Review Article

The Role of HNF1B in Tumorigenesis of Solid Tumours: a Review of Current Knowledge

(HNF1B / tumorigenesis / immunohistochemistry)

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Abstract. Hepatocyte nuclear factor 1-β is a transcription factor which plays a crucial role during ontogenesis in the differentiation of visceral endoderm from primitive endoderm, and is especially important for the normal development of the kidney, urogenital tract, gastrointestinal tract, liver, and pancreas. Despite the growing knowledge about the potential involvement of hepatocyte nuclear factor 1-β in the process of carcinogenesis, the exact underlying mechanism that would explain its rather varied effects in different tumours has not been sufficiently investigated. Most of the data regarding the significance of hepatocyte nuclear factor 1-β arise from genome-wide association studies and is concerned with the influence of single-nucleotide polymorphisms of hepatocyte nuclear factor 1-β on either the increased or decreased susceptibility to certain types of cancer. However, the influence of both the germinal and somatic mutations of this gene on the process of carcinogenesis is still poorly understood. According to current data, in some tumours hepatocyte nuclear factor 1-β acts as a protooncogene, while in others as a tumour suppressor gene, although the reasons for this are not clear. The exact incidence of hepatocyte nuclear factor 1-β mutations and the spectrum of tumours in which they may play a role in the process of carcinogenesis remain unknown. From the practical point of view, immunohistochemical expression of hepatocyte nuclear factor 1-β can be used in differential diagnostics of certain tumours, especially clear cell carcinoma. In our article we review the current knowledge regarding the significance of hepatocyte nuclear factor 1-β in carcinogenesis.

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Abbreviations: aa – amino acid, Atoh1 – atonal basic helix-loop-helix transcription factor 1, BAG1 – B-cell lymphoma 2-associated athanogene, CCA – clear cell adenocarcinoma, CCC – clear cell carcinoma, CD24 – cluster of differentiation 24, CD44 – cluster of differentiation 44, CHK1 – checkpoint kinase 1, CpA1 – carboxypeptidase A1, CRC – colorectal carcinoma, DDR1 – discoidin domain receptor tyrosine kinase 1, DFS – disease-free survival, EC – endometrial carcinoma, ERBB4 – human epidermal growth factor receptor 4, ESR1 – oestrogen receptor 1, GATA4 – GATA4 binding protein, GS – Gleason score, GWAS – genome-wide association study, HCC – hepatocellular carcinoma, HNF1A – hepatocyte nuclear factor 1-α, HNF1B – hepatocyte nuclear factor 1-β, Hnf6 – hepatocyte nuclear factor 6, HSPD1 – heat-shock protein family D1, ICC – intrahepatic cholangiocarcinoma, IGFBP2 – insulin-like growth factor binging protein 2, IGFBP5 – insulin-like growth factor binging protein 5, IGF2 – insulin-like growth factor 2, IHC – immunohistochemistry, i1 – intron 1, i2 – intron 2, i4 – intron 4, i6 – intron 6, i8 – intron 8, Jag1 – jagged 1, MA – metanephric adenoma, MESH – Medical Subject Headings, MTSCC – mucinous tubular and spindle cell carcinoma, MODY – maturity-onset diabetes of the young, NCBI – National Center for Biotechnology Information, NF-κB – nuclear factor κ-light-chain-enhancer of activated B cells, NLS – nuclear localization signal, NR4A1 – nuclear receptor subfamily 4 group A member 1, OCCC – ovarian clear cell carcinoma, OR – odds ratio, OS – overall survival, PAWR – pro-apoptotic Wilms tumour 1 regulator, PCDH10 – protocadherin 10, PDAC – pancreatic ductal adenocarcinoma, PIK3CG – phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit γ, RCAD – renal cysts and diabetes, PDX1 – pancreatic and duodenal homeobox 1, POU – Pit-1/Oct-1/Unc-86 transcription factor, POU_H – POU homeodomain, POU_S – POU-specific domain, PTF1A – pancreas-associated transcription factor 1a, RAP2A – Ras-related protein Rap-2a, RCC – renal cell carcinoma, RCT – renal cell tumour, RT-PCR – real-time polymerase chain reaction, RUNX3 – runt-related transcription factor 3, SFRP5 – secreted frizzled related protein 5, SNP – single-nucleotide polymorphism, SOX9 – sex-determining region Y-related high mobility group box, TPD52 – tumour protein D52, UTR – untranslated region.

Introduction

Hepatocyte nuclear factor 1-β (HNF1B, which is encoded by the *HNF1B* gene, also known as *TCF2*) is a transcription factor that plays a crucial role during ontogenesis in the differentiation of visceral endoderm from primitive endoderm (Barbacci et al., 2004; Cereghini, 1996). The HNF1B protein regulates expression of multiple genes involved in the cell cycle modulation, susceptibility to apoptosis, and glucose metabolism (Pontoglio, 2000; Suzuki et al., 2015; Tsuchiya et al., 2003). Despite the growing number of studies demonstrating the potential involvement of HNF1B in the process of carcinogenesis, the exact underlying mechanism that would explain its rather varied effects in different lesions of different anatomical settings has not been sufficiently investigated and is still poorly understood. Furthermore, even the very question of its basic oncogenic or tumour suppressor effects does not seem to have a unified answer, as HNF1B has been reported to act in either capacity in different lesions, or even in the same lesions when examined by different authors. Given the inconsistent findings, the aim of this study was to summarize the current and previous findings concerning the role of HNF1B in selected solid tumours, with a special focus on tumours of the female and male genital tract, the urinary tract, pancreatic cancer, and colorectal cancer.

Material and Methods

A comprehensive review of all available literature published on the subject of HNF1B and its involvement in several selected solid tumours was performed, including the results of our studies focusing on HNF1B. The data was obtained through a database search using the MESH (Medical Subject Headings) terms “HNF1B”, “cancer”, “solid tumours”, “prostate cancer”, “colorectal cancer”, “female genital tract cancer”, “ovarian cancer”, “pancreatic cancer”, “SNP variants”, “cancer risk association”, “immunohistochemistry” and their combinations. Data was mined from the PubMed/MEDLINE, ScienceDirect, and Web of Science databases. Once a thorough search was performed, all the articles obtained were reviewed by the authors in order to determine their relevance. The results of the studies reviewed were not a criterion for exclusion, as both positive and negative associations needed to be taken into account. Studies were only excluded if they focused on *HNF1B* mutations leading to congenital anomalies of the kidneys and urinary tract, as only the association with cancer was relevant for this review. A strong emphasis was placed on studies with well-conducted methodology, based on large patient cohorts. Special care was taken to seek out studies focusing on full mutation analyses with a clearly defined clinical significance. Following analysis of all the available data, this paper undertook to comprehensively summarize the current state of knowledge of the involvement of HNF1B in the development and progression of selected solid tumours.

HNF1B isoforms

HNF1B is located at chromosome 17q12 and comprises nine coding exons that span around 60 kb (MIM#189907). It is a member of the homeodomain-containing gene superfamily of transcription factors and encodes transcription factor Pit-1/Oct-1/Unc-86 (POU), which is involved in a number of signalling pathways and plays a significant role in endodermal development (Alvelos et al., 2015). The resulting protein has three functional domains: the DNA-binding domain (POU-specific domain and an atypical homeodomain, POU homeodomain), the transactivation domain, and the dimerization domain (El-Khairi and Vallier, 2016).

According to the current knowledge, there are three fully characterized HNF1B isoforms reported by NCBI and Ensemble databases. The wild-type transcript variant 1 (GenBank NM_000458) is 2815 bp long and gives rise to 557 amino acid (aa) protein isoform 1 (UniProtKB P35680). The other two alternatively spliced transcripts are transcript variant 2 (2746 bp; GenBank NM_001165923) encoding 531 aa long protein with a shorter form of exon 3, which, compared to isoform 1, lacks aa 183-208. The transcript variant 3 (2432 bp; GenBank NM_001304286) codes for 457 aa long protein isoform 3, also with a shorter form of exon 3 compared to isoform 1 with missing aa 420-551 in the C-terminus, which leads to the skipping of exons 7 and 8. Other protein isoforms are reported in the Ensemble database, but the reference mRNA sequence is missing, as well as their more detailed analysis. The precise characterization of all HNF1B isoforms in different lesions is missing, and both the literature data and the comparison of isoform variants among the databases are equivocal. There is huge inconsistency in the literature regarding the described HNF1B isoforms, resulting from differently described sequences of particular protein isoforms, named 1, 2, and 3 or A, B, and C, in various investigated tissues. Previously described isoforms are inconsistent with the ones currently declared in the databases of NCBI and Ensemble according to the data gained from projects devoted to massive parallel sequencing of genomes and transcriptomes, which is probably based on more accurate data. A comprehensive schematic diagram of the current HNF1B transcripts and their domains is provided in Fig. 1.

The expression of the HNF1B protein is altered in several tumour types, and both down- and up-regulation has been described thus far based on the tumour type. However, only one study analysed expression of particular mRNA HNF1B transcripts and noted a switch in the expression of HNF1B transcripts in prostate cancer tissues compared to non-malignant prostate tissue (Harries et al., 2010). In this study, the authors described changes in the expression of the B form and C form comparing 39 non-malignant benign prostatic hyperplasia samples and 21 prostate adenocarcinoma samples. However, the impact of this work is questionable, since the C variant (RefSeq NM_006481) was not proved to exist and in the

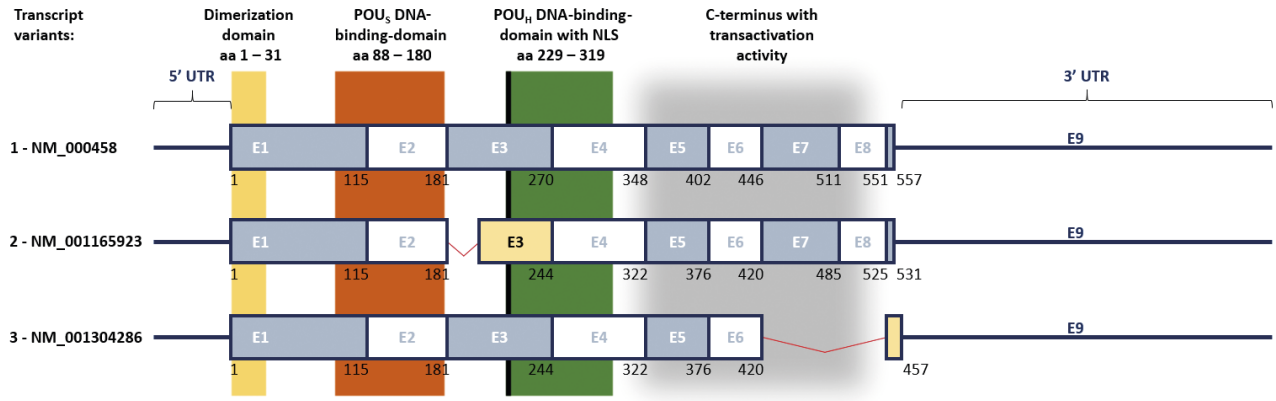

Fig. 1. Schematic diagram of the HNF1B transcripts (according to the RefSeq database)
The blue and white boxes illustrate the coding parts of reference exons, with the amino acid (aa) numbers indicated below the corresponding exons. The yellow boxes represent alternative exons. The yellow, brown, black, green, and grey areas illustrate the functional domains across the HNF1B transcripts. The lengths of exons are represented proportionally to scale. Transcript 2 lacks 26 aa (78 bp) at the 5' end of exon 3. Transcript 3 lacks the entire exons 7 and 8, and contains an alternative STOP codon in exon 9 due to a frame-shift (110 bp after the original STOP codon). Abbreviations: UTR – untranslated region, NLS – nuclear localization signal, POU_S – POU-specific domain, POU_H – POU homeodomain.

NCBI database was permanently suppressed. Data about mRNA expression of the individual HNF1B transcripts is still missing.

Germline *HNF1B* mutations

Germline mutations in the *HNF1B* gene are linked with a number of diseases associated with defects in kidney development, kidney and liver disorders, pancreatic atrophy, genital malformations, a complex syndrome known as renal cysts and diabetes (RCAD), and maturity-onset diabetes of the young type 5 (MODY5) or neonatal diabetes (Bellanne-Chantelot et al., 2004; Edghill et al., 2006; Lokmane et al., 2008; Adalat et al., 2009). Mutations in *HNF1B* have also been associated with hypomagnesemia and renal magnesium wasting, suggesting that HNF1B plays a role in both nephrogenesis and maintenance of renal tubular functions (Adalat et al., 2009). Other described associated phenotypes include autism, epilepsy, gout, and primary hyperparathyroidism. However, the precise description of germline mutations of *HNF1B* and associated non-neoplastic diseases is out of the scope of this review. With respect to the association of *HNF1B* germline mutations and tumours, only rare (Lebrun et al., 2005; Rebouissou et al., 2005) case studies describing cases of chromophobe renal carcinoma associated with a combination of monoallelic germline and somatic mutations have been published (Clissold et al., 2015; Bockenhauer and Jaureguiberry, 2016; Verhave et al., 2016).

Somatic *HNF1B* mutations

According to the results of our comprehensive search, so far no data showing somatic variants in the coding sequence of the *HNF1B* gene in different types of cancer (including pancreatic, prostate, kidney and colorectal cancer) has been published, as a vast majority of the reviewed studies focus on analysing the intronic sequences and potentially significant single-nucleotide polymorphisms (SNPs). The SNPs of highest significance according to the literary data are summarized in Table 1, along with their location in the *HNF1B* gene. Alterations detected in tumour samples are collected in the cosmic database, including data from peer-reviewed published variants or from large systematic screen studies. Some alterations of the *HNF1B* gene have been described, including mutations, copy number variations, and methylations in different types of tumours. However, the information about the somatic or hereditary origin is often missing, and just a few of these were confirmed to be somatic (Forbes et al., 2015). In our recent study, screening of genetic variants in the whole coding sequences of *HNF1B* revealed four mutations in 30 endometrioid carcinomas and one missense variant among 12 ovarian clear cell carcinomas (OCCC) (Nemejcova et al., 2015a). With the exception of these, there are no other studies focusing on the genetic changes of *HNF1B* in solid tumours.

A new potential biomarker of selected solid cancers

The genetic and epigenetic changes of *HNF1B* apparently play a pleiotropic role in tumorigenesis. Surprisingly, despite the growing knowledge of the importance of *HNF1B* SNPs in several cancer types, little is known about the mutations of this gene in various tumours, except for some SNPs in the non-coding intronic sequences of the *HNF1B* loci associated with the risk of kidney cancer (Rebouissou et al., 2005), endometrial cancer (Setiawan et al., 2012; Painter et al., 2015), and ances-

Table 1. Summarization of the HNF1B SNPs with the highest reported significance in the literary data, with their location in the HNF1B gene and the studied cohort where they were of statistical significance concerning their effect on carcinogenesis

SNP	Location in *HNF1B*	Chromosome 17 position, GRCh37	Studied cohort	References
rs4430796	intron 2	g.36098040G>A	endometrial cancer	Spurdle et al., 2011; Setiawan et al., 2012; Mandato et al., 2015
			ovarian clear cell carcinoma	Shigetomi et al., 2014
			prostate cancer	Sun et al., 2008; Thomas et al., 2008; Elliott et al., 2010; Harries et al., 2010; Berndt et al., 2011; Kim et al., 2011; Grisanzio et al., 2012; Chornokur et al., 2013; Hu et al., 2013; Zhao et al., 2015
rs757210	intron 2	g.36096515T>C/G	ovarian cancer	Ross-Adams et al., 2016
rs7501939	intron 1	g.36101156T>C	prostate cancer	Elliott et al., 2010; Kim et al., 2011; Chornokur et al., 2013
rs11649743	intron 4	g.36074979G>A	prostate cancer	Harries et al., 2010; Sun et al., 2008
rs7405696	intron 1	g.36102035G>C	prostate cancer	Berndt et al., 2011; Kim et al., 2011
rs4794758	intron 4	g.36080428C>T	prostate cancer	Berndt et al., 2011
rs1016990	intron 4	g.36088915G>C	prostate cancer	Berndt et al., 2011
rs3094509	intron 6	g.36062299A>G	prostate cancer	Berndt et al., 2011
rs11868513	intron 8	g.36052692G>A	prostate cancer	Kim et al., 2011
rs2074429	intron 6	g.36061297C>T	prostate cancer	Kim et al., 2011

try-specific *HNF1B* variants associated with prostate cancer (Berndt et al., 2011; Hindorff et al., 2011). There is also emerging evidence about the involvement of HNF1B in pancreatic cancer, while for colorectal cancer no relationship has been confirmed as yet (Elliott et al., 2010; Janky et al., 2016).

Rather than somatic variants in cancer tissues, what has been the focus of several studies are epigenetic changes, pointing to the complex nature of HNF1B's influence. Epigenetic silencing of the *HNF1B* gene has been reported in several types of human cancers, including breast cancer, colorectal carcinoma (CRC), and ovarian cancer (Bubancova et al., 2017). For example, the development of sporadic colorectal cancer has been traditionally described as the result of accumulated genetic and epigenetic alterations, and studies have suggested that the common event in the carcinogenesis of CRC is the association of global hypomethylation with discrete hypermethylation at the promoter regions of various specific genes. *HNF1B* was one of the five genes that had the highest average hypermethylation percentage (50 %) in the test group of CRC, and the authors suggest that DNA methylation may serve as a non-invasive epigenetic marker of this tumour (Silva et al., 2013). The other hypermethylated genes included *RUNX3* (58.9 %), *PCDH10* (55.5 %), *SFRP5* (52.1 %), and *IGF2* (50.4 %).

The exact mechanism by which HNF1B participates in the process of cancerogenesis is unknown and probably differs in various types of tumours. In one study, knockdown of *HNF1B* in OCCC led to induction of apoptosis (Tsuchiya et al., 2003). This correlates with the results of a recent study showing that up-regulation of HNF1B by inflammatory cytokine NF-κB/p65 decreases OCCC susceptibility to apoptosis (Suzuki et al., 2015). Depending on the relevant genetic and epigenetic changes, *HNF1B* can probably serve as either a tumour suppressor gene or an oncogene in different cancers. It has been shown that down-regulation of HNF1B in clear cell renal cell carcinoma and prostate carcinoma is associated with tumour progression and poor prognosis (Buchner et al., 2010; Noto et al., 2010).

A different situation is present in OCCC. We and others have found that the promoter of *HNF1B* in OCCC is typically unmethylated and gene expression is increased compared with other ovarian cancer types (Tsuchiya et al., 2003; Shen et al., 2013; Nemejcova et al., 2015a). In our study, we have not identified any methylated case in a group of 15 OCCCs. In contrast, we detected methylation of the *HNF1B* gene promoter in 4/30 endometrioid carcinoma samples (Nemejcova et al., 2015a). Methylation of the *HNF1B* gene promoter was also found in some cancer cell lines derived from pancreatic, colorectal, gastric, and ovarian tumours (Terasawa et al., 2006). All of these diverse findings support the suggestion that *HNF1B* may in fact represent one of the emerging "lineage-dependent" oncogenes, which while acting as master transcriptional regulators in normal cell lines show abnormal expression in tumours derived from these lines (Cuff et al., 2013). In a cancerous setting, their expression favours tumour survival.

The specific mechanisms through which *HNF1B* exerts its influence are yet to be fully uncovered. Some authors have termed it to be a pro-differentiation factor, which in healthy tissues suppresses the epithelial-mes-

enchymal transition and has potent tumour-suppressive activity (Ross-Adams et al., 2016). In tissues that have undergone malignant transformation, *HNF1B* was, however, suggested as a new oncogene inducing the cancerous phenotype (which was enhanced by co-expression of *ERBB2*) and activating epithelial-mesenchymal transition and formation of invasive phenotypes (Matsui et al., 2016). Other suggestions are related to HNF1B's association with stem/progenitor cells – several studies have hypothesized that HNF1B could regulate expression of the genes associated with these cells. For instance, HNF1B was reported to activate the *CD24* gene (recently identified as a marker of renal progenitor population) in OCCC, osteopontin (associated with liver progenitors), and *CD44* (associated with cancer stem cells in various types of tumours) (Yu et al., 2015).

Female genital system

The role of *HNF1B* SNPs in the development and risk of endometrial carcinoma (EC) has already been established, especially demonstrating the relationship between SNP rs4430796 (located in the intron 2 (i2) of *HNF1B*, also known as rs17626333 or rs58756954) and a decreased risk of endometrial cancer (Spurdle et al., 2011; Setiawan et al., 2012). In recent years, new data has emerged focusing on the relationship between HNF1B and prognosis of endometrial cancer. One study analysing association between the rs4430796 polymorphism and overall survival (OS) in 191 patients with endometrial cancer found rs4430796 (particularly the GG genotype) to be an independent risk factor for OS in EC patients (with the GG genotype showing the worst prognosis) (Mandato et al., 2015). Interestingly, this study demonstrated a significant interaction between rs4430796 and the success of adjuvant therapy, as the polymorphism was found to be related to OS only in patients who received a combined treatment with both radiotherapy and chemotherapy. These findings therefore suggest that rs4430796 could play a key role in tumour chemosensitivity and represent a useful predictive marker capable of distinguishing the patient population that would profit from the administration of a sensitization agent (as the presence of the A allele of rs4430796 in EC patients treated with chemotherapy may be responsible for an increased response to therapy). With the G allele being named as a possible culprit for the reduced chemosensitivity through HNF1B overexpression, it could be useful in the future to select those patients who are candidates for chemotherapy and adjust the therapy options accordingly. Some of the suggested explanations for these results are connected to the effect of HNF1B overexpression on some of the key regulators of the cell cycle, especially the aberrant retention of the G2 checkpoint. This conclusion is further supported by similar results reported in the setting of OCCC, where the overexpression of HNF1B may also cause aberrant retention of the G2 checkpoint, leading to chemoresistance of this particular tumour. This study demonstrated chemosensitization by a CHK1 inhibitor in CCA (Shigetomi et al., 2014).

Another SNP associated with ovarian cancer risk is rs757210 (located in i2, also known as rs3786124 or rs60456671), which was linked to increased promoter methylation in high-grade serous carcinoma of the ovary and also to increased HNF1B expression in OCCC (Ross-Adams et al., 2016).

In regard to protein expression, HNF1B was identified in 2003 as the first positive, relatively specific immunohistochemical marker of OCCC (Tsuchiya et al., 2003). An example of immunohistochemical evaluation of HNF1B in selected tumours is provided in Fig. 2. Recently, one study suggested that the association of HNF1B with a clear cell phenotype might reflect derivation from the secretory endometrium (Cuff et al., 2013). Since the first implication of HNF1B, its expression in non-neoplastic tissue and neoplasms of the female genital tract has been analysed in several studies (Kato et al., 2006; Yamamoto et al., 2007; Kato and Motoyama, 2009; Park et al., 2011; Kenny et al., 2012). These studies analysed HNF1B expression in endometriosis, normal endometrium, and lesions and tumours of the cervix and ovary. Most of the early studies found that the expression of HNF1B is mostly restricted to clear cell carcinoma (CCC) (Tsuchiya et al., 2003; Kato et al., 2006; Yamamoto et al., 2007; Ye et al., 2016). However, more recent studies have described HNF1B expression not only in CCC, but also in other tumour types including serous, endometrioid, and mucinous carcinomas and most types of borderline tumours (Tomassetti et al., 2008; Kalloger et al., 2011; Park et al., 2011; Fadare and Liang, 2012). In our study, we have recently described expression of HNF1B in cervical adenocarcinomas, and we have also observed its expression in atypical polypoid adenomyomas of the uterus (Nemejcova et al., 2015a, b).

Furthermore, down-regulation of HNF1B may contribute to drug resistance in ovarian cancer, which was suggested by a bioinformatic analysis of the mRNA expression data from online databases of ovarian cancer cases and in platinum-resistant ovarian cancer cells (Li et al., 2014). This study also suggested several protein/gene, protein/protein, or gene/gene partners that were also proved to be involved in drug resistance in cancer.

In the endometrial lesions, ours and other recent studies have found expression of HNF1B in some cases of endometriosis and in normal endometrium, especially in the secretory phase or gestational state (Yamamoto et al., 2007; Kato and Motoyama, 2009; Nemejcova et al., 2015a). Based on these results, HNF1B is not a specific marker of CCC and can be commonly found in other non-tumour and tumour lesions. However, among the tumours, strong expression of HNF1B is usually found in CCC only. In our study, we observed differing HNF1B expression in ECs depending on the degree of tumour differentiation (Nemejcova et al., 2016). Poorly differentiated (grade 3) ECs seldom express HNF1B compared with well or moderately differentiated (grade 1 or 2) ECs.

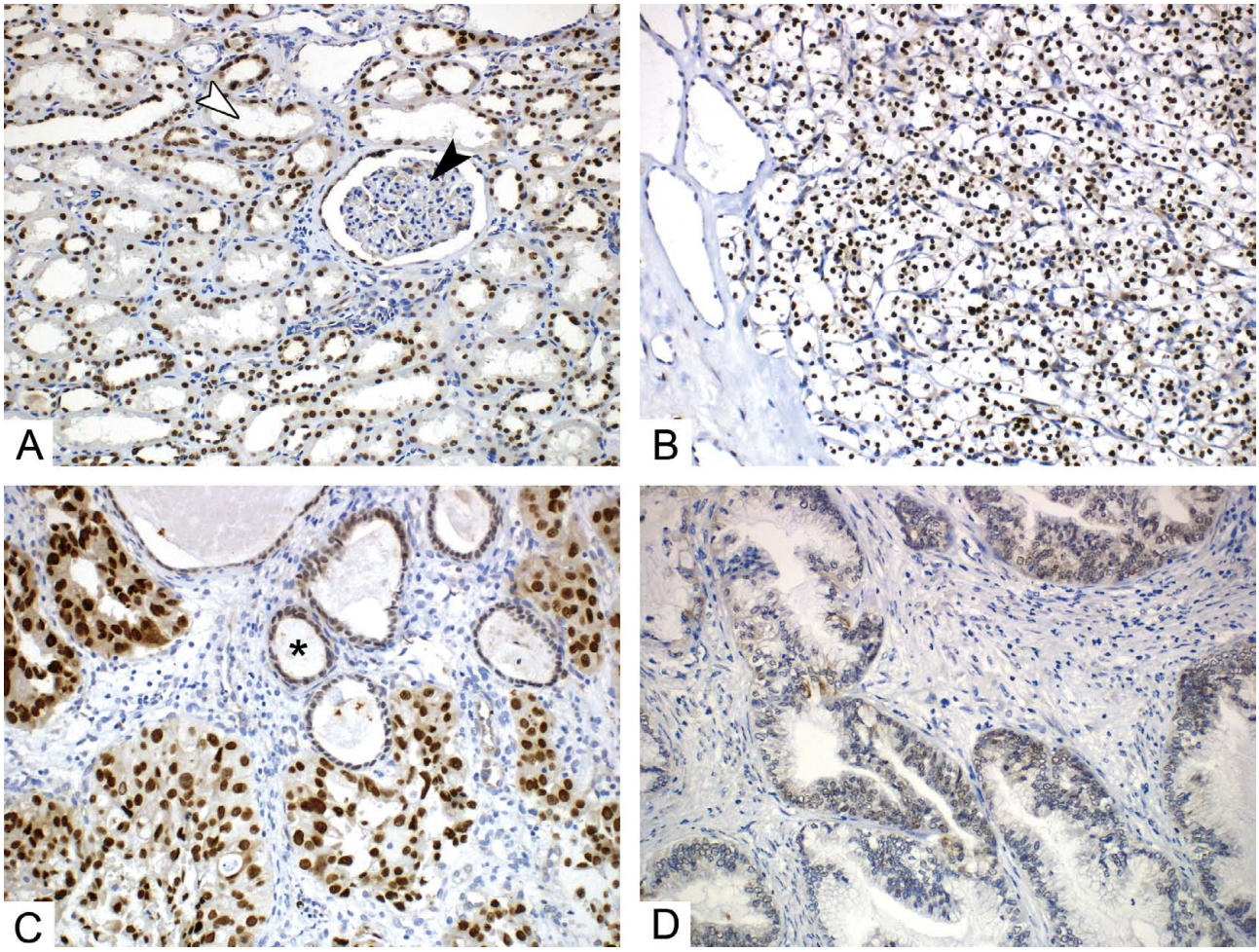

Fig. 2. Immunohistochemical evaluation of HNF1B expression in various lesions
(A) Normal renal parenchyma, showing strong diffuse nuclear positivity in tubular cells (white arrow). Note the negativity of the glomerulus (black arrow) (200×). **(B)** Clear cell renal cell carcinoma demonstrating diffuse, strong nuclear positivity of the tumour cells (200×). **(C)** Clear cell endometrial carcinoma. Note the strong nuclear positivity of HNF1B in tumour cells when compared to the weak nuclear positivity of the atrophic endometrial glands (black asterisk) (200×). **(D)** Endometrioid endometrial carcinoma with mucinous differentiation. Only a portion of the tumour cells show weak nuclear HNF1B positivity (200×).

Colorectal cancer

HNF1B plays a significant role in the terminal differentiation and cell fate commitment of intestinal epithelial cells, where it works together with HNF1A in directly controlling expression of Jag1 and Atoh1 upstream of the Notch pathway (D'Angelo et al., 2010). In colorectal carcinoma, only some epigenetic alterations of *HNF1B* were found, with the conclusion that this marker may be used as a biomarker for detection of the early stage of colorectal cancer, but its expression has not yet been investigated (Silva et al., 2013). The significance of HNF1B expression in colorectal cancer was also evaluated as a part of collaborative analysis of data from 19 genome-wide association studies (GWAS) focusing on a number of diverse cancers (prostate cancer, colorectal cancer, breast cancer, lung and pancreatic cancer, melanoma); however, for the two potentially significant *HNF1B* SNPs (rs4430796 and rs7501939, located in i2 and i1, respectively), there was no statistically significant association (Elliott et al., 2010). Prostate cancer was the only malignancy that has shown a significant relationship with these two *HNF1B* SNPs.

Pancreatic cancer

HNF1B is one of the transcription factors that take part in directing differentiation of pre-pancreatic foregut endodermal cells into pancreatic progenitors via sequential activation of the Hnf1b – Hnf6 – Pdx1 cascade (Poll et al., 2006). HNF1B thus controls pancreatic morphogenesis, and its experimental early deletion leads to a reduced pool of pancreatic multipotent progenitor cells as a result of increased apoptosis and decreased cellular proliferation (De Vas et al., 2015). HNF1B has also been described as one of the molecular markers of pancreatic progenitor cells, along with CpA1 and Sox9 (Pinho et al., 2011). It has also been suggested that HNF1B plays a critical role in the development and differentiation of

at least the dorsal part of the pancreas, as agenesis of the pancreatic body and tail are both parts of the systemic disease phenotype in *HNF1B* mutation carriers (Haldorsen et al., 2008).

Pancreatic ductal adenocarcinoma (PDAC) is still relatively poorly understood at the genetic level. However, according to a recent study based on 27 microdissected surgical samples, there are three distinct molecular subtypes of PDAC (classical, exocrine-like, and quasi-mesenchymal) associated with type-specific gene signatures, which have been defined as PDAssign (Collisson et al., 2011). The authors also provide evidence for the clinical outcome and therapeutic response differences between these subtypes, with the classical subtype being associated with best survival, while the quasi-mesenchymal subtype showed the worst survival.

The prognostic relevance of molecular subtypes of PDAC was subsequently recently confirmed on a larger cohort of samples (whole tumour tissue obtained from 118 surgically resected PDAC) and possible master regulators were evaluated, naming *HNF1B* as a good candidate master regulator of pancreatic differentiation (Janky et al., 2016). In this study, the immunohistochemical expression of HNF1B in PDAC and normal pancreatic tissue was assessed. While both the normal acinar parenchyma and ductal cells showed clear nuclear staining, a gradual loss of nuclear expression was observed through the well-differentiated and poorly differentiated PDAC (correlating to the recorded down-regulation of HNF1B in tumour samples). The samples of well-differentiated PDAC demonstrated low expression of HNF1B, with minimal to no expression observed in poorly differentiated pancreatic ductal adenocarcinoma. The malignant ductal cells of the pancreas therefore lose expression of HNF1B at the protein level, suggesting that *HNF1B* may play a role as a tumour suppressor in pancreatic cancer.

Similar results concerning HNF1B expression in pancreatic tumours were also reported in another study dealing with the master regulatory genes of pancreatic embryonic development (Kondratyeva et al., 2017). In their work the authors studied 21 samples of pancreatic tumours of various histogenesis and found that compared to samples of normal pancreatic tissue, the tumour samples showed significantly reduced expression of *HNF1B* and other master regulatory genes. For *HNF1B*, this reduction is linked to promotor hypermethylation. The authors further stated that *HNF1B*, along with *SOX9*, *GATA4*, *PDX1* and *PTF1a*, form a single regulatory module with complex interregulatory interactions, all involved in the regulation and development of pancreatic ductal adenocarcinoma. The fact that the expression suppression was observed in all the mentioned master regulatory genes leads to the proposition that these may in fact either all be required to work together in order to sustain the cell identity of the tumour cell progenitor, or that they act in a hierarchical order, with one gene controlling the expression of the others in determining the malignant transformation of pancreatic cells.

Prostate cancer

Prostate cancer susceptibility is one of the most heritable (with genetic factors being estimated to account for 42 % of the risk), and with the increasing availability of GWAS the number of reported risk associations is growing steadily. To date, over 40 risk loci with a strong association with prostate cancer have been reported (Grisanzio et al., 2012). Several of these loci are located at the 17q12 chromosome, where *HNF1B* is placed, and therefore have been the focus of attention of studies dealing with prostate cancer for more than a decade. However, despite the large number of published results (often based on tremendously large patient cohorts) the conclusions remain largely inconsistent. There is no doubt that several intron-situated SNPs have been proved to be statistically significantly associated with prostate cancer, although their role and clinical meaning are often either unclear or not explained at all. The reported associations also seem to differ significantly concerning the authors' conclusions about the biological significance of their findings, as will be discussed below, the same SNPs are frequently reported as either being associated with increased prostate cancer susceptibility or as a protective factor, further underlying the complexity of this issue.

The most commonly studied *HNF1B* SNP in association with prostate cancer is rs4430796 (located in i2), which also well represents the significant differences between the published results. Although several GWAS reported the SNP variant rs4430796 as the most significant in men of European ancestry (a finding that was later confirmed in a study of Japanese men as well), the significance of this variant does not seem to be consistent at all. Some authors describe it as a protective factor leading to a decreased risk of prostate cancer development (Thomas et al., 2008; Elliott et al., 2010; Berndt et al., 2011; Kim et al., 2011; Chornokur et al., 2013; Zhao et al., 2015), others report it as being associated with increased prostate cancer susceptibility (Sun et al., 2008; Grisanzio et al., 2012; Hu et al., 2013). Additional studies confirmed the significance of rs4430796 and named the *HNF1B* intronic variants rs11649743 (intron 4; i4), rs7405696 (i1), rs4794758 (i4), rs1016990 (i4), and rs3094509 (i6) as the best model for risk in this region (Sun et al., 2008; Thomas et al., 2008; Harries et al., 2010; Berndt et al., 2011). However, the inconsistencies in the estimated biological significance of these remain. For instance, SNP variant rs7405696 is reportedly associated both with an increased odds ratio (OR) (Berndt et al., 2011) and with a decreased OR as a protective factor (Kim et al., 2011). Similarly, rs7501939 was associated with both increased (2.42 in men of African American descend) and decreased (0.71) ORs as well (Kim et al., 2011; Chornokur et al., 2013). Interestingly, one study suggested that *HNF1B* rs7501939 (i1) was associated with increased prostate cancer risk exclusively in obese men of African American descent, concluding that variations in *HNF1B* may influence

prostate cancer risk in this specific population (Chornokur et al., 2013). As the burden of prostate cancer varies between certain racial and ethnic groups (with African American men demonstrating 1.6 times higher incidence and 2–3 times higher mortality rates), this finding is of particular interest. As these authors further discuss, studies that investigated prostate cancer risk and its association with *HNF1B* SNPs report mixed results when stratified by ethnicity.

A statistically significant association was also reported in Korean prostate cancer patients, in a study naming 14 SNPs and three haplotypes that were significantly associated with prostate cancer risk (Kim et al., 2011). Nine of these SNPs were associated with a lower risk of prostate cancer and five SNPs were linked with an increased risk of the disease, all located in the intronic region of *HNF1B*. Of these, one SNP in particular (rs11868513, located in *HNF1B* i8) was more frequently found in patients with tumours of a greater stage, while two SNPs (rs4430796, rs2074429, located in i2 or i6, respectively) and one haplotype (Block3_htl) were more common in patients with a higher Gleason score ($GS \geq 7$) than in those with $GS < 6$. At the moment, there is no single plausible explanation for these discrepancies, other than that the association between different SNP variants in the 17q12 region and the development of prostate cancer exists, but it appears to be highly complex and it is probable that the reported SNPs work in a combined fashion rather than on their own. This would be in accordance with the observation of Berndt et al., who stated that the significance of *HNF1B* SNPs increases with an increased number of risk alleles (with OR of 0–2 risk alleles being set as 1.0 and for a combination of 8–10 risk alleles increased to 1.88) (Berndt et al., 2011).

Although the biological mechanism by which HNF1B may be implicated in increased prostate cancer risk has not yet been determined, differential levels of HNF1B expression have been associated with prostate cancer recurrence (Glinsky et al., 2004). A number of recent forays into the topic of HNF1B and its functional role and mechanistic effects highly support the idea of pleiotropy as the driving underlying force. One recent genetic and functional analysis performed by Grisanzio et al. (2012) on one of the largest cohorts of European American, African American, and Japanese men also suggests that the suppression of HNF1B expression affects cellular phenotypes associated with tumour-related properties (colony formation, proliferation, viability) of prostate cancer cells, implying other mechanisms through which HNF1B may contribute to prostate cancer pathogenesis. One of the recent studies also presents an interesting hypothesis – that HNF1B is involved in prostate cancer risk via modulating androgenic hormone effects and coordination with other genes (namely, *BAG1, DDR1, ERBB4, ESR1, HSPD1, IGFBP2, IGFBP5, NR4A1, PAWR, PIK3CG, RAP2A*, and *TPD52*) (Hu et al., 2013). According to this study, HNF1B was highly expressed in an androgenic hormone-dependent cell line, pointing to its possible association with steroid hormone metabolism.

In prostatic tissue, the immunohistochemical expression of HNF1B was assessed in only one study in relation to tumour progression and aggressiveness, and the authors found that the expression of this marker was strongly associated with cancer cell proliferation (Debiais-Delpech et al., 2014). Nuclear HNF1B staining was significantly increased in the castration-resistant prostatic cancer and prostatic cancer with metastases groups when compared with clinically localized forms. In patients with clinically localized prostatic cancer, the expression of HNF1B was strongly associated with cancer cell proliferation.

Kidney cancer

Tumours of the kidney should be of special interest when it comes to alterations of *HNF1B*, given the enormous significance of this transcription factor in the normal development of the renal tubular system. According to one study, there is a high frequency of chromosome 17q DNA alterations, leading to changes in HNF1B expression and therefore pointing to a possible role of HNF1B in papillary renal cell tumour (RCT) development (Szponar et al., 2011). These alterations combine specific duplication of the large chromosome 17q21.31-qter region, and more importantly, duplication/amplification of the chromosome 17q12 region, which among others contains the *HNF1B* gene. The authors studied the genetic changes and expression of HNF1B in papillary RCTs, metanephric adenoma (MA), mucinous tubular and spindle cell carcinoma (MTSCC) and their precursor lesions, as well as Wilms' tumour. The results acquired by RT-PCR showed that HNF1B is overexpressed in adult tumours of embryonal origin (papillary RCTs, Mas, and MTSCCs) and in their precursors – embryonic rests. In comparison, conventional RCCs, renal oncocytomas, chromophobe RCCs, and Wilms' tumours all showed only low levels of HNF1B expression, if any, which is particularly surprising given the above-mentioned results of other authors, who found conventional RCCs to show strong expression. The increased levels of expression in papillary RTCs were not only demonstrated via quantitative RT-PCR analysis, but also confirmed immunohistochemically. Immunohistochemical analysis showed strong positive nuclear staining in 56 % of papillary RTCs (38/67), and 100 % of metanephric adenomas (5/5) and mucinous tubular and spindle cell carcinomas (5/5). Strong nuclear positivity was also observed in all the precursor lesions (nephrogenic rests) associated with papillary RCTs and MTSCCs, suggesting that the overexpression of HNF1B in these lesions may lead to subsequent delayed tubular differentiation. This decreased cellular differentiation could represent the underlying factor in the development of the precursor lesions, which persist during life and lead to the development of papillary RTCs, MAs and MTSCCs. Wilms' tumour demonstrated negative

staining for HNF1B in the stromal and blastemal cells, but there was strong nuclear positivity in the differentiating tubules of surrounding nephrogenic rests. Renal oncocytomas showed isolated (1/18) positivity and chromophobe RCCs were negative for HNF1B staining, while conventional RCCs displayed only scattered nuclear positivity in 7 % (7/98) of cases. However, a different study focusing on renal cell carcinoma showed that the HNF1B mRNA expression correlated with malignant transformation and progression from normal renal tissue to primary tumour and to metastasis (Buchner et al., 2010). The same authors also performed immunohistochemistry (IHC) in order to localize HNF1B expression, which showed specific nuclear staining confined to the tumour cells of the primary tumours and metastases.

Contrary to the results published by Szponar et al. (2011), several studies identified HNF1B as not only a biomarker of OCCC, but as a broad marker of the clear cell phenotype in general. One such study further explored the relation between HNF1B and carcinomas of clear cell histology across both gynaecologic and renal carcinomas and found it to be strongly associated with both, similarly to their association with hypomethylation of the *HNF1B* promoter (Cuff et al., 2013). Furthermore, these authors also demonstrate a link between positive HNF1B immunostaining and an increased risk of clinically significant venous thrombosis (3-fold increase in the gynaecologic cohort and 2.3-fold increase in a combined gynaecologic and renal carcinoma cohort). HNF1B may therefore not be associated only with glycogen accumulation, but also with thrombosis.

Liver cancer

The significance of HNF1B in liver tumours is largely unknown. However, recently, the expression of HNF1B in hepatocellular carcinoma (HCC) has been more closely examined, and so far the results suggest that the expression of HNF1B predicts disease recurrence and HCC-specific death after liver transplantation in patients with HCC (Shim et al., 2013). Several interesting conclusions are presented in another recent clinicopathological study that focused on evaluating HNF1B expression in different pathologic subtypes of primary liver cancer (hepatocellular carcinoma and cholangiocarcinoma – ICC) (Yu et al., 2015). The immunohistochemically evaluated expression was associated with the pathological subtype of primary tumour (as HCC with strong nuclear expression displayed biliary phenotype) and was positively correlated with the expression of hepatocyte progenitor cell/biliary markers. Moreover, the results suggest that the HNF1B expression in HCC tumour tissue may be associated with the change of phenotype on recurrence. Out of the 183 cases of HCC examined in this study, 15 showed the ICC phenotype on recurrence, with all the cases showing strong immunohistochemical HNF1B positivity (with 3+ values in 80 % of them). Given these findings, HNF1B-positive tumour cells may in fact represent bipotential cells that are capable of giving rise to both hepatocellular and biliary cell lineages, which is supported by the fact that in rare instances a combined hepatocellular-cholangiocarcinoma containing malignant cells of both of these origins may develop. Regarding prognostic factors, the authors found the expression of HNF1B to be an independent risk factor for both disease-free survival (DFS) and OS in HCC (but not in ICC), associated with poorer disease outcome.

Conclusion

With the rise of genome-wide association studies and their use in human cancer genetics, it is increasingly apparent that a significant proportion of the identified risk alleles are located in the non-protein-coding parts of the human genome, and therefore probably represent regulatory elements acting through other regulatory protein transcripts. Given that most of the intron sequences remain unmapped, the number of emerging potentially significant variants is steadily increasing, although their interpretation and assessment of their meaning and biological effects are highly complicated and not always possible. According to the results of several recent studies referenced in this review, HNF1B may be one of these factors implicated in the pathogenesis of a number of solid tumours. Its role in the development of carcinomas with clear cell morphology (in both gynaecologic and non-gynaecologic setting) has already been established; however, as HNF1B also plays a distinctive role in the morphogenesis of other tissues, it is worth exploring whether differing expression and genetic changes of this factor could be implicated in the pathogenesis of other tumours of both the male and female genital tract, the urinary tract, and colorectal and pancreatic carcinoma. From the practical point of view, the immunohistochemical analysis of the HNF1B protein expression may be used in the differential diagnosis of several tumours. However, because of the paucity of knowledge regarding its expression in several tumour types, its use is currently limited mostly to tumours of the female genital tract and clear cell carcinomas of other origins. Despite its high sensitivity for clear cell carcinomas, the specificity is relatively low, and in routine practice a panel of other markers should be used, the correlation with tumour morphology being essential.

A comprehensive analysis of the morphological, genetic, immunohistochemical, and epigenetic changes of *HNF1B* is needed to better understand the role of HNF1B in the process of carcinogenesis, but such a complex analysis has yet to be undertaken.

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