C H A P T E R

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Successful stories of drug repurposing for cancer therapy in hepatocellular carcinoma

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O U T L I N E

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a major health problem with devastating consequences associated with treatment failure. It has an estimated global incidence of more than 850,000 new cases annually [1]. HCC is currently the second leading cause of cancer-related death worldwide and accounts for 90% of cases with primary liver cancer [2]. Several risk factors contribute to HCC development such as liver cirrhosis, viral hepatitis including hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, fatty liver, and alcohol abuse. Smoking and the fungal carcinogen, aflatoxin B1, are also well-known contributors to HCC [3]. Recent advances in HBV vaccination and antiviral therapeutics have remarkably contributed to a decrease in incidence. However, nonalcoholic fatty liver disease (NAFLD) and its progressive form, nonalcoholic steatohepatitis (NASH), at its current pace of growing prevalence approaching epidemic proportions is projecting as the most common underlying etiology of HCC, presented in almost 60% of cases, which made NAFLD-associated HCC an emerging indication for liver transplantation [4−6]. Additionally, HCC has a notable gender predilection where incidence in men is threefold that in women [1,7].

Molecular pathogenesis of HCC

Over the past decade, substantial progress has been achieved in understanding how HCC develops and progresses in an attempt to improve treatment options [8]. HCC is a very heterogeneous disease in terms of both phenotype and genotype. This heterogeneity could be attributed, in part, to the divergent nature of the contributing factors, the complexity of the liver microenvironment, and the stage at which HCC turns to be clinically evident/detectable.

Malignant transformations in liver cells are driven by several factors such as chronic injury or inflammation due to oxidative stress that may lead to genetic and epigenetic modification. These modifications consequently lead to disrupted cellular signaling pathways leading to an overexpression in several growth factors and their receptors. This inevitably results in cell resistance to apoptotic signals, stimulation of angiogenesis, and uncontrollable proliferation besides the acquisition of a metastatic phenotype [9,10].

Since HCC almost exclusively develops in patients with chronic liver diseases, injury of liver cells can promote the progression to HCC over a long period of time [11] driven by a number of cytokines and inflammatory mediators along with aberrant activity of several signaling pathways, as shown in Fig. 8.1 (will be discussed later).

Therapeutic management of HCC

HCC mostly exhibits resistance to conventional chemotherapy. Besides, patients with HCC are usually intolerant to treatment due to an underlying hepatic dysfunction.
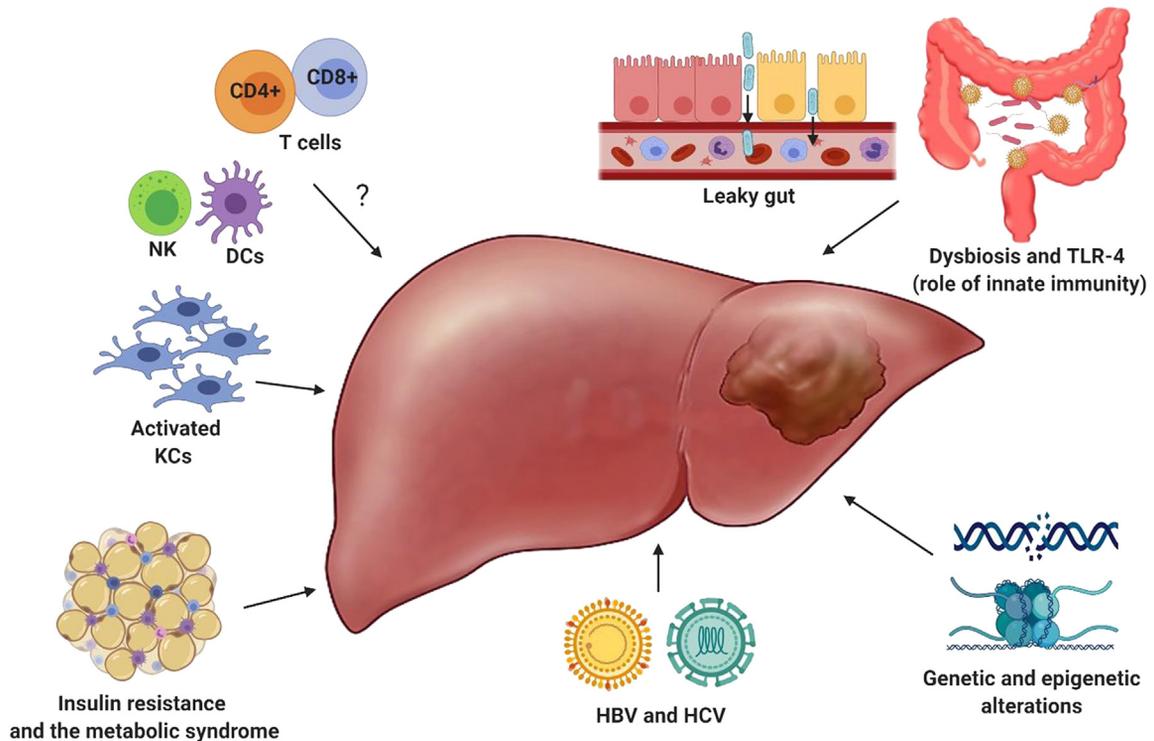
FIGURE 8.1   Proposed mechanisms for the progression of HCC. These mechanisms include viral infections by hepatitis B and C viruses (HBV and HCV), genetic and epigenetic alterations, dysbiosis and leaky gut, role of innate immunity through Toll-like receptors-4 (TLR4) and Kupffer cells (KCs), metabolic syndrome and its hepatic manifestation (nonalcoholic fatty liver disease), and adaptive immune cells (CD4+ and CD8+ T cells) whose role is still quite debatable. Also, dendritic cells (DCs) and natural killer T cells (NK T cells) bridge innate with adaptive immune systems.

However, almost half of HCC patients still receive chemotherapy at some point during the course of the disease [12,13]. Chemotherapeutic intervention mainly relies on the multitargeted tyrosine kinase, sorafenib, that acts by effectively blocking the Ras/Raf/MAPK pathway impeding cancer cell ability to circumvent apoptotic signals and induce angiogenesis, proliferation, and invasion. Sorafenib was found to extend the median survival in advanced HCC patients for up to 3 months with manageable adverse effects; however, it lacks predictive biomarkers to reflect on responsiveness [14]. Sorafenib remained the only approved systemic treatment for HCC between 2007 and 2016. Yet, promising outcomes were reported in randomized phase III trials using other multiple kinase inhibitors such as lenvatinib [15], regorafenib [16], cabozantinib [17], and ramucirumab [18,19], where regorafenib has received FDA approval in the second-line setting. Moreover, nivolumab, a monoclonal immunotherapy-based antibody targeting the immune checkpoint programmed cell death protein 1, showed also positive response rates and mean overall survival durations in a phase I—II trials performed on patients who were formerly treated with sorafenib [20] for which it has been successfully granted an accelerated FDA approval. On the other hand,

several other kinase inhibitors such as sunitinib and erlotinib failed to show comparable improved survival rates especially in unrespectable HCC patients [21].

Sorafenib efficacy over other proposed interventions is most likely attributed to its ability to target several molecules and pathways in tumor cells as well as the microenvironment. Also, the heterogeneous nature of HCC may limit the efficacy of other targeted therapeutic approaches possessing higher selectivity [22].

HCC is undoubtedly a resistant type of cancer making treatment more challenging. Despite the fact that systemic therapy enhanced survival rates in HCC patients, therapeutic outcomes are still incremental and inadequate, especially if compared to other types of cancer. Sorafenib was the first FDA-approved drug for treatment of patients with advanced HCC for its ability to increase the median overall survival. However, with the use of the newly developed and approved multiple kinase inhibitors, the median overall survival still remains almost 1 year. Moreover, de novo resistance developing to sorafenib has been recently heavily reported impeding its beneficial clinical applications [23—26]. Resistance to sorafenib involves a cross talk between several pathways such as Janus kinase/signal transducer and activator of transcription 3 (STAT3), phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR), and hypoxia-inducible pathways beside others [27]. Providing new treatment options for HCC, therefore, still remains an unmet medical need. Accordingly, further insights into the molecular targets affiliated with the pathophysiology of HCC will be dissected in the following section.

HCC drivers and affiliated molecular targets

To date, we still have very few identified "druggable" drivers for HCC. However, most of the preclinical and clinical attempts to identify pharmacological interventions, whether new or repositioned ones, focused on signaling pathways implicated in disease progression. Hence, investigating effect on oncogenic drivers for the identification of new molecular targeted therapies in HCC needs to be revisited. In order to provide pharmacological interventions for HCC, the main drivers and pathways involved have to be identified first. As it is the case with solid tumors, a simultaneous alteration in at least three signaling pathways and five to eight driver genes should take place for HCC to develop [28].

The main drivers and pathways contributing to HCC development and progression will be discussed in the following section in more details. These include, but not limited to, Ras/Raf/MAPK, PI3K/AKT/mTOR, Wnt/β-catenin, hedgehog (Hh), and IL-6/STAT3 pathways. Moreover, the role of the following key players will be explored: tumor necrosis factor-alpha (TNF-α), nuclear factor kappa-B (NF-κB), c-Jun N-terminal kinase (JNK), gut microbiome, and toll-like receptors and also adaptive immunity. All these molecular targets and signaling pathways represent potential candidates for targeting HCC, as shown in Fig. 8.2.

Ras/Raf/MAPK pathway

Among the most extensively investigated pathways implicated in HCC development and progression is the Ras/Raf/MAPK pathway. Cell surface receptor tyrosine kinases such as insulin-like growth factor (IGF) receptor, endothelial and vascular epidermal growth factor receptor (EGFR), c-Met, and platelet-derived growth factor receptor send signals that are transmitted to the nucleus via this pathway to control cell survival, growth, and differentiation. Upregulation of the Ras/Raf/MAPK pathway in liver cells induces cell growth augmenting antiapoptotic signals leading ultimately to HCC [29]. Aberrant upstream IGF and EGFR signaling, suppression of Raf kinase inhibitor protein,
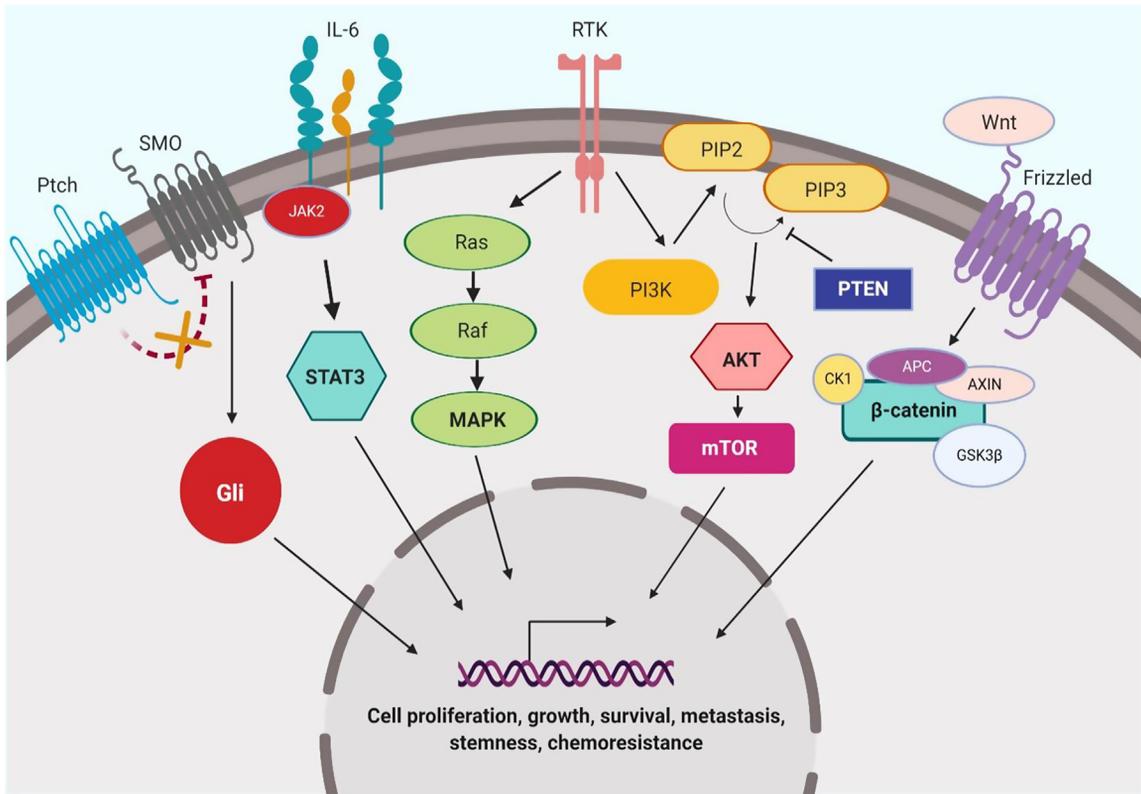
FIGURE 8.2 Molecular targets and pathways affiliated with HCC. Ras/Raf/MAPK, IL-6/STAT3,Wnt/β-catenin, PI3K/ AKT/mTOR, and hedgehog signaling pathways are among the most important pathways implicated in HCC, which are presented as potential targets for treatment. *APC*, adenomatosis polyposis coli; *CK1*, caseinkinase 1; *JAK2*, janus kinase-2; *mTOR*, mammalian target of rapamycin; *PI3K*, phosphoinositide3-kinases; *PIP2*, phosphatidylinositol 4,5-bisphosphate; *PIP3*, phosphatidylinositol (3,4,5)-trisphosphate; *RTK*, receptor tyrosine kinase; *STAT3*, signal transducer and activator of transcription.

and HBV- and HCV-related proteins induction are among the mechanisms by which the Ras/ Raf/MAPK pathway can be activated toward HCC development [30].

PI3K/AKT/mTOR pathway

Mounting evidence over the past years suggested the aberrant upregulation of PI3K/ AKT/mTOR pathway in HCC where mTOR phosphorylation and a subsequent upregulation of its downstream effector, p70S6k, were reported in almost 50% of HCC patients [31]. Likewise, almost 40% of patients encountered an

activated mTOR [32]. Aberrant PI3K/Akt/ mTOR pathway was also correlated with poor prognosis, especially in advanced-stage HCC [33]. The underlying mechanism by which PI3K/AKT/mTOR pathway is activated in HCC is not yet fully deciphered, yet overly expressed upstream IGF, c-Met, or EGFR is likely [34–36]. Hepatitis viral infections are also capable of activating PI3K/AKT/mTOR pathway in liver [37], where HCV infection was found to increase neuroblastoma (N)-Ras expression in HCC, which turns on the PI3K/AKT/mTOR pathway [38]. Loss of *PTEN*, a tumor suppressor gene, by

mutations was also evident in HCC patients [39], contributing to AKT activation. Apart from *PTEN*, mutations in PI3K catalytic subunits were also reported [40]. Based on the previously presented sound evidence from previous studies, different agents that target the main key players of this pathway such as PI3K, AKT, or mTOR are being tested on HCC animal models and others currently enrolled in clinical trials [41,42].

Wnt/β-catenin pathway

The Wnt pathway comprises a canonical or β-catenin—dependent pathway as well as a non-canonical or β-catenin—independent one. The classic or canonical pathway consists of the Wnt protein and Wnt protein ligand, also known as frizzled protein, besides other proteins such as β-catenin and glycogen synthase kinase 3 [12,43]. Aberrant activation of Wnt/β-catenin pathway was found to support tumor growth. When the upstream Wnt protein complexes with its ligand, β-catenin builds up in cells and gets transferred to the nucleus where it dimerizes with LEF/TCF, a downstream transcription factor that regulates the transcription of other genes such as cyclin D [44]. Abnormal upregulation of Wnt/β-catenin pathway was linked to HCC development and cancer stem cell maintenance. Aberrant β-catenin was detected in almost 90% of liver cancers [12].

Hedgehog pathway

Hh signaling pathway is among the most important pathways implicated in HCC. It consists of Hh ligand, Ptch and Smo (two transmembrane receptors), and Glib nuclear transcription factor along with downstream genes. Once activated, Hh ligands bind to Ptch receptors blocking Ptch inhibitory effect on Smo. Smo then moves to the cytoplasm activating Gli, to induce specific genes upregulation, thereby controlling growth and division. The Hh pathway is rarely activated in normal liver cells; however, in HCC, it is abnormally active [45,46].

In HCC, the inhibition of the Gli2 gene can also cause Bcl-2 and c-Myc downregulation, while increasing the expression of p27, turning off the cell cycle, and thus impeding tumor growth [47].

NF-κB and JNK

TNF-α is a pivotal protumorigenic cytokine as it is capable of stimulating both NF-κB and JNK signaling pathways [48,49]. It is currently widely believed that inflammation is the fuel that feeds the genetic aberration sparks that inaugurate the tumorigenic process. Moreover, the wide spectrum of chronic injury etiologies in the liver can sufficiently act as both initiators and promoters of HCC. In this last decade, several inflammatory mediators were identified and their roles were extensively elucidated in the pathogenesis of chronic liver disease [50]. Most of these inflammatory mediators act as activators or targets for NF-κB [51,52]. NF-κB is considered a key transcriptional factor for almost all chronic liver diseases such as viral hepatitis, alcoholic liver disease, and NASH [53—57]. It also finely tunes crucial functions in liver cells such as Kupffer cells (KCs; hepatic resident macrophages) and hepatic stellate cells (HSCs). Inhibition of NF-κB—related signaling cascades, however, may lead to liver fibrosis and tumorigenesis and that is why NF-κB is considered very essential in maintaining homeostasis and wound-healing processes in the liver [58]. Accordingly, NF-κB is thought of as a two-edged sword since inhibition may be sometimes beneficial; however, it may also influence other essential processes pertaining to liver homeostasis.

Regarding JNK, two isoforms are expressed in liver cells, namely, JNK1 and JNK2, where the former being linked to carcinogenesis. JNK1 protumorigenic potential is defined by its ability to induce the proliferative potential of HCC [28]. It also regulates the proliferation of HCC cells via downregulation of p21 and upregulation.of c-Myc [59]. Moreover, apoptosis induced by caspase-8 was found to activate JNK and hence

promote proliferation of liver cells [60]. These findings propose that hepatocyte apoptosis is what triggers JNK activation. Although, the role of JNK in HCC is controversial, JNK1 and 2 knockout attenuated HCC in an animal model of DEN, exhibited p21 upregulation, and decreased c-Myc [61]. Overall, TNFα, NF-κB, and JNK pathways can either have prosurvival or proapoptotic potentials augmenting HCC proliferation and growth. Thus, treatment approaches involving NF-κB and JNK inhibition should act moderately to avoid a complete shutdown in hepatocytes leading to liver injury and subsequent HCC.

IL-6/STAT3 pathway

IL-6, as a mediator of STAT3 activation, is an essential driver of liver cell proliferation, which may consequently lead to HCC [62]. Moreover, overactive STAT3 coinciding with high levels of IL-6 was found in patients with HCC [63]. Likewise, STAT3 upregulation had promoted DEN-induced HCC experimentally [64]. IL-6 autocrine production is essential for malignant transformations in HCC, which once develops, IL-6 paracrine production from KCs start initiating growth and proliferation of HCC cells [65]. Activation of STAT3 may also take place via IL-22, produced by Th17 cells, that is overly expressed in HCC patients. Similar to IL-6, IL-22 also plays a role in promoting DEN-induced HCC in mice via STAT3 pathway [66]. Besides IL-6 and IL-22, IL-17 can also activate STAT3. This action, however, is IL-6-dependent [67] Collectively, these findings propose a role for IL-6/STAT3 pathway in promoting hepatic carcinogenesis suggesting it as a potential therapeutic target for treatment of HCC.

Innate and adaptive immunity

Overwhelming evidence from the past few decades have underlined the role of dysbiosis in the development of chronic liver disease and HCC. Moreover, the role of activating the innate immune system—related receptors in general and toll-like receptors (TLRs) in particular, in HCC development, and progression was also explored [68]. Due to its anatomical location, the liver is considered the first organ exposed to gut-derived microbial products translocated through the portal vein that in turn leads to TLR activation in the liver [68]. TLR-4 is expressed in different liver cells such as KCs, HSCs, and hepatocytes. It was previously reported that activation of TLR-4 on liver cells by bacterial lipopolysaccharides, a gram-negative cell wall component, leads to subsequent fibrotic and carcinogenic events [69]. The immune surveillance hypothesis suggests that the immune system may protect against nascent tumors by destroying malignant cells early on, before they develop into detectable tumors. The T-cell antitumor role is facilitated through immune surveillance by CD4+ and CD8+ T cells. Interestingly, an animal model of NASH-associated HCC was found to cause a depletion of CD4+ T cells impairing immune surveillance [70]. Other studies, however, reported a protumorigenic potential of CD8+ T, natural killer T cells, and T-helper (Th) 17 cells in an animal model of choline-deficient high fat diet [71]. Yet still debatable, these findings suggest a role for adaptive immunity in HCC development and progression. Thus, targeting it in the appropriate context may offer a chemopreventive strategy for HCC patients.

Drug repositioning: drug discovery tool

Drug discovery process involves multidisciplinary integrating experiences starting with in silico computational modeling to design scaffolds of interest based on the affiliated therapeutic molecular targets to different clinical diseases. Typically, this is followed by synthetic methodologies optimization to assemble final ligands ready for biological evaluation [72]. Preclinical biological evaluations involving in vitro and

in vivo assessments create significant portion for getting the potential ligands to be considered as potential drug candidates or active pharmaceutical ingredients; however, there are different discovery stories that failed to make it into the market due to intellectual property, efficacy, safety, toxicity, biopharmaceutical compatibility, cost/benefit feasibility, regulatory affairs, etc. The whole discovery process typically takes 10—15 years to get a drug into the market at a cost close to 1 billion US dollars.

In the last decade (2009—19), drug repurposing/repositioning approach has been an emerging drug discovery tool to overcome the associated challenges faced by the pharmaceutical industry. Conceptually, the whole drug—receptor binding theory focuses on two approaches, whether key-lock theory or induced-fit theory [73], where most of the pharmaceutical ingredients exhibit on-target effects targeting receptors/proteins of interest in a drug positioning fashion, as shown in Fig. 8.3.

Therefore, existing drugs might show potential binding interactions along with other molecular targets, where drug repositioning shows off-target effects to pronounce newly alternative therapeutic applications. Drug repositioning offered excellent opportunity to offer more pharmaceutical candidates to the market with established postmarketing surveillance safety data, toxicity, and pharmacokinetics profile [74]. This approach was adopted by the industrial, funding, and academic bodies to evaluate assets on the shelves by having a trilateral funding mechanism integrating universities along with pharmaceutical industry through National Institute of Health to support preclinical/clinical investigations for already existing drugs/assets with established efficacy and safety for novel clinical applications. Most recently, the outbreak of the new strain of COVID-19 virus has prompted the researchers to adopt the repurposing strategy to offer rapid therapeutic regimens targeting polymerase, protease, or spike protein.
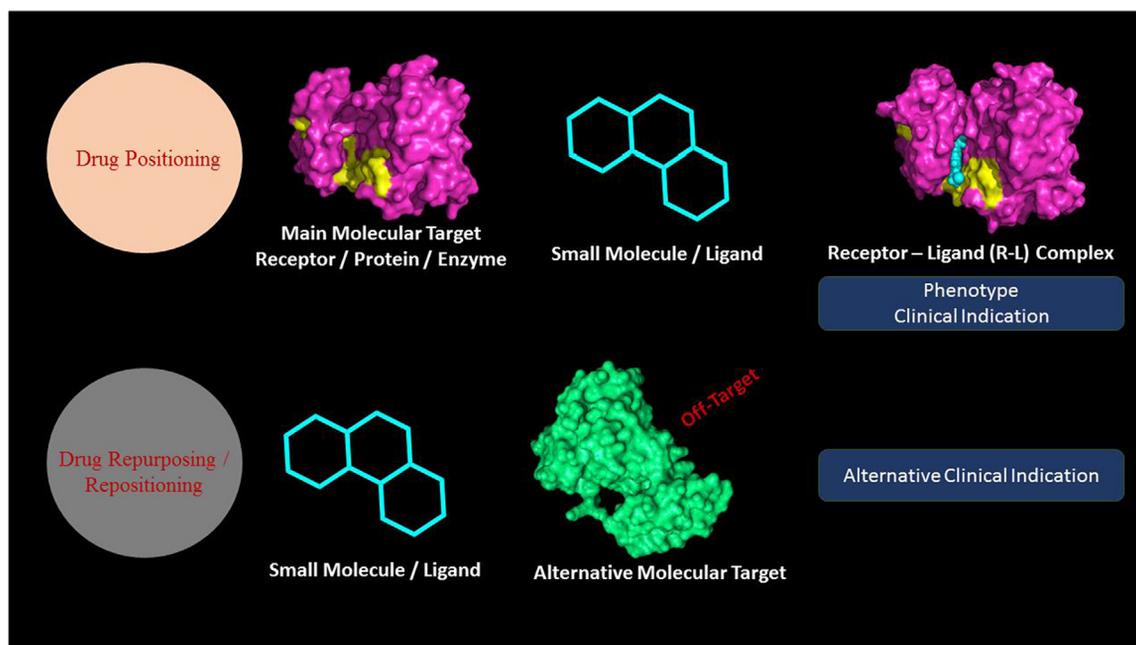

FIGURE 8.3   Drug positioning approach, where the receptor—ligand complex is known for certain clinical indication. Drug epositioning approach, where already existing molecule is repurposed for alternative molecular target (off-target mechanism) whether structurally or biochemically to reveal alternative therapeutic application.

Drug repositioning strategies development

The biological system is complicated enough to repurpose different pharmaceutical drugs for various clinical indications blindly based on off-targets screening, adverse effects reporting, serendipity, or clinical trials. In this section, there is a general chronological highlight for the drug development strategies development, as shown in Fig. 8.4. The whole arise of the drug repositioning integration started with **blinded serendipity—based repositioning** stories, such as sildenafil, minoxidil, and everolimus [75,76]. Consequently, the strategic progress led to **disease network—based repositioning** where certain diseases share same histological, pathological, pharmacological, or biochemical phenotypes; this certainly could be potential opportunity for testing the already established drugs for identified diseases to be evaluated for other different diseases having the same histopathological/biochemical features. The advancement of biological sciences made a huge transition from -ology era to -omics era; consequently, this showed huge impact on the transformation of the drug repositioning strategy; where the integration of proteomics, metabolomics, transcriptomics, and genomics heavily participated in the evolution of the drug repositioning. Ultimately, this gave the opportunity for the **high-throughput screening—based repositioning** to evolve; where the phenotypic assessment using cell lines, fluorescence assays, or enzyme kinetics and the genomic/proteomic-based assays by analyzing multigenomics profiling, mRNA, and protein sequencing offered molecular signature for different molecular targets [77]. This strategy helped potential drugs, such as metformin, digoxin, and statins to be repurposed for different types of cancer. The integration of computational tools has the adequate
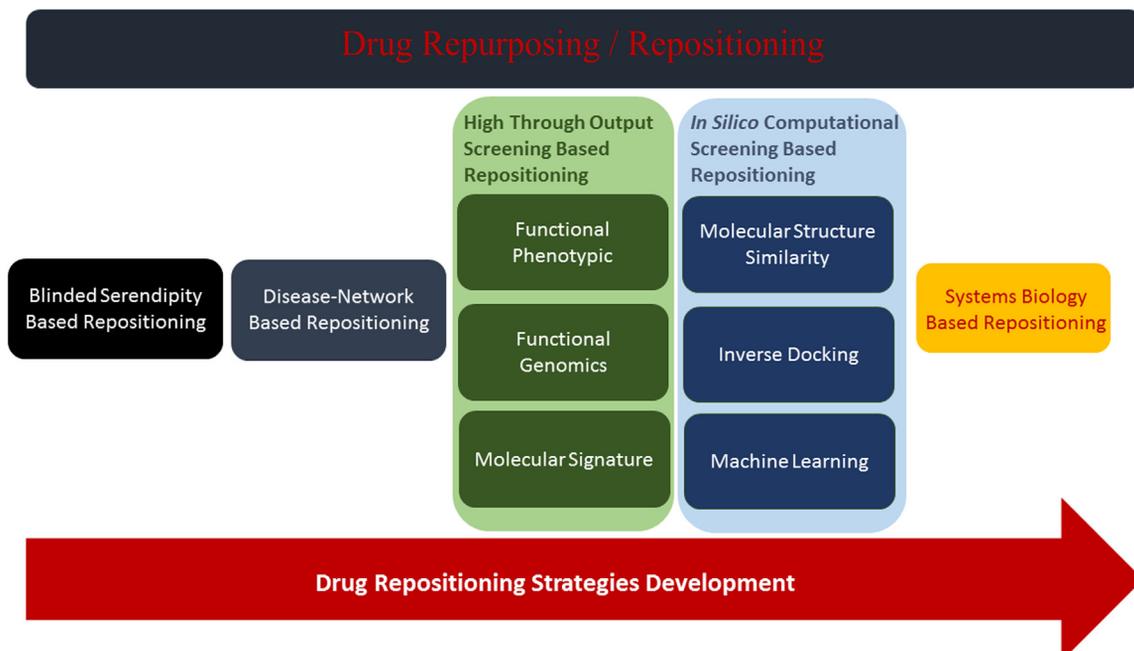

FIGURE 8.4 Drug repositioning strategies development categories.

share for the advancement and validation of the drug repositioning strategy to evolve in silico **computational screening—based repositioning** [72]. This strategy helped the repositioning of nonsteroidal antiinflammatory drugs and proton-pump inhibitors for different types of cancer [78,79]. The main in silico screening involves different tactics:

(1) Molecular structure similarity assessment using Jaccard index or Tanimoto coefficient calculations.
(2) Molecular docking to validate the drug—receptor(s) interactions with respect to binding mode/energy with respect to enthalpy and entropy.
(3) Dealing with huge consortium of data on the molecular and structural levels applying artificial intelligence network to create algorithm connecting the structural input along with molecular output.

The association of omics-based repositioning coupled with computational-based repositioning led to the appearance of **systems biology— based repositioning**, for instance, digoxin was repurposed for medulloblastoma [76].

Drug repositioning stories for hepatocellular carcinoma

In this chapter, the authors will focus on drug repositioning stories to target HCC, showing the affiliated molecular targets identifying the repurposing strategy, as shown in Table 8.1. The complexity of HCC leads to multiple mutations or malfunction of different receptors, proteins, and molecular targets.

Centrally acting nervous system drugs

Pimozide

Pimozide is an antipsychotic agent that showed potential inhibitory in vitro effect against cell proliferation of HCC cell lines via induction of apoptosis at G0/G1 phase. In addition, pimozide exhibited inhibitory profile for HCC stem-like cells, particularly the CD133-positive cells side population. Pimozide was found to target STAT3 expression via luciferase assay activity along with downregulation of the transcription levels of downstream oncogenes for STAT3 signaling. The antitumor activity for pimozide was further validated in vivo in nude mice [80].

Valproate

Valproic acid (VPA), a potent and specific histone deacetylase (HDAC) inhibitor, is widely used antiepileptic drug. HDAC has been recognized for its significant role as identified target with known molecular signature in the progression of different types of cancer. The mono- and adjuvant therapeutic in vitro effects of VPA and doxorubicin (DOX) managed to identify specific, efficient, and antiproliferative profiles of VPA and DOX combination against HepG2 cell line in a synergistic manner. Molecular levels of caspase-3 and poly (ADP-ribose) polymerase (PARP) activation validated the synergism caused by VPA and DOX to induce apoptosis. Dual treatment of VPA and DOX increased the levels of reactive oxygen species (ROS) [81].

Anthelmintic drugs

Niclosamide ethanolamine

The antitumor activity of niclosamide and its ethanolamine salt (NEN) were discovered by computational identification of drugs that can increase the mRNA expression of downregulated genes in HCC and reduce the mRNA expression of the upregulated ones. This was followed by in vitro evaluation to show the antiproliferative activity of niclosamide and NEN in various HCC cell lines and primary human hepatocytes. This was further validated by in vivo evaluation against two mouse models

TABLE 8.1 List of repurposed drugs targeting hepatocellular carcinoma.

Drug	Original therapeutic indication	HCC molecular target	Repositioning strategy	Investigation status
Pimozide	Antipsychotic	STAT3	Blinded screening	In vivo
Valproate	Antiepileptic	HDAC	Molecular signature	In vitro
Niclosamide ethanolamine	Anthelmintic	STAT3	Systems biology	In vivo
Amiodarone	Antiarrhythmic	mTOR	Disease network	In vivo preclinical
Lanatoside C	Antiarrhythmic	MMP, PKCδ, AIF	Systems biology	In vivo
Simvastatin	Antihypercholesterolemia	AMPK, STAT3	Phenotype screening Artificial intelligence	In vivo
Guanabenz acetate	Antihypertensive	DNA damage-inducible, p34, eukaryotic initiation factor 2α	Phenotype screening	In vitro
Fenofibrate	Antihypercholesterolemia	PPARa, AKT, CTMP	Serendipity	In vitro
Metformin	Oral hypoglycemic	KLF6/p21, AMPK	Systems biology	In vitro
Canagliflozin	Oral hypoglycemic	ERK, p38, AKT	Blinded screening	In vivo
Linagliptin	Oral hypoglycemic	ADORA3	Structure-based docking	In vitro
Atovaquone	Pneumonia	DNA double-stranded breaks	Blinded screening Phenotype screening	In vivo
Ketoconazole	Antifungal	PTGS2	Disease network Phenotype screening	In vivo
Obeticholic acid	Primary biliary cholangitis	IL-6/STAT3 pathway	Blinded screening Phenotype screening	In vivo

(genetically induced liver tumors and patient-derived xenografts [PDXs]) for HCC to show significant reduction in the tumor growth after oral administration of NEN compared to niclosamide. The dual administration of NEN and sorafenib also showed significant improvement to reduce the progression of PDX compared to either sole drug treatment. In HepG2 cell lines and PDX models, administration of niclosamide or NEN showed unique molecular signature in terms of gene expression compared to HCC molecular signature. Administration of NEN to PDX model reduced expression of proteins in the Wnt/β-catenin, STAT3, AKT/mTOR EGFR/Ras/Raf signaling pathways, and disruptive interactions along with heat shock protein 90 [82].

Cardiovascular system-acting drugs

Amiodarone

Amiodarone, a class III antiarrhythmic agent and a potent mTOR inhibitor, was found to suppress liver tumor formation through induction of autophagy activity in the rat orthotropic model and in the mouse xenograft model. Furthermore, a big data analysis of 32,625 case−control provided by Taiwan's National Health Insurance

program revealed that long-term regular amiodarone usage significantly decreases the risk of HCC. Amiodarone, as a repurposed drug, has antitumor potential to suppress liver tumor formation and prevent HCC incidence through induction of the autophagy activity [83].

Lanatoside C

Lanatoside C is an antiarrhythmic agent, naturally occurring compound extracted from *Digitalis lanata*. Integration of systems biology repositioning approach showed the potential of lanatoside C to tackle HCC at the in vitro and in vivo levels with significant reduction in tumor growth. The molecular mechanistic investigation revealed the ability of lanatoside C to trigger mitochondrial membrane potential (MMP) loss, followed by activation of apoptotic markers to induce cell death. Inhibition of Thr505 phosphorylation for protein kinase delta (PKCδ) reversed lanatoside C—induced MMP loss and apoptosis validating the molecular mechanism for lanatoside C, where AKT/mTOR pathway is involved via modulation of PKCδ activation [84].

Statins (simvastatin)

Statins are HMG-CoA reductase inhibitors, to intervene with the cholesterol synthesis via inhibiting mevalonate pathways targeting different cardiovascular system complications. Statins were identified as potential drugs tackling various types of cancer, including breast cancer, prostate, head, and HCC using high-throughput activity—based screens of disease phenotypes and in silico data-driven algorithms. Simvastatin induced cell cycle arrest at G0/G1 phase accompanied by series of molecular changes via promoting AMP-activated protein kinase (AMPK), leading to induction of p21 and p27 accumulation. Using the transcriptomics, simvastatin showed significant reduction in the Skp2 expression, resulting in p27 accumulation by preventing proteasomal degradation, mediated by STAT3 inhibition. In addition, simvastatin significantly decreased tumor growth in HepG2 xenograft mice [85].

Guanabenz acetate

Guanabenz acetate, an antihypertensive drug, was screened as a potential candidate for HCC via a phenotypic screening assay by in vitro antiproliferative activity against HCC cell lines using high-throughput screening—based repositioning approach. Guanabenz acetate reduced HCC cell viability via inhibition of growth and induction of DNA damage, leading to increased phosphorylation of eukaryotic initiation factor 2α, increased activation of transcription factor 4, and induction of apoptosis [86].

Fenofibrate

Fenofibrate is known for its lipid lowering capability; however, it has shown huge potential to target various types of cancer recently. Fenofibrate showed significant reduction in the viability of human HepG2 cells via necrosis, but not apoptosis. The mechanistic investigation for the fenofibrate revealed an increase in the levels of ROS and a decrease in glutathione (GSH, an important cellular antioxidant) accompanied with impairment for the mitochondrial function in HepG2 cell lines. In other studies, fenofibrate showed cell cycle arrest for Huh-7 cell lines at G2/M phase through downregulation of cyclins group with upregulation of p27. Fenofibrate also activated endogenous peroxisome proliferator-activated receptor (PPAR)α in Huh7, HepG2, and Li7 cell lines, but the antiproliferative activity induced by fenofibrate was not affected by the PPARα inhibitor GW6471 or the knockdown of the expression of PPARα by siRNA. Moreover, fenofibrate suppressed AKT phosphorylation and increased the expression of C-terminal modulator protein, which binds specifically to AKT [87,88].

Oral hypoglycemic drugs

Metformin

Metformin is the drug of choice toward treatment of type II diabetes. The in vitro antiproliferative activity for metformin showed multiple

phenotypic events starting with induction of cell cycle arrest at G0/G12 phase accompanied with significant reduction in cell growth with elevated levels of KLF6/p21 protein content in HepG-2 cell line. Metformin played a unique role via modulating the microenvironment of tumors by reducing cellular lipid accumulation and promoting AMPK activity. Metformin downregulated the expression of IGF I and II [89].

Canagliflozin

Canagliflozin, a sodium—glucose cotransporter 2 inhibitor (SGLT2-I), is known as an antidiabetic agent. Canagliflozin was selected for the phenotypic screening based on the given fact of high expression levels of SGLT2 in HCC cell lines. Initially, the antiproliferative ability of canagliflozin against HCC cell lines was assessed to show inhibitory profile in a dose-dependent manner along with induction of apoptosis at G2/M phase with elevated levels of caspase-3 and inhibition of ERK. This suggested that canagliflozin can inhibit glycolytic metabolism including glucose uptake, lactate, and intracellular ATP production. This was validated by in vivo screening for tumor growth assay after oral administration of canagliflozin (10 mg/kg/day) to show significant reduction in the tumor size and attenuated intratumor vascularization in HepG2-derived xenograft tumors in BALB/c nude mice [90].

Linagliptin

Linagliptin is known as antidiabetic class targeting dipeptidyl peptidase-4, where the scaffold of 3,7 dihydro-1H-purine-2,6-dione functional group was an attractive scaffold to be structurally repositioned in a trial to target HCC by modulation of adenosine 3 receptors (ADORA3) via in silico molecular modeling simulations coupled with in vitro analysis. Linagliptin and its degradation product showed antiproliferative activity against HepG-2 and Huh-7 cell lines inducing cell cycle arrest at G2/M phase with elevated levels of apoptotic

marker, caspase-3. This was validated by monitoring the expression levels of ADORA3 [91].

Respiratory system targeting drugs

Atovaquone

Atovaquone, an FDA-approved drug for pneumocystis pneumonia, significantly inhibited hepatoma cell proliferation via S phase cell cycle arrest and induced both extrinsic and intrinsic apoptotic pathways associated with upregulation of p53 and p21. Molecular investigations demonstrated that atovaquone inhibits hepatoma cell proliferation by inducing double-stranded DNA breaks, leading to sustained activation of ataxia telangiectasia mutated and its downstream molecules such as cell cycle checkpoint kinase-2 and H2AX. In addition, atovaquone also induced apoptosis, inhibited both cell proliferation and angiogenesis in vivo, and prolonged the survival time of tumor-bearing mice, without any obvious side [92].

Others

Ketoconazole

Ketoconazole is a broad-spectrum antifungal agent, exhibiting antiproliferative activity against HCC cell lines by worsening mitophagy in vitro and in vivo. Ketoconazole-induced suppression of prostaglandin-endoperoxide synthase 2 led to PINK1-PRKN—mediated mitophagy, resulting in mitochondrial dysfunction and induction of apoptosis [93].

Obeticholic acid

Obeticholic acid (OCA) was granted accelerated approval from the FDA in 2016 for the treatment of primary biliary cholangitis. OCA is a synthetically modified bile acid agonist for the nuclear farnesoid X receptor (FXR). A cross talk between IL-6/STAT3 pathway and the hepatic FXR in HCC was previously reported [94]. In this study, activating FXR using OCA

interfered with HCC cell growth by downregulating IL-6/STAT3 pathway in vitro. These effects were hampered in the presence of an FXR antagonist.

Future opportunities and limitations

Drug repositioning has become a significant emerging shortcut approach to deliver chemical entities into the pharmaceutical market cutting cost with an eye on the established toxicity profile, pharmacokinetics behavior, and postmarketing safety surveillance. However, repurposing novel clinical indications for already existing molecules should not grab our attention from investing in assembly of novel chemical entities to different molecular targets for different pathological conditions. Conceptually, the repositioning approach should be good platform for novel scaffold repurposing to synthesize novel ligands and pharmacophores.

In this chapter, we have outlined the repositioning as an excellent opportunity to bypass the toxicity issues, where the FDA-approved drugs show clinical safety profiles including cardiac, hepatic, and metabolic safety profiles. The authors tried to cover the serious repurposing trials in that field, where most of the repositioned drugs for HCC were revealed via phenotypic screening or systems biology without rational approaches with respect to structural or ligand-based drug design. However, the literature is full of random repositioning studies without solid understanding for fundamental basics for in vitro/in vivo biological assessment coupled with in silico molecular modeling simulations with no revealing for molecular target validation. The authors believe that despite the huge efforts for drug repositioning targeting HCC, amiodarone can be considered as a potential therapeutic drug for HCC due to its repositioning strategy counting on disease network, where data analysis of 32,625 case—control provided by Taiwan's NHI program revealed that long-term regular amiodarone usage significantly decreases the risk of HCC. Technically, the integration of artificial intelligence to get business solutions, servers, and healthcare platforms, such as Watson developed by IBM, can create potential opportunity for drug repositioning to offer robust methodology driving more drugs to clinical trials for novel therapeutic applications.

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