

# Controversial roles of hepatocyte nuclear receptor 4 $\alpha$ on tumorigenesis (Review)

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Received September 8, 2020; Accepted February 9, 2021

DOI: 10.3892/ol.2021.12617

**Abstract.** Hepatocyte nuclear receptor 4  $\alpha$  (HNF4 $\alpha$ ) is known to be a master transcription regulator of gene expression in multiple biological processes, particularly in liver development and liver function. To date, the function of HNF4 $\alpha$  in human cancers has been widely investigated; however, the critical roles of HNF4 $\alpha$  in tumorigenesis remain unclear. Numerous controversies exist, even in studies from different research groups but on the same type of cancer. In the present review, the critical roles of HNF4 $\alpha$  in tumorigenesis will be summarized and discussed. Furthermore, HNF4 $\alpha$  expression profile and alterations will be examined by pan-cancer analysis through bioinformatics, in order to provide a better understanding of the functional roles of this gene in human cancers.

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## 1. Introduction

Hepatocyte nuclear receptor factor 4  $\alpha$  (HNF4 $\alpha$ ; NR2A1) is a highly conserved orphan member of the nuclear receptor superfamily. It was first cloned by Frances Sladek in James Darnell's laboratory for nuclear factor expression in the liver and was demonstrated to act as a central regulator of gene

expression in certain types of cells that play critical roles in metabolic homeostasis, including hepatocytes, enterocytes and pancreatic  $\beta$  cells (1). In humans, HNF4 $\alpha$  gene is widely expressed in the liver, kidney, pancreas, stomach, small intestine and colon (2), is located on chromosome 20 and comprises at least 12 exons. Two promoters of HNF4 $\alpha$ , named P1 and P2, have been identified to drive the expression of at least six different splicing variants, HNF4 $\alpha$ 1- $\alpha$ 3 and HNF4 $\alpha$ 7- $\alpha$ 9 (2-4). Four variants have been well characterized: HNF4 $\alpha$ 1 and HNF4 $\alpha$ 2 from the P1 promoter and HNF4 $\alpha$ 7 and HNF4 $\alpha$ 8 from the P2 promoter. HNF4 $\alpha$ 3 (P1-driven) and HNF4 $\alpha$ 9 (P2-driven), which both have a different F domain, are less well characterized, although some recent studies reported that these isomers are expressed in human pancreas and may play a role in the development of diabetes (4).

HNF4 $\alpha$  is classified as an orphan nuclear receptor for which ligand has not been found yet, and its regulatory role remains unclear (5). Previously, research on HNF4 $\alpha$  ligands have reported conflicting evidence. For example, long-chain fatty acids have been shown to bind as acyl-CoA thioesters to the ligand binding pocket of HNF4 $\alpha$  and to act as transactivators or antagonists, depending on their chain length and saturation (6-9). However, these fatty acids are bound firmly and not exchangeable, and seem to bind irreversibly to the receptor, suggesting that they might act more as structural co-factors than classical regulatory ligands (10,11). Conversely, HNF4 $\alpha$  expressed in mammals was shown to be bound to the essential fatty acid linoleic acid (LA; C18:2), which is considered as a potential endogenous ligand of HNF4 $\alpha$  (12). Although this binding is reversible, it does not appear to have any significant effects on the transactivation function of HNF4 $\alpha$  (5,12). Recently, several synthetic HNF4 $\alpha$  antagonists were reported to bind to the ligand binding domain (LBD) of HNF4 $\alpha$  with high affinity and to regulate the expression of known HNF4 $\alpha$  target genes. In particular, these antagonists were found to be selectively cytotoxic to cancer cells *in vitro* and *in vivo* (1,13). For example, one small molecule, 1-(2'-chloro-5'-nitrobenzene sulfonyl)-2-methylbenzimidazole (BIM5078), which has been discovered by a novel high-throughput screening for insulin promoter modulators (14) exhibited a dose-response inhibition of the expression of known HNF4 $\alpha$  target genes (1). BIM5078 was demonstrated to be structurally similar to FK614, which is a PPAR $\gamma$  agonist formerly described as a therapeutic agent for type II diabetes (15). BIM5078 may directly bind and interact

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**Key words:** hepatocyte nuclear receptor 4  $\alpha$ , nuclear receptor, oncogene, tumor repressor

with the LBD of HNF4 $\alpha$  repress target genes and may be cytotoxic to hepatocellular carcinoma cells (1).

HNF4 $\alpha$  is a highly conserved member of the nuclear receptor superfamily of ligand-dependent transcription factors, acts as a homodimer and plays a critical role in early liver morphogenesis, fetal liver development, liver differentiation and metabolism by regulating the transcription of genes involved in each of these biological processes (5,16-18). Numerous studies have also reported the central role of HNF4 $\alpha$  in regulating a number of genes, such as cytochrome P450 genes (CYP2C8, CYP2C9, CYP2C19, CYP7A1, CYP3A4 and CYP8B1) that essential for the xenobiotic and drug metabolism, to protect individuals from toxic effects and provide key building blocks and nutrients to promote the growth or maintain the survival of the organism (5,19-21). Due to its multiple functions, HNF4 $\alpha$  is described as a master regulator in multiple signal channels. For example, HNF4 $\alpha$ -deficient embryonic livers showed decreased expression of most hepatic factors, including apolipoprotein B, liver fatty acid-binding protein and microsomal triglyceride transfer protein as well as retinol-binding protein, which indicated that HNF4 $\alpha$  is a hepatocyte differentiation factor critical for maintaining normal liver structure and normal liver development (12,22-25).

Similarly, HNF4 $\alpha$  was demonstrated to promote the differentiation of intestinal epithelial cells (26) and embryonic development of the colon in mice (27). HNF4 $\alpha$  also serves an important role in hepatic progenitor cells differentiation by governing the expression of the transcription factors, including forkhead box protein A2, (T-Box transcription factor 3), hematopoietically-expressed homeobox protein, GATA4 and GATA6 that control the hepatocyte cell development and regeneration (28). Other studies reported that ectopic expression of HNF4 $\alpha$  significantly inhibits the proliferation of HEK293 (29) and pancreatic INS-1 $\beta$ -cells (30). Furthermore, HNF4 $\alpha$  may act as a mesenchymal-to-epithelial transition (MET)-inducing factor in hepatocytes (31-33), and accumulated evidence indicates that HNF4 $\alpha$  is involved in inflammatory networks (34,35).

The present review will summarize the expression patterns, alterations and regulatory effects of HNF4 $\alpha$  in human cancers, and introduce the novel functions of this ancient receptor.

## 2. Expression pattern of HNF4 $\alpha$ in human cancers

Using the Broad Institute Fire Browse portal (<http://firebrowse.org/>), we investigated the expression patterns of HNF4 $\alpha$  gene in 38 human cancers compared with normal controls. The results demonstrated that the levels of HNF4 $\alpha$  transcription varied among different types of cancer (Fig. 1). Compared with the matched normal tissues, HNF4 $\alpha$  expression was upregulated in 11 types of tumors tissue and downregulated in 14 types of tumors tissue. The expression of HNF4 $\alpha$  was the highest in liver hepatocellular carcinoma and the lowest in pheochromocytoma and paraganglioma. In both types of cancer, HNF4 $\alpha$  was decreased in cancer tissues compared with normal tissues. These findings indicated that the expression of HNF4 $\alpha$  may be regulated in tissue-specific and cancer type-dependent manners. Because there are two promoters (P1 and P2) of HNF4 $\alpha$  gene, the differential expression pattern and dysregulation of HNF4 $\alpha$  in cancer cells could be partially

due the alternative promoter use and splicing (36-38). The distribution of differential promoter-driven HNF4 $\alpha$  isoforms have been well defined using immunohistochemical analysis, which was associated with the pathogenesis of certain types of cancer (36). For example, downregulation of P1-driven HNF4 $\alpha$  isoforms expression is involved in tumor metastasis and poor prognosis of colorectal cancer (CRC) (37). Some studies on gastric cancer suggested that P1-driven HNF4 $\alpha$  isoforms negative expression may be considered as a useful marker for mucin phenotypic classification (38). In addition, HNF4 $\alpha$  appears to encode multiple isoforms from both promoters by selective splicing, with different transcriptional functions (39-41). However, the precise regulation mechanism of differential promoter-driven HNF4 $\alpha$  isoforms in human carcinogenesis is not fully understood.

## 3. Alterations of HNF4 $\alpha$ in human cancers

The frequency of HNF4 $\alpha$  gene alterations, including mutation, deletion, fusion and amplification, was determined across multiple types of cancer using the cBioPortal for Cancer Genomics database (<http://www.cbioportal.org/>), which contains 147 common cancer studies that included the clinicopathological characteristics of almost 23,000 patients. All searches were carried out according to the online instructions of cBioPortal website. By pan-cancers analysis, we found that amplifications and mutations were the most common alterations of HNF4 $\alpha$  in human cancers, particularly in colorectal and uterine cancers (Fig. 2). Notably, HNF4 $\alpha$  alterations were mostly observed in one of the CRC studies (TCGA, Pan-Can) (42), in which HNF4 $\alpha$  was altered in 59 cases of 594 patients (9.93%), where 48 cases (81.4%) of these alterations were amplifications. As a core transcription factor, HNF4 $\alpha$  was demonstrated to be associated with the tumorigenesis and development of CRC (43). However, the expression and functional role of HNF4 $\alpha$  in CRC was controversial (44). It was reported that HNF4 $\alpha$  expression is downregulated in CRC specimens and is positively correlated with pT typing, lymph node metastasis, distant metastasis and clinical stage in patients with CRC (45). Furthermore, HNF4 $\alpha$  plays an inhibitory role in the progression of colon cancer by interacting with Wnt/ $\beta$ -catenin/transcription factor 4 (TCF4) pathway and influencing apoptosis and cell cycle progression (45). In addition, P2-driven HNF4 $\alpha$  has been shown to promote inflammation and carcinogenesis in colon (40,44). Numerous proteins, including RAD50, PARP1 (double strand break repair protein, poly(ADP-ribose) polymerase 1) and DNA-PKCS (DNA-dependent protein kinase) have been demonstrated to interact with HNF4 $\alpha$  in order to improve the response of CRC cells to DNA damage (46). These different functional roles and controversial reports may be due at least in part to the different transcriptional or translational changes in HNF4 $\alpha$  gene.

The protein expression level, sub-nuclear distribution and post-translational modifications (PTMs) of HNF4 $\alpha$  are also critical determinants of its transactivation potency. Yokoyama *et al* (47) analysed the PTMs in HNF4 $\alpha$  proteins by mass-spectrometry (MS) and identified eight PTMs, including phosphorylation sites (S142, T166, S167 and S436), ubiquitylation sites (K234 and K307) and an ubiquitination

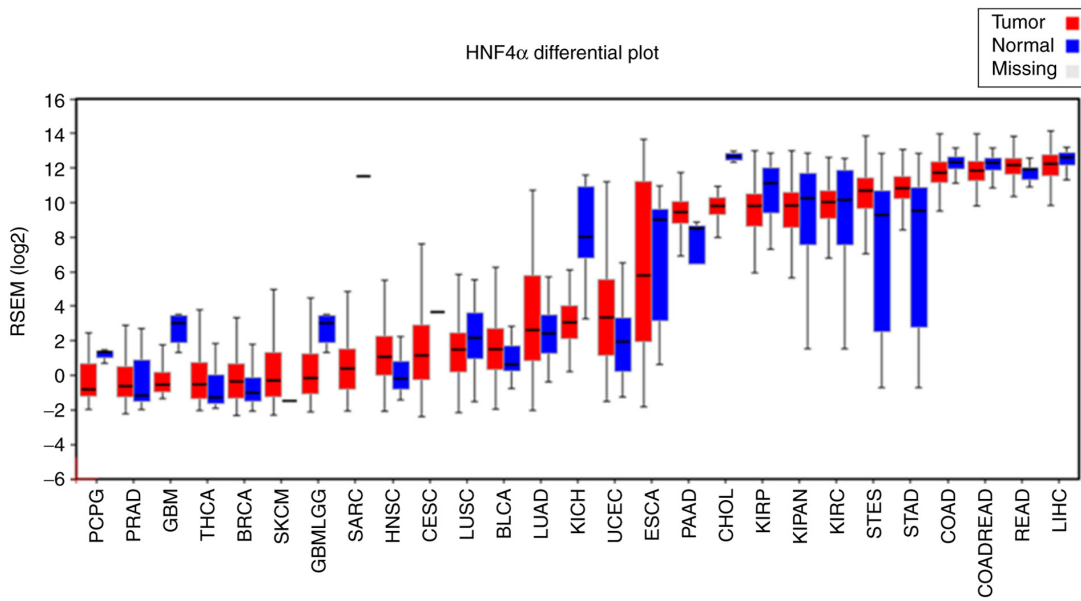


Figure 1. HNF4 $\alpha$  differential plot. HNF4 $\alpha$  was surveyed in 38 human cancers through the Broad Institute Fire Browse portal (<http://firebrowse.org/>). Columns represented the accurate quantification of gene and isoform expression from RNA-Seq data. Results showed that the level of HNF4 $\alpha$  transcripts varied in different types of cancer. The highest level of HNF4 $\alpha$  expression was observed in (LIHC). The expression of HNF4 $\alpha$  was up-regulated in 11 cancers and down-regulated in 14 cancers as compared with their matched normal tissues. LIHC, liver hepatocellular carcinoma; HNF4 $\alpha$ , hepatocyte nuclear receptor 4  $\alpha$ ; RSEM, RNA-Seq by expectation-maximization.

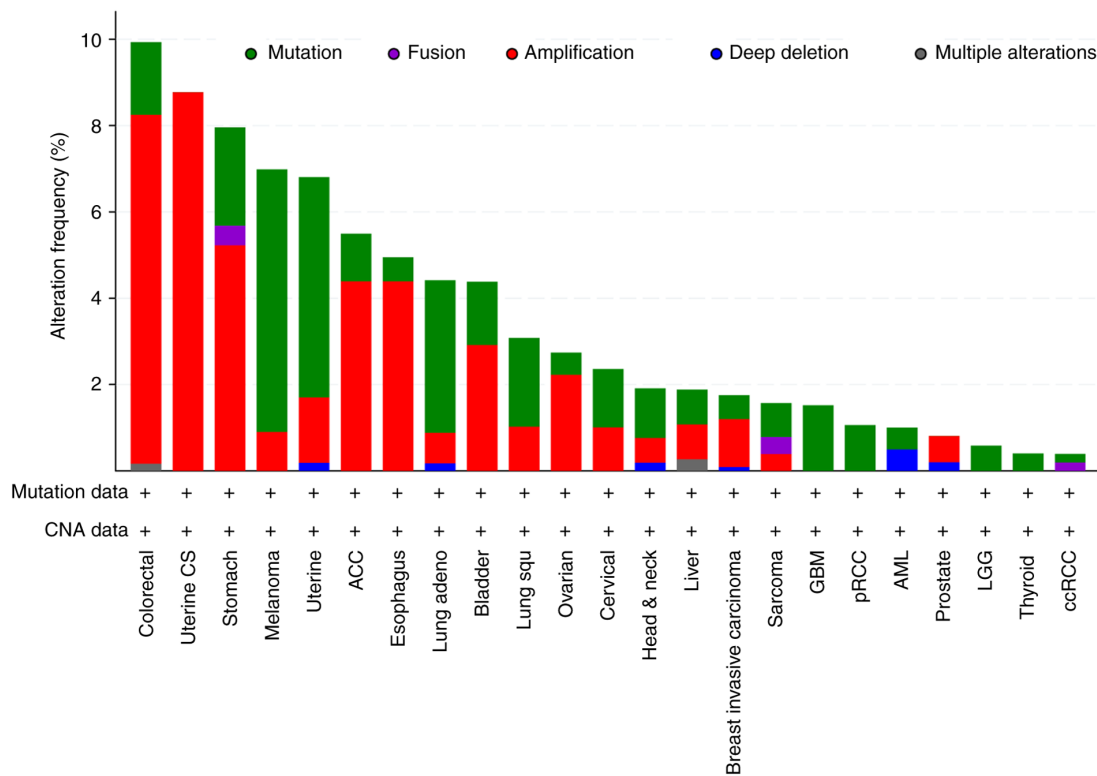


Figure 2. HNF4 $\alpha$  alterations in cancer. HNF4 $\alpha$  alterations, including mutations, deletions, copy number gains and amplifications, were performed across multiple cancer types using the cBioPortal for Cancer Genomics database (<http://www.cbioportal.org>). Results showed that the alterations of HNF4 $\alpha$  mainly included amplification and mutations. HNF4 $\alpha$  alteration was mostly observed in one colorectal cancer study (TCGA, Pan-Can), in which HNF4 $\alpha$  was notably altered in 59 out of 594 patients (9.93%), and 48 cases (81.4%) of these alterations were amplifications. HNF4 $\alpha$ , hepatocyte nuclear receptor 4  $\alpha$ .

and acetylation site at K458. Zhou *et al* (48) reported that the stability of HNF4 $\alpha$  was regulated by SUMOylation in a human embryonic stem cell-based model, and that it serves a critical role during hepatocellular differentiation.

Daigo *et al* (49) validated and identified several phosphorylation sites (Ser<sup>134</sup>, Ser<sup>133</sup>, Ser<sup>158</sup> and Thr<sup>420</sup> + Ser<sup>427</sup>) of HNF4 $\alpha$  by MS/MS neutral loss ion spectra analysis, suggesting a contribution of phosphorylation status alterations to the

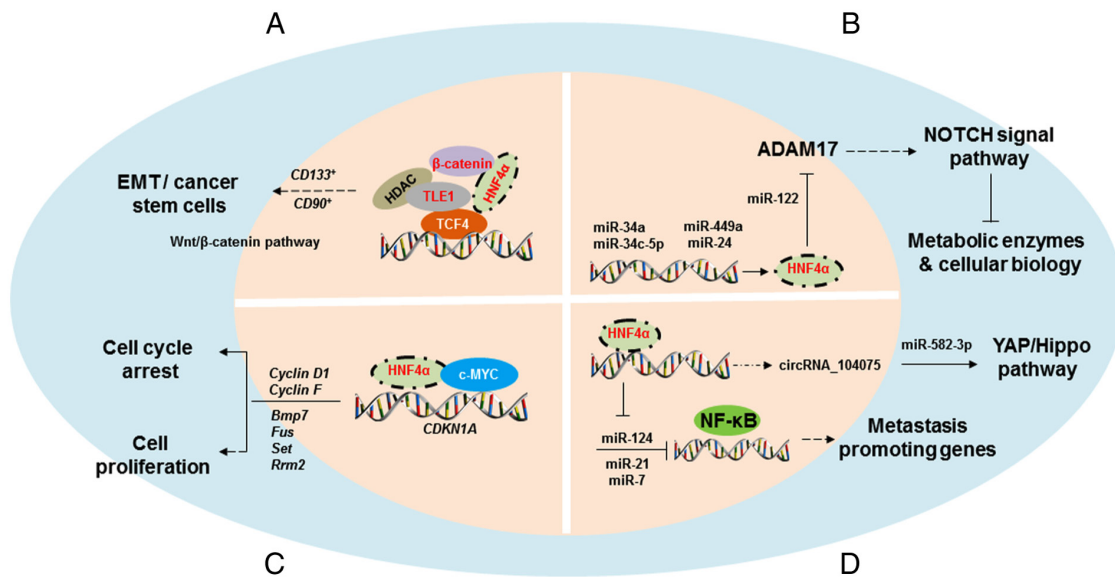


Figure 3 Tumor suppressive role of HNF4 $\alpha$ . Schematic diagram depicting the published mechanisms of the tumor suppressive role of HNF4 $\alpha$  in cancer. (A) HNF4 $\alpha$  is involved in EMT and stemness regulation through Wnt/ $\beta$ -catenin signalling pathway. (B) HNF4 $\alpha$  is regulated by miRNAs, affecting the metabolic enzymes and cellular biology via NOTCH signalling pathway. (C) HNF4 $\alpha$  could induce cell cycle arrest and inhibit cell proliferation by competing binding of p21 promoter region to c-Myc protein. (D) HNF4 $\alpha$  regulates non-coding RNA expression to control the metastasis gene expression through YAP/Hippo pathway and NF- $\kappa$ B signalling. ADAM17, ADAM metallopeptidase domain 17; Bmp7, bone morphogenetic protein 7; CDKN1A, Cyclin Dependent Kinase Inhibitor 1A; EMT, epithelial-mesenchymal transition; Fus, FUS RNA binding protein; HNF4 $\alpha$ , hepatocyte nuclear receptor 4  $\alpha$ ; miR, microRNA; Rrm2, ribonucleotide reductase regulatory subunit M2; Set, SET nuclear proto-oncogene.

multi-functional roles of HNF4 $\alpha$ . Due to the development and application of high-throughput genomic and proteomic technologies, the expression and modification of HNF4 $\alpha$  have become the focus of attention in the recent decades. However, the post-transcriptional role of HNF4 $\alpha$  in the carcinogenesis process remains unclear.

#### 4. Regulatory roles of HNF4 $\alpha$ in human cancers

Increasing evidence indicated that disruption of HNF4 $\alpha$  expression is widely involved in the initiation and development of numerous types of human cancer, including gastric, hepatocellular and colorectal carcinomas (36). Many studies have focused on clarifying the regulatory role and underlying mechanism of HNF4 $\alpha$  in cancer. However, there have been conflicting reports about the role of HNF4 $\alpha$  in promoting and inhibiting cancer in humans.

**Tumor suppressive role of HNF4 $\alpha$ .** HNF4 $\alpha$  is the main regulator of liver specific gene expression and has strong tumor suppressive activity. The tumor suppressive effect of HNF4 $\alpha$  was determined after discovering that HNF4 $\alpha$  expression was lost or significantly decreased in several human cancers, and that restoration of HNF4 $\alpha$  expression could inhibit cancer cell proliferation in different types of cancer, including mouse liver cells (50-53), intestinal cancer (54), lung endothelial cells and embryonic cancer (55), islet tumor cells (30) and embryonic kidney cells (29). However, the underlying mechanism of HNF4 $\alpha$ -mediated tumor inhibition is not fully understood. The mechanisms by which HNF4 $\alpha$  can inhibit cancer in humans are therefore summarized in the present review (Fig. 3 and Table I).

A recent study from our group demonstrated that HNF4 $\alpha$  expression is decreased in prostate cancer cells, and that ectopic

overexpression of HNF4 $\alpha$  could significantly inhibit prostate cancer cell proliferation, induce cell-cycle arrest at G2/M phase and trigger the cellular senescence via activation of p21 signal pathway in a p53-independent manner and direct transactivation of cyclin-dependent kinase inhibitor 1, suggesting that HNF4 $\alpha$  might have a tumor suppressor role in prostate cancer cells (56). Hwang and Sladek (57) reported that HNF4 $\alpha$  competes with the oncoprotein c-Myc for targeting the p21 promoter in order to activate its expression, which could significantly inhibit HCC and colorectal carcinoma cell proliferation. These findings confirmed the critical role of p21 protein in HNF4 $\alpha$ -mediated tumor growth inhibition. The loss of HNF4 $\alpha$  expression may therefore be considered as a key event in the development and progression of cancer; however, its underlying mechanism remains to be further investigated. Previous studies on the HNF4 $\alpha$  gene knockout mouse model reported the negative correlation between deletion/deletion expression of HNF4 $\alpha$  and activation of c-MYC network, which involves many pro-growth genes, such as bone morphogenetic protein 7 (58), FUS RNA binding protein, SET nuclear proto-oncogene, ribonucleotide reductase regulatory subunit M2 and Myc (59,60). Cyclin D1 was also demonstrated to directly bind to HNF4 $\alpha$  and cause a decrease in downstream genes expression (61). Previous studies in renal cell carcinoma reported that decreased HNF4 $\alpha$  expression is positively correlated with e-cadherin expression, suggesting a poor prognosis in patients (62-64). Furthermore, HNF4 $\alpha$  expression is blocked by mutated IDH, which could promote biliary cancer progression (65). Previous studies in hepatocellular carcinoma (HCC) demonstrated that HNF4 $\alpha$  exhibits a decreased expression pattern, which could inhibit hepatocellular carcinoma growth by downregulating miR-122 expression and inhibition of the ADAM metallopeptidase domain 17 and NOTCH signal pathway (52,66,67).

Table I. Tumor suppressive roles of HNF4 $\alpha$ .

Cancer type	Expression pattern	Main findings	Refs.
Hepatocellular carcinoma	Downregulated	i) HNF4 $\alpha$ expression is progressively decreased in rat model of liver carcinogenesis (50,53). ii) HNF4 $\alpha$ -NF- $\kappa$ B feedback circuit, including miR-124, miR-7 and miR-21, exhibits a powerful predictive efficiency of patient prognosis (70).	Lazarevich <i>et al</i> (50) Ning <i>et al</i> (53,70)
Colon carcinoma	Downregulated	i) Decrease expression of HNF4 $\alpha$ plays a critical role in the aggravation of colon carcinoma by promoting epithelial-mesenchymal transition via the Wnt/ $\beta$ -catenin signaling pathway and by affecting apoptosis and cell cycle progression (80). ii) HNF4 $\alpha$ exerts a tumor suppressor function in the colon and its loss of function facilitates tumor progression (54).	Yao <i>et al</i> (80) Saandi <i>et al</i> (54)
Prostate cancer	Downregulated	HNF4 $\alpha$ has a tumor suppressor role in prostate cancer via a mechanism of p21-driven cellular senescence (56).	Wang <i>et al</i> (56)
RCC	Downregulated	HNF4 $\alpha$ expression is downregulated in RCC tissues compared with normal tissues, is positively correlated with E-cadherin expression, and indicates a poor prognosis in patients (62).	Gao <i>et al</i> (62)
Biliary cancer	Downregulated	Mutated isocitrate dehydrogenase blocks HNF-4 $\alpha$ expression and promotes biliary cancer (65).	Saha <i>et al</i> (65)

HNF4 $\alpha$ , hepatocyte nuclear receptor 4  $\alpha$ ; RCC, renal cell carcinoma; miR, microRNA.

Previous studies demonstrated that non-coding RNAs (ncRNAs), including micro (mi)RNAs, long ncRNAs and circular (circ)RNA, serve important roles in the regulation of HNF4 $\alpha$ -mediated transcriptional level (68-70) (Fig. 3B). Takagi *et al* (71) reported that HNF4 $\alpha$  expression is decreased in HepG2 cells and is negatively regulated by miR-24-mediated mRNA degradation and miR-34a-mediated transcriptional suppression, affecting therefore the expression of metabolic enzymes and cellular biology (68,72). miR-34a, miR-34c-5p and miR-449a are reported to share the same target elements located at two distinct locations within the 3'-UTR of HNF4 $\alpha$ , which overexpression could significantly repress HNF4 $\alpha$  protein level by blocking mRNA translation (68,73). Koh *et al* (74) demonstrated that high expression level of miRNAs from let-7 family could regulate self-renewal and differentiation pathways by suppressing the downstream target HNF4 $\alpha$ . It was reported that HNF4 $\alpha$  expression is decreased in HCC via nuclear factor kappa B (NF- $\kappa$ B) mediated miR-21 upregulation (70). In addition, decreased expression of HNF4 $\alpha$  could promote HCC metastasis by regulating the expression and translocation of RelA and affecting NF- $\kappa$ B activation (70). Zhan *et al* (75) demonstrated that HNF4 $\alpha$  could bind to the promoter region of circRNA\_104075 to stimulate its expression, and that circRNA\_104075 can act as a ceRNA able to upregulate YAP-Hippo pathway by absorbing miR-582-3p. ncRNAs are the most abundant regulatory factors that possess great post-transcriptional regulatory potential. However, the

underlying mechanism by which miRNAs act and regulate HNF4 $\alpha$  expression remains to be further investigated.

HNF4 $\alpha$  has been reported to suppress hepatocyte epithelial-mesenchymal transition (EMT) and cancer stem cell generation via inhibition of  $\beta$ -catenin signalling pathway (45,53). EMT is a critical developmental process during cancer invasion and metastasis. Wnt- $\beta$ -catenin signalling pathway plays a crucial role in triggering EMT progression in both embryonic development and tumorigenesis (33,76,77). Previously, HNF4 $\alpha$  was reported to be a potential EMT regulator in HCC cells (77), since its ectopic expression induces MET and blocks HCC progression (25). Previous studies demonstrated that the repression of mesenchymal program of HNF4 $\alpha$  is subsequent to inhibition of Snail (31) and competition with  $\beta$ -catenin for binding to TCF4 in HCC cells (77,78). Other studies also indicated that HNF4 $\alpha$  expression is lost or significantly decreased in cirrhotic tissues and decreased in HCC tissues compared with healthy tissues (50,53,79). Restoration of HNF4 $\alpha$  expression by an adenovirus-mediated gene delivery system could attenuate hepatocyte EMT during hepatocarcinogenesis through inhibition of Wnt/ $\beta$ -catenin signalling pathway (77,80,81), significantly reducing the proportion of cells with stem cell gene expression and CD133<sup>+</sup> and CD90<sup>+</sup> cells, which are considered as tumor stem cells in the start and development of HCC (82). These findings highlighted the central role of HNF4 $\alpha$  in the Wnt- $\beta$ -catenin/snail signalling pathway involved in the EMT/MET progression,

Table II. Oncogenic role of HNF4 $\alpha$ .

Cancer type	Expression pattern	Main findings	Refs.
HCC	Upregulated	HNF4 $\alpha$ is upregulated in HCC samples compared with distal non-tumorous tissues from the same patients (51). Expression of P2 promoter derived-HNF4 $\alpha$ (P2-HNF4 $\alpha$ ) is significantly increased in HCC tissues compared with non-tumorous tissues. High P2-HNF4 $\alpha$ expression is significantly associated with HCC poor differentiation and vascular invasion (86).	Xu <i>et al</i> (51) Cai <i>et al</i> (86)
Gut neoplasia	Upregulated	HNF4 $\alpha$ is upregulated in colorectal cancer tissues compared with adjacent normal epithelial tissues. HNF4 $\alpha$ could be a potential therapeutic target during intestinal tumorigenesis (88).	Darsigny <i>et al</i> (88)
NB	Upregulated	HNF4 $\alpha$ exhibits oncogenic activity that affects the aggressiveness and angiogenesis of NB through activating the transcription of matrix metalloproteinase-14 (91).	Xiang <i>et al</i> (91)
GC	Upregulated	HNF4 $\alpha$ is specifically upregulated in GCs (96) and regulates GC metabolism through directly targeting isocitrate dehydrogenase 1 gene (95).	Chang <i>et al</i> (96) Xu <i>et al</i> (95)
Prostate cancer	Upregulated	HNF4 $\alpha$ is upregulated in LNCaP- and VCaP-derived prostatespheroids. HNF4 $\alpha$ may play a role in stemness regulation of prostate cancer stem cells (100).	Wang <i>et al</i> (100)
PDAC	Upregulated	HNF4 $\alpha$ expression is increased in PDAC tissues. Patients with higher HNF4 $\alpha$ expression display worse prognosis (94).	Sun <i>et al</i> (94)
Paediatric neuroblastoma	Upregulated	Expression of HNF4 $\alpha$ is increased in paediatric neuroblastoma tissues. HNF4 $\alpha$ expression is negatively regulated by miR-34a, which promotes cell proliferation and invasion (93).	Li and Chen (93)

HNF4 $\alpha$ , hepatocyte nuclear receptor 4  $\alpha$ ; HCC, hepatocellular carcinoma; NB, neuroblastoma; GC, gastric cancer; PDAC, pancreatic ductal adenocarcinoma.

which could be a critical inhibitory mechanism for tumorigenesis.

*Oncogenic roles of HNF4 $\alpha$ .* Considering the differences in the biologic properties of experimental systems and tumor samples, increased conflicting reports about the role of HNF4 $\alpha$  in HCC progression and in several other types of cancer (51,83-87; Table II) have been observed. The known oncogenic roles of HNF4 $\alpha$  in human cancers are summarized in Fig. 4.

Darsigny *et al* (88) demonstrated that HNF4 $\alpha$  is significantly upregulated in CRC tissues compared with adjacent normal epithelial tissues and serves a critical role in promoting gut tumorigenesis by directly targeting the promoter of cytochrome P450 family 2 subfamily B member 6 protein and glutathione S-transferase kappa 1 gene against spontaneous and 5-fluorouracil chemotherapy-induced production of ROS in CRC cell lines, indicating the oncogenic role of HNF4 $\alpha$  in human carcinogenesis (89). Another study on human primary mucinous tumors demonstrated that increased HNF4 $\alpha$  expression is associated with higher tumor grade, suggesting a carcinogenic role of HNF4 $\alpha$  in mucinous tumors (90). Similarly, a study on neuroblastoma (NB) reported that HNF4 $\alpha$  expression is

significantly upregulated in clinical neuroblastoma tissues as an independent prognostic factor for poor patient outcomes (91). HNF4 $\alpha$  knockdown can suppress the invasion, metastasis and angiogenesis of NB cells *in vitro* and *in vivo* through down-regulating matrix metalloproteinase 14 (MMP-14) by direct binding to the promoter region of MMP-14, an inhibitory effect that can be neutralized by ectopic expression of HNF4 $\alpha$  (91). HNF4 $\alpha$  was also found to be upregulated in all invasive mucinous adenocarcinoma (IMC) of the lung determined according to tissue array, indicating that HNF4 $\alpha$  could be considered as a useful marker for IMC of the lung (92). HNF4 $\alpha$  expression was also found to be increased in paediatric NB tissues and negatively regulated by miR-34a to promote cancer cell proliferation and invasion (93). A study in pancreatic cancer (PDAC) indicated that HNF4 $\alpha$  is increased in cancer tissues compared with adjacent tissues (94). HNF4 $\alpha$  is required for pancreatic cancer cell proliferation and promotes resistance to gemcitabine by downregulating hENT1 (94).

Recent studies have reported that HNF4 $\alpha$  is significantly upregulated in gastric cancer (GC), head and neck squamous cell carcinoma and pancreatic adenocarcinoma tissues compared with normal tissues (94,95). In particular, HNF4 $\alpha$

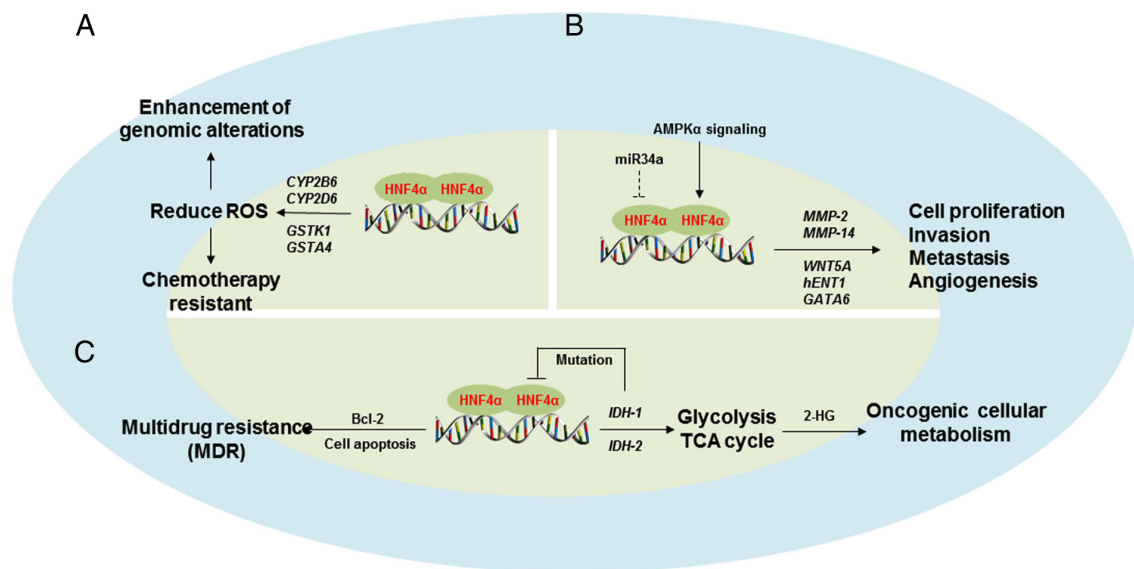


Figure 4 Oncogenic roles of HNF4 $\alpha$ . Schematic diagram depicting the published mechanisms of the oncogenic role of HNF4 $\alpha$  in cancer. (A) HNF4 $\alpha$  could reduce levels of ROS to enhance genomic alterations and chemotherapeutic-resistance of cancer cells. (B) Increased expression of HNF4 $\alpha$  could promote cell proliferation, invasion, metastasis and angiogenesis via increasing MMP-2 and MMP-14 expression. (C) Overexpression of HNF4 $\alpha$  induces multidrug-resistance via Bcl-2 mediated cell apoptosis and promotes oncogenic cellular metabolism by directly targeting the IDHs related to glycolysis and TCA cycle. CYP2B6, cytochrome P450 family 2 subfamily B member 6; CYP2D6, cytochrome P450 family 2 subfamily D member 6; GATA6, GATA binding protein 6; GSTA4, GATA binding protein 4; GSTK1, glutathione S-transferase kappa 1; hENT1, human equilibrative nucleoside transporter 1; IDH-1, isocitrate dehydrogenase 1; IDH-2, isocitrate dehydrogenase 2; MMP-14, matrix metalloproteinase 14; MMP-2, matrix metalloproteinase 2; ROS, reactive oxygen species; TCA, tricarboxylic acid; WNT5A, Wnt family member 5A.

acts by sustaining an oncogenic metabolism in GC via the direct binding to the promoter region of isocitrate dehydrogenase-1 (IDH-1), which is a key enzyme for TCA cycle required for GC development (95). However, mutated IDH-1 and IDH-2 can inhibit HNF4 $\alpha$  to block hepatocyte differentiation and promote the development of premalignant biliary lesions and progression to metastatic HCC (65). Chang *et al* (96) reported that HNF4 $\alpha$  is upregulated by AMPK signalling and acts as an upstream regulator of the WNT signal pathway through its target gene Wnt family member 5A in GC. In addition, the overexpression of HNF4 $\alpha$  in GC tissues is significantly associated with tumor stage and lymph node metastasis in patients with GC, which may cause drug resistance to multiple chemotherapeutics due to regulation of cell apoptosis and Bcl-2 expression (97). Nakajima *et al* (98) demonstrated that HNF4 $\alpha$  is a direct target gene of kruppel like factor 5/GATA binding protein (GATA)4/GATA6 that can interact with GATA6 and contribute to the development of mucinous-type lung adenocarcinomas and GC (99). A previous study from our laboratory demonstrated that HNF4 $\alpha$  can be significantly upregulated in prostate cancer cells-derived prostatespheroids, suggesting that HNF4 $\alpha$  may also work in the regulation of prostate cancer stem cells (100). In summary, HNF4 $\alpha$  had demonstrated an upregulation pattern and an oncogenic role in many types of cancer, suggesting that HNF4 $\alpha$  may be considered as a therapeutic target in numerous human carcinomas.

## 5. Conclusions

As an important regulator of tumorigenesis and tumor development, HNF4 $\alpha$  is expressed at different levels in different types

of tumor and serves various roles that are tissue-specific. This suggests the ubiquitous expression patterns of HNF4 $\alpha$  and the changes in HNF4 $\alpha$  expression, as well as the controversial mechanisms that may be involved in cancer progression, which provide further clues to the better understanding of HNF4 $\alpha$  role in cancer. Over the years, numerous studies have provided significant advances in the role of HNF4 $\alpha$  in human cancers; however, the underlying mechanisms involved remain unclear and require urgent further investigation. Considering the different roles of HNF4 $\alpha$  isoforms in the transcriptional control of cell proliferation, EMT, stemness and other cellular processes in different types of cancer, further research should focus on the potential therapeutic approaches of targeting HNF4 $\alpha$ .

## Acknowledgements

Not applicable.

## Funding

This work was partially supported by the Shenzhen Science and Technology Program (Basic Research Project; grant no. JCYJ20180228163919346), the National Natural Science Foundation of China (grant no. 81802566) and Longhua Science and Technology Innovation Fund (grant nos. 2020013 and 2020003).

## Availability of data and materials

The data that support the findings of this study are available from The Cancer Genome Atlas (<http://cancergenome.nih.gov/>).

### Authors' contributions

ZW and YZ drafted the manuscript. ZW obtained funding, drafted and revised the manuscript. QD, JZ and HL helped to revise the manuscript for important intellectual content. ZW and HL confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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