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REVIEW ARTICLE

Molecular Pathogenesis of Hepatocellular Carcinoma

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ABSTRACT

Liver cancer, which consists predominantly of hepatocellular carcinoma, is one of the most frequently occurring cancers and the third most common cause of cancer death worldwide. Hepatocarcinogenesis follows a multistep process evolving from cirrhosis / chronic hepatitis to dysplastic nodules and ultimately malignant tumours. Recent advances in molecular methods have led to a growing understanding of the underlying mechanisms of hepatocarcinogenesis. Hepatocarcinogenesis is closely associated with allelic losses, chromosomal changes, gene mutations, and epigenetic alterations. Some of these alterations show a stepwise increase at different stages of hepatocarcinogenesis. Chromosomal, genetic, and epigenetic abnormalities can lead to deregulation in many cell signalling pathways implicated in tumour proliferation, progression, and survival. Discoveries and insight into these complex pathways have created opportunities for targeted agents and new therapeutic approaches for this disease. More recent evidence also suggests the involvement of aberrant microRNA expression and the concept of liver-specific cancer stem cells in hepatocarcinogenesis. Detailed understanding of the molecular pathogenesis is crucial for the development of new therapeutic approaches against hepatocellular carcinoma. This article summarises the molecular mechanisms currently implicated in the pathogenesis of hepatocellular carcinoma and their potential roles in improving the clinical management of this disease.

Key Words: *Carcinoma, hepatocellular; Gene expression regulation, neoplastic; Liver neoplasms; Mutation; Signal transduction*

中文摘要

肝細胞癌的分子發病機制

吳呂愛蓮

肝癌主要包括肝細胞癌，是最常見的癌症之一，亦在全球致命癌症中排行第三。肝癌的發病是從肝硬化或慢性肝炎演變成非典型增生的結節而最終造成惡性腫瘤的一個牽涉多個步驟的過程。分子生物學的最新發展使人們對肝癌發病的潛在機制有更多認識。肝癌與等位基因損失、染色體變化、基因突變和表觀遺傳改變密切相關，而這些改變在肝癌的不同階段續漸遞增。染色體、遺傳和表觀遺傳異常可導致許多牽連細胞通訊路線出現不規則、腫瘤增殖、惡化及生存。發現和洞悉這些複雜的分子病變機制造就了標靶藥物和新療法的誕生。最新的實證亦顯示了microRNA的異常表達和肝臟特有的癌幹細胞有參與肝癌發病機制。詳細認識分子發病機制對肝癌的新治療方案的發展至為重要。本文總結了肝癌病發所牽涉的分子機制，以及它們在改善肝癌臨床管理中的角色。

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INTRODUCTION

Liver cancer, which consists predominantly of hepatocellular carcinoma (HCC), is the fifth most common cancer in men and the seventh in women, responsible for approximately 700,000 deaths annually. The incidence of HCC varies widely depending on geographical location. Countries in East and South East Asia have the highest prevalence of HCC, with China alone accounting for more than 50% of the global HCC morbidity and mortality burden. Despite current progress with the treatment of cancers, existing therapies are limited in their abilities to cure HCC and fatality remains high, making it the third most common cause of death from cancer worldwide.[1]

The development of HCC is believed to follow a multistep hepatocarcinogenesis process. Most cases of HCC develop from a background of liver cirrhosis or chronic hepatitis due to hepatitis B virus and / or hepatitis C virus infection or alcoholism. Hepatocyte proliferation can be accelerated with chronic liver inflammation or cirrhosis, resulting in the production of aberrant hepatocytes and the formation of dysplastic nodules.[2]

Dysplastic nodules are precancerous lesions commonly detected in the cirrhotic liver and are considered the intermediate steps of hepatocarcinogenesis. Histologically, dysplastic nodules can be further classified as low grade or high grade according to the degree of atypia. Low-grade dysplastic nodules may show mild cytologic atypia compared with surrounding hepatocytes and a slightly raised nucleus-to-cytoplasm ratio. In contrast, high-grade dysplastic nodules have a high cell density and nucleus-to-cytoplasm ratio, with cytological or architectural atypia that approach, but do not quite reach, those of HCC.[3,4] With further accumulation of mutational events and aberrant growth, dysplastic nodules can further transform into primary HCC and finally metastatic HCC.[2] Progression of hepatocarcinogenesis is associated with multiple molecular mechanisms that involve genetic, epigenetic, and cell signalling alterations. Recent research also indicates the involvement of aberrant microRNA (miRNA) expression and the concept of liver-specific cancer stem cells (CSCs) in HCC development. Understanding the mechanisms underlying HCC pathogenesis is of fundamental importance to optimising the clinical management of HCC and the development of new therapeutic approaches to this disease.

CHROMOSOMAL AND GENETIC ALTERATIONS

Using genome-wide allelotyping to evaluate chromosomal alterations at different stages of hepatocarcinogenesis, we have previously reported a stepwise increase in allelic losses from cirrhosis, through dysplastic nodules (low grade to high grade), to primary and metastatic HCC.[5] Loss of heterozygosity (LOH) was uncommon in cirrhotic livers (n = 24; mean fractional allelic loss [FAL] index, 0.09). In contrast, LOH was common in HCC nodules (n = 74; mean FAL index, 0.4). The stepwise increase in frequency of allelic losses provides further evidence for the hypothesis of multistep hepatocarcinogenesis. Furthermore, high-grade dysplastic nodules were found to have FAL indices significantly higher than those of low-grade dysplastic nodules ($p = 0.031$) and close to that of HCC, indicating that high-grade dysplastic nodules were genetically closer to HCC.[5] The close association of high-grade dysplastic nodules with HCC suggests a more aggressive treatment approach may be beneficial for high-grade dysplastic nodules to prevent further progression to HCC.

By quantitative comparisons of HCC tumours to non-tumours using comparative genomic hybridisation (CGH) arrays and LOH, HCC has been shown to harbour a high degree of chromosomal instability. Recurrent allelic losses are common in chromosomes 1p, 4q, 8p, 13q, 16q, and 17p, and allelic gains are most often observed in 1q, 8q, 16p, and 20q.[6-9] The regions of recurrent chromosomal deletions may harbour the loss or mutation of putative tumour suppressor genes,[10-12] while regions of recurrent chromosomal gains may be associated with gain of function mutations and oncogenes.[13] Thus, chromosomal gains and losses may result in deregulation of signalling pathways in HCC leading to tumourigenesis and metastasis. Correlating chromosomal aberration data from CGH arrays with gene expression data may be a feasible method to identify novel oncogenes and tumour suppressor genes.[13]

EPIGENETIC ALTERATIONS

In addition to genetic and chromosomal mechanisms of mutations, epigenetic alterations have been implicated to play an important role in human carcinogenesis. Epigenetic alterations refer to the reversible and heritable changes in gene expression that occur without alteration to the DNA sequence. DNA methylation is an example of such changes and a key epigenetic event in cancer. DNA methylation in the mammalian

genome is found at the cytosine residues of CpG dinucleotides, often associated with promoter-related CpG islands. Although DNA methylation is essential for normal development and differentiation, aberrant hypomethylation in many human cancers can lead to the expression of oncogenes,[14] or similarly, hypermethylation can lead to the silencing of tumour-suppressor genes.[15]

In HCC, aberrant DNA hypermethylation has been reported in promoter regions of tumour suppressor genes, such as p16INK4A, E-cadherin, RAS-association domain family (RASSF1A), suppressor of cytokine signalling (SOSC-1) and phosphatase and tensin homolog (PTEN). The frequency of aberrant DNA methylation increases from precancerous lesions to dysplastic nodules and finally HCC, signifying their importance in tumour progression.[16] Demethylation agents such as DNA methyltransferase inhibitors are being developed and utilised as anticancer drugs as they allow re-expression of the aberrantly methylated genes to restore normal tumour-suppressive functions. Azacytidine and decitabine are two DNA methyltransferase inhibitors currently approved by the US Food and Drug Administration (FDA) for chemotherapy against myelodysplastic syndrome.[17]

DEREGULATED CELL SIGNALLING PATHWAYS IN HEPATOCELLULAR CARCINOMA

Genetic and epigenetic changes can lead to altered gene expression patterns, resulting in the activation of oncogenes and / or inactivation of tumour suppressor genes and disruption of normal cell signalling pathways. Deregulation of various signalling pathways have been implicated in pathogenesis of HCC, including proliferation and survival pathways (e.g. epidermal growth factor, insulin-like growth factor [IGF], and hepatocyte growth factor), differentiation pathways (e.g. Wnt and Hedgehog pathways), inflammation pathways (e.g. interleukin-6 [IL-6] and interferon [IFN]), and growth factor-regulated angiogenic signalling (e.g. vascular endothelial growth factor and platelet-derived growth factor).[18,19]

Analyses of many previous genome-wide gene expression-profiling studies of HCC have revealed altered gene expression patterns (signatures) that correlate with clinical outcomes. Distinct gene signatures and molecular aberrations can be associated with aggressive and less aggressive forms of HCC.[20] These distinct molecular characteristics, combined with traditional clinical variables associated with prognosis — such as tumour size, vascular invasion, and distant metastasis — can enable better classification of patients into prognostic subgroups for targeting adequate treatment. A recent meta-analysis showed that aggressive HCC subclass was associated with larger tumour size, poor histological differentiation, and specific molecular alterations such as the activation of transforming growth factor–alpha and –beta, MYC and AKT, overexpression of AFP and IGF2, and downregulation of IFN-related genes. In contrast, less aggressive tumours were well differentiated, and associated with CTNNB1 mutation and Wnt activation.[21,22] Specific oncogenes required for tumour progression have yet to be identified in HCC.

The improved understanding of the molecular basis of hepatocarcinogenesis has opened up opportunities for targeted therapies in HCC. Many agents targeting signalling pathways implicated in the pathogenesis of HCC and other cancers are now in preclinical or clinical trials for HCC therapy (Table).[22-24] Sorafenib, a tyrosine kinase inhibitor, is currently the only targeted agent with demonstrable clinical efficacy to be approved by the FDA for HCC treatment.[25] Other promising agents in phase II / III clinical trials will likely expand the therapeutic armamentarium for HCC in the future. With further research and identification of new targets / targeted agents, management strategies for HCC will be better defined and personalised to maximise efficacy and cost benefit.

miRNA DYSREGULATION

In recent years, the aberrant expression of miRNAs has been implicated in a wide variety of cancers. miRNAs are a class of small (20-23 nucleotides) endogenous non-coding RNAs that negatively regulate gene expression by targeting mRNA for translational repression or cleavage. Through downregulation of target gene expression, miRNA is involved in the regulation of a variety of cellular processes, including cell proliferation, differentiation, apoptosis, and stem cell maintenance.[26] Depending on the genes they target, miRNAs can function as oncogenes or tumour suppressor genes. Aberrant miRNA expression is suggested to be involved in hepatocarcinogenesis, as the expression of many miRNAs is dysregulated in HCC, resulting in aberrant gene expression.[27]

By comparing miRNA expression profiles of

Table. SMolecular targeted agents for hepatocellular carcinoma in phase II/III clinical trials.[24]

Agent	Target
Sorafenib®	BRAF, VEGFR, PDGFR, c-KIT, Flt3
Phase III	
Brivanib	VEGFR, FGFR
Erlotinib	EGFR
Linifanib	VEGFR, PDGFR
Orantinib	VEGFR, FGFR, PDGFR
PI-88	FGF, VEGT
Sunitinib	VEGFR, PDGFR, c-KIT
Ramucirumab	VEGFR-2
Phase II	
AMG-386	Angiopoietin 1/2
AVE-1642	IGFR1
ARQ-197	cMet
AZD-6244	MEK1/2
Bevacizumab	VEGF
BIBF-1120	VEGFF, PDGFR, FGFR
BIIB-022	IGFR1
Bortezomib	26S proteasome
Cetuximab	EGFR
Cediranib	VEGF
Cixutumumab	IGFR1
Dasatinib	BCR/ABL
Dovitinib	VEGFR, PDGFR, FGFR, Flt3, c-KIT, CSF-1R
E-7080	VEGF, FGF, SCF
Erlotinib	EGFR
Everolimus (RAD-001)	mTOR
Gefitinib	EGFR
Lapatinib	EGFR, HER2/neu
Linifanib	VEGFR-2, PDGFR-b, CSF-1R
LY-2181308	Survivin
Mapatumumab	TRAIL
MK-2206	Akt
OSI-906	IGFR1, IR
Regorafenib	BRAF, VEGFR, PDGFR-b, c-KIT, Flt3, Tie2
Temsirolimus	mTOR
Vandetanib	VEGFR, RET, EGFR
XL-184	c-MET, RET, VEGFR2

primary human HCC tumours with those of non-tumours, we previously identified miRNAs that were underexpressed (n = 44) and overexpressed (n = 122) in HCC compared with non-tumours. Among these, miR-139 was frequently downregulated in primary HCC tumours and further downregulated in metastatic HCC tumours. Downregulation of miR-139 in HCC was significantly associated with poor prognosis and features of metastatic tumours, including venous invasion, microsatellite formation, absence of tumour encapsulation, and reduced differentiation. Re-expression of miR-139 in HCC cells significantly reduced cell migration and invasion in vitro, and HCC metastasis in vivo. Our data also suggested that miR-139 interacted with the untranslated region of Rho-kinase 2 (ROCK2) and reduced its expression to regulate HCC migration and metastasis.[28] Further insight into the mechanisms of miRNA dysregulation in HCC may enhance understanding of the molecular pathogenesis and the development of new therapeutic approaches in treating advanced HCC.

LIVER CANCER STEM CELLS

Recent research suggests that CSCs may be involved in the development of HCC. Traditionally, all cells within a tumour were believed to be biologically homogenous and, therefore, had equal capacity to regenerate the tumour.[29] However, accumulating evidence indicates that only a small subset of tumour cells, designated CSCs, within a tumour exhibits the capacity to initiate and sustain tumour growth. Cancer progression is believed to be driven by CSCs through their capacity for self-renewal, differentiation, and production of heterogeneous progeny.[30] CSCs were first isolated in acute myelogenous leukaemia and, subsequently, also in solid tumours such as those of the breast, brain, colon, pancreas, lung, and liver. Liver-specific CSCs have been isolated in HCC by several cell surface antigens including CD133, CD90, CD44, OV6, CD24, and the epithelial cell adhesion molecule (EpCAM), or by selecting for the side population cells in Hoechst dye-staining.[31] The capacity of CSCs for self-renewal and tumourigenesis is thought to involve various cancer-related signalling pathways that may serve as molecular targets for novel cancer treatment strategies. These pathways and potential targets include self-renewal (e.g. Wnt/β-catenin, Bmi-1, Notch and Sonic Hedgehog), cell growth (e.g. PTEN and IL-6), survival (e.g. ABC multidrug efflux transporters) and differentiation (e.g. hepatocyte nuclear factor–4α).[31,32]

Successful eradication of cancer requires therapies that kill differentiated cancer cells and the potential CSC population. Current conventional therapies, including chemotherapy, radiation, and immunotherapy, kill fast-growing, differentiated tumour cells but may leave behind CSCs, which are more resistant to treatment. Therapies that fail to eradicate CSCs might ultimately result in relapse and the proliferation of resistant and more aggressive tumour cells. Anticancer agents targeting liver CSCs through their surface antigens or related signalling pathways could potentially provide more selective treatment for HCC, enabling disruption of the self-renewal potential of CSCs while reducing toxic side-effects for other cell types. Potential

strategies useful against liver CSCs include antibodies targeting CSC markers (e.g. anti-CD133, anti-CD44, and anti-EpCAM antibodies) and agents that block CSC pathways involved in self-renewal, growth, survival, and differentiation (e.g. anti-Wnt antibody, DAPT or GSI-18 inhibitors against the Notch pathway, and LY294002 inhibitor targeting PTEN).[30] Additionally, we have reported that lupeol, a natural triterpene found in fruits and vegetables, targets liver CSCs through specific cell surface markers and sensitizes HCC tumours to chemotherapeutic agents through the PTEN-Akt-ABCG2 pathway.[33] Lupeol exerts a significant synergistic and cytotoxic effect without adverse effects when combined with low-dose chemotherapeutic drugs. As such, lupeol is a CSC-targeting dietary phytochemical that may potentially complement other therapies for HCC.[33]

CONCLUSION

Hepatocarcinogenesis involves the accumulation of chromosomal, genetic, and epigenetic alterations. Some of these alterations occur at different stages of hepatocarcinogenesis and some show a stepwise increase as the disease progresses. These alterations can perturb important cell signalling pathways, and result in cancer progression and metastasis. The role of miRNA has been recently implicated in cancers and evidence supports that miRNA expression is dysregulated in HCC. Modulation of miRNA expression may be an attractive treatment approach for HCC. Additionally, understanding the role of liver CSCs and the associated regulatory pathways holds promise for developing new therapeutic strategies that may spare the normal cell population. Further insight into the complex molecular pathways underlying pathogenesis is integral to developing new treatment approaches that will translate into improved clinical management and outcomes in HCC.

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