Hepatocyte nuclear factor 4-alpha involvement in liver and intestinal inflammatory networks

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Abstract

Hepatocyte nuclear factor 4-alpha (HNF4-α) is a nuclear receptor regulating metabolism, cell junctions, differentiation and proliferation in liver and intestinal epithelial cells. Mutations within the HNF4A gene are associated with human diseases such as maturity-onset diabetes of the young. Recently, HNF4A has also been described as a susceptibility gene for inflammatory bowel disease following results obtained from knockout mice and human genome-wide association studies. However, no review has been published on the subject yet and no link with its known role in liver inflammation has been discussed. This review will gather for the first time all the current knowledge about the role of HNF4-α in gut inflammation, the potential impact of its isoforms in its role and hypotheses about the possible biological mechanisms involved.

Key words: Hepatocyte nuclear factor 4-alpha; Inflammatory bowel diseases; Colitis-associated cancer; Gastrointestinal tract; Intestinal epithelium barrier; Inflammation

Core tip: Hepatocyte nuclear factor 4-alpha (HNF4-α) is an important regulator of liver and intestinal epithelial cells function. Over the last years, HNF4-α has been associated with inflammatory bowel diseases following results obtained from knockout mice and human genome-wide association studies. However, no review has been published on the subject yet and no link with its known role in liver inflammation has been discussed. This review will gather for the first time all the current knowledge about the role of HNF4-α in gut inflammation, the potential impact of its isoforms in its role and hypotheses about the possible biological mechanisms involved.

INTRODUCTION

Hepatocyte nuclear factor 4-alpha (HNF4-α) is an important regulator of liver and intestinal epithelial cells function. Over the last years, HNF4-α has been associated with inflammatory bowel diseases following results obtained from knockout mice and human genome-wide association studies. However, no review has been published on the subject yet and no link with its known role in liver inflammation has been discussed. This review will gather for the first time all the current knowledge about the role of HNF4-α in gut inflammation, the potential impact of its isoforms in its role and hypotheses about the possible biological mechanisms involved.
has been widely associated with the transcriptional regulation of hepatocyte genes specifically implicated in lipid metabolism, glucose metabolism, differentiation and morphogenesis. However, HNF4-α expression is also detected in epithelia of the pancreas, kidneys, stomach, and intestine, for which it exerts functional roles in regulating epithelial junctions and cell proliferation. In addition to these multiple known functions in epithelium, HNF4-α has been shown to play a role in inflammation processes of the liver and recent evidences suggest the involvement of this regulator in the pathophysiology of inflammatory bowel diseases (IBD). This review will focus on the recent uncovered roles for HNF4-α during intestinal inflammation with regard on conclusions that can be made from earlier studies obtained in the liver context. Moreover, since the nature of HNF4-α isoforms expressed in the liver and the intestine differs, we will highlight the potential contribution of these isoforms in the inflammation process and, to some extend, to the inflammation-associated pathology of cancer.

HNF4-α P1 AND P2 CLASSES OF ISOFORMS

HNF4-α has been generally involved in inflammation processes occurring in liver and the gastrointestinal tract. Importantly, these organs do not express the same HNF4-α isoforms, which leads to the fundamental question of whether these isoforms are functionally redundant during inflammation. HNF4A is coded by a single gene located on the long arm of the chromosome 20 in human[1]. HNF4A locus is transcriptionally regulated through the use of two distinct promoters that are physically separated by more than 45 kb[2]. Isoforms produced by the activity of the closer promoter are designated P1 whereas isoforms produced by the second and more distant promoter are designated P2 (Figure 1). The P1 isoforms class regroups six distinct isoforms (α1 to α6) that are generated through alternative splicing of HNF4-α pre-mRNA while P2 isoforms class regroups three other isoforms (α7 to α9) generated through the same process[3-4]. The functional relevance for production of such a variety of HNF4-α isoforms still remains unclear. Although these classes of isoforms share 90% of homology in their overall protein structures, an important difference is noted between their respective N-terminal domains (Figure 1). This difference causes P2 isoforms to be shorter than P1 isoforms and, more importantly, to lack the cofactor interacting domain designed as activating function (AF)-1. These structural differences suggest that P1 and P2 isoforms could harbour distinct roles by interacting differently with specific cofactors and being differently regulated in specific contexts of physiological importance. In support of this, an elegant study performed in “knock-in” mice has revealed that P1-only mice generated by exon swapping are phenotypically different when compared to P2-only mice[7].

The expression of HNF4-α is restricted to the epithelial compartment in both the liver and intestine. However, adult hepatocytes express P1-only isoforms while intestinal epithelial cells express both P1 and P2 isoforms[8,9]. Many studies have revealed that HNF4-α expression can vary depending on the environmental context. It has been notably demonstrated that the expression profile of P1 and P2 isoforms is modified in many cancers such as hepatocellular carcinoma where P1 isoforms expression is inhibited and P2 isoforms re-expressed[10]. Modulation of HNF4-α isoforms expression is the result of a complex regulatory circuit that involves transcriptional, post-transcriptional and post-translational mechanisms. For instance, P1 and P2 promoters are regulated by different transcription factors[11,12] and are differently targeted by epigenetic regulation[13]. In addition, HNF4-α mRNA is targeted by multiple miRNA[14-18] and its protein function and stability by phosphorylation[19,20], acetylation[21] and nitrosylation[22]. Although most of these studies did not specifically investigate whether P1 or P2 isoforms share the same post-translational modifications, it is predictable that some of them could be isoforms specific. Indeed, it has been recently demonstrated that HNF4-α phosphorylation by the Src kinase preferentially targets P1 isoforms to lead to their degradation without influencing P2 isoforms stability[23]. Thus, HNF4-α isoforms production is regulated by a wide variety of mechanisms that will connect their cellular functions to environmental specific needs.

HNF4-α ACTIVITY IS A TARGET FOR CYTOKINES EFFECTS ON HEPATOCYTES

HNF4-α is crucial for the early embryonic development and function of the adult liver as supported with the generation of mouse models with specific and conditional deletion of HNF4-α in hepatocytes[19,20]. There is an overall agreement on the idea that HNF4-α could represent a central regulator of gene transcription in hepatocytes, making it thus a crucial transcription factor in liver physiology[21-22]. The liver is strongly involved during the acute-phase response (APR) by the synthesis of many acute-phase proteins. Therefore, it is not surprising that HNF4-α has been confirmed to play an important role in inflammation through the regulation of acute-phase protein gene transcription. In fact, many studies demonstrated that HNF4-α is massively targeted by cytokines under these conditions. There is a general consensus supporting a negative regulatory role for cytokines on transcriptional HNF4-α action on its target genes. However, some exceptions highlight the fact that these cytokines effects could be sometimes context dependent. Studies performed in the liver hepatocellular cell line HepG2 support this phenomenon. In the context of an inflammatory redox state, interleukin (IL)-1 was shown to stimulate p38MAPK-dependent phosphorylation of HNF4-α and to increase its affinity to DNA as well as to the PC4 cofactor[23]. This had
the consequence of increasing iNOS expression in the redox context\textsuperscript{32}. On the other hand, IL-1 was shown to promote the reduction of HNF4-\( \alpha \) mRNA and protein levels in the same cell line through c-Jun effect on the P1 promoter as well as through proteasomal degradation\textsuperscript{33}. This effect was reported to occur only for a transient period of time and to resume to normal HNF4-\( \alpha \) level after 12 h of treatment. Similar opposite examples apply to the pro-inflammatory role of transforming growth factor (TGF)-\( \beta \), a cytokine released by the liver during stress or injury. On one hand, TGF-\( \beta \) is able to reduce the expression of its target genes by inhibiting HNF4-\( \alpha \)\textsuperscript{24,34}. Indeed, TGF-\( \beta \) stimulation in hepatic cell lines activate a signalling cascade where Extracellular signal Regulated Kinase 5 (ERK5) will phosphorylate and inactivate Glycogen Synthase Kinase (GSK)-3\( \beta \) kinase that usually phosphorylates HNF4-\( \alpha \), a step that will promote HNF4-\( \alpha \) interaction with target gene promoters\textsuperscript{34}. In addition, the TGF-\( \beta \) pathway causes post-translational modifications of HNF4-\( \alpha \) that ultimately lead to its degradation by the proteasome\textsuperscript{34}. By opposition to these observations, HNF4-\( \alpha \) can also poteniate TGF-\( \beta \) signalling pathway and its downstream effect on gene transcriptional activation. More than the third of identified SMAD2/3 binding regions in HepG2 genes displayed an overlapping binding site for HNF4-\( \alpha \) under specific TGF-\( \beta \) influence\textsuperscript{35}. Coincidentally, binding of HNF4-\( \alpha \) to these SMAD2/3 dependent gene promoters was able to transcriptionally synergize them, as illustrated for the Mix paired-like homeobox (MIXL1) gene\textsuperscript{36}.

Another argument to sustain HNF4-\( \alpha \) involvement during hepatocyte inflammatory stimulation relates to its reported sensitivity to the NF-\( \kappa \)B pathway, a crucial signalling relay that dictates cellular inflammatory response. For example, it has been reported that stimulation of HepG2 cells with tumour necrosis factor (TNF)-\( \alpha \) inhibits Apolipoprotein C3 expression through the influence of NF-\( \kappa \)B that targets HNF4-\( \alpha \) DNA binding affinity and transactivation activity under these circumstances\textsuperscript{37}. Other evidences suggest that the control of HNF4-\( \alpha \) activity by cytokines could be at the level of its natural interactions with cofactors that were reported necessary to promote its optimal transcriptional activity. A study performed in HepG2 cells revealed that the induction of an acute-phase response from the concomitant action of IL-1\( \beta \), TNF-\( \alpha \) and IL-6 reduced the expression of HNF4-\( \alpha \)-dependent APR genes by inhibiting its interaction with the coactivator Peroxisome proliferator-activated receptor-gamma coactivator-1\textsuperscript{38}. Although most of these studies have been performed \textit{in cellulo}, there is also
evidence that these mechanisms are relevant in a physiological context. In support of this, Bauzá et al. showed that the induction of APR in mice following a burn injury was leading to an increase of IL-6 serum level and a concomitant decrease of liver HNF4-α capacity to bind to its DNA response element.

**HNF4-α PROTECTS THE INTESTINAL EPITHELIUM AGAINST INFLAMMATION**

HNF4-α regulates many intestinal epithelial functions. Acting as a morphogen, HNF4-α expression was shown to be required during mouse embryonic colon development. In adult, HNF4-α was also reported to be important in sustaining proper intestinal epithelial cell differentiation, lipid metabolism and epithelial junctions. With such described roles, HNF4-α is now considered as an important regulator of intestinal epithelial cell homeostasis and mucosal barrier integrity. Since perturbation of the epithelial barrier is a recognized important step for initiation of IBD, HNF4-α appeared to be a logical candidate gene to be investigated in relation to these inflammatory diseases.

The first suggestion implying HNF4-α as a possible protector during IBD came from the analysis of ulcerative colitis (UC) and Crohn’s disease (CD) patient biopsies where its expression was found drastically reduced as compared to non-disease controls. While it was not clear whether HNF4-α reduction of expression was the cause or the consequence of inflammation, it nevertheless pointed out that the expression of this transcriptional regulator could be influenced during intestinal inflammation. The generation of intestine specific HNF4-α null mouse models was able to partly resolve this question. Although it was chronologically concluded that deletion of intestinal HNF4-α did not lead to significant defects in young adult mice, these mice were found to be prone to increased susceptibility to dextran sulfate sodium (DSS) model of colitis based on the record of clinical and morphological features of the disease. The exact nature of the mechanisms involved in such susceptibility remained however speculative in this context.

It was further discovered that mice lacking intestinal expression for both HNF4-α P1 and P2 isoforms were able to develop, on the long term, spontaneous inflammation similar to human IBD. The first signs of inflammation appeared around 6 mo of age and consisted of important leukocytes infiltration in the colonic mucosa. The inflammation worsened with time with the appearance of regions of acute inflammation and epithelial destruction, crypt hyperplasia and, in rare cases, early signs of neoplasia. Many cytokines were still induced in the colon of mutant mice of 12 mo of age, supporting then a chronic status of the disease. The progressive appearance of inflammation in this model suggested that the loss of HNF4-α did not immediately provoke IBD, but rather induced modifications in the epithelium that eventually tilted the balance toward chronic inflammation. Exact nature of the molecular cascades involved in this progressive state of disease remains unclear. Early modification of claudin-15 expression in the mutant mice, a direct gene target of HNF4-α, suggested that an alteration in ionic transport could be part of the processes. While all these evidences suggest that long-term reduction of HNF4-α activity promote IBD, it remains unclear whether mechanisms are in place to down-modulate HNF4-α expression from the action of inflammatory signals. DSS-induced colitis caused a reduction in colonic HNF4-α expression at both the transcript and protein levels. As exemplified above, the influence of cytokines on HNF4-α activity is well documented in the liver but remains to be explored in the gut.

Whether these mouse studies could imply that interference on HNF4-α integrity can predispose to IBD in humans still remain an active debate. Interestingly, a human genome-wide association study has identified HNF4-A locus as a susceptibility gene for ulcerative colitis, a finding that was corroborated by an independent study using a Dutch cohort. In addition, the single-nucleotide polymorphism rs1884613 found in the P2 promoter of HNF4A has also been associated with the risk to develop pediatric CD. The functional relevance of these findings on a human biology perspective awaits to be addressed.

**HNF4-α IS IMPLICATED IN AN INFLAMMATORY-CANCER REGULATION LOOP**

HNF4-α is implicated in liver and intestine inflammation. Sustained inflammation can favour cancer development in both tissues and HNF4-α represents a strong candidate to be involved in linking these processes. An elegant study by Hatziapostolou et al. supported a functional role for HNF4-α during inflammation-associated liver cancer. This study identified HNF4-α as a repressor of the inflammatory IL-6/STAT3 pathway. HNF4-α was able to maintain STAT3 in an inactive state by inhibiting the expression of the IL-6 receptor (IL6R) through activation of miR-124 transcription (Figure 2). However, when the expression of HNF4-α was inhibited in non-transformed immortalized human hepatocytes, STAT3 was activated to turn up expression of miR-64 and miR-629 that eventually turned down HNF4-α mRNA level. This kept HNF4-α expression inhibited in order to prevent its retro-inhibition feedback on the STAT3 inflammatory pathway. It was thus concluded that when this inflammatory molecular feedback loop is altered, hepatocytes initiate transformation that will contribute to cancer. Such molecular pathways linking HNF4-α to inflammation-associated cancer have not yet been identified in the intestine. However, a recent study from Koukos et al. reported a similar mechanism where STAT3
activity was also under the control of miR-124 in the context of pediatric UC. It is thus logical to extrapolate that HNF4-\(\alpha\) could also inhibit the ILR6/STAT3 pathway through regulation of miR-124 expression in intestinal epithelial cells. Indeed, DSS-induced colitis resulted in a decrease of HNF4-\(\alpha\) expression in intestinal epithelial cells\[47,48\] while it increased STAT3 mRNA level\[52\]. By extension, colorectal cancer is associated with a decrease of HNF4-\(\alpha\) P1 isoforms expression\[8,25\] and activation of the STAT3 pathway through IL-6\[53,54\]. The existence of such a retro-inhibition loop of HNF4-\(\alpha\) on the ILR6/STAT3 pathway represents thus an interesting possibility to explore in the context of IBD and colitis-associated colorectal cancer.

The results obtained from Hatziapostolou et al\[16\] suggest that the inflammatory-cancer regulation loop could be dependent on HNF4-\(\alpha\) P1 isoforms. While the authors did not specifically establish which isoforms were expressed in their models, it is well documented that P1 isoforms are the major ones expressed in hepatocytes as well as hepatocyte-derived cultured cells. Whether P2 isoforms could also be involved in the inflammatory-cancer loop of IL6R/STAT3/HNF4-\(\alpha\) will be important to establish. In hepatocellular carcinoma, the expression of P1 isoforms is reduced while it has been reported that P2 isoforms are often transiently up-regulated\[8,55\]. In addition, the intestinal epithelium expresses both P1 and P2 isoforms. In that context, it is tempting to extrapolate that P1 isoforms would exert tumour suppressor roles in hepatocytes\[56-58\] and intestinal epithelial cells while P2 isoforms would assume different roles.

CONCLUSION
HNF4-\(\alpha\) appears to be a transcriptional sensor of inflammation and some of the mechanisms and interacting pathways involved in the liver context have been elucidated. Although there are growing evidences for intestinal epithelial HNF4-\(\alpha\) to participate in the pathophysiological consequences of gut inflammation, the exact molecular and biological mechanisms involved are still to be defined. There are several non-exclusive hypotheses to explore the functional roles for HNF4-\(\alpha\) in this context (Figure 3). As observed in hepatocytes, intestinal epithelial HNF4-\(\alpha\) could sense and regulate cytokines effect on gene transcription. In addition, HNF4-\(\alpha\) could play an active role in the maintenance of adequate mucosal barrier properties. HNF4-\(\alpha\) could

Figure 2 Cytokines effect on hepatocyte nuclear factor 4-alpha activity and inflammation associated-cancer in hepatocytes. Cytokines regulate hepatocyte nuclear factor 4-alpha (HNF4-\(\alpha\)) function through its proteosomal degradation, DNA binding affinity, transcriptional activity and cofactor interaction (left panel). HNF4-\(\alpha\) participates also to a retro-inhibition feedback loop of the oncogenic interleukin (IL)-6/STAT3 pathway (right panel). In hepatocytes, HNF4-\(\alpha\) binds to miR-124 promoter and activates its transcription. MIR-124 targets IL-6 receptor transcript to decrease its expression and then block the activation of IL-6 pathway. On the other hand, stimulation of hepatocytes by IL-6 activates the phosphorylation of STAT3 that binds and transactivates the promoters of miR-24 and miR-629. These two microRNA target HNF4-\(\alpha\) transcript and decrease its expression to relieve inhibition of the IL-6 pathway. The perturbation of this molecular circuit ultimately results in the sustained activation of the oncogenic STAT3 pathway and the loss of the tumour suppressor effect of HNF4-\(\alpha\) P1 isoforms.
also indirectly control the microbiota to prevent the progressive emergence of chronic inflammation based on previous results showing that Paneth cells integrity is affected in HNF4-α knockout mice\(^{[41]}\). Finally, HNF4-α could be part of regulatory signals closely involved in the maintenance of the regenerative mucosal properties during chronic injuries (Figure 3). In contrast to hepatocytes that express P1-only isoforms, intestinal epithelial cells express both P1 and P2 isoforms. It is plausible that these isoforms harbour distinct roles during intestinal inflammation based on the fact that inflammation can influence HNF4-α activity through cofactors interaction\(^{[38]}\) and that P2 isoforms lack the cofactor interacting domain AF-1. Moreover, the fact that HNF4-α isoforms are differently expressed in the course of intestinal diseases such as colorectal cancer\(^{[8,25]}\) strengthens the idea that this might occur during inflammation. Exploration of these possibilities will turn out to be of clinical relevance in the context of targeting HNF4-α for diagnostic and/or therapeutically strategies designed to control pathophysiological conditions of IBD.

REFERENCES

1. Krits AA, Argyroskastiritis A, Moschonas NK, Power S, Katrakili N, Zannis VI, Coreghi S, Talianidis I. Isolation and characterization of a third isoform of human hepatocyte nuclear factor 4. Gene 1996; 173: 275-280 [PMID: 8964514 DOI: 10.1016/0378-1119(96)00183-7]

2. Thomas H, Jaschikowitz K, Bulman M, Frayling TM, Mitchell SM, Roosen S, Lingott-Frieg A, Tack CJ, Ellard S, Ryffel GU, Hattersley AT. A distant upstream promoter of the HNF-4alpha gene connects the transcription factors involved in maturity-onset diabetes of the young. Hum Mol Genet 2001; 10: 2089-2097 [PMID: 11590126 DOI: 10.1093/hmg/10.19.2089]

3. Drewes T, Senkel S, Holewa B, Ryffel GU. Human hepatocyte nuclear factor 4 isoforms are encoded by distinct and differentially expressed genes. Mol Cell Biol 1996; 16: 925-931 [PMID: 8622695]

4. Furuta H, Iwasaki N, Oda N, Hinokio Y, Horikawa Y, Yamagata K, Yano N, Sugahiro J, Ogata M, Ohgawara H, Omori Y, Iwamoto Y, Bell GI. Organization and partial sequence of the hepatocyte nuclear factor-4 alpha/MODY1 gene and identification of a missense mutation, R127W, in a Japanese family with MODY. Diabetes 1997; 46: 1652-1657 [PMID: 9313765 DOI: 10.2337/diacare.46.10.1652]

5. Hansen SK, Parrizas M, Jensen ML, Pruhova S, Ek J, Bej SF, Johansen A, Maestro MA, Rivera F, Eiberg H, Andel M, Lebl J, Pedersen O, Ferrer J, Hansen T. Genetic evidence that HNF-1alpha-dependent transcriptional control of HNF-4alpha is essential for human pancreatic beta cell function. J Clin Invest 2002; 110: 827-833 [PMID: 12235114 DOI: 10.1172/JCI15085]

6. Nakhei H, Lingott A, Lemm I, Ryffel GU. An alternative splice variant of the tissue specific transcription factor HNF-4alpha predominates in undifferentiated murine cell types. Nucleic Acids Res 1998; 26: 497-504 [PMID: 9421506 DOI: 10.1093/nar/26.2.497]
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Briançon N, Weiss MC. In vivo role of the HNF4α alpha-1 activation domain revealed by exon swapping. *EMBO J* 2006; 25: 1253-1262 [PMID: 16498401 DOI: 10.1038/ sj.emboj.7601021]

Tanaka T, Jiwan S, Hotta H, Takano K, Iwanari H, Sumi K, Daigo K, Ohashi R, Sugai M, Ikegame C, Umezue H, Hirayama Y, Midorikawa Y, Hippo Y, Watanabe A, Uchiyama Y, Hasegawa G, Reid P, Aburatani H, Hamakubo T, Sakai J, Naito M, Kodama T. Dysregulated expression of P1 and P2 promoter-driven hepatocyte nuclear factor-α in the pathogenesis of human cancer. *J Pathol* 2006; 208: 662-672 [PMID: 16400631 DOI: 10.1002/path.1928]

Torres-Padilla ME, Fougeré-Deschatrette C, Weiss MC. Expression of HNF4α in mouse liver development is regulated by sequential promoter usage and constitutive 3’ end splicing. *Mech Dev* 2001; 109: 183-193 [PMID: 11737232 DOI: 10.1016/S0925-4773(01)00521-4]

Boj SF, Parrizas M, Maestro MA, Ferrer J. A transcription factor regulatory circuit in differentiated pancreatic cells. *Proc Natl Acad Sci USA* 2001; 98: 14481-14486 [PMID: 11717395 DOI: 10.1073/pnas.241349398]

Briançon N, Bailly A, Clotman F, Jacquinon P, Lemaigre AF, Weiss MC. Expression of the α7 isoform of hepatocyte nuclear factor (HNF) 4 is activated by HNF6/OC-2 and HNF1 and repressed by HNF4α in the liver. *J Biol Chem* 2004; 279: 33398-33408 [PMID: 15159395 DOI: 10.1074/jbc.M405312200]

Hatzis P, Talianidis I. Regulatory mechanisms controlling human hepatocyte nuclear factor 4α gene expression. *Mol Cell Biol* 2001; 21: 7320-7330 [PMID: 11585914 DOI: 10.1128/MCB.21.21.7320-7330.2001]

Rammamourthy A, Li L, Gaedigk A, Bradford LD, Benson EA, Fleckhaur B, Skaar TC. In silico and in vitro identification of microRNAs that regulate hepatic nuclear factor 4α expression. *Drug Metab Dispos* 2012; 40: 726-733 [PMID: 22232426 DOI: 10.1124/dmd.111.040329]

Takagi S, Nakajima M, Kida K, Yamaura Y, Fukami T, Yokoi T. MicroRNA regulate human hepatocyte nuclear factor 4α, modulating the expression of metabolic enzymes and cell cycle. *J Biol Chem* 2010; 285: 4415-4422 [PMID: 20081894 DOI: 10.1074/jbc.M109.054341]

Wirsing A, Senkel S, Klein-Hitpass L, Ryffel GU. A systematic analysis of the 3’UTR of HNF4A mRNA reveals an interplay of regulatory elements including miRNA target sites. *PLoS One* 2011; 6: e27438 [PMID: 22144041 DOI: 10.1371/journal.pone.027438]

Hatziapostolou M, Polyarchou C, Aggelidou E, Drakaki A, Poulissides GA, Jaeger SA, Ogata H, Karin M, Struhl K, Hadjioannou G, Flockhart DA, Skaar TC. In silico and in vitro identification of hepatocyte nuclear factor regulatory circuit in differentiated pancreatic epithelial cells. *Mol Cell Biol* 2001; 21: 1393-1403 [PMID: 11585324 DOI: 10.1128/MCB.21.4.1393-1403.2001]

Parviz F, Mutullo C, Garrison WD, Savatski L, Adamson JW, Ning G, Kaestner KH, Rossi J, Zaret KS, Duncan SA. Hepatocyte nuclear factor 4α controls the development of a hepatic epithelium and liver morphogenesis. *Nat Genet* 2003; 34: 292-296 [PMID: 12680453 DOI: 10.1038/ng1175]

Bolotin E, Liao H, Ta TC, Yang C, Hwang-Verslues W, Evans JR, Jiang T, Sladek FM. Integrated approach for the identification of human hepatocyte nuclear factor 4α liver target genes using protein binding microarrays. *Hepatology* 2010; 51: 642-653 [PMID: 20054869 DOI: 10.1002/hep.23357]

Battle MA, Konopka G, Parviz F, Gaggil AL, Yang C, Sladek FM, Duncan SA. Hepatocyte nuclear factor 4α orchestrates expression of cell adhesion proteins during the epithelial transformation of the developing liver. *Proc Natl Acad Sci USA* 2006; 103: 8419-8424 [PMID: 16714383 DOI: 10.1073/pnas.0606246103]

Wang Z, Bishop EP, Burke PA. Expression profile analysis of the inflammatory response regulated by hepatocyte nuclear factor 4α. *BMC Genomics* 2011; 12: 128 [PMID: 21352552 DOI: 10.1186/1471-2164-12-128]

Odom DT, Zizziplerger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DG, Farnenkel L, Bell GI, Young RA. Control of pancreas and liver gene expression by HNF transcription factors. *Science* 2004; 303: 1378-1381 [PMID: 14988562 DOI: 10.1126/science.1089769]

Guo H, Gao C, Mi Z, Wai PY, Kuo PC. Phosphorylation of Ser158 regulates inflammatory redox-dependent hepatocyte nuclear factor-4α transcriptional activity. *Biochem J* 2006; 394: 379-387 [PMID: 16351573 DOI: 10.1042/BJ20051730]

Simó R, Baroisa-Desonges A, Hernandez C, Selva DM. IL1β down-regulation of sex hormone-binding globulin production by decreasing HNF-4α via MEK-1/2 and JNK MAPK pathways. *Mol Endocrinol* 2012; 26: 1917-1927 [PMID: 22902540 DOI: 10.1210/me.2012-1152]
47 Darsigny M, Babeu JP, Dupuis AA, Furth EE, Seidman EG, Levy E, Verdu EF, Gendron FP, Boudreau F. Loss of hepatocyte-nuclear-factor-4alpha affects colonic ion transport and causes chronic inflammation resembling inflammatory bowel disease in mice. PLoS One 2009; 4: e7699 [PMID: 19896810 DOI: 10.1371/journal.pone.0007690]

48 Ahn SH, Shah YM, Inoue J, Morimura K, Kim I, Yim S, Lambert G, Kurotani R, Nagashima K, Gonzalez FJ, Inoue Y. Hepatocyte nuclear factor 4alpha in the intestinal epithelial cells protects against inflammatory bowel disease. Inflamm Bowel Dis 2006; 14: 908-920 [PMID: 17358728 DOI: 10.1002/ibd.20141]

49 Barrett JC, Lee JC, Lees CW, Prescott NJ, Anderson CA, Phillips A, Wesley E, Parnell K, Zhang H, Drummond H, Nimmo ER, Massey D, Blaszczyk K, Elliott T, Cotterill L, Dallal H, Lobo AJ, Mowat C, Sanderson JD, Jewell DP, Newman WG, Edwards C, Ahmad T, Mansfield JC, Satsangi J, Parkes M, Mathew CG, Donnelly P, Peltonen L, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Craddock N, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CC, McCarthy MI, Palmer CN, Plomin R, Rautanen A, Sawyer SJ, Samani N, Trembath RC, Viswanathan AC, Wood N, Spencer CC, Barrett JC, Bellenguez C, Davison D, Freeman C, Strange A, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilim R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Perez ML, Potter SC, Ravindranarajah R, Ricketts M, Waller M, Weston P, Widdas S, Whitaker P, Deloukas P, Peltonen L, Mathew CC, Blackwell JM, Brown MA, Corvin A, McCarthy MI, Spencer CC, Attwood AP, Stephens J, Sambrook J, Ouwehand WH, McArthur WL, Ring SM, Strachan DP. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. Nat Genet 2009; 41: 1330-1334 [PMID: 19915572 DOI: 10.1038/ng.483]

50 van Sommeren S, Visschedijk MC, Festen EA, de Jong DJ, Ponsioen CY, Wijmenga C, Weersma RK. HNF4α and CDH1 are associated with ulcerative colitis in a Dutch cohort. Inflamm Bowel Dis 2011; 17: 1714-1718 [PMID: 21744425 DOI: 10.1002/ibd.21541]

51 Marcil V, Sinnett D, Seidman E, Boudreau F, Gendron FP, Beaulieu JF, Menard D, Lambert M, Bitton A, Sanchez R, Amre D, Levy E. Association between genetic variants in the HNF4A gene and childhood-onset Crohn’s disease. Genes Immun 2012; 13: 556-565 [PMID: 22914453 DOI: 10.1038/gene.2012.37]

52 Koukos G, Polytauchou C, Kaplan JL, Morley-Fletcher A, Gras-Miralles B, Kokotou E, Baril-Dore M, Pothoulakis C, Winter HS, Iliopoulos D. MicroRNA-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis. Gastroenterology 2013; 145: 842-52.e2 [PMID: 23856509 DOI: 10.1053/j.gastro.2013.07.001]

53 Rollith J, Phesse TJ, von Burstin VA, Poutcziw T, Bennecke M, Bateman T, Nebelsieck T, Lundgren-May T, Canli O, Schwatilla S, Matthews V, Schmid RM, Kirchner T, Arkan MC, Ernst M, Guten FR, gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumourigenesis. Cancer Cell 2009; 15: 91-102 [PMID: 19185844 DOI: 10.1016/j.ccancl.2009.01.002]

54 Grivennikov S, Karin E, Terzic J, Muicda D, Yu GY, Vallabhappanuru S, Scheller J, Rose-John S, Cherouste H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 2009; 15: 103-113 [PMID: 19185845 DOI: 10.1016/j.ccr.2009.01.001]
Babeu JP et al. HNF4-α in liver and gut inflammation

tumors progression. Exp Oncol 2010; 32: 167-171 [PMID: 21403612]

56 Bonzo JA, Ferry CH, Matsubara T, Kim JH, Gonzalez FJ. Suppression of hepatocyte proliferation by hepatocyte nuclear factor 4α in adult mice. J Biol Chem 2012; 287: 7345-7356 [PMID: 22241473 DOI: 10.1074/jbc.M111.334599]

57 Rieck S, Zhang J, Li Z, Liu C, Naji A, Takane KK, Fiaschi-Taesch NM, Stewart AF, Kushner JA, Kaestner KH. Overexpression of hepatocyte nuclear factor-4α initiates cell cycle entry, but is not sufficient to promote β-cell expansion in human islets. Mol Endocrinol 2012; 26: 1590-1602 [PMID: 22798294 DOI: 10.1210/me.2012-1019]

58 Walesky C, Gunewardena S, Terwilliger EF, Edwards G, Borude P, Apte U. Hepatocyte-specific deletion of hepatocyte nuclear factor-4α in adult mice results in increased hepatocyte proliferation. Am J Physiol Gastrointest Liver Physiol 2013; 304: G26-G37 [PMID: 23104559 DOI: 10.1152/ajpgi.00064.2012]

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