REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Hepatocellular carcinoma pathogenesis: Epigenetics and relationship with cancer hallmarks

Tohada M. AL-Noshokaty[a]*, Noha M. Mesbah[b], Dina M. Abo-Elmatty[b], Ahmed I. Abulsoud[a], Asmaa R. Abdel-Hamed[b]

[a] Department of Biochemistry, Faculty of Pharmacy, Heliopolis University, Cairo, Egypt; [b] Department of Biochemistry, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt

Received: 15. 10. 2022

Revised: 20. 10. 2022

Accepted: 21. 10. 2022

*Correspondence Author:

Tel: +201016342428

E-mail address:

Tohada.mohamed@hu.edu.eg

Abstract

On the global scale, primary liver cancer (PLC) is the second leading cause of death due to cancer in men, and the third most prevalent cause of death in both sexes. Hepatocellular carcinoma (HCC) accounts for almost 75% of the total cases among primary liver malignancies. Curative operations for HCC include liver resection, ablation, and transplantation. However, a timely diagnosis is required for these operations to be successful. Hepatocarcinogenesis is a multistep process and can be induced by molecular alterations at both the genetic and epigenetic levels. The characteristic pathogenesis mechanisms of HCC are abnormal signal transduction resulting in uncontrolled cell proliferation, loss of apoptosis or programmed cell death, tissue invasion and metastasis allowing the spread of cancer, and finally angiogenesis which leads to the enhanced blood supply of tumors. In addition to epigenetic control of tumorigenesis. The p53 cell-cycle pathway, mutations in oxidative stress pathways, PI3K/AKT/mTOR, and Ras/Raf/MAPK signaling pathways are of great important pathways in HCC pathogenesis. Crosstalk between these pathways may be targeted by anti-cancer medications.

Keywords: Hepatocellular carcinoma; pathogenesis; apoptosis; angiogenesis; proliferation; metastasis.

1. Hepatocellular carcinoma

Primary liver cancer (PLC), a rising public health concern, is the third most lethal malignancy worldwide, according to the GLOBOCAN 2020 database (https://gco.iarc.fr). Liver cancer remains a global health challenge, with an estimated incidence of >1 million cases by 2025. Hepatocellular carcinoma (HCC) is the most common form of liver cancer and accounts for ~90% of cases **(Llovet et al., 2021)**.

Mortality due to HCC in last fifteen years has increased. Liver resection, ablation, and liver transplantation are potentially curative but require diagnosis at a sufficiently early stage. However, up to 80% of HCCs are diagnosed in an advanced stage when the ultimate treatment choice is interventional systemic therapies **(Bhoori and Mazzaferro, 2014; Singh et al., 2018)**.

1.1. Epidemiology

According to the latest Global Cancer Statistics (GLOBOCAN 2020), liver cancer is the 6th most common cancer, with more than 900,000 estimated annual new cases (4.7%). Its risk factors are well-known; however, it accounts for 8.3% of deaths of all cancers globally, being the third leading cause of worldwide cancer death **(Sung et al., 2021)**. It is

estimated that, by 2025, more than one million individuals will have liver cancer annually **(Llovet et al., 2021)**.

In Egypt, the relation between Hepatitis C virus (HCV) and HCC is an important research area as Egypt used to be the country with the heaviest hepatitis C virus (HCV) burden. Firstly, Egypt has a high recorded HCV transmission rate. According to the Egyptian ministry of health (MOH) screening program that took place over 7 months with a target population of 62.5 million, 49.6 million persons were screened for HCV antibodies, 2.4 million seropositive persons were identified, and 1.6 million viremic patients were identified **(Ezzat et al., 2021)**. Secondly, there is known to be a relationship between HCV and HCC development. Thirdly, the programmed screening and follow up that was initiated by the government detected cases of individuals having both diseases **(Ezzat et al., 2021)**. According to a study carried out by Ziada et. al,108 out of 514 patients (21%) diagnosed with HCV infection in the mid Delta area had focal lesions detected by ultrasound (US) **(Ziada et al., 2016)**.

The improvement of HCC prevention requires the governmental health administration to implement health policies. Although the diagnosis of Egyptian HCC patients follows the international guidelines, HCC treatment options are limited in terms of cost. In addition, there are limited Egyptian reports about HCC survival and relapse. Both basic and clinical HCC research in Egypt are still limited compared to the world **(Rashed et al., 2020)**.

1.2. Risk factors/ Etiology

The main risk factors for HCC are chronic infection with hepatitis B virus (HBV) or HCV, aflatoxin-contaminated foods, heavy alcohol consumption, obesity, type 2 diabetes, and smoking **(Sagnelli et al., 2020)**. Also, geographical factors have a direct impact on the various etiological features of HCC patients and make HCC an extremely complex condition associated with poor prognosis **(Alqahtani et al., 2019)**. Despite substantial advances in therapeutic strategies, the prognosis of late-stage HCC remains hopeless because of the high recurrence rate **(Morishita et al., 2021)**. There is heterogeneity in the distribution of these risk factors between low/middle-income countries and high-income countries. According to the International Agency for Research in Cancer 2020, the age-standardized (for all ages, standardized to the world population) incidence rate of liver cancer in low and medium human development index countries per 100,000 persons is 6.9, and 3.2 in male and female, respectively. Meanwhile, the age-standardized mortality rate is 6.7 and 3.1 in males and females, respectively **(Sung et al., 2021)**. Its incidence is higher with chronic liver diseases, especially viral hepatitis, alcoholic hepatitis or non-alcoholic fatty liver disease **(Ozakyol, 2017)**.

The World Health Organization (WHO) revealed in 2015 that 257 million people worldwide had chronic HBV infection, with the African and western Pacific regions bearing the brunt of the burden with a 15–40% lifetime risk of cirrhosis, liver failure, or HCC **(Moonsamy et al., 2022)**.

In 2018, 71.1 million people were estimated as worldwide chronic carriers of HCV; approximately 18 million of them were in Africa and are at risk of HCC development **(Kamali et al., 2022)**. HCV prevalence in North Africa is estimated at 2.3–7.7% **(Sonderup et al., 2020)**. Chronic HCV infection has also been identified as the primary cause of HCC in North Africa (particularly Egypt) **(Ezzat et al., 2021)**. Being the country with the highest worldwide prevalence of HCV, the Egyptian MOH launched a large national screening program that intended to screen the whole country **(Esmat et al., 2018)**. All screened subjects with proven HCV infection were referred for engagement in a government-funded treatment program employing direct-acting antiviral. However, there is still no national campaign for HCC surveillance **(Ezzat et al., 2021)**. Given the magnitude of Egypt's HCV and HCC problems, the extensive HCV treatment program could significantly impact the country's HCC figures shortly **(El Kassas et al., 2019; El-Kassas and Elbadry, 2022)**.

1.3. Progressive stages of liver disease

Chronic liver diseases that predispose to HCC are generally characterized by steatosis and hepatocellular damage or death, followed by inflammation and fibrosis. However, if the injury is persistent, liver disease may progress to end-stage complications such as cirrhosis and HCC **(Fausto and Campbell, 2010) (Figure 1)**.

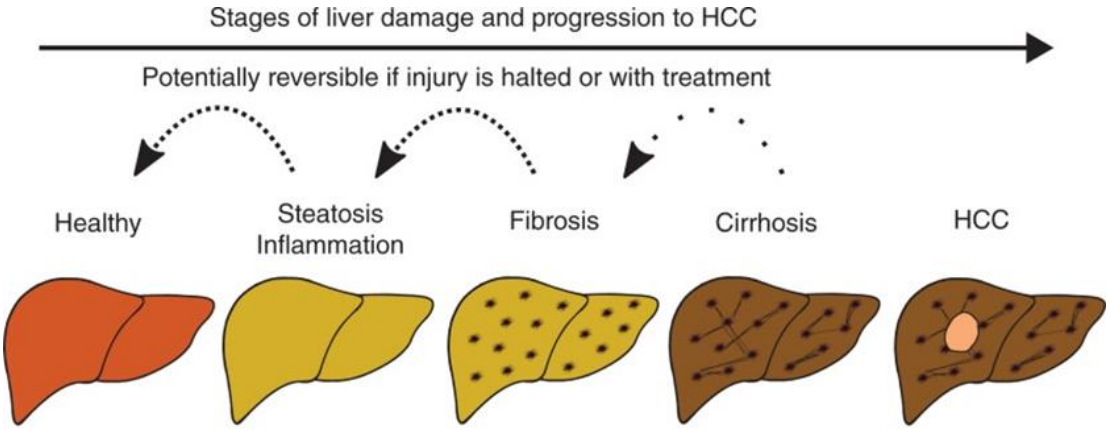

Figure 1: Progressive stages of liver disease to HCC development **(Fausto and Campbell, 2010).** (HCC: Hepatocellular carcinoma).

1.4. Screening and surveillance

Hepatocellular carcinoma continues to have a dismal prognosis, with 5-year survival below 20%. This poor prognosis can be in part attributed to failures along the cancer screening process continuum such as underuse of screening in at risk patients and appropriate treatments for patients with HCC. Better understanding these process failures, and how they compare to those seen in other cancer types, can help inform potential intervention targets and strategies to reduce HCC-related mortality **(Singal et al., 2022a)**.

Screening of HCC is typically performed using semi-annual abdominal US with or without alpha fetoprotein (AFP). Some providers use contrast-enhanced computed tomography (CT) or magnetic resonance irradiation (MRI) for HCC screening in practice, although concerns about cost, patient acceptability, access for large populations, and potential harms have precluded these tests from being incorporated into professional society guidelines **(Marrero et al., 2018; Tzartzeva et al., 2018)**. Follow-up evaluation is dictated by results of screening tests. If US and AFP are both normal, patients should repeat screening in six months. Patients with an abnormal screening result (solid lesion ≥1 cm on US, AFP ≥20 nanogram (ng) / milliliter (mL), or rising AFP) should undergo diagnostic evaluation with multi-phase CT or dynamic contrast-enhanced MRI **(Marrero et al., 2018)**. Patients with indeterminate results (solid lesion <1 cm on US) are recommended to undergo short interval screening in 3 months given low risk of HCC in lesions of this size and poor accuracy of cross-sectional imaging to characterize sub-centimeter lesions. Patients who undergo CT- or MRI-based HCC screening bypass this step as these modalities serve as both screening and diagnostic tests **(Singal et al., 2022a)**.

1.5. Diagnosis

During surveillance, finding a suspicious lesion using US in cirrhotic liver is followed by diagnostic confirmation using contrast enhanced helical CT or dynamic MRI. Also, non-pathological confirmation of HCC diagnosis is achieved by AFP testing combined with imaging techniques **(Dimitroulis et al., 2017; Singal et al., 2022b)**.

1.6. Treatment approach

Precise staging of HCC initially is very useful for determination of the therapeutic options and the overall prognosis of the disease. There are certain clinical features upon which most staging systems use for HCC assessment **(Rashed et al., 2020)**. These clinical features are size and local extent of the tumor, metastasis of the tumor, severity of the liver disease, and the overall patient performance status (PS) **(Tellapuri et al., 2018)**.

There are two common staging systems; Tumor-Node-Metastasis (TNM) system which is maintained by the American Joint Committee on Cancer and The Barcelona Clinic Liver Cancer (BCLC) system **(Henderson et al., 2003)**. The TNM system characterizes tumor features, lymph node involvement, and metastases. While the BCLC depends on the combination of tumor

features, severity of the liver disease, and patient PS. In comparison to other prognostic systems, BCLC system has the best correlation with the patient outcome **(Marrero et al., 2005)**. The BCLC system for HCC includes prognostic stage, treatment methods and survival period are represented in **Figure 2**.

Many systemic cytotoxic chemotherapy drugs are used in HCC treatment as single agents, e.g.: cisplatin, doxorubicin, 5-flurouracil, or combined regimen. All these chemotherapeutic agents are available in the Egyptian market. These systemic treatments have several disadvantages **(Chen et al., 2015b; Yim et al., 2015)**.

They have between 10 to 25% response rate with marginal survival improvement. Also, patients with underlying liver cirrhosis are poorly tolerating these treatments. And finally, HCC is highly resistant to single agent regimen. **Figure 4** represents HCC treatment related to stages **(Ahmad et al., 2019)**.

Currently, there is a paradigm shift in HCC treatment by the introduction of immune checkpoint inhibitors in addition to molecular targeted therapies **(Kudo, 2019)**. Many therapeutic agents for HCC target different pathways implicated in hepatocarcinogenesis. **Figure 4** demonstrates currently approved drugs for advanced HCC treatment and timeline of pivotal clinical trials.

Table 1 represents the list of Food and Drug Administration (FDA) - approved targeted and immune therapies for HCC that are available in the Egyptian market. There is an economical burden in the treatment of HCC in general and in using these expensive targeted therapies in particular. So, using targeted therapies in HCC treatment for Egyptian patients are limited to patients who can afford the cost **(Rashed et al., 2020)**.

1.7. Pathogenesis of cancer

In the year 2000 Douglas Hanahan and Robert Weinberg had a very well-known article published that tried to answer the question of "What properties does a cancerous cell have ?" **(Figure 5) (Hanahan and Weinberg, 2000)**. Reducing the vast complexity of cancer into six key properties or hallmarks as they called them. self-sufficiency in growth signals, insensitivity to antigrowth signals, avoiding apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Each of these physiologic changes' novel capabilities acquired during tumor development represents the successful breaching of an anticancer defense mechanism hardwired into cells and tissues. A more recent version of the hallmarks of cancer was published in 2011 with addition of reprogramming of energy metabolism, evading immune destruction, genomic instability and mutation and finally tumor promoting inflammation **(Hanahan and Weinberg, 2011)**. It has been proposed that changes in cellular redox status contribute in a major way to each of the hallmarks of cancer **(Hornsveld and Dansen, 2016)**.

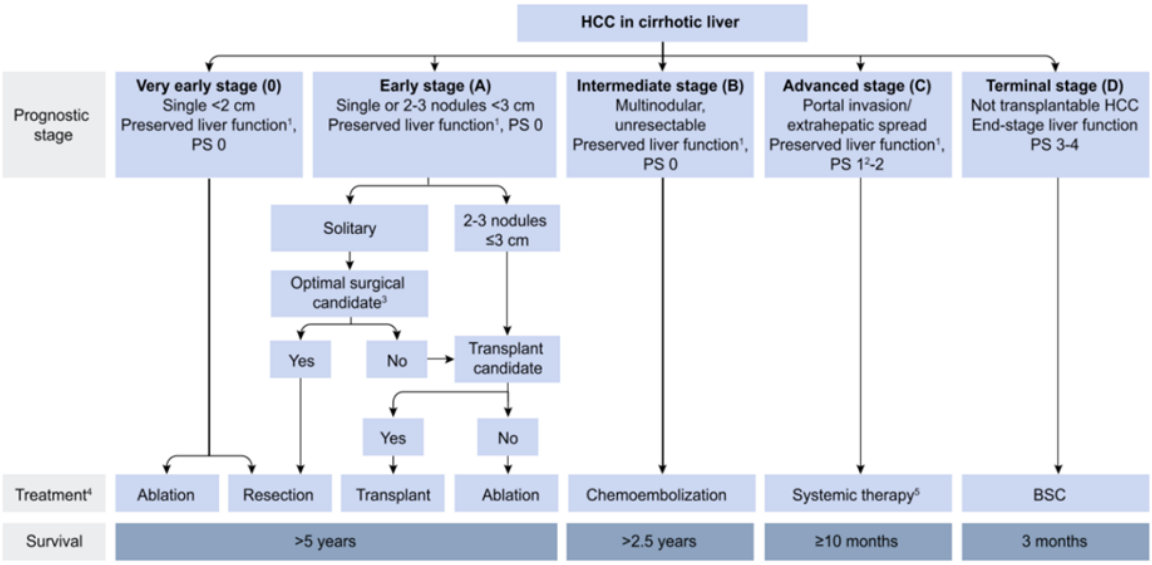

Figure 2: The BCLC staging system for HCC. Prognostic stage, treatment methods and survival period **(European Association for The Study of The Liver, 2018)**. (HCC: Hepatocellular carcinoma, PS: Performance status, BSC: Best supportive care).

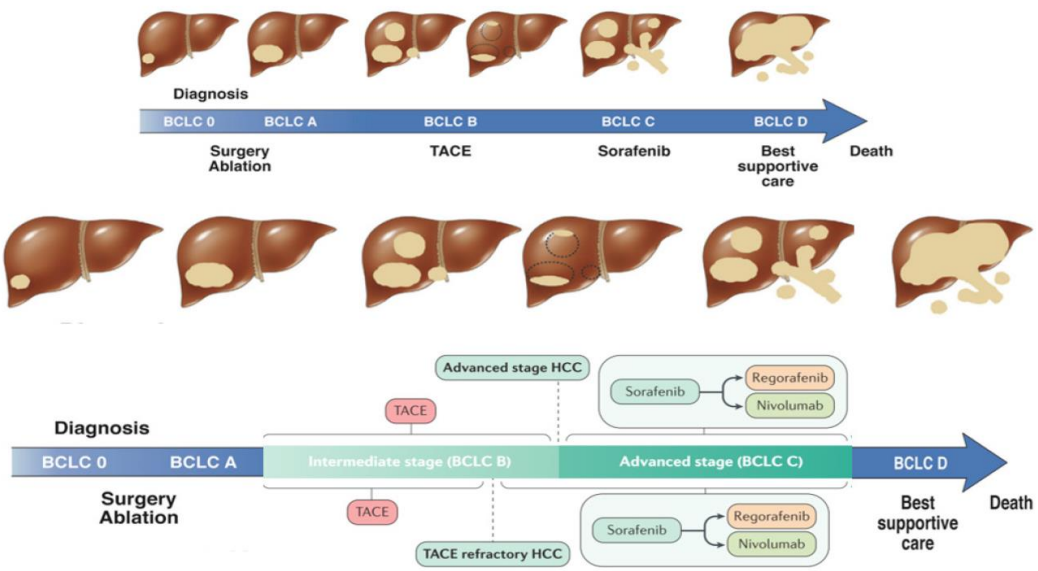

Figure 3: HCC treatment related to stages **(Ahmad et al., 2019)**. (BCLC: The Barcelona Clinic Liver Cancer system; TACE: Transcatheter arterial chemoembolization).

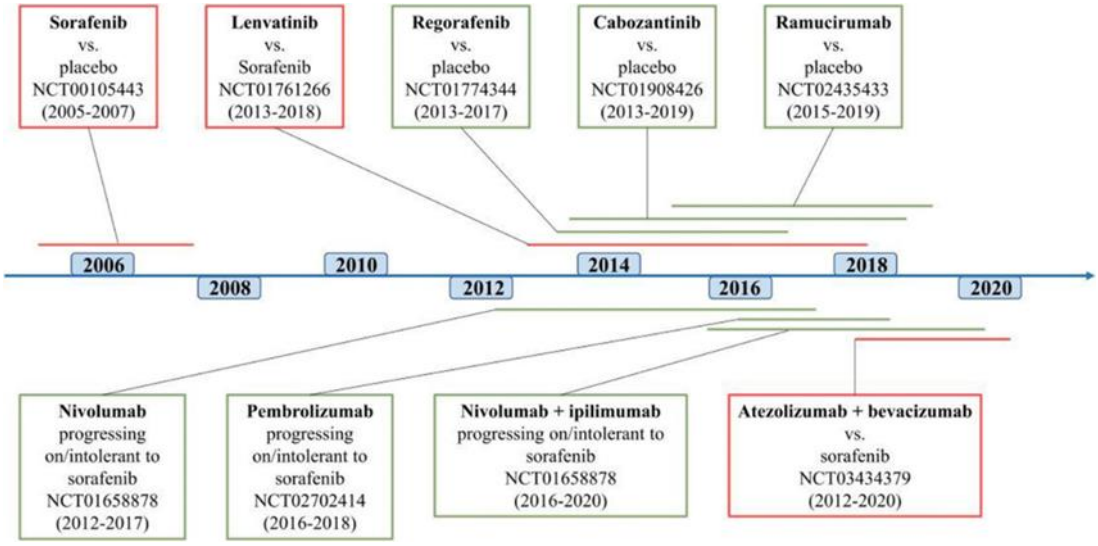

Figure 4: Currently approved drugs for advanced HCC and timeline of pivotal clinical trials **(Zhang et al., 2022).** The lines along the timeline indicate the time from the actual study start to FDA approval. The red boxes represent first-line therapies, and the green boxes represent second-line therapies.

Table 1 : FDA-approved targeted and immune therapies for HCC (Rashed *et al.*, 2020).

Generic drug	Brand name	Target
Sorafenib	Nexavar®	Multikinase inhibitor
Cabozantinib	Cabometyx® and Cometriq®	Multikinase inhibitor
Lenvatinib	Lenvima®	Multikinase inhibitor
Regorafenib	Stivarga®, and Regonix®	Multikinase inhibitor
Nivolumab	Opdivo®	Immune check point inhibitors
Pembrolizumab	Keytruda®	Immune check point inhibitors
Ramucirumab	Cyramza®	Human monoclonal antibody against VEGFR 2

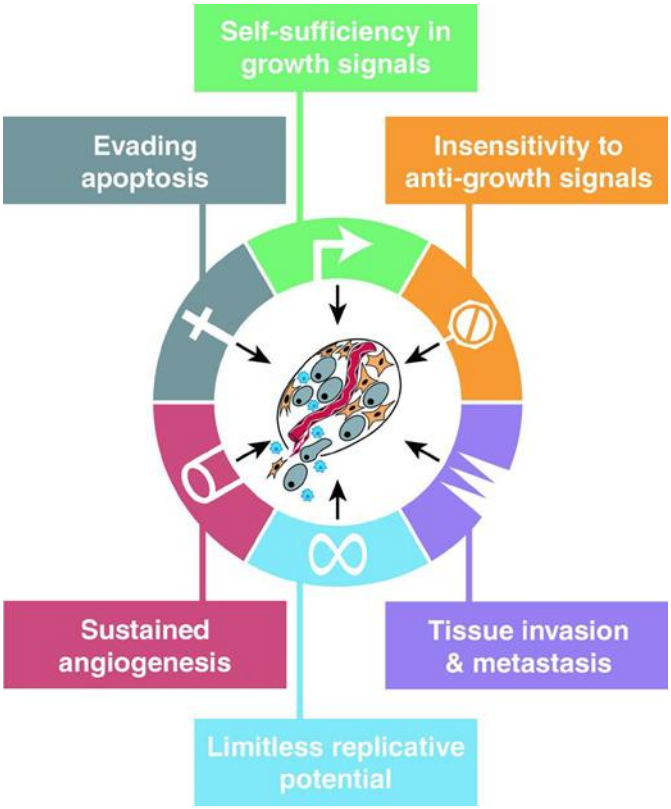

Figure 5: Properties of cancerous cells **(Hanahan and Weinberg, 2000).**

Despite the huge efforts employed to implement novel chemotherapeutic strategies for the treatment of different types of cancer, still the disease remains one of the major concerns worldwide. Consequently, there is an urgent need to explore newer classes of therapeutics with selective action against cancer cells **(Malik et al., 2021)**. The regulation of the cell proliferations and apoptotic pathways associated with cell death is known as an important approach to understand about these cells. Therefore, the identification of cell-cycle regulators and apoptotic stimuli to combat cancer cells represents an attractive strategy for the discovery and development of potential antitumor agents **(Asati et al., 2016)**.

1.8. Hepatocellular carcinoma pathogenesis

Hepatocellular carcinogenesis has been attributed to many biological aberrations, e.g., mutations, epigenetic dysregulations, and chromosomal anomalies **(Zucman-Rossi et al., 2015)**. Six predominant molecular pathways have been identified in HCC by whole-exome sequencing which include telomerase reverse transcriptase promoter mutation, Wnt/β-catenin, the p53 cell-cycle pathway, epigenetic modifiers in histone methylation and chromatin remodeling, mutations in oxidative stress pathways, PI3K/AKT/mTOR, and Ras/Raf/MAPK signaling pathways **(Zucman-Rossi et al., 2015)**. It is a multistep process involving the progressive accumulation of molecular alterations pinpointing different molecular and cellular events **(Ho et al., 2016)**. Dysregulation of the balance between proliferation and cell death represents a pro-tumorigenic principle in human hepatocarcinogenesis **(Fabregat, 2009)**.

2. Abnormal cellular transduction in hepatocellular carcinoma progression

2.1. Loss of apoptosis

Cell death is a normal process in multicellular organisms, playing an important role in homeostasis. There are two types of cell death: necrosis (or accidental cell death), and apoptosis. Apoptosis is a physiological process of programmed cell death that works to control cell clusters and is characterized by specific morphological changes **(Galluzzi et al., 2018)**. It is responsible for the removal of damaged or unnecessary cells throughout the lifecycle, including normal cell turnover, cell loss during embryogenesis, removing cells between fingers,

negative selection by the immune system and nervous system development **(Marquardt and Edlich, 2019)**.

The different stages of apoptotic cell death **(Figure 6)** start by cellular shrinkage and chromatin condensation, concomitant with formation of membrane blebs. Organelles and nucleus fragment and the blebs begin formation of apoptotic bodies which are eventually engulfed by macrophages or neighboring cells by endocytosis / phagocytosis. The lack of release of cellular components to the extracellular fluid results in the absence of inflammation **(Abou-Ghali and Stiban, 2015)**.

The prevention of cancer is one of the main functions of apoptosis. The loss of apoptotic control allows cancer cells to survive longer and gives more time for the accumulation of mutations which can increase invasiveness during tumor progression, stimulate angiogenesis, lead to cell over-proliferation and interfere with differentiation which can give rise to tumor development or tumorigenesis **(Pfeffer and Singh, 2018)**.

The apoptotic signaling pathway is regulated by a diversity of factors and is established on the equilibrium between cell death and survival factors **(Marquardt and Edlich, 2019)**. Most HCC cells display strong resistance to stimuli that prompt apoptosis in other cells. Consequently, disabling apoptotic resistance has become important for the development of current therapeutic lines for HCC treatment **(Lohitesh et al., 2018; Rahmani et al., 2020)**. **Figure 7** represents an overview of the apoptosis pathways **(Grilo and Mantalaris, 2019)**.

Five main pathways have been identified. Extrinsic pathway activated by external stimuli, internal pathway activated by mitochondrion outer membrane permeabilization (MOMP) promoted by Bax channels, deoxyribonucleic acid (DNA)-damage-induced CASP2-dependent pathway, granzyme B induced pathway where this molecule acts as a CASP3 inducer and granzyme A induced pathway where no CASPs are involved in addition to granzyme A induces a DNase, which induces DNA fragmentation and cell death **(Grilo and Mantalaris, 2019)**.

In apoptosis, internal and/or external stimuli initiate a series of highly controlled reactions, which ultimately lead to cell death. CASPs and the Bcl2 family of proteins are the most important groups of proteins involved in apoptosis which participate in all pathways of apoptotic cell death **(McIlwain et al., 2015)**.

Caspases are cysteine-dependent endoproteases that catalyze the breaking of the peptide bond. Most CASPs are produced as monomeric species with little or no activity, generally known as pro-CASPs or zymogens (McIlwain et al., 2015). Upon activation, they homodimerize forming biologically active proteins. Most CASPs can be classified, according to their role, in three main groups. Initiator CASPs (8 and 9), executioner CASPs (3, 6 and 7) in addition to CASPs involved in inflammation (1, 4, 5, 11 and 12) **(McIlwain et al., 2015)**.

The function of CASP2 in apoptosis has remained enigmatic. A number of recent studies suggest that CASP2 plays an important role in the regulation of p53 in response to cellular stress and DNA damage to prevent the proliferation and accumulation of damaged or aberrant cells **(Lim et al., 2021)**.

CASP3 is instrumental in apoptosis and can be activated by initiator CASP8 and 9 or by Granzyme-B. Once CASP3 is activated, it will back-activate upstream CASPs to ensure the continuity of the process **(Grilo and Mantalaris, 2019)**. CASP3 can activate CASP-activated DNase (CAD) which, in proliferative cells, is complexed with its inhibitor. When CASP3 is overexpressed, this complex is cleaved, and CAD degrades chromosomal DNA. During late apoptosis, CASP3 acts as a cytoskeletal reorganization and disintegration inducer leading to the formation of apoptosis bodies **(Elmore, 2007; Grilo and Mantalaris, 2019)**.

Proteins of the Bcl2 family are heavily involved in the regulation of apoptosis both with pro- and anti-apoptotic activities and can be divided into three sub-groups. The anti-apoptotic group comprises of Bcl2 and other proteins. These proteins have four Bcl2 homology domains (BH1, BH2, BH3 and BH4) and are typically associated with either cellular, nuclear or mitochondrial membranes **(Grilo and Mantalaris, 2019)**. While the pro-apoptotic group is composed of Bax and other proteins which lack the BH4 domain in addition to the last subgroup which has only the BH3 domain **(Taylor et al., 2008; Grilo and Mantalaris, 2019)**.

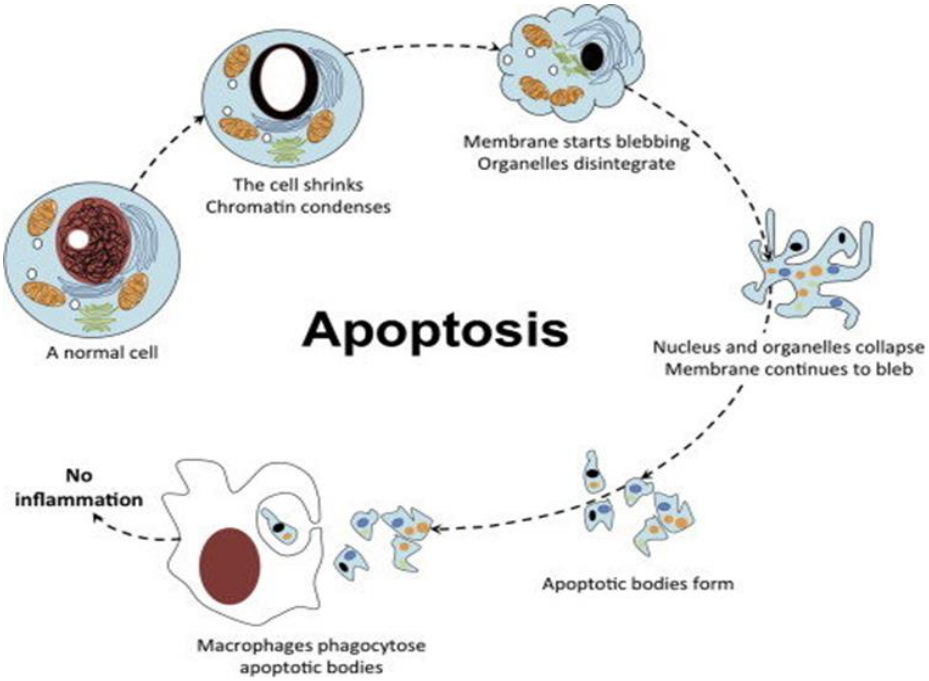

Figure 6: Cytology of apoptosis **(Abou-Ghali and Stiban, 2015).**

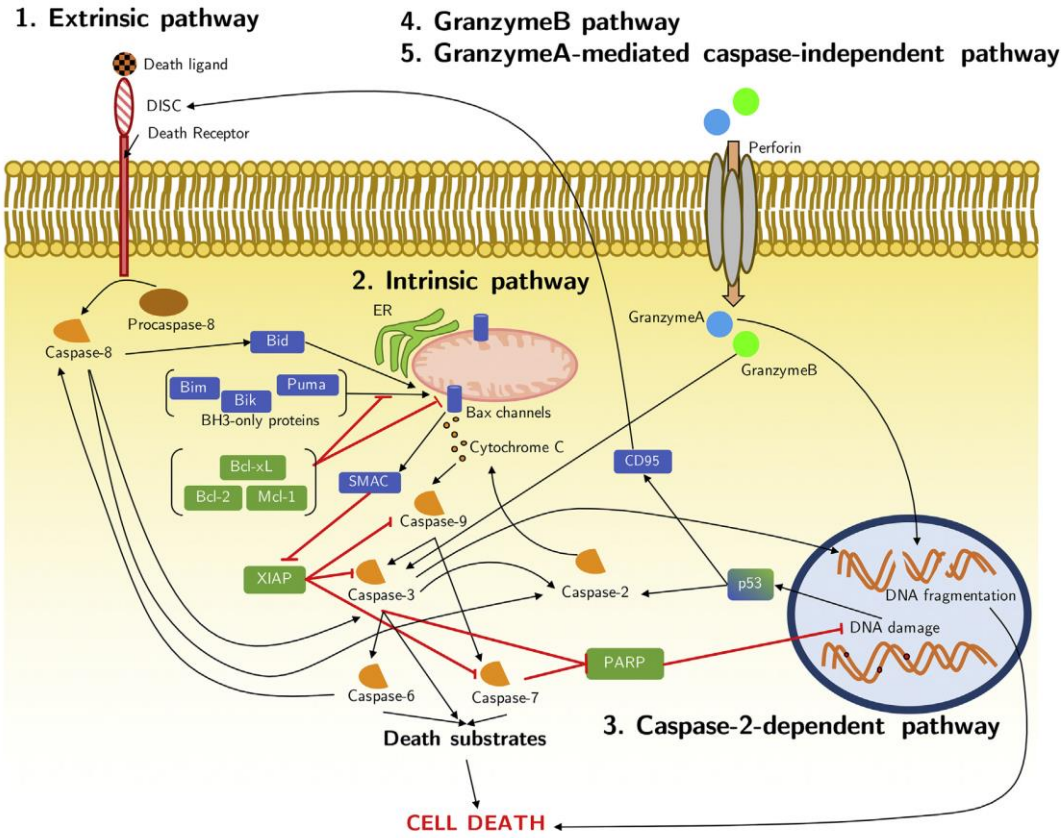

Figure 7: Overview of the apoptosis pathways **(Grilo and Mantalaris, 2019)**. (DISC: Death-inducing signaling complex, ER: Endoplasmic reticulum, Bid: BH3 interacting-domain death agonist, Bik: Bcl2-interacting killer, Bim: Bcl2-like protein 11, Puma: p53 upregulated modulator of apoptosis, Bax: Bcl2 associated X protein, Bcl-xl: B-cell lymphoma extra-large, Bcl2: B- Cell Leukemia/Lymphoma 2, Mcl-1: induced myeloid leukemia cell differentiation protein, SMAC: second mitochondria-derived activator of caspases, XIAP: X-linked inhibitor of apoptosis protein, p53: Tumor suppressor gene).

The extrinsic pathway (often referred as Type I) is triggered by extracellular stimuli and activate CASP8, which initiates apoptosis. The intrinsic pathway (often referred to as Type II or mitochondrial pathway) is activated by internal stimuli (metabolic or hypoxic stresses) and can also be triggered by endoplasmic reticulum (ER) stress **(Shalini et al., 2015)**. Many of the genetic alterations observed in HCC lead to an imbalance in the pro- and anti-apoptotic members of the Bcl2 family **(Knight et al., 2019)**. In contrast, pro-apoptotic members of the family, such as Bax are downregulated in HCC with dysfunction in the p53 pathway **(Booth et al., 2020)**. Accumulating evidence indicates that the RAF/ Mitogen-activated protein kinase kinase (MEK)/ Extracellular signal-regulated kinase (ERK) cascade also has diverse effects on key molecules involved in apoptosis signaling, such as the anti-apoptotic regulatory molecule Bcl2 and apoptotic regulatory molecules including CASP3 **(McCubrey et al., 2007)**.

The p53 tumor suppressor gene is a transcription factor that regulates the cell cycle and is the most widely mutated gene in human tumorigenesis **(Kontomanolis et al., 2020; Boutelle and Attardi, 2021)**. The critical role of p53 is evident by the fact that it is mutated in over 50% of all human cancers. p53 can activate DNA repair proteins when DNA has sustained damage, can hold the cell cycle at the G1/S regulation point on DNA damage recognition, and can initiate apoptosis if the DNA damage proves to be irreparable **(Gohil and Noolvi, 2019; Mijit et al., 2020; Franjić, 2022)**. Tumorigenesis occurs when the p53 gene is damaged and therefore tumor suppression is reduced. The p53 gene can be damaged by radiation, various chemicals, and viruses **(Gu et al., 2001; Katerji and Duerksen-Hughes, 2021; Merlin et al., 2021)**.

Apoptosis plays a key role in HCC carcinogenesis at the molecular level. Although the expression of some pro-apoptotic genes is decreased, the balance between death and survival is dysregulated in HCC mainly due to overactivation of anti-apoptotic pathways **(Moreno-Càceres and Fabregat, 2015)**. Also, some growth factors that mediate cell survival are up regulated in HCC, as well as the molecules involved in the machinery responsible for cleavage of their pro-forms to an active peptide **(Scaggiante et al., 2014)**. The expression and / or activation of Janus Kinase (JAK) / signal transducer and activator of transcription (STAT), PI3K/AKT and RAS/ERK pathways are enhanced in many HCC cells, conferring on them resistance to apoptotic stimuli **(Hung et al., 2014; Hu et al., 2021)**. Finally, recent evidence indicates that inflammatory processes, as well as the epithelial-mesenchymal transitions (EMT) that occur in HCC cells to facilitate their dissemination, are related to cell survival. Therefore, therapeutic strategies to selectively inhibit anti-apoptotic signals in liver tumor cells have the potential to provide powerful tools to treat HCC **(Fabregat, 2009; Giannelli et al., 2016; De Las Rivas et al., 2021)**.

2.2. Uncontrolled cell proliferation

Cell proliferation plays a key role in HCC carcinogenesis. The protein kinases regulate cellular functions such as transcription, translation, proliferation, growth and survival by the process of phosphorylation **(Tomazic et al., 2021)**. Over activation of signaling pathways play a major role in oncogenesis. The PI3K signaling pathway is dysregulated almost in all cancers due to the amplification, genetic mutation of PI3K gene and the components of the PI3K pathway themselves. Stimulation of the PI3K/AKT/mTOR and Ras/Raf/MEK/ERK pathways enhances growth, survival, and metabolism of cancer cells (**Asati et al., 2016; Yang et al., 2019)**.

Recently, the PI3K/AKT/mTOR and Ras/Raf/MEK/ERK signaling pathways have been identified as promising therapeutic targets for cancer therapy. The kinase inhibitors with enhanced specificity and improved pharmacokinetics have been considered for design and development of anticancer agents **(Asati et al., 2016; Yang et al., 2019)**. **Figure 8** demonstrates PI3K/AKT/mTOR and Ras/Raf/MEK/ERK pathways.

Alterations of signal transduction pathways leading to uncontrolled cellular proliferation, survival, invasion, and metastases are the basis of the carcinogenic process. PI3K/AKT/mTOR and the Ras/Raf/MEK/ERK signaling pathways are critical for normal human physiology and alteration in regulation leading to several human cancers **(He et al., 2021)**.

Recent studies have suggested that the PI3K/AKT/mTOR and Raf/MEK/ERK cascades are interconnected with multiple points of convergence, crosstalk, and feedback loops **(Saini et al., 2013)**. Inhibition of any one of the above pathways can still result in the maintenance of signaling via the other (reciprocal) pathway. The

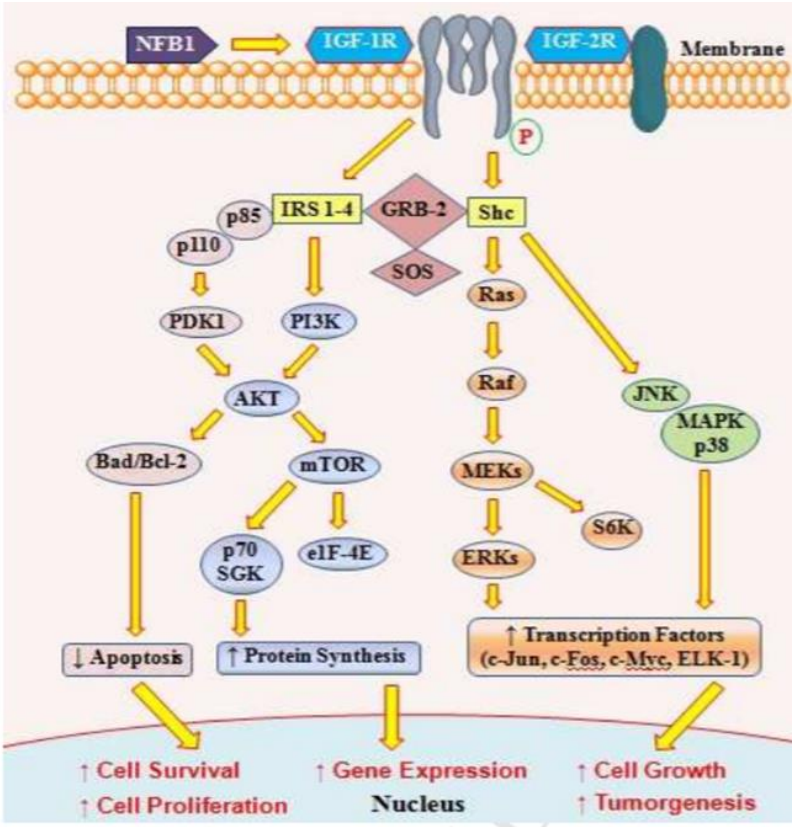

Figure 8: PI3K/AKT/mTOR and Ras/Raf/MEK/ERK pathways **(Asati et al., 2016)**. (NFB1:Nuclear factor kappa B1, IGF-1R: Insulin-like growth factor receptor 1, IGF-2R: Insulin-like growth factor receptor II, IRS: Insulin Receptor Substrate, p85: regulatory subunit of PI3K, p110: catalytic subunit of PI3K, IRS 1-4: Insulin Receptor Substrate 1-4, PI3K: Phosphatidylinositol-3-kinase, PDKI: phosphoinositide-dependent kinase-1, AKT: The serine/threonine kinase, Bad: BCL2 antagonist of cell death, Bcl2: B- Cell Leukemia/Lymphoma 2, mTOR: The mammalian target of rapamycin, p70 SGK: P70-S6 kinase, elF-4E: eukaryotic initiation factor 4E, GRB-2: Growth factor receptor bound protein 2, SOS: Son-of-sevenless, Shc: SHC-transforming protein 1, Ras: Protein from "Rat sarcoma virus", Raf: Rapidly Accelerated Fibrosarcoma, MEKs: MAPK kinase/ERK kinase, ERKs: Extracellular signal-regulated kinase, S6K: Ribosomal S6 kinases, c-Jun: JNK substrates, c-Fos: Polyclonal antibodies to AKT, c-Myc: c-myelocytomatosis oncogene, ELK-1: Erythroblast transformation specific (ETS) Like-1 protein, JNK: c-Jun N-terminal kinase, MAPK: Mitogen-activated protein kinase, p38 MAPK: p38 mitogen-activated protein kinase).

existence of such ‘‘escape’’ mechanisms implies that dual targeting of these pathways which may lead to superior efficacy and better clinical outcome in selected patients **(Saini et al., 2013)**.

The PI3K/AKT/mTOR signaling pathway is frequently in a dysregulated state in tumors, and has now become an important anticancer target **(Thorpe et al., 2015)**. The PI3K/AKT/mTOR signaling pathway itself serves a major role in regulating cell physiology and pathology, including cell proliferation, survival and invasion **(Figure 9)**. Some of the activating mutations in PI3K/AKT/mTOR are also common in human tumors, and thus may promote tumor growth **(Wong et al., 2010; Rodon et al., 2013)**.

The mTOR is a serine/threonine kinase ubiquitously expressed in mammalian cells. It picks up and integrates signals initiated by nutrient intake, growth factors, and other cellular stimuli to regulate downstream signaling and protein synthesis **(Ferrín et al., 2020)**. Through its downstream effectors, Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and Ribosomal protein S6 kinase beta-1 (P70S6K), it is involved in the initiation of ribosomal translation of messenger RNA (mRNA) into proteins necessary for cell growth, cell cycle progression, and cell metabolism **(Wang et al., 2015; Chen and Zhou, 2020)**.

Somatic mutations, gains or losses of key genes, are among a number of genetic alterations affecting these pathways in a number of different solid and hematological tumors **(Olafsson and Anderson, 2021)**. The activation of the PI3K/AKT/mTOR

pathway results in a profound disturbance of control of cell growth and survival, which ultimately leads to a competitive growth advantage, metastatic competence, angiogenesis, and therapy resistance. Thus, this complex pathway has been taken into consideration as one of the most attractive targets for the development of anticancer agents **(Peng et al., 2022)**.

The PI3K/AKT/mTOR can regulate the NF-κB pathway/family of transcription factors that modulate inflammation, cellular stress, and innate and adaptive immune responses, which, in turn, regulate the survival, proliferation, migration, and invasion of hepatocytes, Kupffer cells, and hepatic stellate cells **(Luedde and Schwabe, 2011; Sun et al., 2021)**. The PI3K/AKT/mTOR pathway activates the NF-κB pathway through various mechanisms. The most common is the phosphorylation of inhibitor of NF-κB (IκB) and IκB kinase (IKK) by AKT causing dissociation of IκB from the NF-κB dimers. AKT can also promote IKK activity indirectly through mTOR and the MAPK pathways **(Sun et al., 2021)**.

3. Angiogenesis

Hepatocellular carcinoma is a hyper-vascular tumor characterized by neovascularization, which plays an important role in the growth and progression of HCC **(Jiang et al., 2022)**. A switch to an angiogenic phenotype is a prerequisite for the development from a premalignant stage to an invasive tumor. The neovascularization provides not only nutrients for tumor growth but also a large surface area of leaky vessels with incomplete basement membrane that facilitate intravasation of tumor cells **(Azizi et al., 2020)**. Tumor cells seeding in distant organs develop initially as avascular dormant micro metastasis. Angiogenic switch leads to secondary angiogenesis and tumor growth, resulting in overt metastasis **(Poon et al., 2001; Tsilimigras et al., 2021)**.

By studying liver tissues and serum samples from HCC patients, the enhanced expression of VEGF is found to be correlated with aggressive behavior of HCC, resulting in a poor prognosis **(Hilmi et al., 2019)**. Since HCC is a highly vascularized tumor, the targeting of well-known angiogenic factors, such as VEGF, fibroblast growth factor (FGF), and platelet derived growth factor (PDGF), is appealing for molecular therapy **(Qin et al., 2019)**.

Important angiogenic factors involved in the regulation of angiogenesis in HCC have been identified, although the exact molecular pathways are far from clear. Current data suggest that vascular endothelial growth factor (VEGF) plays a critical role in angiogenesis of HCC (**Morse et al., 2019)**. **Figure 10** shows role of angiogenesis in cancer development, growth, and metastasis.

Receptors for growth factors as VEGFR, fibroblast growth factor receptor (FGFR) and PDGFR activate intracellular receptor tyrosine kinases (RTKs) and the downstream RAS/RAF/MAPK and MEK/ERK signaling pathway, and promote the growth, migration and morphogenesis of vascular endothelial cells, thus increasing vascular permeability **(Huang et al., 2021; Kciuk et al., 2022)**.

Figure 11 illustrates how the PI3K/AKT/mTOR pathway functionally interacts with VEGFR, PDGFR, and EGFR to exert cellular processes, including cell proliferation and survival (He et al., 2021). This pathway is often targeted in therapeutic intervention in HCC because activating it can cause aggressive tumor behavior and a reduction in survival **(Moon and Ro, 2021)**. The RAS/RAF/MEK/ERK pathway, continuously activated in HCC, controls many essential cellular processes by interacting with EGFR, FGFR and c-mesenchymal-epithelial transitionfactor-1) c-Met) **(Dimri and Satyanarayana, 2020; Guo et al., 2020b)**.

Angiogenesis provides a target for novel prognostic and therapeutic approaches to HCC. Assessment of micro vessel density using immunohistochemical staining for specific endothelial cell markers such as CD34 has been shown to provide prognostic information independent of conventional pathological parameters in HCC patients **(Paschoal et al., 2014)**. The development of neovasculature in the tumor provides two essential functions for the growth and metastasis of a cancer. First, the vessels provide a route for supply of nutrient and oxygen to sustain tumor growth, and excretion of metabolic waste **(Pang and Poon, 2006)**. Second, the neovessels provide access for tumor cells to enter the circulation. The new capillaries formed in tumors have incomplete basement membrane, facilitating penetration by tumors cells into the circulation **(Paschoal et al., 2014; Park et al., 2021)**.

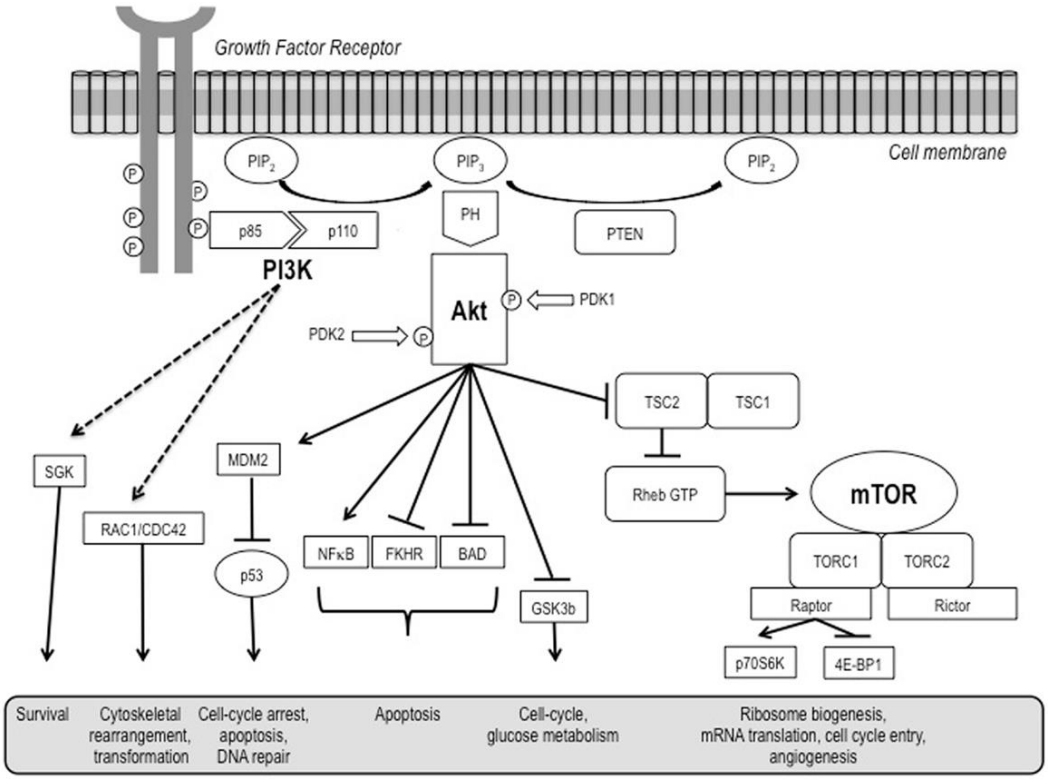

Figure 9: A schematic representation of the PI3K/AKT/mTOR pathway **(Porta et al., 2014)**. (PIP2: Phosphatidylinositol 4,5-bisphosphate, PIP3: Phosphatidylinositol (3,4,5)-trisphosphate, PH: Pleckstrin homology domains, PTEN: phosphatase and tensin homolog, p85: regulatory subunit of PI3K, p110: catalytic subunit of PI3K, PI3K: Phosphatidylinositol-3-kinase, SGK: Serum- and glucocorticoid-regulated kinase, RAC1 / CDC42: Ras-related C3 botulinum toxin substrate 1/ Cell division control protein 42 homolog, AKT: Protein kinase B , a set of three serine/threonine-specific protein, PDK1: phosphoinositide-dependent kinase-1, PDK2: phosphoinositide-dependent kinase-2, MDM2: Mouse double minute 2 homolog, p53: A tumor suppressor gene, NF-κB: Nuclear factor kappa B, FKHR: Forkhead protein, BAD: BCL2 Antagonist of Cell Death , GSK3b: glycogen synthase kinase 3β, TSC1: tuberous sclerosis complex 1, TSC2: tuberous sclerosis complex 2, Rheb GTP: Ras homolog enriched in brain which is a GTP-binding protein, mTOR: The mammalian target of rapamycin, TORC1: mTOR complex 1, TORC2: mTOR complex 2, Raptor: Regulatory-associated protein of mTOR, Rictor: Rapamycin-insensitive companion of mammalian target of rapamycin, p70S6K: Ribosomal protein S6 kinase beta-1 (S6K1), 4E-BP1: Eukaryotic translation initiation factor 4E (eIF4E)-binding protein).

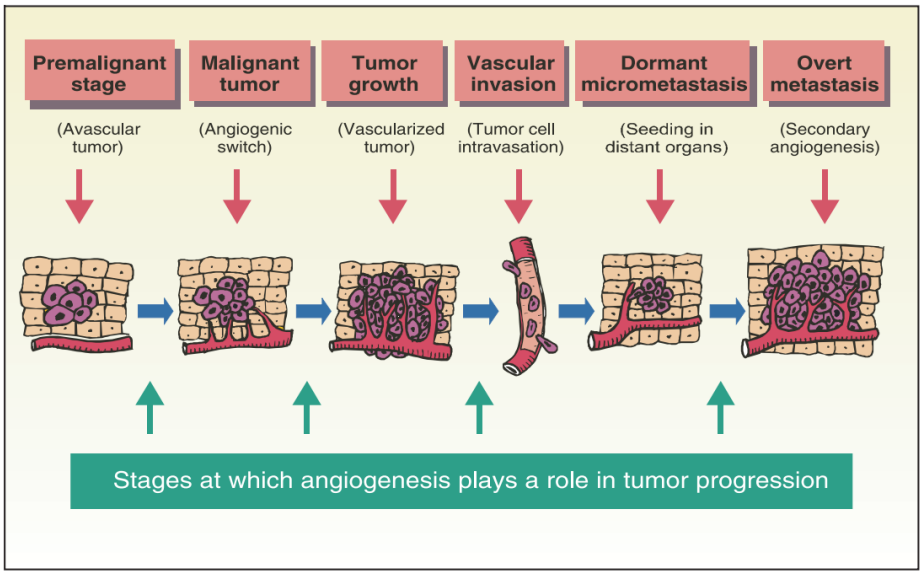

Figure 9: Role of angiogenesis in cancer development, growth and metastasis **(Poon et al., 2001).**

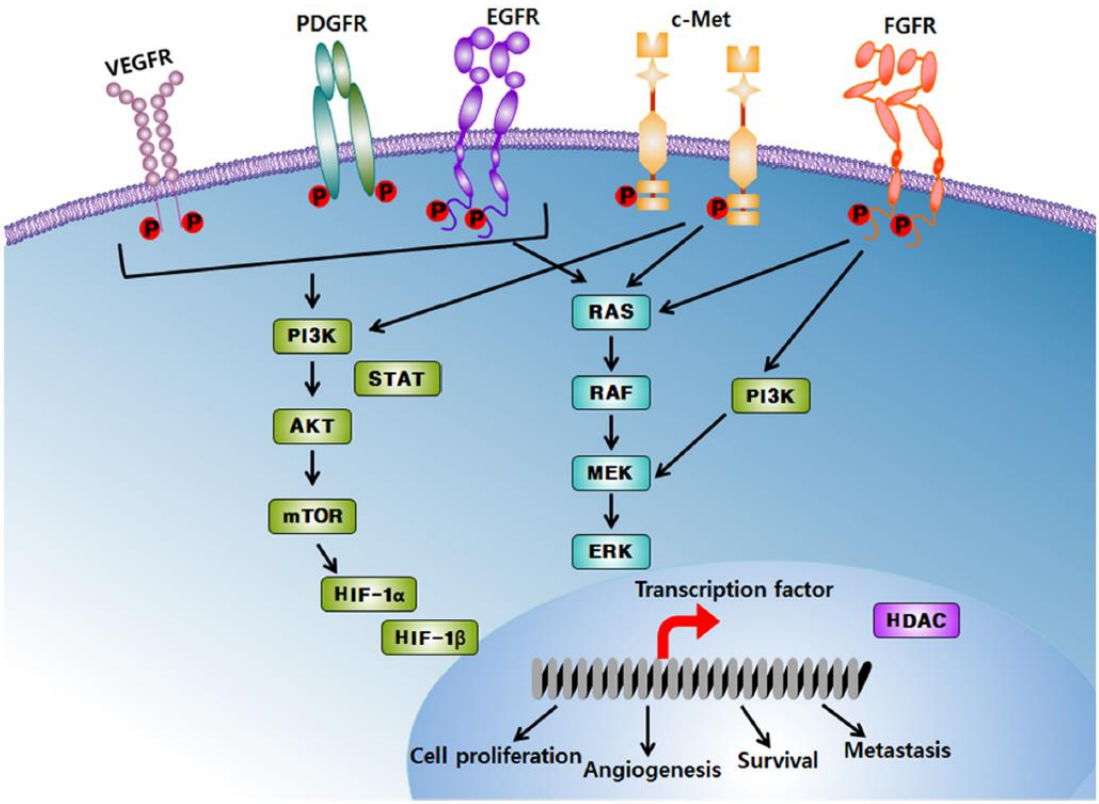

Figure 11: Schematic diagram of key functional pathways and their roles in HCC development **(Choi et al., 2015)**. (VEGFR: Vascular endothelial growth factor receptor, PDGFR: Platelet derived growth factor receptor, EGFR: Epidermal growth factor receptor, c-Met: c-mesenchymal-epithelial transition factor-1, FGFR: fibroblast growth factor receptor, PI3K: Phosphatidylinositol-3-kinase, STAT: Signal transducer and activator of transcription, AKT: Protein kinase B, a set of three serine/threonine-specific protein kinases, mTOR: The mammalian target of rapamycin, HIF-1α: Hypoxia inducible factor-1α, HIF-1β: Hypoxia inducible factor-1β, RAS: Protein from "Rat sarcoma virus", RAF: Rapidly accelerated fibrosarcoma, MEK: MAPK kinase, ERK: Extracellular signal-regulated kinase, HDAC: Histone deacetylases).

4. Tissue invasion and Metastasis

In the process of drug resistance of liver cancer cells, the ability of cells to migrate is enhanced, which brings great difficulties to the treatment of liver cancer patients **(Hu et al., 2019)**. HCC metastasis is a multi-step process. HCC metastasis begins with the invasion of HCC cells from the primary HCC tumor to the extracellular matrix (ECM) of the neighboring stroma **(Elgundi et al., 2020)**. HCC cells then disseminate into the circulation system through intravasation **(Sznurkowska and Aceto, 2021)**. Being able to survive in the circulatory system, HCC cells then extravasate, eventually enter and colonize the secondary tissue site. In intrahepatic metastasis, the portal vein transports HCC cells from the primary site to other parts of the liver **(Tsilimigras et al., 2021)**. In extrahepatic metastasis, hepatic vein transports HCC cells from the primary site in the liver to the systemic blood stream. The heart then pumps the circulating cancerous cells to the lung, and eventually to other organs such as bones, brain, and adrenal glands. Each step of HCC metastasis is supported by different pro-metastatic genes **(Wong et al., 2014) (Figure 12)**.

The PI3K/AKT/mTOR signaling pathway plays an important role in promoting tumor invasion and metastasis via activation of AKT which enhances the transcriptional activity of NF-kB, promotes the transport of tumors, and supports the invasion of tumors **(Chin and Toker, 2010)**. Also, through the activation of matrix metalloproteinases (MMPs), a group of proteolytic enzymes that participate in degrading the ECM, promotes cell invasion and metastasis **(Cheng et al., 2017)**.

Metastasis is an important aspect of HCC progression, and EMT is considered as the first

step in the metastatic cascade **(Nowak and Bednarek, 2021)**. The expression of GPC3 in HCC tissue was upregulated during HCC progression from BCLC stage A or B to stage C. The increased expression of GPC3 in tumor tissues was closely related to the level of EMT markers, as well as to the cancer vascular invasion **(Wu et al., 2015)**.

The GPC3 is a member of the heparan sulfate proteoglycans family, which is highly expressed in the majority of HCCs and correlated with poor outcome of HCC patients **(Shih et al., 2020)**. GPC3 could interact with a variety of growth factors, chemokines, and cytokines to form a concentration gradient on the surface of the cell membrane which promotes these ligands binding to their related receptors **(Hassan et al., 2021)**. In this sense, GPC3 acts as an extracellular signal "recruiter" in various signaling pathways, playing a crucial role in maintaining the concentration of extracellular ligands and promoting ligand-receptor interactions **(Guo et al., 2020a)**. Studies have shown that the loss of GPC3 during development leads to changes in downstream signals such as WNT and Hedgehog **(Hassan et al., 2021)**.

Glypican 3 is an important regulator of WNT signaling pathway in the initial stage. Studies have shown that GPC3 can help the activation of WNT signaling by promoting the formation of membrane surface complexes in liver cancer **(Guo et al., 2020a)**. Both the GPC3 protein and the heparan sulfate side chain can interact with WNTs and its receptor which is known as frizzled (FZD), and acts as a "signal recruiter" in the initial activation-stage of the WNT signaling **(Guo et al., 2020a)**. In addition, the presence of GPC3 can further stabilize the binding of WNTs to FZD, thereby positively regulate WNTs downstream signal transduction **(Li et al., 2020b) (Figure 13)**.

5. Epigenetic regulation through long non-coding ribonucleic acid (LncRNA)

The molecular mechanisms underlying HCC pathogenesis have not been fully understood **(Huang et al., 2020)**. LncRNAs are a type of non-coding RNAs longer than 200 nucleotides. lncRNAs are suggested to play critical roles in the tumorigenesis and development of human HCC. To date, dysregulation of many HCC-related lncRNAs such as Highly up-regulated in liver cancer (HULC), Hox transcript antisense intergenic RNA (HOTAIR), Metastasis-associated lung adenocarcinoma transcript-1 (MALAT1), and long noncoding RNA H19 (H19) have been identified **(Xie et al., 2021)**.

Various HCC-related lncRNAs have been shown to possess aberrant expression and play critical regulatory roles in cancerous phenotypes (e.g., persistent proliferation, evading apoptosis, accelerated vessel formation and gain of invasive capability) through their binding with DNA, RNA or proteins, or encoding small peptides **(Huang et al., 2020)**. Thus, a deeper understanding of lncRNA dysregulation would provide new insights into HCC pathogenesis and novel tools for the early diagnosis and treatment of HCC **(Huang et al., 2020)**. There is evidence that lncRNAs have important roles in promoting metastasis by targeting factors such as VEGF, MMP9, CASP3/8, Bax, Bcl2, Bcl-xL, and interleukin 11 (IL- 11) **(Giannelli et al., 2014; Xue et al., 2019; Li et al., 2020a; Alarcón-Sánchez et al., 2021; Su et al., 2021; Heydarnezhad Asl et al., 2022)**.

The lncRNA-AF085935 has been proved to be a potential biomarker for HCC screening. Studies have shown that lncRNA-AF085935 was significantly upregulated in HCC patients' serum when compared with normal controls **(Lu et al., 2015; Sherif et al., 2020)**. Interestingly, lncRNA–AF085935 is transcribed in antisense orientation with respect to GPC3. Moreover, lncRNA–AF085935 was upregulated and coexpressed with GPC3 in HCC cells and tissues **(Yuan et al., 2014; Sabry et al., 2019)**. Many studies have shown that GPC3 enhances HCC cell proliferation, migration, and invasion, and inhibits HCC cell apoptosis **(Zhang et al., 2018; Wang et al., 2019)**. However, the functions of lncRNA-AF085935 in HCC and its regulation mechanisms regarding GPC3 remain largely unknown.

To conclude, the characteristic pathogenesis mechanisms of HCC are abnormal signal transduction resulting in uncontrolled cell proliferation, loss of apoptosis or programmed cell death, tissue invasion and metastasis allowing spread of the cancer and finally angiogenesis which leads to enhanced blood supply of tumors. In addition to epigenetic control of tumorigenesis, many of the components of these mechanisms are potential targets of anti-HCC therapies.

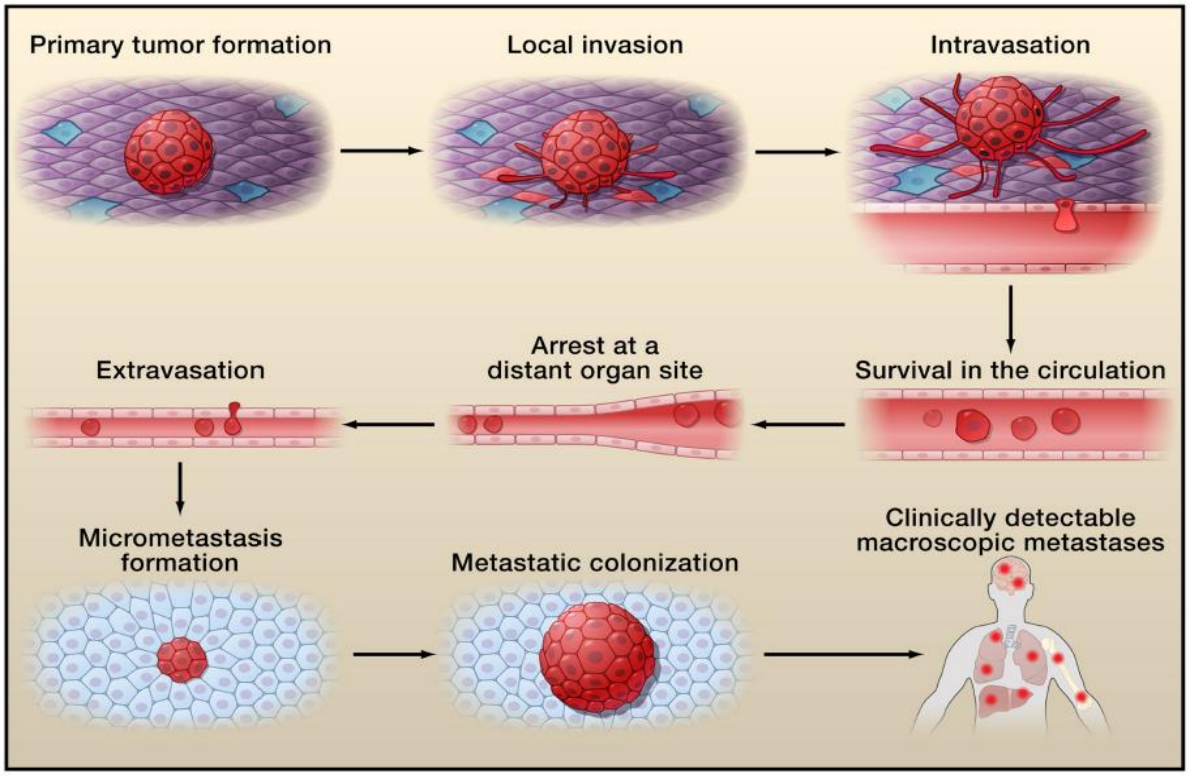

Figure 12: The invasion–metastasis cascade **(Valastyan and Weinberg, 2011)**.

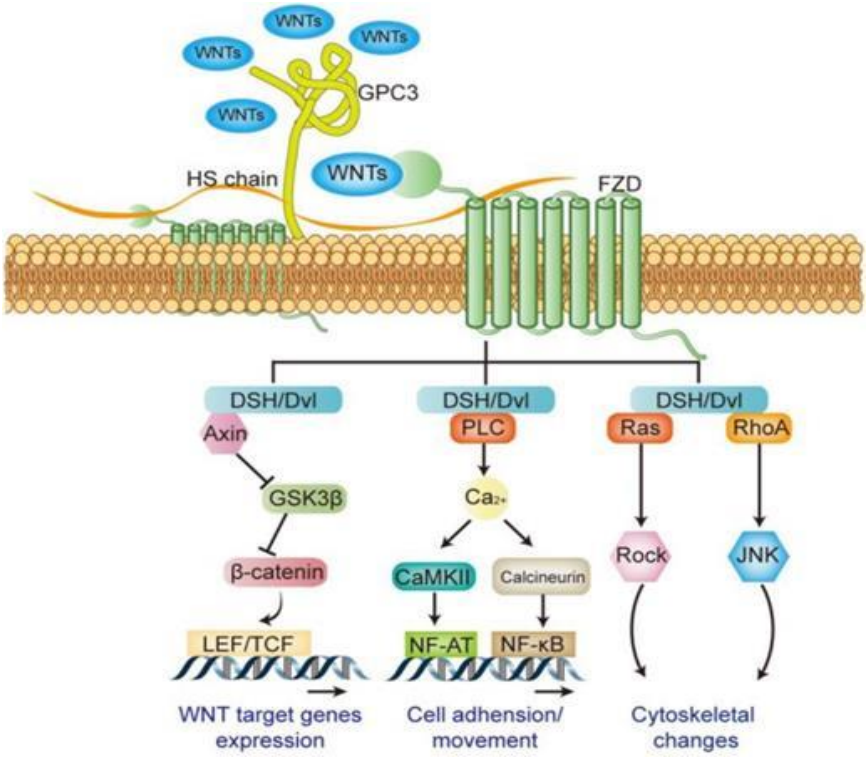

Figure 13: Regulatory effect of GPC3 on the WNT signaling pathway **(Guo et al., 2020a)**. (WNT: Wingless/Integrated, GPC3: Glypican 3, HS chain: heparan sulfate chain, FZD: Frizzled, DSH/Dvl: Dishevelled, GSK3β: Glycogen synthase kinase 3β, β-catenin: Beta catenin, LEF/TCF: lymphoid enhancer factor/ T-cell factor, PLC: Primary liver cancer, Ca2+: Calcium, CaMKII: Ca2+/Calmodulin-dependent protein kinase II, NF-AT: Nuclear factor of activated T cells, Ras: Protein from "Rat sarcoma virus", Rock: RhoA/Rho kinase, RhoA: Ras homolog family member A, JNK: c-Jun N-terminal kinase).

References

Abou-Ghali, M., & Stiban, J. (2015). Regulation of ceramide channel formation and disassembly: Insights on the initiation of apoptosis. Saudi Journal of Biological Sciences, 22(6), 760-772.

Ahmad, M. S., Suardi, N., Shukri, A., Mohammad, H., Oglat, A. A., Abunahel, B. M., Mohamed, A.M. and Makhamrah, O. (2019). Current status regarding tumour progression, surveillance, diagnosis, staging, and treatment of HCC: a literature review. Journal of Gastroenterology and Hepatology Research, 8(2), 2841-2852.

Alarcón-Sánchez, B. R., Pérez-Carreón, J. I., Villa-Treviño, S., & Arellanes-Robledo, J. (2021). Molecular alterations that precede the establishment of the hallmarks of cancer: An approach on the prevention of hepatocarcinogenesis. Biochemical Pharmacology, 194, 114818.

Alqahtani, A., Khan, Z., Alloghbi, A., S. Said Ahmed, T., Ashraf, M., & M. Hammouda, D. (2019). Hepatocellular carcinoma: molecular mechanisms and targeted therapies. Medicina, 55(9), 526.

Asati, V., Mahapatra, D. K., & Bharti, S. K. (2016). PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways inhibitors as anticancer agents: Structural and pharmacological perspectives. European Journal of Medicinal Chemistry, 109, 314-341.

Azizi, M., Dianat-Moghadam, H., Salehi, R., Farshbaf, M., Iyengar, D., Sau, S., Iyer, A.K., Valizadeh, H., Mehrmohammadi, M. and Hamblin, M.R. (2020). Interactions between tumor biology and targeted nanoplatforms for imaging applications. Advanced Functional Materials, 30(19), 1910402.

Bhoori, S., & Mazzaferro, V. (2014). Current challenges in liver transplantation for hepatocellular carcinoma. Best Practice & Research Clinical Gastroenterology, 28(5), 867-879.

Booth, L. A., Roberts, J. L., & Dent, P. (2020). The role of cell signaling in the crosstalk between autophagy and apoptosis in the regulation of tumor cell survival in response to sorafenib and neratinib. In Seminars in Cancer Biology (66, 129-139). Academic Press.

Boutelle, A. M., & Attardi, L. D. (2021). p53 and tumor suppression: it takes a network. Trends in Cell Biology, 31(4), 298-310.

Chen, K. W., Ou, T. M., Hsu, C. W., Horng, C. T., Lee, C. C., Tsai, Y. Y., Tsai, C.C., Liou, Y.S., Yang, C.C., Hsueh, C.W. and Kuo, W.H. (2015b). Current systemic treatment of hepatocellular carcinoma: A review of the literature. World Journal of Hepatology, 7(10), 1412.

Chen, Y., & Zhou, X. (2020). Research progress of mTOR inhibitors. European Journal of Medicinal Chemistry, 208, 112820.

Cheng, T. C., Din, Z. H., Su, J. H., Wu, Y. J., & Liu, C. I. (2017). Sinulariolide suppresses cell migration and invasion by inhibiting matrix metalloproteinase-2/-9 and urokinase through the PI3K/AKT/mTOR signaling pathway in human bladder cancer cells. Marine Drugs, 15(8), 238.

Chin, Y. R., & Toker, A. (2010). The actin-bundling protein palladin is an Akt1-specific substrate that regulates breast cancer cell migration. Molecular Cell, 38(3), 333-344.

Choi, K. J., Baik, I. H., Ye, S. K., & Lee, Y. H. (2015). Molecular targeted therapy for hepatocellular carcinoma: Present status and future directions. Biological and Pharmaceutical Bulletin, 38(7), 986-991.

De Las Rivas, J., Brozovic, A., Izraely, S., Casas-Pais, A., Witz, I. P., & Figueroa, A. (2021). Cancer drug resistance induced by EMT: Novel therapeutic strategies. Archives of Toxicology, 95(7), 2279-2297.

Dimitroulis, D., Damaskos, C., Valsami, S., Davakis, S., Garmpis, N., Spartalis, E., Athanasiou, A., Moris, D., Sakellariou, S., Kykalos, S. and Tsourouflis, G. (2017). From diagnosis to treatment of hepatocellular carcinoma: An epidemic problem for both developed and developing world. World Journal of Gastroenterology, 23(29), 5282.

Dimri, M., & Satyanarayana, A. (2020). Molecular signaling pathways and therapeutic targets in hepatocellular carcinoma. Cancers, 12(2), 491.

El Kassas, M., Elbaz, T., Salaheldin, M., Abdelsalam, L., Kaseb, A., & Esmat, G. (2019). Impact of treating chronic hepatitis C infection with direct-acting antivirals on the risk of hepatocellular carcinoma: The debate continues–A mini-review. Journal of Advanced Research, 17, 43-48.

El-Kassas, M., & Elbadry, M. (2022). Hepatocellular Carcinoma in Africa: Challenges and Opportunities. Frontiers in Medicine, 9, 899420.

Elgundi, Z., Papanicolaou, M., Major, G., Cox, T. R., Melrose, J., Whitelock, J. M., & Farrugia, B. L. (2020). Cancer metastasis: the role of the extracellular matrix and the heparan sulfate proteoglycan perlecan. Frontiers in Oncology, 9, 1482.

Elmore, S. (2007). Apoptosis: a review of programmed cell death. Toxicologic Pathology, 35(4), 495-516.

Esmat, G., El-Sayed, M. H., Hassany, M., Doss, W., & Waked, I. (2018). One step closer to elimination of hepatitis C in Egypt. The Lancet Gastroenterology & Hepatology, 3(10), 665.

European Association for The Study of The Liver. (2018). EASL clinical practice guidelines: management of hepatocellular carcinoma. Journal of Hepatology, 69(1), 182-236.

Ezzat, R., Eltabbakh, M., & El Kassas, M. (2021). Unique situation of hepatocellular carcinoma in Egypt: A review of epidemiology and control measures. World Journal of Gastrointestinal Oncology, 13(12), 1919.

Fabregat, I. (2009). Dysregulation of apoptosis in hepatocellular carcinoma cells. World Journal of Gastroenterology, 15(5), 513-520.

Fausto, N., & Campbell, J. S. (2010). Mouse models of hepatocellular carcinoma. In Seminars in liver disease. (30(1),087-098). © Thieme Medical Publishers.

Ferrín, G., Guerrero, M., Amado, V., Rodríguez-Perálvarez, M., & De la Mata, M. (2020). Activation of mTOR signaling pathway in hepatocellular carcinoma. International Journal of Molecular Sciences, 21(4), 1266.

Franjić, S. (2022). Briefly about Apoptosis. International Journal of Cancer Research & Therapy, 7 (2), 82-86.

Galluzzi, L., Vitale, I., Aaronson, S. A., Abrams, J. M., Adam, D., Agostinis, P., Alnemri, E.S., Altucci, L., Amelio, I., Andrews, D.W. and Annicchiarico-Petruzzelli, M. (2018). Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death & Differentiation, 25(3), 486-541.

Giannelli, G., Rani, B., Dituri, F., Cao, Y., & Palasciano, G. (2014). Moving towards personalised therapy in patients with hepatocellular carcinoma: the role of the microenvironment. Gut, 63(10), 1668-1676.

Giannelli, G., Koudelkova, P., Dituri, F., & Mikulits, W. (2016). Role of epithelial to mesenchymal transition in hepatocellular carcinoma. Journal of Hepatology, 65(4), 798-808.

Gohil, C., & Noolvi, M. N. (2019). p53 Protein: Master Regulator of Apoptosis and its Application in Cancer Therapy. International Journal of Pharmaceutical & Biological Archive, 10(3), 154-164.

Grilo, A. L., & Mantalaris, A. (2019). Apoptosis: A mammalian cell bioprocessing perspective. Biotechnology Advances, 37(3), 459-475.

Gu, J., Kawai, H., Wiederschain, D., & Yuan, Z. M. (2001). Mechanism of functional inactivation of a Li-Fraumeni syndrome p53 that has a mutation outside of the DNA-binding domain. Cancer Research, 61(4), 1741-1746.

Guo, M., Zhang, H., Zheng, J., & Liu, Y. (2020a). Glypican-3: a new target for diagnosis and treatment of hepatocellular carcinoma. Journal of Cancer, 11(8), 2008.

Guo, Y. J., Pan, W. W., Liu, S. B., Shen, Z. F., Xu, Y., & Hu, L. L. (2020b). ERK/MAPK signalling pathway and tumorigenesis. Experimental and Therapeutic Medicine, 19(3), 1997-2007.

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. Cell, 100(1), 57-70.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. Cell, 144(5), 646-674.

Hassan, N., Greve, B., Espinoza-Sánchez, N. A., & Götte, M. (2021). Cell-surface heparan sulfate proteoglycans as multifunctional integrators of signaling in cancer. Cellular Signalling, 77, 109822.

He, Y., Sun, M. M., Zhang, G. G., Yang, J., Chen, K. S., Xu, W. W., & Li, B. (2021). Targeting PI3K/Akt signal transduction for cancer therapy. Signal Transduction and Targeted Therapy, 6(1), 425.

Henderson, J. M., Sherman, M., Tavill, A., Abecassis, M., Chejfec, G., & Gramlich, T. (2003). AHPBA/AJCC consensus conference on staging of hepatocellular carcinoma: consensus statement. HPB, 5(4), 243-250.

Heydarnezhad Asl, M., Pasban Khelejani, F., Bahojb Mahdavi, S. Z., Emrahi, L., Jebelli, A., & Mokhtarzadeh, A. (2022). The various regulatory functions of long noncoding RNAs in apoptosis, cell cycle, and cellular senescence. Journal of Cellular Biochemistry, 123(6), 995-1024.

Hilmi, M., Neuzillet, C., Calderaro, J., Lafdil, F., Pawlotsky, J. M., & Rousseau, B. (2019). Angiogenesis and immune checkpoint inhibitors as therapies for hepatocellular carcinoma: current knowledge and future research directions. Journal for Immunotherapy of Cancer, 7(1), 333.

Ho, D. W. H., Lo, R. C. L., Chan, L. K., & Ng, I. O. L. (2016). Molecular pathogenesis of hepatocellular carcinoma. Liver Cancer, 5(4), 290-302.

Hornsveld, M., & Dansen, T. B. (2016). The hallmarks of cancer from a redox perspective. Antioxidants & Redox Signaling, 25(6), 300-325.

Hu, B., Cheng, J. W., Hu, J. W., Li, H., Ma, X. L., Tang, W. G., Sun, Y.F., Guo, W., Huang, A., Zhou, K.Q. and Gao, P.T. (2019). KPNA3 confers sorafenib resistance to advanced hepatocellular carcinoma via TWIST regulated epithelial-mesenchymal transition. Journal of Cancer, 10(17), 3914.

Hu, X., Fu, M., Zhao, X., & Wang, W. (2021). The JAK/STAT signaling pathway: From bench to clinic. Signal Transduction and Targeted Therapy, 6(1), 402.

Huang, X. L., Khan, M. I., Wang, J., Ali, R., Ali, S. W., Kazmi, A., Lolai, A., Huang, Y.L., Hussain, A., Bilal, M. and Li, F. (2021). Role of receptor tyrosine kinases mediated signal transduction pathways in tumor growth and angiogenesis—New insight and futuristic vision. International Journal of Biological Macromolecules, 180, 739-752.

Huang, Z., Zhou, J. K., Peng, Y., He, W., & Huang, C. (2020). The role of long noncoding RNAs in hepatocellular carcinoma. Molecular Cancer, 19(1), 77.

Hung, M. H., Tai, W. T., Shiau, C. W., & Chen, K. F. (2014). Downregulation of signal transducer and activator of transcription 3 by sorafenib: a novel mechanism for hepatocellular carcinoma therapy. World Journal of Gastroenterology, 20(41), 15269 - 15274.

Jiang, X., Xu, Y., Chen, D., Wang, M., Qiu, M., Xiong, L., Zhang, L., Yu, H. and Xiong, Z. (2022). A Novel Angiogenesis-Related Prognostic Signature Associated with the Hepatocellular Carcinoma Immune Microenvironment and Survival Outcome. International Journal of General Medicine, 15, 311.

Kamali, I., Shumbusho, F., Barnhart, D. A., Nyirahabihirwe, F., Gakuru, J. D. L. P., Dusingizimana, W., Nizeyumuremyi, E., Habinshuti, P., Walker, S., Makuza, J.D. and Serumondo, J. (2022). Time to complete hepatitis C cascade of care among patients identified during mass screening campaigns in rural Rwanda: a retrospective cohort study. BMC Infectious Diseases, 22(1), 272.

Katerji, M., & Duerksen-Hughes, P. J. (2021). DNA damage in cancer development: special implications in viral oncogenesis. American Journal of Cancer Research, 11(8), 3956.

Kciuk, M., Gielecińska, A., Budzinska, A., Mojzych, M., & Kontek, R. (2022). Metastasis and MAPK Pathways. International Journal of Molecular Sciences, 23(7), 3847.

Knight, T., Luedtke, D., Edwards, H., Taub, J. W., & Ge, Y. (2019). A delicate balance–The BCL-2 family and its role in apoptosis, oncogenesis, and cancer therapeutics. Biochemical Pharmacology, 162, 250-261.

Kontomanolis, E. N., Koutras, A., Syllaios, A., Schizas, D., Mastoraki, A., Garmpis, N., Diakosavvas, M., Angelou, K., Tsatsaris, G., Pagkalos, A. and Ntounis, T. (2020). Role of oncogenes and tumor-suppressor genes in carcinogenesis: a review. Anticancer Research, 40(11), 6009-6015.

Kudo, M. (2019). Targeted and immune therapies for hepatocellular carcinoma: predictions for 2019 and beyond. World Journal of Gastroenterology, 25(7), 789.

Li, B., Yang, L., Peng, X., Fan, Q., Wei, S., Yang, S., Li, X., Jin, H., Wu, B., Huang, M. and Tang, S. (2020). Emerging mechanisms and applications of ferroptosis in the treatment of resistant cancers.

Biomedicine & Pharmacotherapy, 130, 110710.

Li, N., Spetz, M. R., & Ho, M. (2020). The role of glypicans in cancer progression and therapy. Journal of Histochemistry & Cytochemistry, 68(12), 841-862.

Lim, Y., Dorstyn, L., & Kumar, S. (2021). The p53-caspase-2 axis in the cell cycle and DNA damage response. Experimental & Molecular Medicine, 53(4), 517-527.

Llovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., Lencioni, R., Koike, K., Zucman-Rossi, J., & Finn, R. S. (2021). Hepatocellular carcinoma. Nature Reviews. Disease Primers, 7(1), 6.

Lohitesh, K., Chowdhury, R., & Mukherjee, S. (2018). Resistance a major hindrance to chemotherapy in hepatocellular carcinoma: an insight. Cancer Cell International, 18(1), 44.

Lu, J., Xie, F., Geng, L., Shen, W., Sui, C., & Yang, J. (2015). Investigation of serum lncRNA-uc003wbd and lncRNA-AF085935 expression profile in patients with hepatocellular carcinoma and HBV. Tumor Biology, 36(5), 3231-3236.

Luedde, T., & Schwabe, R. F. (2011). NF-κB in the liver—linking injury, fibrosis and hepatocellular carcinoma. Nature reviews Gastroenterology & Hepatology, 8(2), 108-118.

Malik, A., Thanekar, U., Amarachintha, S., Mourya, R., Nalluri, S., Bondoc, A., & Shivakumar, P. (2021). "Complimenting the complement": mechanistic insights and opportunities for therapeutics in hepatocellular carcinoma. Frontiers in Oncology, 10, 627701.

Marquardt, J. U., & Edlich, F. (2019). Predisposition to apoptosis in hepatocellular carcinoma: from mechanistic insights to therapeutic strategies. Frontiers in Oncology, 9, 1421.

Marrero, J. A., Fontana, R. J., Barrat, A., Askari, F., Conjeevaram, H. S., Su, G. L., & Lok, A. S. (2005). Prognosis of hepatocellular carcinoma: comparison of 7 staging systems in an American cohort. Hepatology, 41(4), 707-715.

Marrero, J. A., Kulik, L. M., Sirlin, C. B., Zhu, A. X., Finn, R. S., Abecassis, M. M., Roberts, L.R. and Heimbach, J.K. (2018). Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. Hepatology, 68(2), 723-750.

McCubrey, J. A., Steelman, L. S., Chappell, W. H., Abrams, S. L., Wong, E. W., Chang, F., Lehmann, B., Terrian, D.M., Milella, M., Tafuri, A. and Stivala, F. (2007). Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1773(8), 1263-1284.

McIlwain, D. R., Berger, T., & Mak, T. W. (2015). Caspase functions in cell death and disease. Cold Spring Harbor Perspectives in Biology, 5(4), a008656.

Merlin, J. P., Rupasinghe, H. P., Dellaire, G., & Murphy, K. (2021). Role of dietary antioxidants in p53-mediated cancer chemoprevention and tumor suppression. Oxidative Medicine and Cellular Longevity, 2021, 9924328

Mijit, M., Caracciolo, V., Melillo, A., Amicarelli, F., & Giordano, A. (2020). Role of p53 in the Regulation of Cellular Senescence. Biomolecules, 10(3), 420.

Moon, H., & Ro, S. W. (2021). MAPK/ERK signaling pathway in hepatocellular carcinoma. Cancers, 13(12), 3026.

Moonsamy, S., Suchard, M., Pillay, P., & Prabdial-Sing, N. (2022). Prevalence and incidence rates of laboratory-confirmed hepatitis B infection in South Africa, 2015 to 2019. BMC Public Health, 22(1), 29.

Moreno-Càceres, J., & Fabregat, I. (2015). Apoptosis in liver carcinogenesis and chemotherapy. Hepatic Oncology, 2(4), 381-397.

Morishita, A., Oura, K., Tadokoro, T., Fujita, K., Tani, J., & Masaki, T. (2021). MicroRNAs in the pathogenesis of hepatocellular carcinoma: A review. Cancers, 13(3), 514.

Morse, M. A., Sun, W., Kim, R., He, A. R., Abada, P. B., Mynderse, M., & Finn, R. S. (2019). The Role of Angiogenesis in Hepatocellular CarcinomaRole of Angiogenesis in HCC. Clinical Cancer Research, 25(3), 912-920.

Nowak, E., & Bednarek, I. (2021). Aspects of the epigenetic regulation of EMT related to cancer metastasis. Cells, 10(12), 3435.

Olafsson, S., & Anderson, C. A. (2021). Somatic mutations provide important and unique insights into the biology of complex diseases. Trends in Genetics, 37(10), 872-881.

Ozakyol, A. (2017). Global epidemiology of hepatocellular carcinoma (HCC epidemiology). Journal of Gastrointestinal Cancer, 48(3), 238-240.

Pang, R., & Poon, R. T. (2006). Angiogenesis and antiangiogenic therapy in hepatocellular carcinoma. Cancer Letters, 242(2), 151-167.

Park, W., Cho, S., Ji, J., Lewandowski, R. J., Larson, A. C., & Kim, D. H. (2021). Development and validation of sorafenib-eluting microspheres to enhance therapeutic efficacy of transcatheter arterial chemoembolization in a rat model of hepatocellular carcinoma. Radiology: Imaging Cancer, 3(1), e200006.

Paschoal, J. P., Bernardo, V., Canedo, N. H. S., Ribeiro, O. D., Caroli-Bottino, A., & Pannain, V. L. (2014). Microvascular density of regenerative nodule to small hepatocellular carcinoma by automated analysis using CD105 and CD34 immunoexpression. BMC Cancer, 14(1), 72.

Peng, Y., Wang, Y., Zhou, C., Mei, W., & Zeng, C. (2022). PI3K/Akt/mTOR Pathway and Its Role in Cancer Therapeutics: Are We Making Headway? Frontiers in Oncology, 12, 819128.

Pfeffer, C. M., & Singh, A. T. (2018). Apoptosis: a target for anticancer therapy. International Journal of MolecularSsciences, 19(2), 448.

Poon, R. T. P., Fan, S. T., & Wong, J. (2001). Clinical implications of circulating angiogenic factors in cancer patients. Journal of Clinical Oncology, 19(4), 1207-1225.

Porta, C., Paglino, C., & Mosca, A. (2014). Targeting PI3K/Akt/mTOR signaling in cancer. Frontiers in Oncology, 4, 64.

Qin, S., Li, A., Yi, M., Yu, S., Zhang, M., & Wu, K. (2019). Recent advances on anti-angiogenesis receptor tyrosine kinase inhibitors in cancer therapy. Journal of Hematology & Oncology, 12(1), 27.

Rahmani, F., Ziaeemehr, A., Shahidsales, S., Gharib, M., Khazaei, M., Ferns, G. A., Ryzhikov, M., Avan, A. and Hassanian, S.M. (2020). Role of regulatory miRNAs of the PI3K/AKT/mTOR signaling in the pathogenesis of hepatocellular carcinoma. Journal of Cellular Physiology, 235(5), 4146-4152.

Rashed, W. M., Kandeil, M. A. M., Mahmoud, M. O., & Ezzat, S. (2020). Hepatocellular Carcinoma (HCC) in Egypt: A comprehensive overview. Journal of the Egyptian National Cancer Institute, 32(1), 5.

Rodon, J., Dienstmann, R., Serra, V., & Tabernero, J. (2013). Development of PI3K inhibitors: lessons learned from early clinical trials. Nature Reviews Clinical Oncology, 10(3), 143-153.

Sabry, D., Abdelaleem, O. O., El Amin Ali, A. M., Mohammed, R. A., Abdel-Hameed, N. D., Hassouna, A., & Khalifa, W. A. (2019). Anti-proliferative and anti-apoptotic potential effects of epigallocatechin-3-gallate and/or metformin on hepatocellular carcinoma cells: in vitro study. Molecular Biology Reports, 46(2), 2039-2047.

Saini, K. S., Loi, S., de Azambuja, E., Metzger-Filho, O., Saini, M. L., Ignatiadis, M., Dancey, J.E. and Piccart-Gebhart, M.J. (2013). Targeting the PI3K/AKT/mTOR and Raf/MEK/ERK pathways in the treatment of breast cancer. Cancer Treatment Reviews, 39(8), 935-946.

Sagnelli, E., Macera, M., Russo, A., Coppola, N., & Sagnelli, C. (2020). Epidemiological and etiological variations in hepatocellular carcinoma. Infection, 48(1), 7-17.

Scaggiante, B., Kazemi, M., Pozzato, G., Dapas, B., Farra, R., Grassi, M., Zanconati, F. and Grassi, G. (2014). Novel hepatocellular carcinoma molecules with prognostic and therapeutic potentials. World journal of gastroenterology: WJG, 20(5), 1268.

Shalini, S., Dorstyn, L., Dawar, S., & Kumar, S. (2015). Old, new and emerging functions of caspases. Cell Death & Differentiation, 22(4), 526-539.

Sherif, I. O., Al-Mutabagani, L. A., Sabry, D., & Elsherbiny, N. M. (2020). Antineoplastic activity of chrysin against human hepatocellular carcinoma: New insight on GPC3/SULF2 axis and lncRNA-AF085935 expression. International Journal of Molecular Sciences, 21(20), 7642.

Shih, T. C., Wang, L., Wang, H. C., & Wan, Y. J. Y. (2020). Glypican-3: A molecular marker for the detection and treatment of hepatocellular carcinoma. Liver Research, 4(4), 168-172.

Singal, A. G., Lok, A. S., Feng, Z., Kanwal, F., & Parikh, N. D. (2022a). Conceptual model for the hepatocellular carcinoma screening continuum: current status and research agenda. Clinical Gastroenterology and Hepatology, 20(1), 9-18.

Singal, A. G., Zhang, E., Narasimman, M., Rich, N. E., Waljee, A. K., Hoshida, Y., Yang, J.D., Reig, M., Cabibbo, G., Nahon, P. and Parikh, N.D. (2022b). HCC Surveillance Improves Early Detection, Curative Treatment Receipt, and Survival in Patients with Cirrhosis: A Systematic Review and Meta-Analysis. Journal of Hepatology.77(1), 128-139.

Singh, A. K., Kumar, R., & Pandey, A. K. (2018). Hepatocellular carcinoma: causes, mechanism of progression and biomarkers. Current Chemical Genomics and Translational Medicine, 12, 9-26.

Sonderup, M. W., Spearman, C. W., & Nordien, R. (2020). A decade of hepatitis C at the University of Cape Town/Groote Schuur Hospital Liver Clinic, South Africa, in the pre-direct-acting antivirals era. South African Medical Journal, 110(2), 106-111.

Su, C., Zhang, J., Yarden, Y., & Fu, L. (2021). The key roles of cancer stem cell-derived extracellular vesicles. Signal Transduction and Targeted Therapy, 6(1), 109.

Sun, E. J., Wankell, M., Palamuthusingam, P., McFarlane, C., & Hebbard, L. (2021). Targeting the PI3K/Akt/mTOR pathway in hepatocellular carcinoma. Biomedicines, 9(11), 1639.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 71(3), 209-249.

Sznurkowska, M. K., & Aceto, N. (2021). The gate to metastasis: key players in cancer cell intravasation. The FEBS Journal. 289(15), 4336-4354.

Taylor, R. C., Cullen, S. P., & Martin, S. J. (2008). Apoptosis: controlled demolition at the cellular level. Nature reviews Molecular Cell Biology, 9(3), 231-241.

Tellapuri, S., Sutphin, P. D., Beg, M. S., Singal, A. G., & Kalva, S. P. (2018). Staging systems of hepatocellular carcinoma: a review. Indian Journal of Gastroenterology, 37(6), 481-491.

Thorpe, L. M., Yuzugullu, H., & Zhao, J. J. (2015). PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. Nature Reviews Cancer, 15(1), 7-24.

Tomazic, S. B., Schatz, C., & Haybaeck, J. (2021). Translational regulation in hepatocellular carcinogenesis. Drug Design, Development and Therapy, 15, 4359.

Tsilimigras, D. I., Brodt, P., Clavien, P. A., Muschel, R. J., D'Angelica, M. I., Endo, I., Parks, R.W., Doyle, M., de Santibañes, E. and Pawlik, T.M. (2021). Liver metastases. Nature Reviews Disease Primers, 7(1), 27.

Tzartzeva, K., Obi, J., Rich, N. E., Parikh, N. D., Marrero, J. A., Yopp, A., Waljee, A.K. and Singal, A.G. (2018). Surveillance imaging and alpha fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. Gastroenterology, 154(6), 1706-1718.

Valastyan, S., & Weinberg, R. A. (2011). Tumor metastasis: molecular insights and evolving paradigms. Cell, 147(2), 275-292.

Wang, B., Xian, J., Zang, J., Xiao, L., Li, Y., Sha, M., & Shen, M. (2019). Long non-coding RNA FENDRR inhibits proliferation and invasion of hepatocellular carcinoma by down-regulating glypican-3 expression. Biochemical and Biophysical Research Communications, 509(1), 143-147.

Wang, C., Cigliano, A., Jiang, L., Li, X., Fan, B., Pilo, M. G., Liu, Y., Gui, B., Sini, M., Smith, J.W. and Dombrowski, F. (2015). 4EBP1/eIF4E and p70S6K/RPS6 axes play critical and distinct roles in hepatocarcinogenesis driven by AKT and N-Ras proto-oncogenes in mice. Hepatology, 61(1), 200-213.

Wong, C. C. L., Kai, A. K. L., & Ng, I. O. L. (2014). The impact of hypoxia in hepatocellular carcinoma metastasis. Frontiers of Medicine, 8(1), 33-41.

Wong, K. K., Engelman, J. A., & Cantley, L. C. (2010). Targeting the PI3K signaling pathway in cancer. Current Opinion in Genetics & Development, 20(1), 87-90.

Wu, Y., Liu, H., Weng, H., Zhang, X., Li, P., Fan, C. L., Li, B., Dong, P.L., Li, L., Dooley, S. and Ding, H.G. (2015). Glypican-3 promotes epithelial-

mesenchymal transition of hepatocellular carcinoma cells through ERK signaling pathway. International Journal of Oncology, 46(3), 1275-1285.

Xie, C., Li, S. Y., Fang, J. H., Zhu, Y., & Yang, J. E. (2021). Functional long non-coding RNAs in hepatocellular carcinoma. Cancer Letters, 500, 281-291.

Xue, J., Gao, H. X., Sang, W., Cui, W. L., Liu, M., Zhao, Y., Wang, M.B., Wang, Q. and Zhang, W. (2019). Identification of core differentially methylated genes in glioma. Oncology Letters, 18(6), 6033-6045.

Yang, J., Nie, J., Ma, X., Wei, Y., Peng, Y., & Wei, X. (2019). Targeting PI3K in cancer: mechanisms and advances in clinical trials. Molecular Cancer, 18(1), 26.

Yim, H. J., Suh, S. J., & Um, S. H. (2015). Current management of hepatocellular carcinoma: an Eastern perspective. World Journal of Gastroenterology: WJG, 21(13), 3826.

Yuan, S. X., Tao, Q. F., Wang, J., Yang, F., Liu, L., Wang, L. L., Zhang, J., Yang, Y., Liu, H., Wang, F. and Sun, S.H. (2014). Antisense long non-coding RNA PCNA-AS1 promotes tumor growth by regulating proliferating cell nuclear antigen in hepatocellular carcinoma. Cancer Letters, 349(1), 87-94.

Zhang, H., Zhang, W., Jiang, L., & Chen, Y. (2022). Recent advances in systemic therapy for hepatocellular carcinoma. Biomarker Research, 10(1), 3.

Zhang, Y., Xu, J., Zhang, S., An, J., Zhang, J., Huang, J., & Jin, Y. (2018). HOXA-AS2 promotes proliferation and induces epithelial-mesenchymal transition via the miR-520c-3p/GPC3 axis in hepatocellular carcinoma. Cellular Physiology and Biochemistry, 50(6), 2124-2138.

Zucman-Rossi, J., Villanueva, A., Nault, J. C., & Llovet, J. M. (2015). Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology, 149(5), 1226-1239.