The transcription factor HNF4a: A Key player in haematological disorders

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HNF4a is one of the steroid hormone receptor family of transcription factors with roles in the development of the liver and the regulation of several critical metabolic pathways, such as glycolysis, drug metabolism, apolipoproteins and blood coagulation. The transcriptional potency of HNF4a is well known due to its involvement in diabetes and other metabolic diseases. However, recently HNF4a has been discovered to be closely associated with several haematological disorders, mainly because of genetic mutations, drugs, and hepatic disorders. We review HNF4a structure and function, and its role in haematological disorders. We discuss possible novel therapies that are based on targeting HNF4a.

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Introduction

Hepatocyte nuclear factor 4 alpha (HNF4a) is an orphan member of the nuclear receptor family of ligand-activated transcription factors that are activated by steroid hormones. HNF4a gene is evolutionarily conserved across multicellular organisms and encodes a protein that regulates transcription through homodimer DNA binding to its target genes (1, 2). Research in several mammals shows that the HNF4a protein is a nuclear DNA-binding phosphoprotein that acts as a transcriptional activator or repressor of several target genes (2, 3, 4, 5). HNF4a is involved in the regulation of 40% of hepatic genes and 11% of pancreatic islet genes (6). This transcription factor is necessary for development and differentiation that influence a wide variety of cell types and tissues with roles in gluconeogenesis (7), fatty acid metabolism (8), drug metabolism (9), hepatocyte differentiation (10) and blood coagulation (11). HNF4a was first detected in crude liver extracts by Sladek and colleagues in 1990 and its gene resides on chromosome 20 (12). Phylogenetic research suggests that HNF4a evolved from an explosive burst of gene duplications that occurred about 541 million years ago (13).

HNF4a’s IN DEVELOPMENT AND NORMAL PHYSIOLOGY

A comprehensive explanation of HNF4a’s roles in development and physiology is beyond the scope of this article. Therefore, here we briefly review the
fundamental features. HNF4a plays essential roles in cross-regulatory networks that are essential in the development of the pancreas, liver, and kidneys during the embryonic stage. In typical development, HNF4a is highly expressed initially in the embryonic endothelium where it plays a critical role in the hepatogenesis and the formation of hepatoblast (14, 15,16). Additionally, HNF4a is highly expressed in the embryonic digestive system and neural crest cells during their development stage (17). HNF4a expression decreases with age. However, its expression is not restricted to development as it continues to regulate gene expression in adult hepatocytes and pancreatic islets (18). During the embryonic development of mice, HNF4a is initially expressed in the endodermal cells, particularly the yolk sac; in the hindgut and liver diverticulum. Later on, HNF4 isoforms were detected in the developing kidneys, stomach, and pancreas. HNF4a is essential for organogenesis in which it functions as a development regulator (17). Recently, several studies have shown that HNF4a transcripts regulate each other in a synergistic pattern and regulate the development, integrity, viability and functions of the embryo and numerous adult tissues. With these observations, hepatocyte-specific HNF4a knockout in mice leads to hepatomegaly and an increase in intrahepatic fat that is associated with embryonic lethality (19, 20). HNF4a has also been shown to be involved in intestinal inflammation interactions where it protects the mucosal layer of the intestines from prolonged and extreme inflammatory conditions (21). Inhibition of HNF4a in non-transformed immortalized human hepatocytes leads to the activation of the inflammatory IL-6/STAT3 pathway, which suggests that HNF4-
α acts as a repressor to STAT3 (21, 22). HNF4a has also been identified as a transcriptional activator of factor VII and, therefore, plays a significant role in blood coagulation. A mutation in the HNF4a binding region of the factor VII promoter resulted in a limited interaction between HNF4a and its binding site, which reduced the promoter activity and led to severe factor VII deficiency (23). Moreover, HNF4a is essential for the kidneys as it is expressed in the proximal tubules. In the adult rat nephrons, it plays an important role in the formation of developed proximal convoluted tubules. On the other hand, the loss of HNF4a caused a reduction in the expression of multiple genes that are expressed in the proximal tubules. The aberrant expression of HNF4a in the proximal tubules promotes defective development and accumulation of calcium in the kidneys (24). In the embryonic pancreas of mice, HNF4a has been described as a major regulator of several transcriptional interactions that are essential for the differentiation and specification of the pancreatic cells. HNF4a is constitutively expressed in the pancreatic exocrine glandular cells where it controls the function of mature β-cells. Mutations in HNF4a and HNF1a genes lead to decreased insulin secretion, which causes diabetes (25).
STRUCTURE OF HNF4α PROTEIN

Full-length HNF4α is a 465 amino-acid 78-kDa transcription factor (26). What identifies the nuclear receptor superfamily is a specific DNA-binding domain (DBD) (27). HNF4α is characterized by five working domains (A/B, C, D, E, and F domains). DBD is located in the C domain that is a 60–90 amino-acid domain, which consists of two zinc finger modules followed by a C-terminal extension (Figure 1). The core recognition motif in the DBD consists of a six-base pair sequence, which is responsible for target recognition. This motif is conserved for all members of the nuclear receptor superfamily, whereas other structures vary to serve unique receptor-specific binding sites. The transcriptional response elements within HNF4α target genes result from three different manners of DNA binding: monomers, homodimers, or heterodimers. The ligand-binding domain (LBD), which is located in the D/E domain, is composed of approximately 200 aa. LBD plays several roles, such as protein dimerization, transactivation, and ligand binding. The formation of homodimers or heterodimers in a solution is essential for protein-protein interactions and this is controlled by LBD (26, 27). HNF4α phosphorylation by ERK1 revealed several phosphorylation sites located in the DBD, LBD, and the hinge and the C-terminus of HNF4α. The phosphorylation of HNF4α at multiple sites, such as serine and threonine residues leads to the inhibition of its transcriptional activation capability. The mechanism of HNF4α inhibition by the activated ERK pathway could also be achieved by cytokines,
oxidative stress and growth hormones. This inhibition leads to reduced HNF4a-transactivation abilities and consequently, the downregulation of its target genes (27).

**Figure 1.** A schematic structure of the HNF4a domain. The full-length HNF4a is 465 amino-acid long. Functional sites include the DNA-binding domain (DBD) located in the C domain which consists of two zinc fingers. a ligand-binding domain (LBD) located in the D/E domain.

**INVOLVEMENT OF HNF4a IN HAEMATOLOGICAL DISORDERS**

Over the last decade, HNF4a has been increasingly implicated in the aetiology of different haematological conditions. In 2015, a paper published by Shunsuke et al. showed that HNF4a regulates both iron metabolism and transferrin receptor 2 (Tfr2) and, therefore, plays a significant role in iron deficiency anaemia. This study has shown that the elimination of HNF4a in liver-specific HNF4a-null mice lowered the serum iron levels but did not cause significant variations in the red blood cell (RBC) count, haemoglobin concentration and haematocrit. This means there was no evidence of iron deficiency anaemia in these mice. Nevertheless, there was a remarkable reduction in the levels of hepatic transferrin mRNA and serum transferrin protein in HNF4a-null mice. This low expression resulted from the limited transcriptional activation of transferrin by HNF4a (28). Another study reported that in mice carrying a mutation in the transferrin gene, a hypotransferrinemic status developed resulting in severe iron deficiency anaemia that killed the mice. However, direct injection of serum or purified transferrin
rescued these mice (29). Hence, hypoferremia cannot be fully attributed to the elimination of HNF4a. Therefore, the reduction in the serum transferrin protein that resulted from the lack of HNF4a was not sufficient to induce iron deficiency anaemia (29). A recent study has correlated the production of hepatic transferrin with serum transferrin. They also suggested that HNF4a regulates hepatic transferrin production and HNF4a signalling activity could be reflected through the serological testing of transferrin levels (30). Additionally, the role of HNF4a has been reported to be associated with erythropoiesis in humans (31).

Since the foetal haemoglobin (HbF) levels are high in the cases of sickle cell anaemia. A study evaluated HbF in erythroid progenitor cells, human umbilical cord blood-derived erythroid progenitor-2 (hudep-2) aiming to understand its regulation. Following the induction of HbF production by treating the cells with an antineoplastic drug (decitabine) – a 'hypomethylating agent', they observed an elevation in HNF4a levels. Accordingly, this study has chosen HNF4a as a target for a knockdown, which increased HbF levels. Therefore, the findings in this study suggest that HNF4a might regulate the transcription of γ-globin, which is a component of HbF (32).

Moreover, in acute myelogenous leukaemia (AML), HNF4a was found to be a part of a regulatory mechanism that is essential for AML cell survival. Shi et al described that HNF4a is regulated by LncRNA in non-homologous end-joining pathway 1 (LINP)1 protein, which is overexpressed in AML cells and accelerates disease
progression. The survival and glucose metabolism of AML cells depend on HNF4a and its downstream AMPK/WNT5A-signalling pathway. LINP1 inhibition leads to HNF4a downregulation and, subsequently, reduced AML cellular proliferation and induced apoptosis and senescence. These findings suggest that HNF4a could be used as a biomarker and a target for therapy in AML (33). Another study aimed to compare the patterns of kinetic changes in gene expression between sensitive and resistant multiple myeloma (MM) patients found that HNF4α is one of the activated genes in the resistant patients. Exploration of the association of HNF4a with the therapeutic resistance of (MM) would be an interesting topic for future research (34). (Figure 2)

Increased RBC count (polycythaemia) is another haematological condition where HNF4a expression was apparent. Polycythaemia and its related erythrocytosis increase the risk of blood clots and hyperviscosity. Erythrocytosis is one of the main disorders in hepatocellular carcinoma (HCC) which results from the overexpression of erythropoietin (EPO), which is a hormone produced by the kidneys to stimulate the production of RBCs. HNF4a transcriptionally regulates EBO. Under hypoxic conditions, HNF4a and another DNA-binding transcription factor known as hypoxia-inducible factor 1 (HIF-1) regulate EPO expression In HepG2, a human hepatocellular carcinoma cell line that secretes EPO. HNF4a was found to be maintained by Prolyl-4-hydroxylase 2 (PHD2) induced suppression of the TGF-β1 pathway. Thus, HNF4a enhances the expression of EPO and, thereby, promotes erythrocytosis (35, 36, and 37).
Furthermore, HNF4a is required for optimal gene expression of several blood coagulation factors. For instance, low expression of XII, XII, V, IX and XI coagulation factors was observed in HNF4a-null mice. HNF4a binds directly to its promoter sequence on the genes that encode those coagulation factors and activate their transcription (38, 39, 40). Compared to those wild-type mice, there was a slight reduction in IX and no difference in VII, VIII, and X levels of expression in HNF4a-null mice. On the other hand, the complete absence of HNF4a resulted in low expression of XIII and XII. A recent study has observed an elevation in HNF4a expression that has been induced by histone H4 and DNA. Overexpression in the mRNA of several coagulation factors was partially triggered by the increase in HNF4a expression (41). This suggests that HNF4a transcriptional activity is essential for the gene expression of XIII, XII among other coagulation factors.

Factor VII (FVII) is another coagulation factor that is transcriptionally regulated by HNF4a. Stauffer et al identified the −33 to −50 bp region upstream of the transcription start site within the FVII promoter as HNF4 binding site (39). Two-point mutations at the promoter region of the HNF4a binding site contributed to a severe FVII deficiency. The first point mutation is the transformation of thymine to cytosine at -60 bp. This mutation abolishes efficient HNF4a-FVII promoter interaction and leads to severe FVII deficiency. Arginine replacement to histidine number 348 was identified as the second point mutation in the FVII gene promoter region. HNF4a transcriptional regulation of factor VII is highly significant since it plays an important role in the coagulation cascade. The activation of IX and X
depends on the FVII-tissue factor interaction. Activated factor X transforms fibrinogen to fibrin, which generates thrombin that is essential for stabilizing the initial haemostatic plug (38, 39,42). (Figure 2)

**Figure 2. Summary of the role of HNF4a in Haematological disorders.** In iron deficiency anaemia, HNF4a has been shown to lower serum iron levels, serum transferrin protein and hepatic transferrin mRNA. In sickle cell anaemia, HNF4a siRNA knockdown and chemical induction both enhanced the transcription of γ-globin and HbF levels. HNF4a is regulated by LINP1 in acute myelogenous leukaemia, where the cell survival depends on HNF4a and its downstream AMPK/WNT5A signalling pathway. HNF4a increases EPO expression and erythrocytosis in polycythaemia, EBO is regulated by HNF4a, which is maintained by TGF-β. Under hypoxic conditions, both HNF4a and (HIF-1) regulate EBO production. The elimination of HNF4a results in reduced expression of XIII, XII, V, IX and XI coagulation factors. HNF4a binds directly to its promoter sequence at −33 to −50 bp region on the FVII gene. This binding is disrupted by two-point mutations at the promoter region, thymine to cytosine at -60 bp and Arginine replacement to histidine number 348. Consequently, severe FVII deficiency occurs.

**Potential therapeutic role of HNF4a**

The several findings explained in the previous sections associate HNF4a with numerous aspects of haematological disorders. The enormous body of evidence suggests that the apparent expression of HNF4a does contribute to worse clinical outcomes and is involved in the regulation of signalling pathways that are dysregulated. HNF4a is involved in several biological processes that are relevant
to iron metabolism, HbF levels, AML cell survival, erythrocytosis and the expression of coagulation factors.

Having discussed the association of HNF4a with haematological conditions, HNF4a as a potential target for therapies comes next in this review. For instance, the renewal of HNF4a functions has been proposed as a potential therapeutic strategy in HCC (44). Due to the involvement of HNF4a in polycythaemia, HNF4a and its co-factors constitute a target for novel therapies. As previously described, the apparent expression of HNF4a promotes erythrocytosis, which is one of the complications associated with hepatocellular carcinoma (HCC). The DNA binding activity of HNF4a is maintained by the suppression of TGFβ by PHD2. This enabled HNF4a to continue activating EPO expression, which enhances erythrocytosis (37). TGFβ was found to weaken HNF4a DNA binding activity on HNF4a target genes (45). This TGFβ dominance over HNF4a occurs because of the inactivation of Glycogen synthase kinase 3 beta (GSK3β). The post-translational modifications profile of HNF4a was found to be moderated by the activity of GSK3β. This moderation was the result of the chemical inhibition of the inhibitor BIO by GSK3β. This led to the loss of the negative charge on HNF4a, which impaired its DNA binding activity (45). Therefore, the GSK3β inhibitor could be used to diminish the transcriptional activation of EPO by HNF4a and lower the HCC associated erythrocytosis. In general, targeting HNF4a demonstrated anti-tumor activities in gastric cancer (46), prostate cancer (47) and colorectal cancer (48). Targeting HNF4a caused AMPK to link to the Wnt signalling pathway in the
cases of gastric cancer (GC). Applying metformin, an HNF4a antagonist, on GC cell lines reduced the expression of HNF4a by increasing AMPK expression and induced cell cycle arrest. WNT5A has been reported to be an HNF4a target gene where the downregulation of WNT5A occurred as a result of siRNA-mediated knockdown of HNF4a. This indicates that the use of HNF4a antagonists performed antitumor activities in GC (46). Wnt signalling is essential for several regulatory functions in AML and other leukaemias (49) (Figure 3). In AML, cell survival depends on HNF4a and its downstream AMPK/WNT5A signalling pathway. Similar to GC, the AMPK-HNF4a-WNT5A signalling pathway represents a potential therapeutic target in AML. These findings suggest that introducing a drug combination that incorporates HNF4a antagonists in the AML course of therapy could be a promising development. Overall, Targeting HNF4a is another avenue for the potential treatment of several haematological conditions.

**Figure 3. HNF4a as a target of therapy.** Targeting HNF4a through the downregulation of protein level to restrain the transcriptional activity of hyperactivated HNF4a. Targeting the transcription of HNF4a by GSK3β inhibitor to loosen the negative charges of HNF4a and weaken its DNA binding activity to reduce the transcriptional activation of EPO by HNF4a. The inhibition of HNF4a by applying metformin increases AMPK expression and induces cell cycle arrest. Another way to target HNF4a is by siRNA-mediated HNF4a knockdown, which results in WNT5A downregulation and increases AMPK expression, which also induces cell cycle arrest.
CONCLUSIONS AND FUTURE PERSPECTIVES

The role of HNF4α in several haematological conditions has been well established over the last decade. The ability of HNF4α to regulate the transcription of a broad network of genes involved in growth, differentiation, and epigenetic control are all hallmarks of its physiological potential. To associate a particular gene to a specific type of haematological disorder is extremely unusual in the field of pathology and cancer research. This review has focused on haematological conditions. However, HNF4α is also implicated in a wide range of malignancies and hereditary disorders. It is rational to anticipate that HNF4α will turn out to be implicated in numerous other types of metabolic disorders. The use of chemical inhibitors to disrupt the abilities of HNF4α to interact with other proteins and cofactors or inhibit its DNA binding capacity to its target genes are yet to be studied. More research is needed before the role of HNF4α role in haematological disorders can be fully comprehended. There is no doubt that HNF4α-based prognostic markers and therapeutic interventions will offer new opportunities in the clinical interventions of haematological disorders.
Ethics declarations

The university of Bisha ethics committee reviewed and approved the ethical criteria of this study.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Figure 2
Figure 3

GSK3β chemical inhibitor → transcriptional activation of EPO by HNF4a & erythrocytosis

metformin → HNF4a

AMPK expression → cell cycle arrest

siRNA-HNF4α knockdown → WNT5A