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Intracellular Signaling and Hepatocellular Carcinoma

Polina Iakova, **Lubov Timchenko**[1], and **Nikolai A. Timchenko**[*]
Department of Pathology and Immunology and Huffington Center on Aging

[1]Department of Molecular Physiology and Biophysics and Huffington Center on Aging

Abstract

Liver cancer is the fifth most common cancer and the third most common cause of cancer related death in the world. The recent development of new techniques for the investigations of global change in the gene expression, signaling pathways and wide genome binding has provided novel information for the mechanisms underlying liver cancer progression. Although these studies identified gene expression signatures in hepatocelluar carcinoma, the early steps of the development of hepatocellular carcinomas (HCC) are not well understood. The development of HCC is a multistep process which includes the progressive alterations of gene expression leading to the increased proliferation and to liver cancer. This review summarizes recent progress in the identification of the key steps of the development of HCC with the focus on early events of carcinogenesis and on the role of translational and epigenetic alterations in the development of HCC. Quiescent stage of the liver is supported by several tumor suppressor proteins including p53, Rb and C/EBPα. Studies with chemical models of liver carcinogenesis and with human HCC have shown that the elevation of gankyrin is responsible for the elimination of these three proteins at early steps of carcinogenesis. Later stages of progression of the liver cancer are associated with alterations in many signaling pathways including translation which leads to epigenetic silencing/activation of many genes. Particularly, recent reports suggest a critical role of histone deacetylase 1, HDAC1, in the development of HCC through the interactions with transcription factors such as C/EBP family proteins.

Keywords

liver cancer; C/EBPα; p53; Rb; gankyrin

Introduction

The mechanisms of the liver cancer have been intensively investigated during last decade. These investigations provided huge amounts of the information showing global alterations of gene expression in HCC and alterations of certain signaling pathways. Several recent reviews have summarized the studies of the gene expression signatures [1-4]; therefore, our review mentions a small part of these findings which is related to the focus of the current review. A significant portion of studies of liver cancer was performed using well established

[*]Correspondent author: Nikolai A. Timchenko, Department of Pathology & Huffington Center on Aging, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, Tel: 713-798-1567, Fax: 713-798-4161, nikolait@bcm.tmc.edu.

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chemical carcinogenesis mediated by diethylnitrosamine/phenobarbital (DEN/PB) [5], mouse models of hepatocellular carcinoma [6, 7] and HCC samples from patients with liver cancer [1]. In DEN-mediated carcinogenesis, DEN (5ug/g body weight) is injected to young mice (15 day old) and the development of liver tumors is usually observed at 35 weeks [7]. The development of liver cancer by DEN treatment is associated with the cytochrome P450-medaited conversion of DEN into alkyl diazohydroxide (DNA alkylating agent) which interacts with DNA and causes DNA damage [8]. The DNA damage leads to the multistep alterations in expression of genes and in the development of HCC at 33-35 weeks after initiation of the protocol. In human liver, the major risk factors for the development of HCC are hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol and age [2, 9]. Interestingly, HCV, CBV and alcohol also cause DNA damage via activation of inflammation and reactive oxygen species, ROS [2].

Transgenic animal models of HCC

Although the investigations of chemically initiated liver cancer in rodents and studies of human HCC found several key events in the liver cancer, the generation of genetically engineered mouse models has revealed a causal role of individual proteins and pathways in development of liver cancer. These studies have been recently summarized in several reviews [6, 7]; therefore, we will mention briefly findings which are related to the focus of this review. It has been shown that transgenic mice (TR) expressing SV40 T-Ag from the liver specific promoters, such as albumin and α-1 antitrypsin, develop HCC at age of 8 months [10, 11]. Because SV40 T-Ag neutralizes the tumor suppressor genes p53 and Rb [12, 13], these data showed that the inactivation of the Rb and p53 is one of the key steps in the liver cancer. Generation of single transgenic mouse models for liver-specific expression of c-myc and TGFα showed that single TR mice develop liver cancer starting at 20 mo (c-myc, [14]) and 12 mo (TGFα, [15, 16]). However, the double c-myc /TGFα TR mice develop liver cancer at 8 mo [14] showing that activation of several pathways is required for the fast liver cancer progression. It has been also shown that liver specific double transgenic c-myc/ E2F1 mice develop liver cancer at age of 8 months [17, 18]. Thus, examinations of liver specific transgenic models clearly showed that the inactivation of Rb and p53 and activation of c-myc-TGFα and c-myc-E2F1 pathways are critical events in the development of HCC.

FXR knockout and C/EBPα-S193D knockin mouse models of HCC

The detailed description of animal models for HCC can be found in a recent review by Fausto and Campbell [6]. We will briefly discuss two recently generated animal models of HCC: FXR KO mice and C/EBPα-S193D knockin mice. Farnesoid X receptor (FXR) is a nuclear receptor which is highly expressed in liver and to less extend in small intestine, kidney, vascular smooth muscle and in adipose tissues [19, 20]. FXR might positively or negatively regulate expression of a number of genes depending on the interactions with co-activators or co-repressors. Studies from D. Moore's group have shown that FRX is required for liver proliferation after partial hepatectomy, PH [21]. Surprisingly, further work showed that FXR actually protects livers from development of HCC and that FXR knockout mice spontaneously develop liver tumor at age of 15-17 months [22, 23].

Transcription factor, CCAAT/Enhancer binding protein α, C/EBPα, is a strong inhibitor of liver proliferation [24-29]. Phosphorylation of C/EBPα at serine 193 (S193-ph) is required for the growth inhibitory activity of C/EBPα [29, 30]. The generation and analyses of C/EBPα-S193D mice showed that the phosphomimetic S193D isoform of C/EBPα completely inhibits liver proliferation after PH; however, the treatment of these mice with DEN/PB actually resulted in earlier development of liver tumors [31]. It has been shown that the development of liver cancer requires elimination of S193-ph and S193D isoforms of C/

EBPα by the ubiquitin-proteasome system, UPS [31]. Taken together, these recent findings showed that FXR and C/EBPα are tumor suppressor proteins and that the development of HCC requires elimination of these proteins. This review discusses mechanisms by which tumor suppressor proteins protect liver cancer and signaling pathways by which HCC neutralizes activities of p53, Rb and C/EBPα.

Loss of p53 is the main characteristic of hepatocellular carcinoma

P53 is one of the well recognized tumor suppressor genes. Numerous reports have shown that p53 is mutated or down-regulated in many human HCC [32, 33]. The molecular pathways by which p53 suppresses tumors include the inhibition of cell proliferation mainly through activation of p21 protein and the initiation of apoptosis in malignant cells leading to their death [33]. In addition to p53, two other members of p53 family, p63 and p73, are expressed in the liver and are involved in the maintenance of liver quiescence. The biological functions of this family and their role in HCC have been described by Machado-Silva et al in a recent review [34]. Examination of hepatocellular carcinoma in many animal models clearly demonstrated that the inactivation or/and the reduction of p53 is one of the key steps of carcinogenesis [32, 33]. It has been shown that, in addition to promotion of liver cancer, the loss of p53 and Ink4a/Arf cooperate in the enhancement of metastases of the hepatocellular carcinoma cells [35]. Tannapfel et al have demonstrated that the inhibition of the activity of p53 by a truncated dominant-negative DNp73 molecule leads to the spontaneous development of HCC starting at the age of 12 months [36]. Although protein levels of p53 are not changed in these mice, this development of HCC is associated with the inhibition of p53 regulated genes and with inactivation of Rb [36]. Despite the well established role of inactivation of p53 in the development of HCC, it became clear that the loss of p53 cooperates with other alterations in development of HCC. For example, Farazi et al have shown that p53 mutations, telomere dysfunction and chronic liver damage cooperate in the progression of HCC [37].

In a recent report, Colak et al have performed cross-species comparative analyses of early human HCC using micro array technology and found that the development of HCC is associated with alterations of several pathways including p53, p38 MAPK, ERK/MAPK PI3K/Akt and TGFβ signaling [38]. Together with previous reports, these findings emphasized the role of loss of p53 in development of HCC. It is interesting to note that several other tumor suppressor genes utilize p53 pathway to protect livers from the development of HCC. Teoh et al have shown that livers of K70 knockout mice have accelerated development of HCC and that this development is associated with the loss of p53 via proteosomal degradation [39]. It has been found that a pro-apoptotic Ras effector, NORE1A, inhibits HCC through the promoting p53 nuclear localization and activation of p21 [40]. The inhibitor of growth 1 (ING1) inhibits hepatocellular carcinoma through stabilization of p53 and via increasing p53 acetylation [41]. In addition, a recent paper by Teoh et al has shown that the development of DEN-mediated cancer in mice with deletion of Araxia Telengiectasia Mutated (ATM) kinase occurs much later than in wild type (WT) mice and that this inhibition of liver cancer is associated with the induction of p53 and p19 (ARF) [42]. Investigation of the mechanisms of elimination of p53 showed that liver cancer has several pathways for the neutralization of the activities of p53. One of these pathways includes epigenetic silencing of the p53 promoter by methylation of GC islands [2]. The second pathway is associated with the elevation of DNp73 in hepatocellular carcinoma and following neutralization of activity of p53 [36]. The third pathway utilizes activation of a subunit of 26S proteasome, gankyrin, which activates MDM2 ligase leading to degradation of p53 through UPS [1]. The gankyrin mediated degradation of p53 is discussed below. In summary, numerous literature data show that the development of HCC includes inactivation of p53.

Rb pathway is inhibited in hepatocellular carcinoma

Retinoblastom protein, Rb, is a tumor suppressor protein which is mutated in a number of human hepatocellular carcinomas [43]. Rb is an important regulator of cell cycle progression and it is required for the arrest of proliferating cells in G1 [44]. Rb interacts with a transcription factor E2F and inhibits transcription of E2F-dependent genes [43, 44]. The activity of Rb is regulated by phosphorylation. While de-phosphorylated Rb binds to E2F, the phosphorylated Rb does not interact with E2F and does not inhibit cell proliferation [45, 46]. During the cells cycle progression, cyclin dependent kinases cdk4/cdk6 are activated by D-type cyclins and phosphorylate Rb leading to neutralization of its inhibitory activity and to cell cycle progression [46, 47]. The activity of D-type cyclins-cdk4/cdk6 is also regulated by $p16^{INK4}$ inhibitor which binds to and inhibits the cdk4/cdk6 complexes [48]. Investigations of Rb pathway in human HCC revealed that Rb is inactivated in human HCC cell lines and in significant number of human hepatocellular carcinomas (up to 28%, [49]). It has been also shown that cyclin D1-cdk4 complex, which neutralizes Rb activity, is activated in 58% of HCC [49, 50] and that p16 is reduced in many HCC samples [49, 51]. Experiments with animal models confirmed the critical role of Rb inactivation in the development of hepatocellular carcinomas. Knudsen's group has generated liver-specific Rb knockout mice and has shown that the loss of Rb resulted in dysregulation of E2F targets and in elevation in cell cycle progression during postnatal growth [52]. Further investigations of these mice under conditions of DEN-mediated carcinogenesis revealed that Rb deficiency increases tumor multiplicity in livers exposed to DEN [53]. Therefore, these reports showed that inactivation of Rb is a major event in development of HCC. Although phosphorylation of Rb is considered as the main pathway of its inactivation, growing number of reports shows an additional pathway of neutralization of Rb which is the degradation of Rb by gankyrin (see below).

C/EBPα is a tumor suppressor protein which is neutralized or reduced in hepatocellular carcinoma

Transcription factor C/EBPα is a strong inhibitor of liver proliferation [24-26]. The growth inhibitory activity of C/EBPα is regulated on the multiple levels which include transcription, stability of mRNA, translation, phosphorylation at S193 and stabilization of the protein [9, 27-31]. In addition to this complexity of the regulation, the age also changes the activity of C/EBPα by switching interacting partners [54, 55]. The phosphorylation of C/EBPα at S193 seems to be the main pathway of the regulation of growth inhibitory activity of C/EBPα. Serine 193 residue is located in proline-rich growth inhibitory region of C/EBPα and it is the subject of phosphorylation by two cell cycle kinases cdk4 and cdc2 [30, 31]. This phosphorylation increases the interactions of C/EBPα with cdk2 and Brm and causes growth arrest [29, 30]. It has been shown that S193 de-phosphorylated C/EBPα does not arrest cell proliferation [29, 31]. Moreover, de-ph-S193-C/EBPα is able to interact with Rb and titrate Rb out of E2F-Rb complexes leading to acceleration of proliferation [56]. The tumor suppression activity of C/EBPα has been demonstrated in several animal models. Tan et al have generated C/EBPα knockin mice in which C/EBPα is expressed from alpha-fetoprotein promoter (which is active in HCC) and have shown that the elevated expression of C/EBPα inhibits liver carcinogenesis [27]. A recent work with C/EBPα-S193D knockin mice demonstrated that the elimination of C/EBPα by gankyrin-UPS pathway accelerates development of HCC after DEN treatment [31]. Studies during last 10 years showed that liver cancer neutralizes growth inhibitory activity of C/EBPα using several pathways (Fig. 1). Micro array analyses of HCC have found that the levels of C/EBPα mRNA were down-regulated in HCC [57]. Examination of levels of C/EBPα in liver tumor sections and non-tumor sections of the same patients has found a significant reduction of C/EBPα mRNA in tumor sections [58]. It has been shown that the reduced expression of C/EBPα in hepatocellular carcinoma is associated with advanced tumor stage and with shortened patient

survival [59]. In addition to transcriptional down-regulation of C/EBPα, liver cancer neutralizes the activity of C/EBPα by de-phosphorylation of C/EBPα at S193 [29]. It is interesting to note that de-ph-S193-C/EBPα has been found in prostate cancer where it promotes proliferation via titration of Rb [60]. A recent report has identified a new pathway by which liver cancer eliminates C/EBPα protein. Wang et al have shown that the elevation of gankyrin in liver tumors triggers degradation of C/EBPα by UPS system [31]. Figure 1 summarizes pathways by which liver cancer neutralizes tumor suppressor activities of C/EBPα. Taken together, these studies showed that C/EBPα is a tumor suppression protein and that elimination of growth inhibitory activity of C/EBPα is a critical step in development of liver cancer.

Gankyrin is a killer of tumor suppressor proteins C/EBPα, p53 and Rb

Given the fact that liver is well protected from cancer by expression of tumor suppressor proteins p53, Rb and C/EBPα, one would assume that development of HCC should include activation of a powerful system for the elimination/neutralization of these proteins. Examination of early events in the development of liver tumor cancer in animal models and in human HCC has identified a candidate for this role, which is a protein called gankyrin [1, 5, 61]. Gankyrin has been initially discovered as 26S proteasome regulatory subunit p28 or $p28^{GANK}$ [62]. Further studies have shown that this protein is elevated in HCC [63-65]. It has been also shown that the inhibition of gankyrin by siRNA reduces liver cancer [66]. The investigations of mechanisms of gankyrin-dependent promotion of HCC have revealed several interesting findings. First, gankyrin binds to MDM2 and enhances ubiquitylation and degradation of p53 [67]. This activity of gankyrin has been further confirmed in the studies using zebrafish model [68]. Second, gankyrin has been shown to interact with Rb and to reduce its stability [69]. This interaction is involved in the conferring anchorage-independent growth of NIH 3T3 fibroblasts [69, 70]. Gankyrin also binds to cdk4 and replaces $p16^{INK4A}$ from cdk4 leading to the activation of cdk4 [71]. In addition to p53 and Rb, recent study has identified ph-S193-C/EBPα as a new target of gankyrin [31]. Because C/EBPα is de-phosphorylated at S193 in certain liver tumors [29], Wang et al have generated S193D knockin mice with the goal to protect liver cancer. Surprisingly, examination of DEN-mediated carcinogenesis in these mice showed that S193D and ph-S193 isoforms of C/EBPα were completely eliminated by gankyrin and that this elimination accelerates development of liver cancer [31]. It has been also shown that the development of liver tumors in aged mice involves gankyrin-dependent elimination of C/EBPα [31]. Taking together these observations, we suggest that the elevation of gankyrin is an early event in development of HCC and that this elevation is required for the elimination of three tumor suppressor proteins, p53, Rb and C/EBPα (Fig. 2). Given significant role of each of these proteins in protection of liver from HCC, the elevation of gankyrin seems to be a key step in the release of growth inhibitory control of the liver and in development of HCC (Fig. 2). In agreement with this suggestion, Li et al have shown that the inhibition of gankyrin by RNAi techniques inhibited HCC cell growth and tumorigenesis [66].

The role of gankyrin pathway in development of other cancers

Although gankyrin was first discovered as the protein increased in liver cancer, growing number of recent publications show that gankyrin pathway is also involved in development of cancer in other tissues. Examination of gankyrin expression in colorectral carcinoma (CRC) samples showed that gankyrin is dramatically increased in majority of CRC samples [72]. It has been shown that RNAi-mediated inhibition of gankyrin in the CRC cells inhibits proliferation of these cells [72]. Recent investigations of 64 specimens of primary pancreatic cancer and adjacent non-cancerous tissues revealed that gankyrin expression is significantly increased in pancreatic cancer [73]. Suppression of gankyrin in these pancreatic cancer cells down-regulates expression of many cell cycle genes and restores protein levels of p53 and

Rb [73]. In addition to these observations, a recent paper by Man et al showed that gankyrin is highly expressed in human lung cancers and that gankyrin is involved in the development of lung cancer [74]. Interestingly, the majority of lung cancer cells with elevated levels of gankyrin have Ras mutations [74]. Although mechanisms of gankyrin-mediated cancer in colorectal carcinoma, pancreatic and lung tissues are not well understood, it is likely that gankyrin eliminates Rb, p53 and C/EBPα in these tissues. Taken together, these new observations revealed that gankyrin is involved in tumorigenesis in many tissues and that development of gankyrin-based therapy might be one of the promising approaches to prevent cancer.

Activation of RNA binding protein CUGBP1 in liver cancer leads to elevation of HDAC1-C/EBPβ complexes and C/EBPβ-LIP

The late stages of DEN-mediated carcinogenesis are characterized by the activation of transcription of cell cycle genes and by epigenetic silencing of tumor suppressor genes such as p16 [75]. While the gankyrin-mediated elimination of the tumor suppressor proteins plays a significant role at yearly stages of carcinogenesis, recent reports have emphasized the role of translational regulation in the late stages of liver cancer (Fig. 3). It has been shown that cyclins D1 and D3 are elevated in the majority of human samples with HCC and that these cyclins activate cdk4 in both nucleus and cytoplasm [50, 76]. In cytoplasm, cyclin D1/D3-cdk4 phosphorylates the RNA binding protein CUGBP1 at S302 and enhances its ability to interact with eukaryotic initiation translation factor eIF2 [76-79]. As the result, liver tumors contain abundant CUGBP1-eIF2 complex which is an activator of translation of mRNAs. The translational CUGBP1-eIF2 complex binds to the 5' regions of mRNAs coding for C/EBPβ and HDAC1 and increases translation of these proteins [76, 77]. It has been shown that the translational elevation of C/EBPβ-LAP and HDAC1 leads to the increase of HDAC1-C/EBPβ complexes in liver cancer [76]. Regarding CUGBP1-mediated activation of C/EBPβ, a single mRNA of C/EBPβ produces two isoforms C/EBPβ-LAP and C/EBPβ-LIP. It has been shown that CUGBP1 activates translation of both isoforms via usage of different AUG codons [76-79]. The studies of the targets of C/EBPβ–LAP-HDAC1 complex showed that these complexes bind to C/EBPβ-dependent promoters [76, 80]. Taking together these reports, we suggest that the activation of the translational CUGBP1-eIF2 complex in liver cancer contributes to the development HCC via elevation of HDAC1-C/EBPβ complexes and following silencing of the promoter of tumor suppressor genes (Fig. 3). So far, the available data show that the complex represses the promoter of tumor suppressor protein C/EBPα [76] and the promoter of GSK3β [80]. The repression of C/EBPα promoter is consistent with the reports showing that C/EBPα mRNA is reduced in certain tumor samples [57-60].

The elevation of HDAC1 in human liver tumors [76] suggested that HDAC1 might affect gene expression through a global remodeling of the chromatin structure. While the information for the role of HDAC1 in human liver cancer is limited, several publications have shown that expression and activity of HDAC1 are increased in cancer of other tissues. For example, expression of HDAC1 is increased in invasive carcinoma of the breast and in prostate cancer [81-84]. The studies in animal models strongly suggest that the elevation of HDAC1 might play an important role in hepatocellular carcinomas. Wang et al have generated mouse model with liver-specific overexpression of HDAC1 and have shown that the livers of young HDAC1 TR mice develop steatosis which is known as a high risk factor for development of HCC [85]. Han et al showed that Hepatitis B Virus (HBV)-induced development of hepatocellular carcinoma is mediated by the HBx-dependent repression of estrogen receptor and that this repression involves HDAC1 [86]. It is also important that several reports showed the critical role of HDAC1 in repression of tumor suppressors [87, 88]. Consistent with the elevation of HDAC1 in the liver cancer, breast and prostate cancer;

the histone deacetylase inhibitors are considered as promising candidates for chemotherapeutic drugs [83, 84].

The elevation of translational CUGBP1-eIF2 complex in liver tumor samples also leads to the increase of a truncated isoform of C/EBPβ; C/EBPβ-LIP [76]. C/EBPβ-LIP lacks activation domains and works as a dominant negative molecule by neutralizing activities of full-length C/EBP proteins [89]. It has been shown that C/EBPβ-LIP accelerates liver proliferation after PH by activation of PCNA and cyclin A [90] and that C/EBPβ-LIP is elevated in neoplastic mouse mammary tumors [91]. A recent report identified the mechanisms of C/EBPβ-LIP-mediated promotion of liver proliferation. Orellana et al have shown that C/EBPβ-LIP de-represses E2F-dependent promoters through disruption of E2F1-Rb complexes and through direct interactions with the E2F-dependent promoters and replacement of E2F-Rb complexes [92]. In agreement with putative role of C/EBPβ-LIP in HCC, it has been shown that the elevation of C/EBPβ-LIP leads to aggressive forms of breast cancer [93]. Ewen's group has shown that C/EBPβ-LIP is a critical component of cyclin D1 network and that it is involved in development of human cancer [94]. Based on these observations, we suggest that the translational elevation of C/EBPβ-LIP contributes to the development of liver cancer (Fig. 3).

Alterations of signaling pathways in aged livers create favorable conditions for the development of liver cancer

Many publications have shown that the age is a high risk factor for the development HCC in patients with hepatitis B virus (HBV) infections, hepatitis C virus (HCV) infections, non-alcoholic and alcoholic fatty liver [95-97]. In agreement with these data, experiments with animal models showed that aged livers have a frequent appearance of the liver cancer [31] and that aged livers are characterized by alterations which create favorable conditions for the development of HCC [9]. One of these alterations is the hyper-phosphorylation of C/EBPα at S193. It has been shown that cyclin D3 is stabilized in aged livers by the reduction of GSK3β [80] and that cyclin D3-cdk4 converts C/EBPα into ph-S193 isoform [30], which becomes a target for gankyrin-mediated degradation [31]. It is important to note that ph-S193-C/EBPα is associated with Rb in aged livers [54] and it is likely that, during development of HCC, both Rb and C/EBPα are targeted for degradation by gankyrin as the components of the complex. The other critical alterations in livers of aged mice are the elevation of the translational CUGBP1-eIF2 complex and following accumulation of C/EBPβ-HDAC1 complexes [77, 79]. Since both these complexes are elevated in liver cancer [76], it is likely that down-stream targets of these complexes are also altered in aged liver. Several reports showed that aged livers contained elevated levels of the truncated isoform of C/EBPβ C/EBPβ-LIP [76, 79, 98]. Because C/EBPβ-LIP is involved in promotion of cancer in other tissues [93, 94] and because C/EBPβ-LIP releases Rb-dependent repression of E2F targets [92], the increase of C/EBPβ LIP in livers of old mice suggested that aged livers should be more sensitive for the development of cancer. In agreement with this prediction, Wang et al have shown that DEN treatments cause liver cancer in old mice much early (in 20 weeks) than in young mice (in 35 weeks) [31]. Taken together, these studies demonstrated that the age creates pre-conditions for the liver cancer and that some addition triggers such as elevation of gankyrin are required to utilize these favorable conditions.

Concluding remarks

The development of hepatocellular carcinoma is a multistep process which includes alterations of gene expression at several levels. Studies of DEN-mediated carcinogenesis revealed that the development of hepatocellular carcinoma takes 33-35 weeks. In the course of this development, the key early event is a neutralization of tumor suppressor proteins, Rb, p53 and C/EBPα. Recent reports suggest that the elevation of gankyrin is a mechanism by

which liver cancer eliminates these proteins. Consistent with this hypothesis, the inhibition of gankyrin inhibits liver cancer [66]. Given these observations and the evidence showing the elevation of gankyrin as an early event in human HCC [65], the development of gankyrin-based approaches for prevention of liver cancer might be one of the promising directions. A recent finding that FXR knockout mice spontaneously develop liver cancer [22, 23] suggests that FXR might be also a tumor suppressor protein. Future studies are required to elucidate the mechanisms of liver cancer in FXR knockout mice and mechanisms by which liver cancer neutralizes FXR in animal models of carcinogenesis and in patients. Although the elevation of gankyrin and elimination of C/EBPα, Rb and p53 are important steps in DEN-mediated carcinogenesis, the development of HCC takes place at later time points and is associated with alterations in many signal transduction pathways. One of these pathways is translational elevation of HDAC1, C/EBPβ-HDAC1 complexes and a truncated isoform of C/EBPβ-LIP. Despite the limited information for the role of these proteins in human HCC, studies in animal models suggests that HDAC1 and HDAC1-C/EBPβ complexes might contribute to the development of cancer via epigenetic alteration of gene expression and through growth promotion activities of C/EBPβ-LIP.

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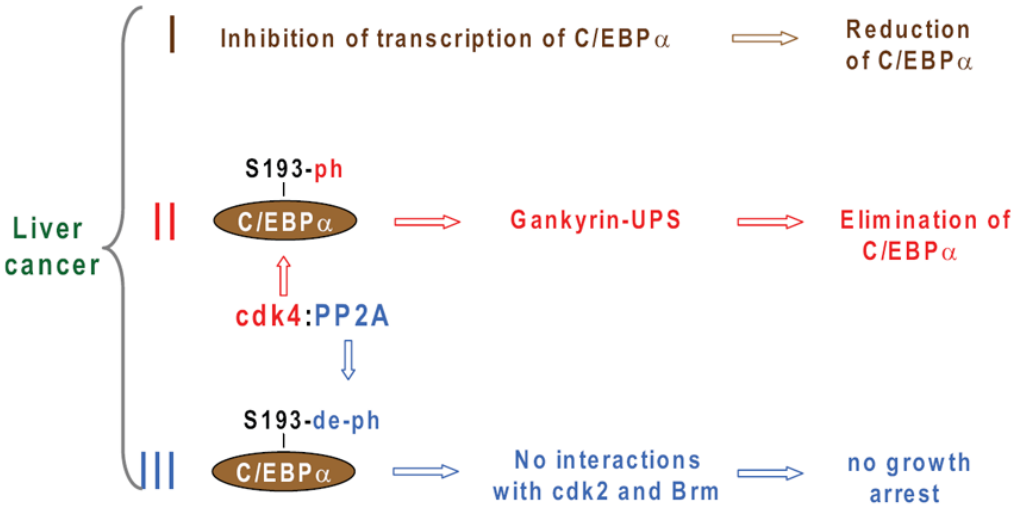

Figure 1. Molecular pathways of neutralization of C/EBPα in liver cancer

There are three known pathways of the elimination or reduction of growth inhibitory activity of C/EBPα. The first pathway (I) includes transcriptional repression of the C/EBPα promoter. The second pathway (II) includes hyper-phosphorylation of C/EBPα at S193 by cdk4/cdc2 and following elimination of S193-ph-C/EBPα by gankyrin-UPS system [31]. The third pathway (III) includes de-phosphorylation of C/EBPα at S193 by a phosphatase PP2A. While protein levels of C/EBPα are not changed (or even increased), the de-ph-S193 molecule does not interact with cdk2 and Brm and does not inhibit liver proliferation.

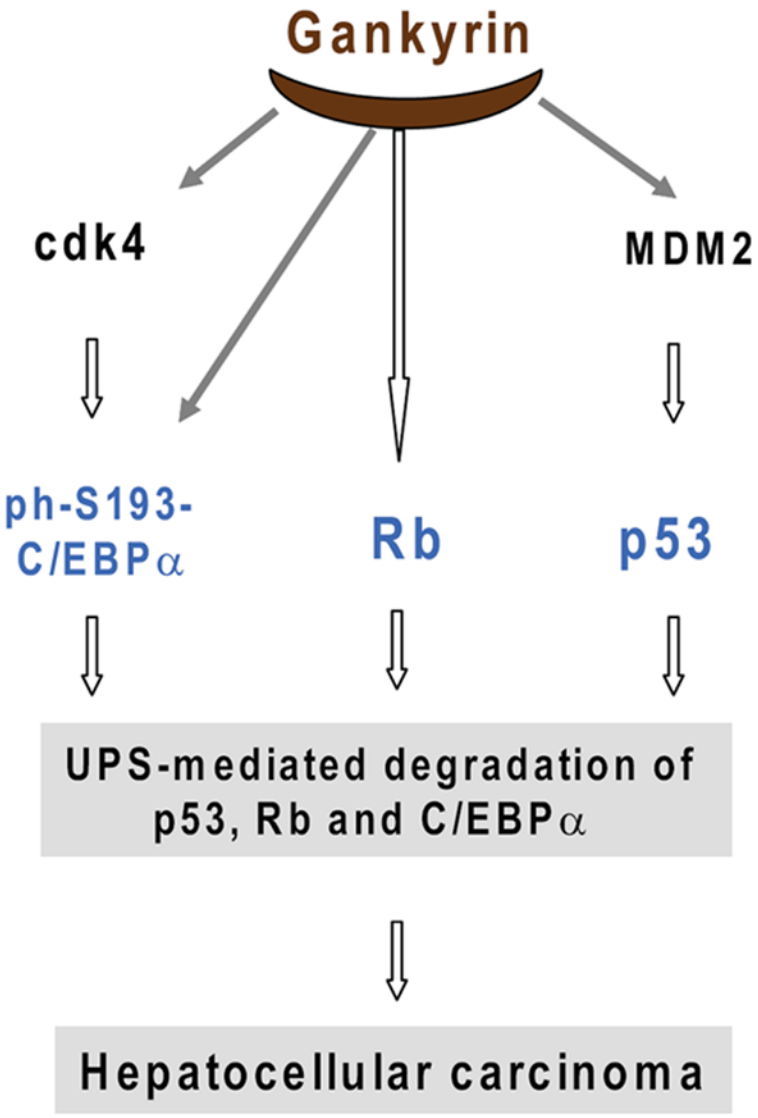

Figure 2. Gankyrin is a tumor suppressor killer

A diagram summarizes literature data showing pathways by which gankyrin triggers degradation of three tumor suppressor proteins: ph-S193-C/EBPα, Rb and p53 at early stages of hepatocellular carcinoma.

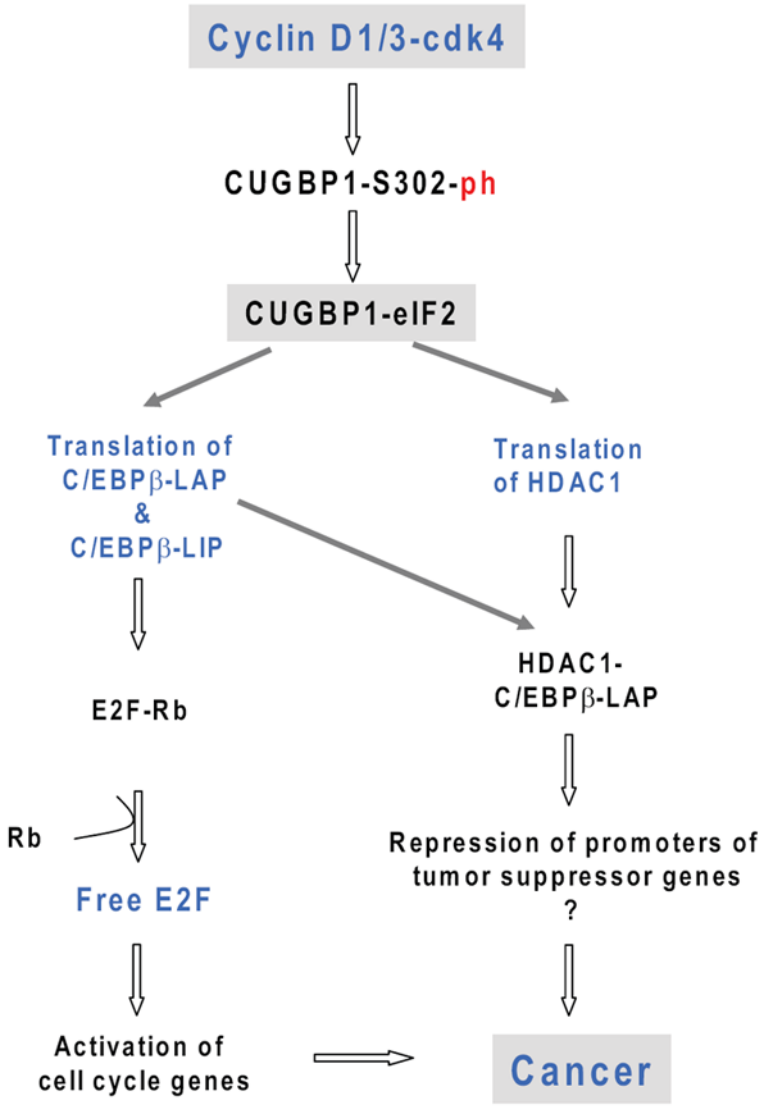

Figure 3. Hypothetical role of translational alterations in the development of liver cancer

Elevation of D-type cyclins during development of HCC activates cdk4 which phosphorylates CUGBP1 and causes the increase of CUGBP1-eIF2 complex. The CUGBP1-eIF2 complex increases translation of HDAC1, C/EBPβ-LAP and C/EBPβ-LIP. HDAC1 and C/EBPβ-LAP form HDAC1-C/EBPβ complexes which are abundant in liver tumors [76]. We hypothesize that these complexes might be involved in the repression of tumor suppress genes such as C/EBPα. The second consequence of the increased translation of C/EBPβ mRNA is the elevation of a truncated C/EBPβ-LIP isoform. In animal models, C/EBPβ-LIP activates cell cycle progression through de-repression of E2F-dependent promoters [92, 94]. We suggest that the increase of C/EBPβ-LIP in liver tumors contributes to the development of cancer.