthia S. Santillan	UC San Diego
An Tang	University of Montreal

Illustrators & figure contributors

Victoria Chernyak	Montefiore Medical Center
Claude B. Sirlin	UC San Diego
An Tang	University of Montreal

Editors

Victoria Chernyak	Montefiore Medical Center
Claude B. Sirlin	UC San Diego

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Hepatocarcinogenesis

Introduction

This chapter reviews, in a questions & answers format, key concepts in hepatocarcinogenesis.

- Knowledge of hepatocarcinogenesis and familiarity with the associated imaging features can encourage radiologists to apply LI-RADS appropriately, diagnose HCC accurately, differentiate HCC and its precursors from other malignant entities that may arise in cirrhosis, and understand the mechanisms by which HCC forms and spreads.
 - Although the focus is on hepatocarcinogenesis, the chapter also reviews the development of two non-HCC malignancies associated with cirrhosis: iCCA and cHCC-CCA.
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What is hepatocarcinogenesis?

- Hepatocarcinogenesis is the process by which HCC forms.
 - It is a complex process characterized by progressive cellular and molecular dedifferentiation of hepatocytes.
 - Eventually, the neoplastic hepatocytes may acquire an overtly malignant phenotype and biological behavior.
 - Overtly malignant cells have the capacity to invade vessels (i.e., penetrate the vessel wall to enter the vessel lumen) and metastasize (i.e., spread via the circulation).
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What is multicentric hepatocarcinogenesis?

- Numerous HCCs may develop independently in different parts of the liver. This is termed multicentric hepatocarcinogenesis, since there are multiple “centers” of HCC emergence.
 - In multicentric hepatocarcinogenesis, each HCC follows its own path, with its own molecular alterations, and its own history of histological precursors. Thus, each HCC may have a unique histologic phenotype and a unique CT or MRI appearance.
 - The HCCs may be far apart and/or they may be clustered in a segment of the liver, presumably reflecting a regional “field effect”.



Multistep vs. De Novo Hepatocarcinogenesis

What is the difference between multistep vs. de novo hepatocarcinogenesis?

- **At a molecular level**, hepatocarcinogenesis is always multistep: i.e., multiple molecular alterations are required to transform a benign hepatocyte into a malignant cell. These alterations may be genetic or epigenetic:
 - Genetic changes are mutations in the actual DNA sequence. These result in changes in the structure and function of expressed proteins.
 - Epigenetic changes are modifications of DNA such as methylation that modify gene activation but do not change the underlying sequence. These result in increased or decreased expression levels of proteins.
- **At a histological level**, hepatocarcinogenesis may be multistep or de novo:
 - **Multistep hepatocarcinogenesis** refers to the successive emergence within larger precursor nodules of histologically definable, more aggressive inner nodules. Each new nodule is thought to represent a clonal population of cells with a survival advantage compared to the surrounding cells. Over time, the more aggressive inner nodules replace the outer nodules. The initial nodules are histologically benign. Subsequently, progressively more neoplastic nodules emerge until an overtly malignant phenotype is achieved (i.e., HCC). For example:
 - RN → LGDN → HGDN → eHCC → pHCC **OR**
 - RN → HGDN → pHCC

Note that histologically defined steps can be “skipped”

- **De novo hepatocarcinogenesis** refers to the emergence of HCC without histologically definable precursor lesions. In this case, overtly malignant pHCCs arise without passing through intermediate histological steps.

Do most HCCs arise from multistep or de novo hepatocarcinogenesis?

- This is unknown.
- Single-center research studies have reported that a substantial proportion of imaging-diagnosed HCCs have no retrospectively detectable precursor lesions on prior imaging exams, but this does not exclude the possibility of histologically recognizable precursors.



Hepatocarcinogenesis and Imaging Correlates

What pathophysiological alterations occur during multistep hepatocarcinogenesis?

Characteristic pathophysiological alterations occur during histologically defined multistep hepatocarcinogenesis, as listed below and illustrated on the figure on the next page.

The characteristic alterations include the following:

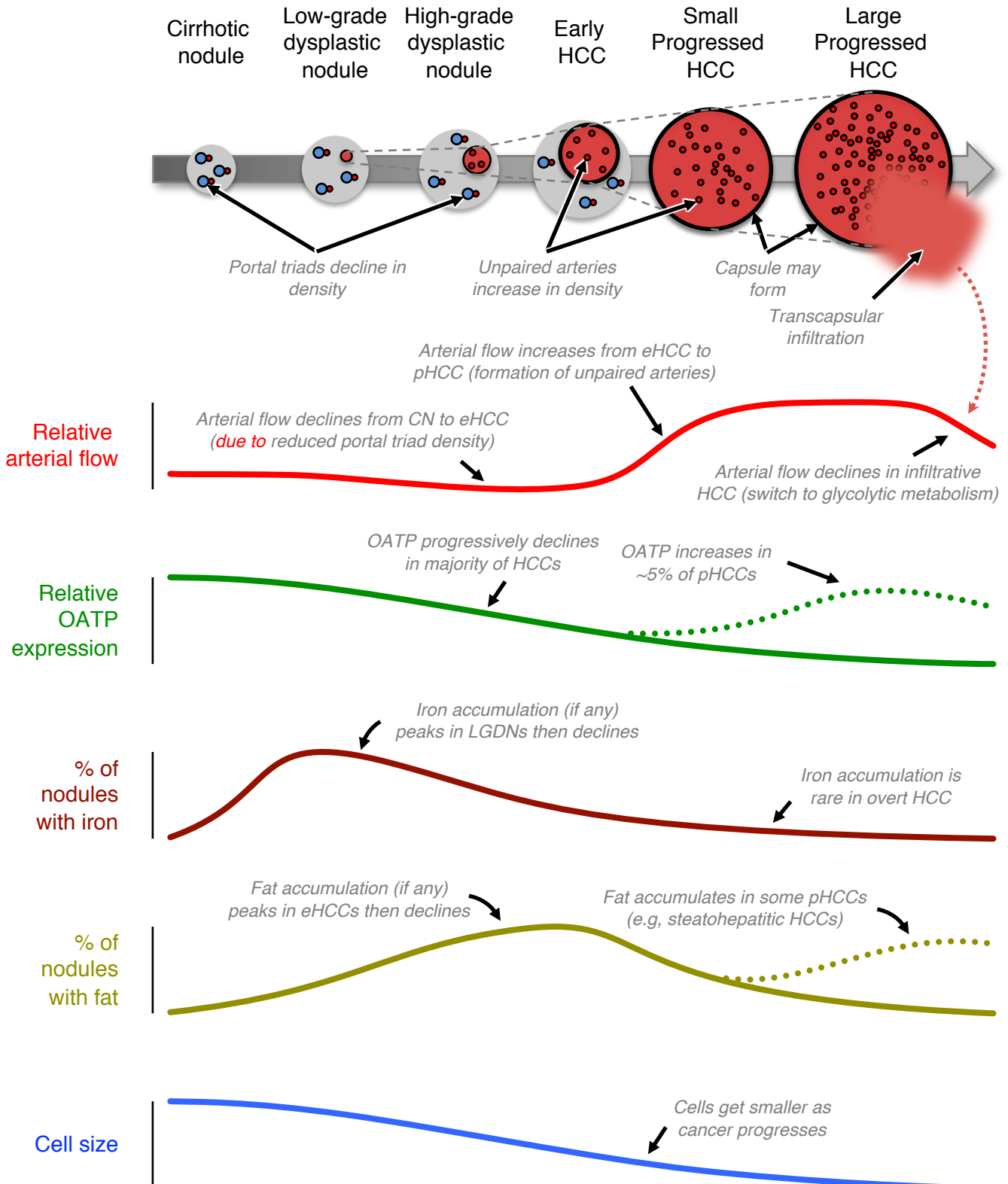
- Nodule growth
- Progressive reduction in portal triads due to stromal invasion with portal triad destruction, as well as other mechanisms.
- Emergence and development of unpaired arteries, usually beginning in HGDN, due to release of angiogenic factors.
- Initial reduction in arterial flow (DNs and eHCC), followed by an increased amount of flow (pHCC), followed by a decrease (infiltrative HCC), reflecting the temporal changes in intranodular density of both normal and tumoral arteries.
- Progressive reduction in relative OATP expression.
 - Exception: Approximately 5% of pHCCs have preserved or elevated OATP expression.
- Capsule formation induced by expansile growth with transformation to overt malignancy (pHCC).
- Capsular penetration with infiltrative growth for very aggressive tumors.
- Propensity to accumulate iron early in hepatocarcinogenesis (some LGDNs and HGDNs) followed by loss of iron or “iron resistance” later (eHCCs and pHCCs).
- Propensity to accumulate fat during early and middle phases of hepatocarcinogenesis (some LGDNs, some HGDNs, and up to 40% of eHCCs) followed by loss of fat with transformation to overt malignancy (pHCC).
 - Exception: Steatohepatic HCC (ShHCC) is a variant of pHCC that accumulates fat.
- Reduction in cell size (small cell change).

Although hepatocarcinogenesis may be de novo, not multistep, understanding the alterations accompanying multistep hepatocarcinogenesis is broadly relevant to HCC imaging, regardless of any individual tumor’s unique hepatocarcinogenesis pathway.

➤ **These alterations are illustrated and their imaging relevance summarized in the figure and table on the next two pages, respectively.**

Hepatocarcinogenesis and Imaging Correlates

Multistep hepatocarcinogenesis and its accompanying pathophysiological alterations



Hepatocarcinogenesis and Imaging Correlates

Alterations of hepatocarcinogenesis and their imaging correlates

Alteration	Imaging correlations
Nodule growth	<p>Size is a predictor of malignancy in cirrhosis</p> <ul style="list-style-type: none"> • < 10 mm: rarely malignant • 10-19 mm: may be malignant • > 20 mm: usually malignant
Initial decrease in arterial flow	Arterial phase hypo- or isoenhancement is characteristic of LGDN, HGDN, and eHCC
Subsequent increase in arterial flow	Arterial phase hyperenhancement (APHE) is characteristic of pHCC
Decline in arterial flow with diffuse growth	Diffuse HCCs may show little or no APHE
Reduction in portal venous inflow within tumor	Contributes to washout appearance
Capsule formation	Contributes to capsule appearance
Small cell change	Contributes to diffusion restriction
Fat accumulation	Intralesional fat favors HGDN, early HCC, or, if nodule has progressed features, steatohepatic pHCC
Iron accumulation	Virtually excludes HCC. Siderotic nodules usually are LGDNs or HGDNs. However, development of an iron sparing inner nodule within a siderotic outer nodule suggests incident HCC.
Clonal expansion	Accounts for nodule-in-nodule appearance, a classic but uncommon imaging feature of HCC



Mechanisms of Carcinogenesis

Why does cirrhosis predispose to HCC formation?

Although cirrhosis is strongly associated with HCC, cirrhosis per se may not be the primary inciting factor. Rather, cirrhosis and hepatocarcinogenesis are parallel processes that progress together over many years or even decades from a common cause – chronic inflammation.

- Chronic inflammation plays a pivotal role in HCC development by causing repeated cycles of cellular injury, death, and regeneration.
- This cycle promotes aberrant cell signaling, epigenetic alterations, and mutations. Hepatocyte proliferation in response to injury leads to clonal propagation of these molecular abnormalities.
- Reactive oxygen species and nitrogen intermediates released by inflammatory infiltrates are thought to be important in inducing the epigenetic and genetic changes.
- These molecular alterations begin during a prolonged preneoplastic phase that begins years or decades before cirrhosis is established.
- As liver disease progresses and cirrhosis becomes established, the genetic and epigenetic alterations escalate during a neoplastic phase, characterized by more extreme genomic changes, including chromosomal deletions and rearrangements, aneuploidy, gene amplifications, other mutations, and extensive DNA methylation. These alterations combine to result in cells which have autonomous growth potential and ability to invade vessels and metastasize.
- Commonly implicated molecular processes include inactivation of the tumor suppressor genes p53 and Rb, activation of the Wnt/ β -catenin and EGFR cellular proliferation signaling pathways, and cirrhosis-related host immunosuppression.

In summary, despite the many etiologies of chronic liver disease, chronic inflammation provides a common pathogenic mechanism that may culminate in HCC.

Why does HBV predispose to HCC formation?

In addition to inciting chronic inflammation and the cascade of events described above, HBV can induce hepatocarcinogenesis directly by integrating into the host genome, resulting in host DNA microdeletions and other mutations. Additionally expression of viral proteins can activate transcription of cellular proliferation genes. These factors combine to increase the risk of developing HCC among HBV carriers even in the absence of cirrhosis or chronic inflammation.

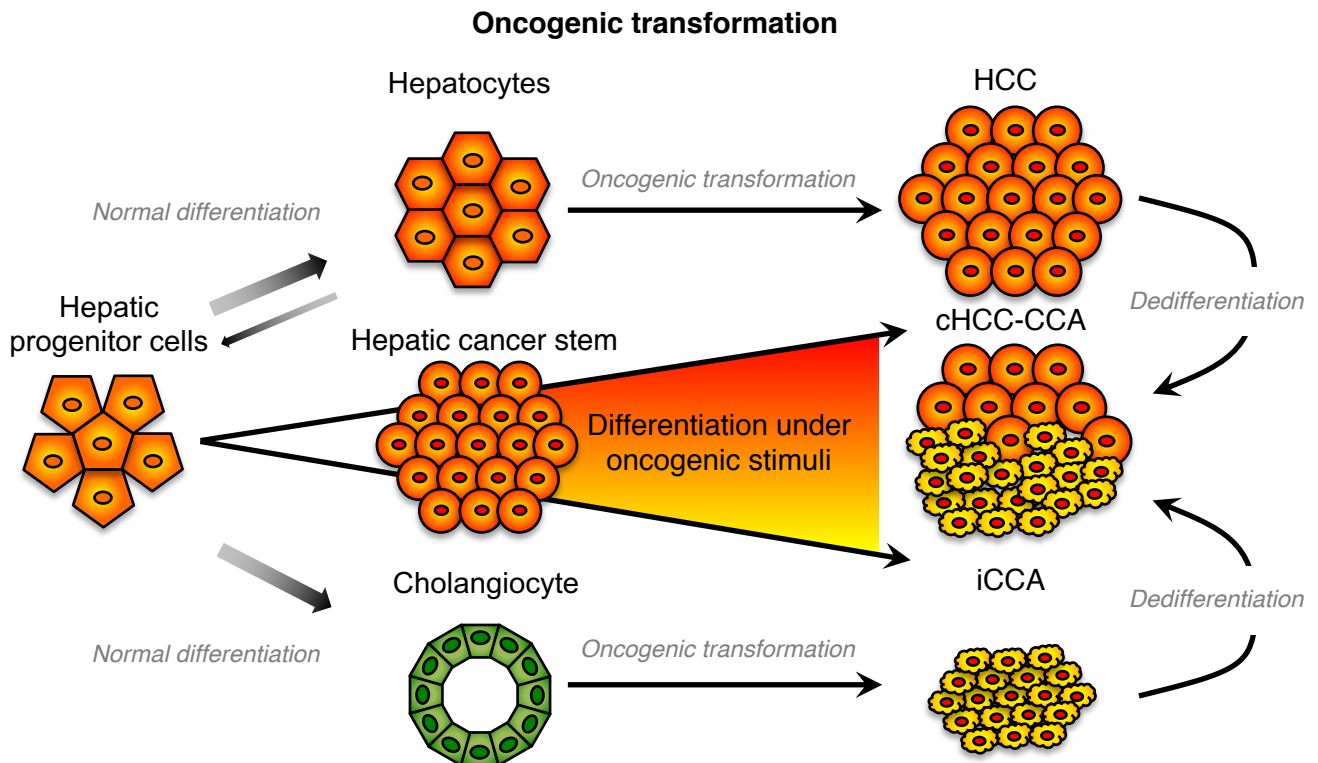
Besides HBV, are there other mutagens that may result in HCC?

Aflatoxin-B1 has a specific mutagenic effect, inactivating the p53 tumor suppressor through modification of a particular amino acid in the protein. Although it does not play a major role in HCC development in the United States, the specific aflatoxin-B1-associated mutation may contribute to HCC formation in up to 30-60% of patients in aflatoxin-endemic areas such as eastern Asia and sub-Saharan Africa.

Mechanisms of Carcinogenesis

Why does cirrhosis predispose to cHCC-CCA and iCCA formation?

- Although not well understood, it is believed that chronic inflammation – the same common pathogenic mechanism driving HCC formation – also drives formation of cHCC-CCA and iCCA.
- cHCC-CCA and iCCA are thought to arise via de novo carcinogenesis from transformation of hepatic progenitor cells or from de-differentiation of mature hepatocytes into progenitor cells. These progenitor cells can differentiate along cholangiocellular or hepatocellular pathways.
- Under the oncogenic conditions associated with chronic liver disease (e.g., chronic inflammation), differentiation and clonal expansion of these progenitors may result in populations of neoplastic cells. Hepatocellular and cholangiocellular differentiation lead to HCC and iCCA, respectively. Sometimes, the differentiation may occur along both pathways simultaneously or in parallel; the resulting tumors have mixed populations of cells, with some resembling HCC and others resembling iCCA – so-called cHCC-CCAs.
- Evidence: many HCCs, iCCAs, and cHCC-CCAs express “stem⁺” like molecular markers, presumably reflecting their origin from progenitor cells or transition through a progenitor state.
- Since they do not arise from multistep hepatocarcinogenesis, iCCAs and cHCC-CCAs do not pass through histologically intermediate stages. Precursor lesions have not been described.

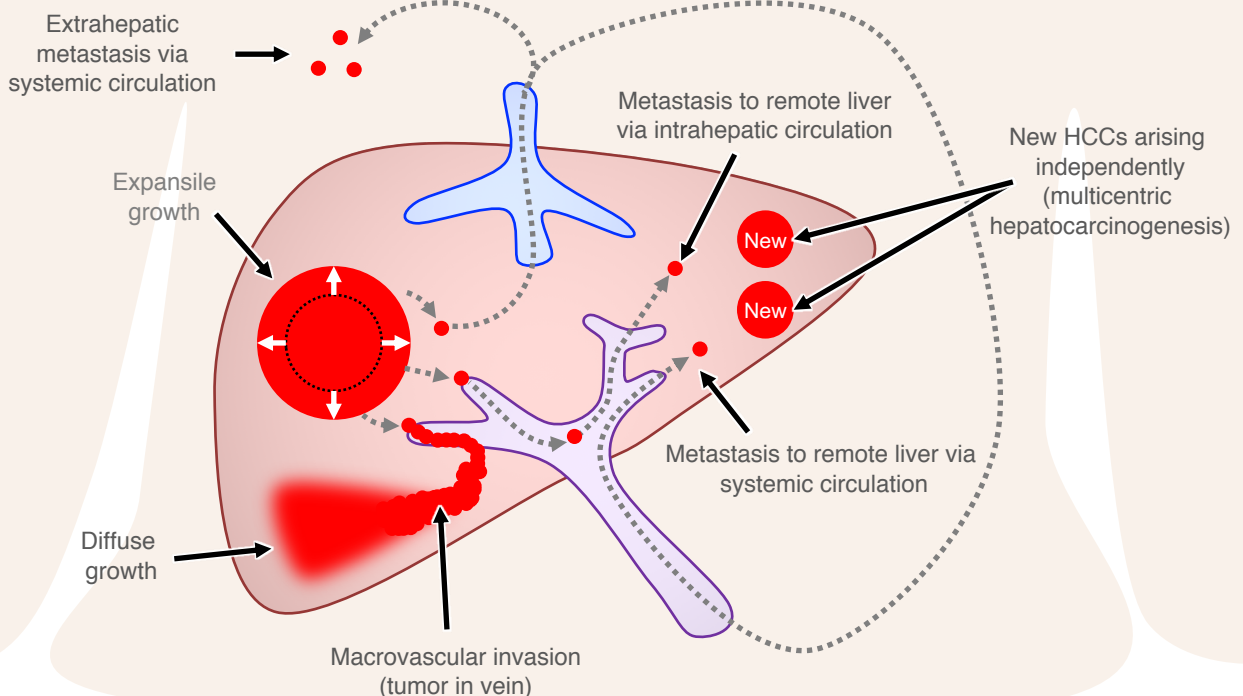


Mechanisms of Spread

How does HCC spread?

Regardless of how they develop (multistep or de novo hepatocarcinogenesis) or from what cell of origin (hepatocyte or progenitor cell) pHCCs can spread through several overlapping pathways:

- Growth – direct extension into surrounding parenchyma. Growth may be expansile or diffuse.
 - Although invasion of adjacent parenchyma is common, invasion by HCC directly through the liver capsule into surrounding organs is unusual.
- Invasion of sinusoids and vessels with subsequent hematogenous dissemination:
 - To the parenchyma, immediately adjacent to the primary tumor (satellite nodules), usually via transtumoral sinusoids and portal venules.
 - To the parenchyma, remote from the primary tumor (distant intrahepatic metastases), usually via altered intrahepatic portal circulation in areas with biphasic or reversed flow
 - To distant organs (lungs, bone, adrenals).
 - Back to the liver via the systemic circulation.
- Lymphatic spread to local or regional lymph nodes.
- Spread within the lumen of a vein.
 - Tumor can spread along the inside of the vein (tumor in vein) and into its branches. This may be microscopic or macroscopic.
 - Portal veins are invaded more commonly than hepatic veins, because blood drains from HCCs via transtumoral sinusoids and portal venules, rather than through hepatic venules.
 - Although not a form of “spread” per se, HCCs may arise at multiple locations in the liver independently (multicentric hepatocarcinogenesis). See [page 6-1](#).





Mechanisms of Spread

How do iCCA and cHCC-CCAs spread?

- iCCAs and cHCC-CCAs spread through similar mechanisms as HCC.
- Both iCCA and cHCC-CCA may be associated with vascular invasion, intrahepatic metastases, and growth within the lumen of a vein (tumor in vein).
- iCCAs tend to metastasize to extrahepatic sites early in their development. Liver transplantation is not done for these lesions except under investigational protocols, because of high post-transplant recurrence risk.
- For cHCC-CCAs, the risk of extrahepatic dissemination is not well known. Conservatively, clinical guidelines assume these tumors have high rates of extrahepatic dissemination.

What processes cause some HCCs, iCCAs, and cHCC-CCAs to be multifocal?

Multifocality may be attributable to one or both of the following basic mechanisms:

- | | |
|---|---|
| <ul style="list-style-type: none"> • Intrahepatic hematogenous metastasis from a primary tumor | <ul style="list-style-type: none"> • The metastatic tumors are usually located near the primary tumor (satellite nodules) but they may be located in remote parts of liver. • Remote intrahepatic metastases may reflect <ul style="list-style-type: none"> • spread of tumor cells within the liver via an altered intrahepatic circulation OR • return to the liver after initial escape into the systemic circulation. |
| <ul style="list-style-type: none"> • Multicentric carcinogenesis | <ul style="list-style-type: none"> • The process of HCC, cHCC-CCA, or iCCA carcinogenesis may occur synchronously or metachronously in different parts of the liver (multicentric carcinogenesis). |

When multiple tumors are detected, can imaging differentiate whether they arose from intrahepatic hematogenous metastasis vs. multicentric carcinogenesis?

Reliable differentiation is usually not possible, but some imaging findings favor hematogenous metastasis and others favor multicentric carcinogenesis:

- Favoring hematogenous metastasis: presence of numerous smaller nodules resembling a larger, presumably primary mass. The smaller nodules have features of overt malignancy despite small size, reflecting their aggressive biology and capability of metastasis.
- Favoring multicentric carcinogenesis: phenotypically diverse nodules spanning the histological spectrum from HCC precursors to progressed HCC. Nodules meeting LR-5 criteria may have different imaging appearances. For example, some may contain fat while others do not. Some nodules may have features of non-HCC malignancy.



Summary

Hepatocarcinogenesis is a complex process by which HCC forms, characterized by progressive cellular and molecular dedifferentiation of hepatocytes.

At a molecular level, hepatocarcinogenesis is always multistep: i.e., multiple molecular alterations are required.

At a histological level, hepatocarcinogenesis may be multistep or de novo. Multistep hepatocarcinogenesis refers to the successive emergence within larger precursor nodules of histologically definable, more aggressive inner nodules. De novo hepatocarcinogenesis refers to the emergence of HCC without histologically definable precursor lesions.

It is not known whether most HCCs arise from multistep or de novo hepatocarcinogenesis.

Numerous HCCs may develop independently in different parts of the liver. This is termed multicentric hepatocarcinogenesis.

Characteristic pathophysiological alterations occur during histologically defined multistep hepatocarcinogenesis.

Although hepatocarcinogenesis may be de novo, not multistep, understanding the alterations accompanying multistep hepatocarcinogenesis is broadly relevant to HCC imaging, regardless of any individual tumor's unique hepatocarcinogenesis pathway.

Chronic inflammation provides a common pathogenic mechanism that may culminate in HCC.

Although HCC occurs most often in cirrhotic livers, cirrhosis is probably not a premalignant condition per se, but a parallel process that occurs in response to the same inflammatory insults that drive hepatocarcinogenesis.

In addition to inciting chronic inflammation, HBV can induce hepatocarcinogenesis directly by integrating into the host genome, resulting in host DNA microdeletions and other mutations.

Aflatoxin-B1 has a specific mutagenic effect. The specific aflatoxin-B1-associated mutation may contribute to HCC formation in up to 30-60% of patients in aflatoxin-endemic areas.

cHCC-CCA and iCCA are thought to arise via de novo carcinogenesis from transformation of hepatic progenitor cells or from de-differentiation of mature hepatocytes into progenitor cells. Under the oncogenic conditions associated with chronic liver disease (e.g., chronic inflammation), differentiation and clonal expansion of these progenitors along hepatocellular or cholangiocellular pathways leads to HCC and/or iCCA, respectively. Sometimes, the differentiation may occur along both pathways simultaneously or in parallel, resulting in cHCC-CCAs.

HCCs, cHCC-CCAs, and iCCAs may spread inside and outside the liver via several mechanisms.

Multifocality of these tumors is common and may be attributable to intrahepatic metastasis from a primary tumor, multicentric carcinogenesis, or both.



References

El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? *Hepatology*. 2014 Nov;60(5):1767-75.

Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat Rev Cancer*. 2006 Sep;6(9):674-87.

International Consensus Group for Hepatocellular Neoplasia. The International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology*. 2009 Feb;49(2):658-64.

Ishigami K, Yoshimitsu K, Nishihara Y, Irie H, Asayama Y, Tajima T, Nishie A, Hirakawa M, Ushijima Y, Okamoto D, Taketomi A, Honda H. Hepatocellular carcinoma with a pseudocapsule on gadolinium-enhanced MR images: correlation with histopathologic findings. *Radiology*. 2009 Feb;250(2):435-43.

Kim CK, Lim JH, Park CK, Choi D, Lim HK, Lee WJ. Neoangiogenesis and sinusoidal capillarization in hepatocellular carcinoma: correlation between dynamic CT and density of tumor microvessels. *Radiology*. 2005 Nov;237(2):529-34.

Kitao A, Matsui O, Yoneda N, Kozaka K, Shinmura R, Koda W, Kobayashi S, Gabata T, Zen Y, Yamashita T, Kaneko S, Nakanuma Y. The uptake transporter OATP8 expression decreases during multistep hepatocarcinogenesis: correlation with gadoxetic acid enhanced MR imaging. *Eur Radiol*. 2011 Oct;21(10):2056-66.

Kitao A, Zen Y, Matsui O, Gabata T, Nakanuma Y. Hepatocarcinogenesis: multistep changes of drainage vessels at CT during arterial portography and hepatic arteriography--radiologic-pathologic correlation. *Radiology*. 2009;252(2):605-14.

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*. 2000 Aug;33(2):282-9.

Marrero JA, Hussain HK, Nghiem HV, Umar R, Fontana RJ, Lok AS. Improving the prediction of hepatocellular carcinoma in cirrhotic patients with an arterially-enhancing liver mass. *Liver Transpl*. 2005 Mar;11(3):281-9.

Matsui O, Kobayashi S, Sanada J, Kouda W, Ryu Y, Kozaka K, Kitao A, Nakamura K, Gabata T. Hepatocellular nodules in liver cirrhosis: hemodynamic evaluation (angiography-assisted CT) with special reference to multi-step hepatocarcinogenesis. *Abdom Imaging*. 2011 Jun;36(3):264-72.

Narsinh KH, Cui J, Papadatos D, Sirlin CB, Santillan CS. Hepatocarcinogenesis and LI-RADS. *Abdom Radiol (NY)*. 2018 Jan;43(1):158-168.



References

Park YN, Kim YB, Yang KM, Park C. Increased expression of vascular endothelial growth factor and angiogenesis in the early stage of multistep hepatocarcinogenesis. *Arch Pathol Lab Med*. 2000 Jul;124(7):1061-5.

Salomao M, Remotti H, Vaughan R, Siegel AB, Lefkowitz JH, Moreira RK. The steatohepatic variant of hepatocellular carcinoma and its association with underlying steatohepatitis. *Hum Pathol*. 2012 May;43(5):737-46.

Sia D, Villanueva A, Friedman SL, Llovet JM. Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis. *Gastroenterology*. 2017;152(4):745-761.

Stevens WR, Gulino SP, Batts KP, Stephens DH, Johnson CD. Mosaic pattern of hepatocellular carcinoma: histologic basis for a characteristic CT appearance. *J Comput Assist Tomogr*. 1996 May-Jun;20(3):337-42.

Tarlow BD, Pelz C, Naugler WE, Wakefield L, Wilson EM, Finegold MJ, Grompe M. Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. *Cell Stem Cell*. 2014 Nov 6;15(5):605-18

Zhang J, Krinsky GA. Iron-containing nodules of cirrhosis. *NMR Biomed*. 2004 Nov;17(7):459-64.
