Pathol. Oncol. Res. (2016) 22:523–530
DOI 10.1007/s12253-015-0037-2

ORIGINAL ARTICLE

Expression, Epigenetic and Genetic Changes of HNF1B in Endometrial Lesions

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Received: 28 October 2015 / Accepted: 15 December 2015 / Published online: 19 December 2015

Abstract Hepatocyte nuclear factor 1-beta (HNF-1-beta) is a transcription factor involved in cancerogenesis of various tumors, including endometrioid carcinoma. We performed comprehensive analysis of HNF-1-beta in lesions of the endometrium, including protein expression and genetic and epigenetic changes. Expression of HNF-1-beta was analyzed immunohistochemically in 320 cases including both tumor and non-tumor endometrial lesions. Promoter methylation and genetic variants were evaluated, using bisulphite and direct sequencing, in 30 (18 fresh frozen, 12 FFPE tumors) endometrioid carcinomas (ECs) and 15 ovarian clear cell carcinomas (OCCCs) as a control group. We detected expression of HNF-1-beta in 28 % of ECs (51/180 cases), 26 % of serous carcinoma (7/27 cases), 83 % of endometrial clear cell carcinoma (15/18 cases), 93 % of hyperplastic polyps with atypias (13/14 cases), 100 % of hyperplastic polyps without atypias (16/16 cases), 88 % of hyperplasias with atypias (14/16 cases), 91 % of hyperplasias without atypias (10/11 cases), and in ≥80 % of different normal endometrium samples. The control group of OCCCs showed HNF-1-beta expression in 95 % (18/19 cases). Methylation in promoter region was detected in 13.3 % (4/30) of ECs, but not in corresponding normal tissue where available, nor in OCCCs (0/15 cases). Mutation analysis revealed truncating variant c.454C > T (p.Gln152X) in one EC and missense variant c.848C > T (p.Ala283Val) was detected in one OCCC. In conclusion, expression of HNF-1-beta was detected in various extents in all types of lesions analyzed, nevertheless its strong expression was mostly limited to clear cell carcinomas. Biological significance of genetic and epigenetic changes needs further investigation.

Keywords Clear cell carcinoma · Endometrioid carcinoma · HNF-1-beta · Immunohistochemistry · Mutation analysis · Methylation

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Introduction

Hepatocyte nuclear factor 1-beta (HNF-1-beta protein is coded 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 [1, 2]. The gene is located at chromosome 17q12, comprises 9 coding exons that spans around 60 kb (MIM#189,907) and encodes four protein isoforms (designated as A, B, C and 4) [3, 4]. The longest A isoform is 557 amino acid (aa) protein that contains a dimerization domain, aa 1–31, and a DNA binding domain,

aa 231–311 (UniProt P35680; http://www.uniprot.org). The B isoform, comparing to A, lacks aa 183–208. The C isoform differs in structure from A in aa 350–400, and in the C terminus aa 401–557 are missing [3, 4]. A and B isoforms are regarded as transcription activators, whereas the C isoform is probably a transcription repressor [3]. HNF-1-beta protein regulates expression of multiple genes involved in cell cycle modulation, susceptibility to apoptosis, and glucose metabolism [5–7]. Hereditary mutations in the *HNF1B* gene are associated with a number of diseases associated with defects in kidney development and a complex syndrome known as renal cysts and diabetes (RCAD) [8, 9]. Expression of the HNF-1-beta protein is altered in several tumor types, with both down- or up-regulations described based on the tumor type. There can also be changes in isoform expressions in benign and malignant conditions [10]. Moreover, genetic and epigenetic changes of *HNF1B* also play role in tumorigenesis. However, only few studies have analyzed epigenetic changes of *HNF1B* in female genital tract tumors and only 6 somatic genetic variants (2 silent, 3 missense and 1 frameshift) were described in a group of 514 endometrioid carcinomas as a part of the large study "Uterine Corpus Endometrioid Carcinoma (TCGA, US) import from ICGC" (Cosmic database; accessed October 2015; http://www.sanger.ac.uk/cosmic) [11, 12]. Immunohistochemical expression of HNF-1-beta was considered as a marker of clear cell carcinomas (CCCs) [5]. However, recent studies have shown that this marker is not entirely specific and its expression can be found in tumors of other histogenesis and also in some non-tumor lesions and normal endometrium [13–21]. In our study, we focused on comprehensive analysis of HNF-1-beta expression in various endometrial lesions and in normal endometrium. Moreover, we analyzed genetic variations of the *HNF1B* coding region and methylation of the promoter region in selected cases of endometrioid carcinoma and ovarian clear cell carcinoma (OCCC) and compared these findings with immunohistochemical results.

Material and Methods

Formalin-fixed paraffin-embedded (FFPE) tissue blocks were obtained from the archive files of our department. Fresh frozen tissue samples were from Bank of Biological Material (BBM) of the First Faculty of Medicine, Charles University in Prague. Histologic review of the hematoxylin and eosin-stained slides was performed in all cases.

In total, 339 FFPE specimens was selected for immunohistochemical analysis, comprising of 320 endometrial lesions; 180 endometrioid carcinomas (77 well differentiated, 73 moderately differentiated, and 30 poorly differentiated), 27 serous carcinomas, 18 endometrial CCC, 14 hyperplastic polyps with atypias, 16 hyperplastic polyps without atypias, 16 hyperplasias with atypias, 11 hyperplasias without atypias, 38 samples from normal endometrium (18 proliferative endometrium, 15 secretory endometrium, and 5 atrophic endometrium); and as control 19 OCCC.

Promoter methylation and gene mutations were examined in 33 cases of endometrioid carcinoma (15 FFPE and 18 fresh frozen (FF) tumors), 15 FF corresponding normal non-tumor tissues and 19 FFPE samples of OCCC. We were able to isolate DNA of sufficient quality for molecular analysis from all fresh frozen tissues and from 12/15 (80 %) FFPE endometrioid carcinoma samples. Regarding OCCC FFPE samples, DNA of sufficient quality was obtained from 15/19 (79 %) cases for methylation analysis and 12/19 (63 %) cases for mutation analysis of whole coding region. Both, methylation and mutation analyses were successful in 10/15 endometrioid carcinomas and 12/19 OCCCs.

In compliance with the Helsinki Declaration, the project has been approved by Ethics Committee of General University Hospital in Prague.

Immunohistochemical Analysis

Immunohistochemical analysis was performed using the avidin-biotin complex method with antibody against the HNF-1-beta (polyclonal, dilution 1:500, Sigma-Aldrich, Prestige Antibodies, St. Louis, United States). Immunohistochemical results were assessed semi-quantitatively and graded on a four-tier scale based on the percentage of positive cells: 0 = <5 %; 1+ = 5–29 %; 2+ = 30–59 %; 3+ = >60 %. Only nuclear staining was regarded as positive. Moreover, the staining intensity of HNF-1-beta was assessed as weak, moderate or strong.

Statistical Analyses

Software R (https://www.r-project.org/) was used to perform Fisher exact test in order to compare different tumor groups based on the immunohistochemical staining intensity or the percentage of positive staining cells. All tests were two sided, and a *P*-value less than 0.05 was considered as significant.

Screening for Genetic and Epigenetic Alterations

Methylation status in promoter region and mutations in coding region and exon-intron boundaries of *HNF1B* were screened. DNA was extracted by using standard procedures implementing GeneRead™ DNA FFPE kit or QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). DNA was quantified fluorometrically by using dsDNA BR Assay Kit and Qubit 3.0 Fluorometer (Invitrogen, Thermo Fisher Scientific Inc., Waltham, MA, USA). Bisulfite modification was analyzed by using EZ DNA Methylation-Lightning Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions,

followed by sequencing. Experiments included two controls, non-methylated DNA and universally methylated DNA (Human HCT116 DKO Non-Methylated DNA and Human HCT116 DKO Methylated DNA; Zymo Research). Primers used for PCR amplification of promoter region and bisulphite sequencing of modified DNA (Table 1) were designed by using software http://www.zymoresearch.com/tools/bisulfite-primer-seeker. Primers for mutation analysis (Table 1) in the exons 1 to 9 and adjacent intron sequences were used for PCR reaction and direct Sanger sequencing. DNA regions of interest were amplified using 5× HOT FirePol EvaGreen HRM Mix NO ROX (Solis Biodyne, Tartu, Estonia) according to the manufacturer's instructions and analyzed by HRMA (High Resolution Melting Analysis). BigDye Terminator v3. 1 Ready Reaction Mix (Applied Biosystems, Thermo Fisher Scientific Inc., Waltham, MA, USA) was used for sequencing reaction and separation was performed on ABI 3130 genetic analyzer (Applied Biosystems). Mutations were detected by both forward and reverse primer. Suspicious fragments were verified by another independent PCR amplification and sequence analysis.

NCBI Reference sequence of the *HNF1B* gene (alternatively TCF2) is NM_000458.3. Promoter region 95–695 bp upstream ATG (start codon) was defined as putative promoter region according RefSeq (Based on UCSC/NCBI Assembly, NCBI Build 35, UCSC Version 17). Nomenclature of variants follow recommendations by the Human Genome Variation Society (HGVS). Mutations which were not found until October 2015 in the literature, the Single Nucleotide Polymorphism Database (http://www.ncbi.nlm.nih.gov/SNP/), the 1000 Genomes Database (http://www.1000genomes.org/), in Exome Sequencing Project (ESP; http://varianttools.sourceforge.net/Annotation/EVS), or in the Catalogue of Somatic Mutations in Cancer (http://www.sanger.ac.uk/cosmic) were considered as novel [22]. To evaluate the predicted effects of missense variants on protein biological function and stability, in silico prediction tools were used: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), Mutation

Table 1 Primer pairs used for PCR amplification and sequence analysis of the *HNF1B* gene

Exon	Name	Primers 5′- > 3′	bp	Amplicon [bp]
Primers for methylation analysis in promoter region				
	HNF1B_mp2F	TTAGGTTGTAAGGTATTGGTTTAATAAGTTTAAAGG	36	160
	HNF1B_mp2R	AAACAAACCTAAAAAACAAAAAACCTAAACC	31	
Primers for mutation analysis of coding region (* long exons were divided in two overlapping amplicons)				
*1	HNF1B_01f	TGCTTGTGAAACTCCCCTCCAC	22	277
	HNF1B_02r	TGGTGAGAGTATGGAAGACCGGC	23	
1	HNF1B_03f	TTGCTGCCATCCCCGAACTTC	21	274
	HNF1B_04r	TCCCCTCCACCTCGCTCTG	19	
2	HNF1B_05f	CCCCAGATGTCTCCCACTAGTACC	24	287
	HNF1B_06r	CACTTCAGGTTGAGGCAGAGGC	22	
*3	HNF1B_07f	GGTGTCTTCGTCCGTTGTCTGTC	23	258
	HNF1B_08r	ATTTGAACCGGTTGCGGCG	19	
3	HNF1B_09f	GTCAACAGAGCCATGGGCCTG	21	234
	HNF1B_10r	TCCTGGGTCTGTGTACTTGCCC	22	
*4	HNF1B_11f	CTGCTGTGATTGTGTGTTTTTGGCC	25	245
	HNF1B_12r	AGTCTGGTTGGAGCTATAGGCGTC	24	
4	HNF1B_13f	TGCAAACCGCAGGAAGGAGG	22	270
	HNF1B_14r	GAGAGCGGCCCTAGGATCATCTC	23	
5	HNF1B_15f	CAGGACCCTGGTGGCACTAATG	22	246
	HNF1B_16r	AGCTCCAGAGCGACAATGGC	20	
6	HNF1B_17f	GGAAACTGCTCTTTGTGGTCCAAGTC	26	241
	HNF1B_18r	TCTTCTTCTCCCTGCCCCCAAG	22	
7	HNF1B_19f	CTCCTTATCCCAGGAGCTGTCTGTG	25	290
	HNF1B_20r	ACTGAGGGTCCTGAGTGCTCC	21	
8	HNF1B_21f	GCCTGTGTATGCACCTTGATTCTG	24	226
	HNF1B_22r	CACATCCATGGCCTTATCACACCC	24	
9	HNF1B_23f	AATGACACAGCTGAGCACCCTC	22	172
	HNF1B_24r	CTCGCAGGTGCTGGTCAGG	19	

Taster (http://www.mutationtaster.org), and MUpro (http://mupro.proteomics.ics.uci.edu).

Results

Immunohistochemical Findings

All the results are summarized in Table 2. Figure 1 shows representative examples of the HNF-1-beta expression in various endometrial lesions.

The group of all CCCs in comparison with the tumors of different histogenesis showed significant difference in the HNF-1-beta expression based on the intensity ($p < 0.001$) as well as the extension of staining ($p < 0.001$) when comparing CCCs with serous carcinomas and comparing CCC with endometrioid carcinomas (EC).

Expression of HNF-1-beta was found in 33/37 (89 %) of all CCCs (18/19 [95 %] ovarian and 15/18 [83 %] endometrial). Fourteen cases OCCCs were 3+ positive, four cases showed 2+ positivity, and only one case was negative. From the group of endometrial CCCs there were eleven cases 3+ positive, two were 2+ positive, two were positive only focally 1+, and three were negative. The intensity of staining was in all HNF-1-beta positive CCCs strong, with the exception of 1 case of endometrial tumor, which showed weak staining in about 7 % of the tumor cells (1+).

ECs showed positivity of HNF-1-beta in 25/77 (32 %) of well differentiated tumors, 24/73 (33 %) of moderately differentiated tumors, and 2/30 (7 %) of poorly differentiated tumors. In total, 51/180 (28 %) ECs were HNF-1-beta positive. Nine ECs were 3+, 14 were 2+, and 28 were 1+ positive. The staining intensity was usually weak, only five cases showed moderate intensity. No EC demonstrated strong HNF-1-beta expression.

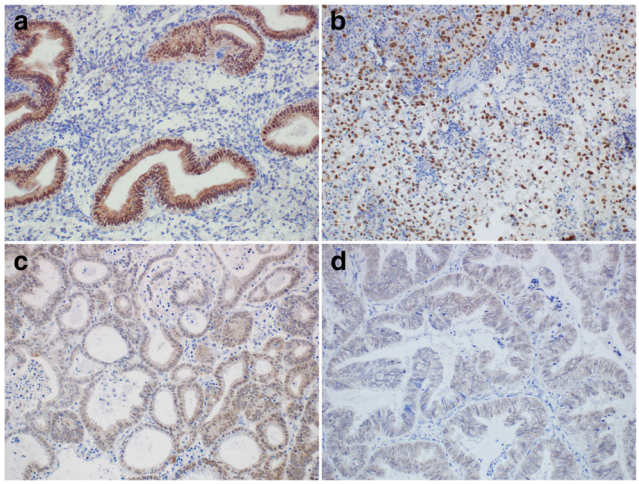

Fig. 1 HNF-1 beta expression in endometrial lesions. Strong HNF-1 beta expression in normal secretory endometrium (1a, 400×) and clear cell carcinoma (1b, 200×). Staining of moderate intensity in grade 1 endometrioid carcinoma (1c, 400×). Weak nuclear staining in grade 1 endometrioid carcinoma (1d, 400×). Note the nonspecific cytoplasmic staining in some cases

Serous carcinomas were HNF-1-beta positive in 7/27 cases (26 %). 2 cases were 1+ positive, 5 cases showed 3+ positivity. The intensity of staining was weak in three cases, moderate in three cases and strong in one case.

All 16 hyperplastic polyps without atypias and 13/14 (93 %) hyperplastic polyps with atypias were HNF-1-beta positive: 4 cases 2+, 12 cases 3+; and 3 cases 1+, 5 cases 2+, 5 cases 3+, respectively. The intensity of staining was mostly weak or moderate.

Weak or moderate intensity of HNF-1-beta was found in 10/11 (91 %) hyperplasias without atypias (2 cases 1+, 3 cases 2+, 5 cases 3+) and in 14/16 (88 %) of hyperplasias with atypias (8 cases 1+, 2 cases 2+, 4 cases 3+).

Samples from normal endometrium showed positivity of HNF-1-beta in 16/18 (89 %) of proliferative endometrium

Table 2 Summary of immunohistochemical results

HNF1B	OCCC	CCCE	SC	EC			HPA	HP	HA	H	Normal endom.		
				G1	G2	G3					Sec.	Pro.	Atr.
Total	19	18	27	77	73	30	14	16	16	11	15	18	5
Positive	18	15	7	25	24	2	13	16	14	10	15	16	4
3+	14	11	5	3	4	2	5	12	4	5	13	11	4
2+	4	2	0	7	7	0	5	4	2	3	1	4	0
1+	0	2	2	15	13	0	3	0	8	2	1	1	0
Negative	1	3	20	52	49	28	1	0	2	1	0	2	1

OCCC Ovarian clear cell carcinomas, *CCCE* Endometrial clear cell carcinomas, *SC* Serous carcinomas of endometrium, *EC* Endometrioid carcinomas, *HPA* Hyperplastic polyps with atypias, *HP* Hyperplastic polyps without atypias, *HA* Hyperplasias with atypias, *H* Hyperplasias without atypias, *Normal endom.* Normal endometrium, *Sec.* Secretory, *Pro.* Proliferative, *Atr.* Atrophic, *G1* Well differentiated, *G2* Moderately differentiated, *G3* Poorly differentiated carcinomas. Immunohistochemical results were assessed semi-quantitatively and graded on a four-tier scale based on the percentage of positive cells: 0 = <5 %; 1+ = 5–29 %; 2+ = 30–59 %; 3+ = >60 %

(1 case 1+, 4 cases 2+, 11 cases 3+), in all 15 cases of secretory endometrium (1 case 1+, 1 case 2+, 13 cases 3+), and in 4/5 (80 %) of atrophic endometrium (4 cases 3+). Intensity of staining in proliferative endometrium was weak in 10 cases and moderate in 8 cases. In secretory endometrium the intensity of staining was mostly moderate or strong, except of two cases which showed only weak intensity. In all cases of atrophic endometrium the intensity of staining was weak.

Genetic and Epigenetic Changes of the *HNF1B* Gene

Epigenetic Analysis

Gold standard method employing bisulphite modification of DNA followed by PCR amplification and direct sequencing of target area was used for the analysis of DNA methylation. We assessed DNA methylation in a part of the promoter region that spans 3 CpG islands (Fig. 2). Methylation in promoter region was detected in 4/30 (13.3 %) ECs, but not in 3 available corresponding normal tissues. Compared to EC, no case of promoter methylation was found in 15 cases of OCCC examined.

Mutation Analysis

Direct DNA sequence analysis of the *HNF1B* gene demonstrates 4 different single nucleotide variants in 4 ECs (one nonsense variant in exon 2, c.454C > T, p.Gln152X; Fig. 3; one silent variant in exon 3, c.585C > T, p.Asp195; two noncoding variants in intron 2 and intron 3, flanking exon 3: c.545-25C > T and c.809 + 27C > T), and one missense variant in exon 4 (c.848C > T, p.Ala283Val) in OCCC. All 3 exon variants were heterozygous transitions C > T, except mutation p.Gln152X where the major variant was mutant allele T. All variants have been reported for the first time. Non-tumor tissue was not available to confirm somatic status of the variant. Truncating mutation p.Gln152X leads to a shorter dysfunctional protein

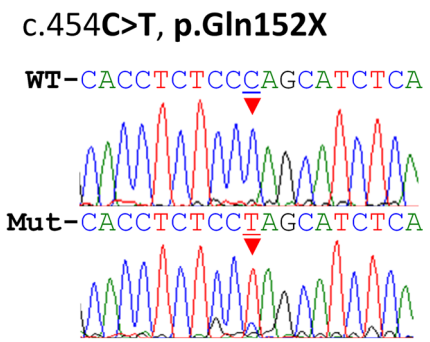

Fig. 2 Bisulfite sequence electropherograms. Fragments show methylation status in three CpG sites from HNF1B promoter. Representative electropherograms of (**a**) methylated, and (**b**) non-methylated are shown. Sodium bisulfite deaminates cytosine into uracil but does not affect 5-methylcytosine. Uracils are detected as thymines after PCR and sequencing reaction. *Triangles* point cytosins in CpG islands

and is generally considered as pathogenic, and p.Ala283Val was evaluated by in silico model as deleterious (Table 3).

Discussion

HNF-1-beta is a transcription factor which plays an essential role during ontogenesis and seems to be involved in cancerogenesis of various tumors, including endometrioid carcinoma [1, 2, 23–25]. As indicated by recent genome-wide association studies, common genetic variants of this gene are associated with risk of type II diabetes and either increased or decreased risk of several cancers including endometrioid endometrial carcinoma and serous ovarian carcinoma [7, 11, 26–29]. However, the exact mechanism by which *HNF1B* participates in the process of cancerogenesis is unknown and probably differs in various types of tumors.

In one study, knock-down of *HNF1B* in OCCC led to the induction of apoptosis [5]. This correlates with results of a recent study which has shown that upregulation of *HNF1B* by inflammatory cytokine NF-κB/p65 decreases OCCC susceptibility to apoptosis [6].

Regarding the fact that *HNF1B* can be either down-regulated or up-regulated, *HNF1B* can probably serve as either a tumor 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 tumor progression and poor prognosis [24, 30]. A different situation is present in OCCC. The promotor of *HNF1B* in OCCC is typically unmethylated and gene expression is increased compared with other ovarian cancer types [5, 11]. This corresponds to our data. We have not identified any methylated case in the control group of 15 OCCCs. On the contrary, methylation of *HNF1B* gene promoter was detected in 4/30 EC samples (in 1/12 FFPE and 3/18 fresh frozen tumors). The corresponding non-tumor tissue available in 15/18 fresh frozen patients was unmethylated. Methylation of the *HNF1B* gene promoter was found in some cancer cell lines derived from pancreatic, colorectal, gastric, and ovarian tumors [12]. DNA methylation is typically associated with the silencing of the gene expression. In our study, it was not possible to correlate *HNF1B* genetic and epigenetic variations with expression pattern of HNF-1-beta because of a small sample set. Nevertheless, we observed in all 4 cases weak or negative HNF-1-beta staining with methylated promoter, while stronger expression was detected in the group with unmethylated promoter.

Surprisingly, despite the growing knowledge about the importance of single nucleotide polymorphisms (SNPs) of *HNF1B* in several cancer types, little is known about mutations of this gene in various tumors, except for some germline mutations associated with kidney cancer [31]. In our study, screening of genetic variants in the whole coding region

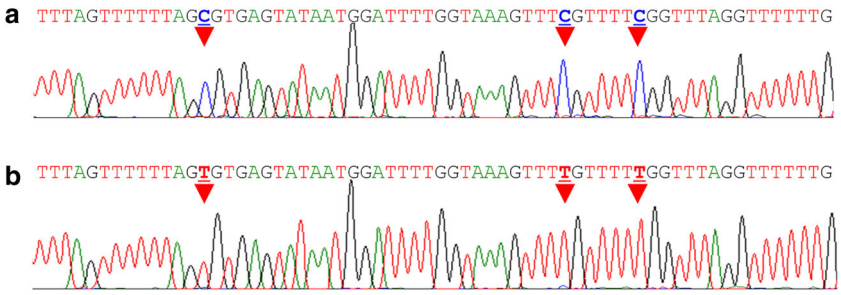

Fig. 3 Novel frame-shift mutation of *HNF1B*. Comparison between wild type (WT) and mutated (Mut) sequence. Changed nucleotide is indicated by a *triangle*

revealed 4 sequence variants in 30 ECs and one missense variant among 12 OCCCs. Nonsense mutation (p.Gln152X) identified in one EC leads to premature translation termination resulting to the aberrant protein formation. This truncated protein has retained epitope detected by our antibody which can explain detection of HNF-1-beta expression. Mutation detected in one OCCC case was missense variant leading to exchange of amino acid Alanine to Valine at the position 283 (p.Ala283Val). This variant affects DNA binding domain (amino acids 231–311) which suggests that this variant affects crucial function of the protein. High probability of damaging effect of this mutation was suggested also by in silico predictive model.

Regarding protein expression, HNF-1-beta was identified in 2003 as the first positive relatively specific immunohistochemical marker of CCC [5]. Since that, expression of HNF-1-beta in non-neoplastic tissue and neoplasms of the female genital tract was analyzed in few studies [13–18]. These studies have analyzed HNF-1-beta expression in endometriosis, normal endometrium, lesions and tumors of cervix and ovary. Most of the early studies found that expression of HNF-1-beta is mostly restricted to CCC [5, 14–16]. However, more recent studies have described HNF-1-beta expression not only in CCC but also in other tumor types including serous, endometrioid, and mucinous carcinomas and most types of borderline tumors [17, 19–21]. We and others have recently described expression of HNF-1-beta in cervical adenocarcinomas and we have also described its expression in atypical polypoid adenomyomas of the uterus [32, 33]. Other recent studies have found expression of HNF-1-beta in some cases of endometriosis and in normal endometrium, especially in the secretory phase or gestational state [13, 14]. The results of our study are in concordance with other recent studies, which have shown that HNF-1-beta is not a specific marker of CCC and can be commonly found in other non-tumor and tumor lesions. However, among tumors, strong expression of HNF-1-beta was in our study found only in CCC, with exception of one case of endometrial serous carcinoma. Moreover, we observed different HNF-1-beta expression in ECs depending on differentiation of tumor. ECs with poor differentiation seldom express HNF-1-beta compared with ECs with well or moderate differentiation.

Despite the fact that HNF-1-beta is a relatively sensitive marker of CCC, specificity is lower than previously thought. For example, in a recent study of ovarian and uterine ECs, ECs with clear cell change and CCC, the authors found sensitivity of HNF-1-beta similar as for Napsin A, but the specificity was lower (55.9 % vs. 93 %) [34]. Another recent study analyzed expression of HNF-1-beta, Napsin A, and AMACR in 279 ovarian carcinomas [35]. HNF-1-beta and Napsin A were expressed in 92 % and 82 % of OCCC, 7 % and 1 % of serous carcinoma, 37 % and 5.3 % of EC, 60 % and 0 % of mucinous tumors, and 100 % and 0 % of yolk sac tumors, respectively. HNF-1-beta expression in 45 endometrial carcinomas has also been assessed in another study analyzing 15 cases each from CCC, serous carcinoma and EC, respectively [25]. In their study, strong expression of HNF-1-beta was found in 100 % of CCC. Three out of 15 cases of EC (grade 3) showed expression in >25 % of tumor cells (50 % tumor cells, weak to moderate intensity). Five serous carcinomas were completely HNF-1-beta negative, 4 showed focal positivity in <25 % of tumor cells, and 6 cases showed strong positivity.

In conclusion, we performed comprehensive analysis of HNF-1-beta in normal endometrium and in various endometrial lesions, including its immunohistochemical expression

Table 3 *In silico* prediction of pathogenicity. Prediction analysis of identified novel variants in the *HNF1B* gene

Exon	Mutation designation*	Predicted effect on protein	Mutation Taster	PolyPhen-2	MUpro
2	c.454C > T	p.Q152X	Disease causing	NA	NA
4	c.848C > T	p.A283V	Disease causing	Probably damaging	Decrease stability

* GenBank Reference Sequence: NM_000458.3: +1 corresponds to the A of the ATG translation initiation codon. NA – in silico prediction tool is not applicable for nonsense mutations

and analysis of epigenetic and genetic changes. Immunohistochemical analysis of HNF-1-beta expression can be of use in the differential diagnosis of female genital tract tumors. We should be aware of some limitations, especially its low specificity for CCC. However, if we consider the character of staining, strong expression of HNF-1-beta was found in our study, with one exception, only in a group of CCCs. Further, molecular analysis revealed relatively common (13.3 %) methylation of the *HNF1B* promotor in EC compared to the unmethylated *HNF1B* promotor in OCCC. We have also found nonsense heterozygous mutations of *HNF1B* in 1 case of EC. Four other single nucleotide variants were identified, 3 of them in ECs (one silent variant in exon 3, and two noncoding variants in intron 2 and intron 3) and one in OCCC (missense variant in exon 4). However, regarding genetic and epigenetic changes, we are well aware of the limitations of our study because of small cohort of cases. The precise role of *HNF1B* in carcinogenesis as well as the importance of molecular targeting of the HNF-1-beta protein for therapeutic purposes remain unknown and require additional studies.

Acknowledgments This work was supported by Ministry of Health, Czech Republic (IGA MZ CR project NT14001-3/2013 and RVO-VFN 64165), by Charles University in Prague (Project PRVOUK-P27/LF1/1, UNCE 204024, and SVV UK 260148/2015), by Bank of Biological Material project BBM LM2010004, and by OPPK (Research Laboratory of Tumor Diseases, CZ.2.16/3.1.00/24509).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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