Review

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The molecular functions of hepatocyte nuclear factors – In and beyond the liver

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Summary

The hepatocyte nuclear factors (HNFs) namely *HNF1α/β*, *FOXA1/2/3*, *HNF4α/γ* and *ONECUT1/2* are expressed in a variety of tissues and organs, including the liver, pancreas and kidney. The spatial and temporal manner of HNF expression regulates embryonic development and subsequently the development of multiple tissues during adulthood. Though the HNFs were initially identified individually based on their roles in the liver, numerous studies have now revealed that the HNFs cross-regulate one another and exhibit synergistic relationships in the regulation of tissue development and function. The complex HNF transcriptional regulatory networks have largely been elucidated in rodent models, but less so in human biological systems. Several heterozygous mutations in these HNFs were found to cause diseases in humans but not in rodents, suggesting clear species-specific differences in mutational mechanisms that remain to be uncovered. In this review, we compare and contrast the expression patterns of the HNFs, the HNF cross-regulatory networks and how these liver-enriched transcription factors serve multiple functions in the liver and beyond, extending our focus to the pancreas and kidney. We also summarise the insights gained from both human and rodent studies of mutations in several HNFs that are known to lead to different disease conditions.

Keywords: Hepatocyte nuclear factor; Liver; Transcription; Regulatory network; Development; MODY; Disease.

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Introduction

The hepatocyte nuclear factors (HNFs) were first identified as liver-enriched transcription factors.[1] However, HNFs are also expressed in a variety of other tissues and organs, such as the pancreas and the kidney, playing important roles in regulating the development and functions of these tissues. Studies have successfully identified the binding motifs of HNFs from different families and these binding motifs do not appear to be exclusively active in the liver. A variety of hepatic and non-hepatic genes contain the matched HNF binding motifs in their promoter and enhancer regions, suggesting the likely presence of multiple transcriptional regulatory networks involving HNFs that are not restricted to liver development and function. Importantly, many of the HNFs are involved in complex auto-regulatory and cross-regulatory circuits, likely acting in a combinatorial manner to determine the development of various tissues including the developing embryo, pancreas, kidney and intestines. Herein, we comprehensively summarise the diverse expression patterns and functions of HNFs in the developing embryo and in adulthood, as well as the HNF cross-regulatory network and mutations in these HNF transcription factors that give rise to disease conditions that implicate different organs. In this review, particular attention is placed on the liver, pancreas and kidney, where the roles of HNFs are best studied. We aim to highlight the significance of the HNFs in the liver and beyond, to increase the appreciation of the pleiotropic effects that these factors can have and to encourage a more holistic approach to the understanding of their role in development and disease.

Molecular structure of the HNF families

HNFs are classified into four families, namely HNF1, FOXA (or HNF3), HNF4 and ONECUT ([OC] or HNF6), each characterised by distinct regions corresponding to functional domains (summarised in Fig. 1). The HNF1 family comprises of HNF1α and HNF1β, whose DNA-binding domain (DBD) is known to bind to the palindromic consensus sequence GTTAATNATTANC (Fig. 1A). The dimerisation domain at the N-terminus allows both HNF1α and HNF1β to form homodimers or heterodimers.[2,3] The *HNF1α* and *HNF1β* genes each encode three isoforms (A, B and C) that appear to have tissue-specific roles.[4,5]

FOXA (formerly known as HNF3) belongs to the subfamily of the Forkhead box (FOX) proteins, comprising FOXA1, FOXA2 and FOXA3 (formerly HNF3α, HNF3β and HNF3γ).[6,7] The FOXA family of proteins contain a winged helix (WH) structure (also referred to as the forkhead domain) flanked by sequences required for nuclear localisation (Fig. 1B).[8] They also share a highly conserved DBD and bind to the target DNA as a monomer.[9] The N- and C-termini of FOXA proteins are conserved and reported to act as the transactivation domains.[10,11]

HNF4 belongs to the orphan nuclear receptor family and comprises HNF4α and HNF4γ (Fig. 1C). The two transactivation function domains, activation function 1 and 2 (AF-1 and

Key point

The liver-enriched HNFs are not restricted to the liver and are expressed in a variety of tissues and organs, playing critical roles in regulating tissue development and function.

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AF-2), located at the N- and C-terminus respectively, activate transcription in a cell type-independent manner.[12,13] A ligand-binding domain (LBD) is located adjacent to AF-2 and transactivates genes in a ligand-dependent manner, providing an additional point of control for regulating protein activity. Of note, HNF4 proteins also contain a repressor domain, region F, that has an inhibitory function,[12] which has not been characterised in other HNF families. *HNF4α* is encoded by two developmentally-regulated promoters (P1 and P2), and differential promoter usage and alternative splicing are now known to generate up to twelve isoforms (P1-derived *HNF4α1-6* and P2-derived *HNF4α7-12*) that are expressed in a temporal and tissue-specific manner.[14–17]

OC1 (or HNF6) belongs to the one cut homeobox gene family. It consists of a single cut domain (CD) and a divergent homeodomain (HD) (Fig. 1D) that form the bipartite DBD.[18,19] The cut homeodomain contains sequences that mediate nuclear localisation and transcriptional activation, in conjunction with the N-terminal STP box (serine/threonine/proline-enriched region).[9,20] The members of this family include OC1 and its two paralogs, OC2[21] and OC3.[22]

It is clear that while the HNF family members harbour common features such as DNA-binding and transactivation capabilities, they are largely defined by distinct, though related, conserved structural domains that account for their functional diversity. The multiple isoforms within each subfamily lend further complexity to their potential to regulate cellular and tissue functions (Fig. 1). The following sections in this review will illustrate overlapping expression patterns among HNF family members and a complex cross-regulatory network connecting several of these HNF isoforms. Studies on rodent gene perturbations and naturally-occurring mutations in humans further reveal their involvement in disease pathophysiology.

Expression profile of HNFs during mammalian embryonic development and in adulthood

Key point

To-date, the expression patterns of HNFs during development have largely been characterised in rodent tissues, given the limited access to human tissues. These studies have provided important information on the spatial and temporal expression patterns of different HNF families and their isoforms, and thus increased our understanding of the role of these HNFs across multiple tissues.

The expression patterns of HNF family members during mammalian embryonic development and in adulthood have been better characterised in rodents than in human tissues. In this section, we highlight and compare the spatial and temporal expression patterns of the various HNFs, with a particular focus on the liver, pancreas and kidney. These organs are known to exhibit high expression levels of many of the HNF family members, are often implicated in diseases resulting from dysregulation of HNF proteins, and provide the tissues in which the respective roles of HNFs are most well-studied. Discussion of the role of HNF family members in other organs is beyond the scope of this review; nonetheless, their expression patterns across other mouse and human tissues are summarised (Tables 1 and 2).

Rodent embryonic development and adulthood

In the mouse, *Hnf1β* is first detected in the primitive endoderm on embryonic day (E)4.5 and is required for specification of the primitive endoderm lineage.[23,24] The expression of *Hnf1β* precedes *Hnf1α* during embryogenesis, as *Hnf1α* transcripts are first detected in the yolk sac on E8.5.[24] *Hnf4α* is expressed in the visceral endoderm from E4.5,[25–29] and is subsequently detected in the liver bud and hindgut starting from E8.5.[25–28,30] On the other hand, *Foxa1*, *Foxa2* and *Foxa3* have distinct temporal expression patterns and appear in partially overlapping domains of the definitive endoderm and notochord.

Hnf1β transcripts are subsequently detected in the foregut endoderm on E9, from which the liver and pancreas develop. During liver development, both *Hnf1α* and *Hnf1β* transcripts can be detected in the liver primordia by E10.5, and they continue to be present in the liver throughout embryonic life.[31] On E9.5, *Foxa1* and *Foxa2* are also highly expressed during liver bud formation while *Foxa3* is expressed at a lower level. The expression of *Foxa1* and *Foxa2* then falls between E12.5 and 15.5 before increasing again in the adult liver. In contrast, *Foxa3* is initially weakly expressed but its expression increases on E10.5 and remains high throughout liver development.[32] Similar to the expression profile observed for *Foxa1* and *Foxa2*, *Oc1* is detected on E9 when liver differentiation occurs, then disappears transiently between E12.5 and E15, before it continues to be expressed within the extrahepatic biliary system and the liver throughout development.[9,20,33] In contrast to *Foxa* and *Oc1*, high levels of *Hnf4* transcripts are localised to the periphery of the liver where hepatocytes develop from E11.5 to E16, but not in the centre where haematopoietic cells differentiate.[29] In the mouse liver, there is a controlled switch from P2 (distal) to P1 (proximal) promoter-driven transcription of *Hnf4α* from foetal life to birth, and P1-derived transcripts continue to be expressed at significantly higher levels in adult liver.[34] The early activation of *Hnf1β*, *Foxa1*, *Foxa2*, *Hnf4α* and *Oc1* in the developing liver between E8.5–9.5 suggests their importance in the early commitment towards the hepatoblast lineage, alongside other transcription factors and signalling molecules.

In the context of pancreatic development, *Hnf1β* and *Hnf4α* are expressed by most epithelial cells of the pancreatic bud from E9.5, and in $Pdx1^+$ pancreatic progenitors followed by $Ngn3^+$ (Neurog3) endocrine precursors (~E12.5) in the early pancreas.[30] In fact, *Hnf1β* is likely to be a key player in early pancreas morphogenesis as it is expressed in the pre-pancreatic foregut endoderm and in early multipotent pancreatic

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