Disturbed Expression of Type 1 Iodothyronine Deiodinase Splice Variants in Human Renal Cancer

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Background: Alternative splicing, one of the sources of protein diversity, is often disturbed in cancer. Type 1 iodothyronine deiodinase (DIO1) catalyzes deiodination of thyroxine generating triiodothyronine, an important regulator of cell proliferation and differentiation. The expression of DIO1 is disturbed in different types of cancer. The aim of the study was to analyze the alternative splicing of DIO1 and its possible disturbance in renal cancer.
Methods: Using real-time PCR, we analyzed 19 tissue samples (T) of renal cancer and 19 matched control samples (C) of the opposite pole of the kidney, not infiltrated by tumor, and 6 control samples (N) (nonneoplastic kidney abnormalities).
Results: Cloning of DIO1 mRNA isoforms revealed 11 different transcripts, among them 7 new splice variants, not previously reported. The expression of all variants of DIO1 was dramatically (>90%) and significantly ($p \leq 0.0003$) lowered in samples T compared to control samples C. The ratio of mRNA isoforms encoding DIO1 protein variants possessing or lacking the active center was lowered in samples T compared with control samples C, suggesting disturbed alternative splicing of DIO1. The expression of mRNA of splicing factors SF2/ASF (splicing factor-2/alternative-splicing factor) and hnRNPA1 (heterogeneous ribonucleoprotein A1), regulating 5′-splice site selection, was significantly but not proportionally lowered in samples T compared to samples C. The mRNA ratio of splicing factors SF2/ASF and hnRNPA1 correlated with the ratio of mRNA isoforms encoding DIO1 protein variants possessing or lacking the active center in controls C but not in samples T.
Conclusions: Our results show that the expression and alternative splicing of DIO1 mRNA is disturbed in renal cancer, possibly due to changes in expression of splicing factors SF2/ASF and hnRNPA1.

Introduction

THE HUMAN TYPE 1 DEIODINASE GENE (*DIO1*; GeneID: 1733) is located in p33–32 region of chromosome 1 (1). Type 1 iodothyronine deiodinase (DIO1; EC 1.97.1.10) is one of the three enzymes regulating bioavailability of thyroid hormone via selective removal of iodine from iodothyronines (2). Iodothyronine deiodinases are selenoproteins with an atypical amino acid selenocysteine in the active center. DIO1 catalyzes two types of deiodination reaction, an outer-ring (i.e., 5′-deiodination) and an inner-ring (5-deiodination). Thus, DIO1 is the only enzyme whose action can both activate (5′-deiodination) and deactivate (5-deiodination) thyroid hormone. The active hormone, triiodothyronine (T3), binds thyroid hormone receptors (TRs) that act as ligand-dependent transcription factors. It has been shown that TRs are involved

in cancerogenesis (3,4) and regulate the expression of oncogenes (5) and tumor suppressors (6). Thus, changes in DIO1 expression may influence the process of cancerogenesis through effecting the level of T3.

DIO1 activity has been detected in liver, kidney, pituitary, and thyroid (2). Previous reports have shown that the expression of DIO1 is disturbed in different types of cancer. DIO1 mRNA and activity were shown to be decreased in papillary thyroid carcinoma (7–10), and increased in follicular adenoma and follicular thyroid carcinoma (7). DIO1 expression has been proposed as a differentiation marker of cancer cells (11,12).

The primary transcript of the *DIO1* gene undergoes alternative splicing in liver, leading to several mRNA variants (13). It has been suggested that proteins encoded by alternatively spliced DIO1 mRNAs could contribute to DIO1 activity in pig kidney (14).

TABLE 1. PRIMERS USED IN THIS STUDY

Primer	Sequence	Target
P1	F: GGAGGTGGCTGTGCATGTG	DIO1
P2	R: CTGCCAACGGACCTTCAAGAC	DIO1
P3	F: CCTGTGTCCCTAGCTGAATC	DIO1
P4	R: GTGCCTGTAGAGTGCAACTG	DIO1
P5	F: GAAGCACATGCATCAGATGG	DIO1
P6	R: CTCTGGTTCTGCATGGTGTC	DIO1
P7	F: TTCCAGACAGAGTCAAGC	DIO1
P8	R: GCCATCTTGCATAAACTCCC	DIO1
P9	F: AGCTTACTCTGGCTTTGC	DIO1
P10	R: GATTCAGCTAGGGACACAG	DIO1
P11	F: TGGATTTGGTAATGATGGAAGC	hnRNPA1
P12	R: TCTCTGGCTCTCCTCTCCTG	hnRNPA1
P13	F: TTAGATCTCATGAGGGAGAAA	SF2/ASF
P14	R: GAGAATAGCGTGGTGATCCT	SF2/ASF
P15	F: CGGCATCGTCACCAACTG	β-Actin
P16	R: GCTGGGGTGTTGAAGGTCTC	β-Actin
T7	F: ATTATGCTGAGTGATATCCCGCT	pGEM-T
SP6	R: CATAAGATATCACAGTGGATTTA	pGEM-T

F, forward primer; R, reverse primer; DIO1, type 1 iodothyronine deiodinase.

Alternative splicing is one of the mechanisms regulating gene expression in eukaryotes and significantly increases the diversity of encoded proteins. Numerous reports have shown that the process of alternative splicing is often disturbed in cancer, resulting in improperly spliced transcript variants (15).

The selection of splice sites is strictly regulated by elements of the spliceosome; for instance, the alternative usage of 5′ splice sites is controlled by the ratio of splicing factors SF2/ASF (splicing factor-2/alternative-splicing factor) and hnRNPA1 (heterogeneous ribonucleoprotein A1) (16).

Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cancer, representing 75–82% of primary malignancies of the kidney (17,18). The molecular basis of ccRCC is not entirely understood; however, several molecular markers have been proposed (17). It has also been reported that alternative splicing of several genes is disturbed in ccRCC (19–22). In a previous work we showed that the expression of DIO1 mRNA and activity are lowered in ccRCC (23), but the alternative splicing of DIO1 in cancer has not been studied yet. Here, we show that alternative splicing of DIO1 primary transcript in kidney tissues results in different transcript variants. We also show that the expression and alternative splicing of DIO1 pre-mRNA is disturbed in ccRCC tissues leading to imbalanced expression of transcript variants. Since we also observed disturbed expression of splicing factors SF2/ASF and hnRNPA1, it seems likely that this may be the reason for the aberrant DIO1 splicing.

Materials and Methods

Tissue specimens

Tissue samples were obtained with the permission of the Ethics Committee of Human Studies (The Medical Centre of

FIG. 1. The gene, transcripts, and protein product for DIO1 according to GeneID: 1733 in Entrez Gene database. (A) DIO1 gene and transcript variants. The upper panel shows the gene structure. The four exons are shown as black boxes. P1–P10: positions of the primers used. ATG and STOP: codons of start and stop of translation, respectively. TGA (Sec): triplet coding for selenocysteine. Below, transcript variants according to reference sequences: variant 1 (NM_000792.4), variant 2 (NM_213593.2), variant 3 (NM_001039715.1), and variant 4 (NM_001039716.1). (B) Protein product of transcript variant 1; the arrows show pivotal regions of the enzyme. DIO1, type 1 iodothyronine deiodinase; TM, transmembrane region; AC, active center; DR, dimerization region; Phe65, rT3 substrate specificity; Sec126, catalytically active residue (according to Refs. 35 and 36).
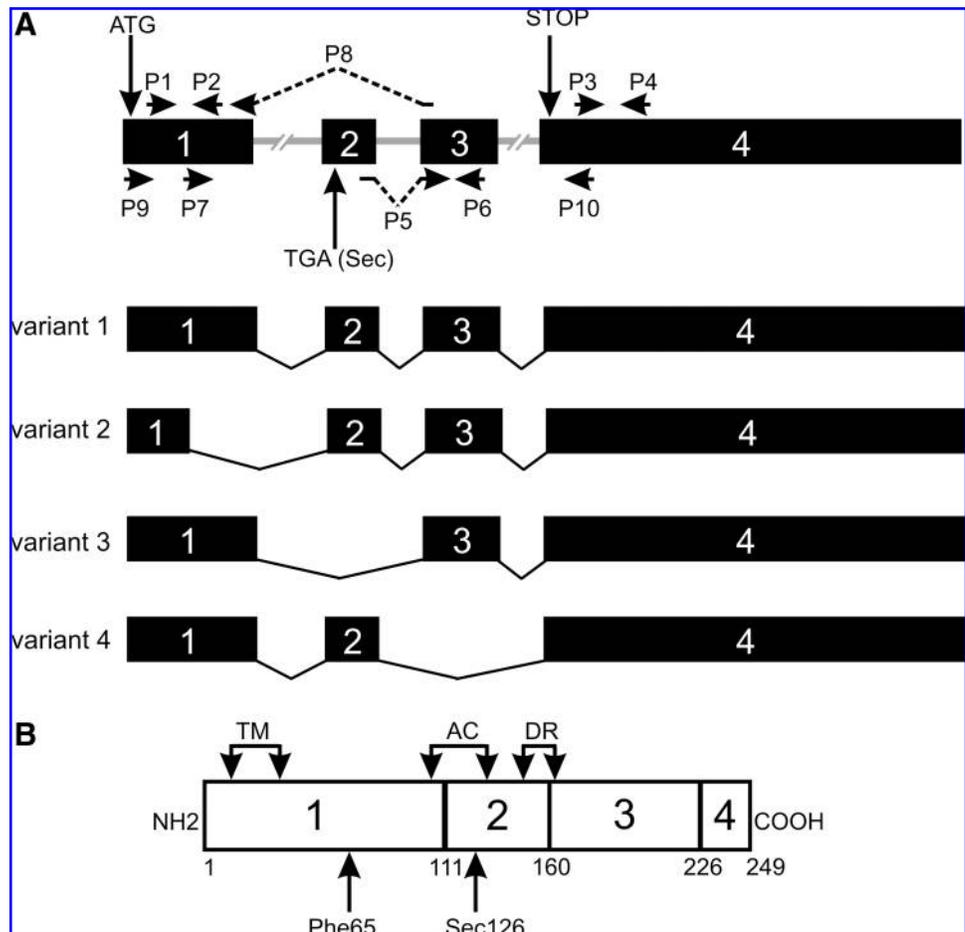
Postgraduate Education) from patients during unilateral ne-phrectomy performed due to kidney cancer (19 patients) and other nonneoplastic abnormalities (nephrolithiasis, renal cir-rhosis, hydronephrosis, and injury—six patients).

The samples were divided into three groups: cancer tissues (19 samples "T") and two types of control tissues—(a) the opposite pole of the cancer kidney with no signs of tumor infiltration (19 samples "C") and (b) tissues from kidneys with other, nonneoplastic abnormalities (six samples "N"). Clear cell renal cell cancer was diagnosed upon histological evalu-ation of the excised tumors according to WHO criteria (24).

RNA isolation

Total RNA was isolated from ~ 100 mg deep-frozen tissue with GeneMATRIX Universal RNA Purification Kit (EURx, Gdansk, Poland) according to manufacturer's instructions.

Real-time PCR

The isolated RNA served as a template in reverse tran-scription reaction, and the obtained cDNA samples were used in semiquantitative real-time PCR analyzes. Reverse tran-scription was performed with RevertAidTM H Minus First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania) using oligo-dT primer, according to manufactur-er's protocol.

Real-time PCRs were performed with QuantiFast SYBR Green PCR Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Primers detecting variants of type 1 deiodinase mRNA were designed on the basis of sequences deposited in GenBank database (variant 1: NM_000792, var-iant 2: NM_213593, variant 3: NM_001039715, variant 4: NM_001039716). The sequences of the primers are shown in Table 1, and their positions relative to mRNA in Figure 1. Primers detecting SF2/ASF and hnRNPA1 mRNAs were designed on the basis of sequences NM_006924.4 and NM_002136.2, respectively. Real-time PCR conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 50 cycles (denaturation at 95°C for 10 seconds and combined annealing/extension at 60°C for 30 seconds); then melting curve analysis: 135 cycles (50°C with 0.3°C temperature increase in each cycle). Expression levels were
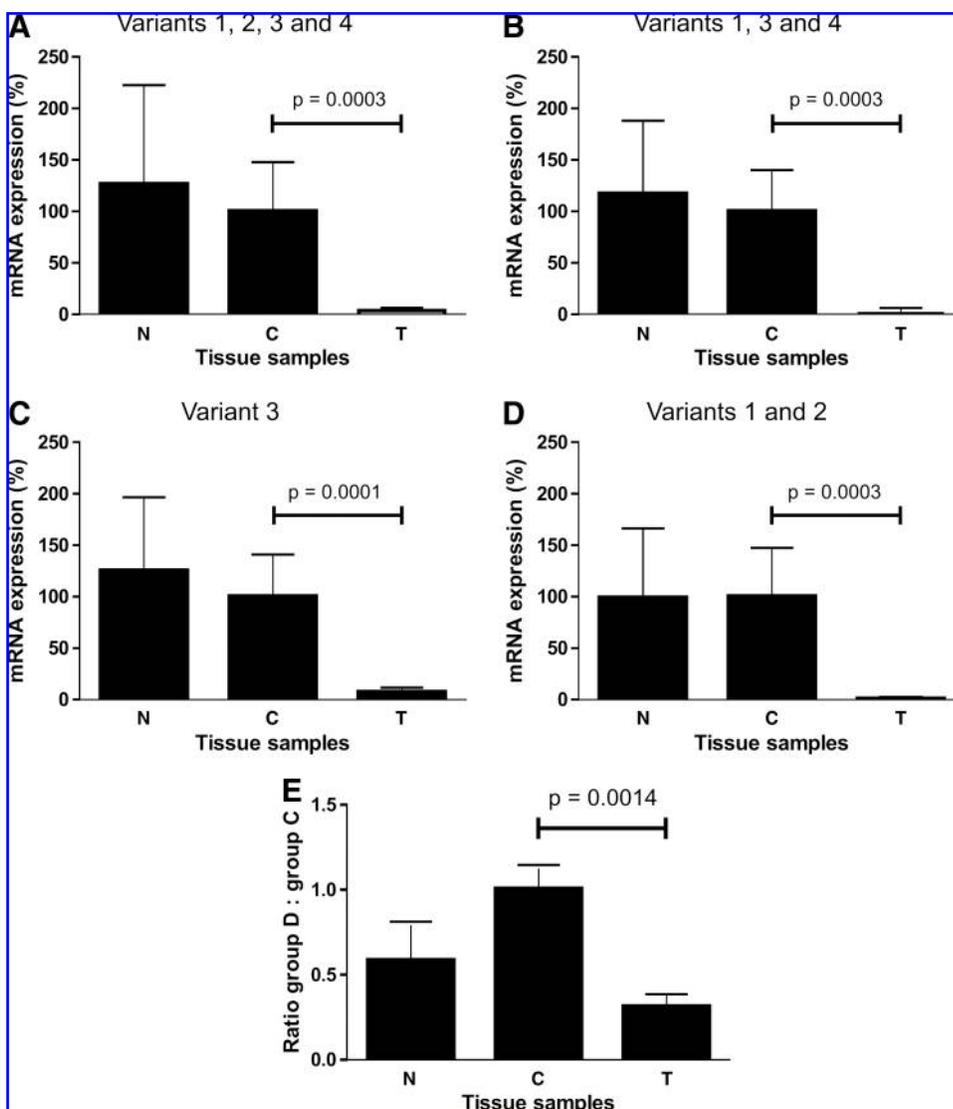

FIG. 2. Expression of type I deiodinase transcript variants. (**A**) Group A of transcripts (all groups tested). (**B**) Group B (transcript variants with lon-ger exon 1). (**C**) Group C (variants lacking exon 2). (**D**) Group D (variants possessing exon 2). (**E**) Expression ratio of transcripts possessing (group D) and lacking (group C) exon 2 encoding the active center of the enzyme. The expression is shown as percentage of con-trol C. The data are given as mean $\pm$ SE ($n = 6$ for N, $n = 19$ for C, and $n = 19$ for T). N, kidney samples of pathology other than cancer; C, control samples from healthy poles of clear cell renal cell carcinoma (ccRCC) kidneys; T, samples from ccRCC tissues.

FIG. 3. Expression of splicing factors (**A**) splicing factor-2/alternative-splicing factor (SF2/ASF) and (**B**) heterogeneous ribonucleoprotein A1 (hnRNPA1). (**C**) Expression ratio of splicing factors SF2/ASF and hnRNPA1. For details, see the legend of Figure 2.
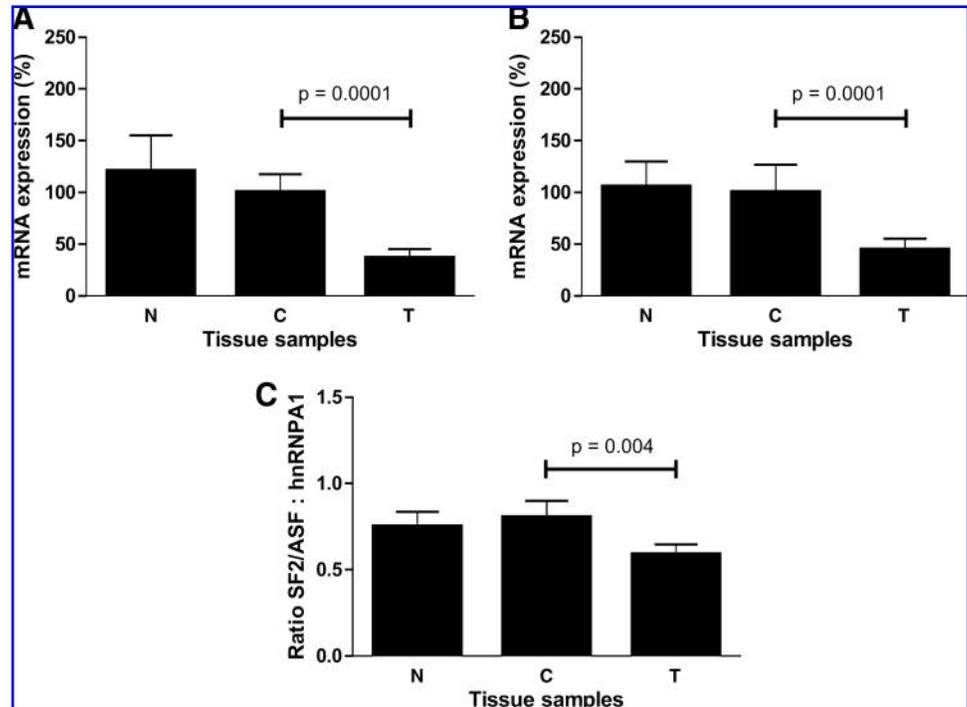
normalized to values obtained for β-actin. The data are given as mean $\pm$ standard error (SE) ($n = 6$ for N, $n = 19$ for C, and $n = 19$ for T).

Cloning of DIO1 splicing variants

Splicing variants of DIO1 were amplified from cDNA with a two-step PCR. The first reaction was performed with primers P9 and P4 (for sequences, see Table 1) under reaction conditions: initial denaturation at 95°C for 10 minutes, followed by 5 cycles (95°C for 20 seconds, 62°C for 20 seconds, and 72°C for 1 minute; the annealing temperature was decreased 0.5°C every cycle), then 40 cycles (95°C for 20 seconds, 57°C for 20 seconds, and 72°C for 1 minute), and final elongation at 61°C for 30 minutes. The PCR products were purified with GeneMATRIX PCR/DNA Clean-Up Purification Kit (EURx) and served as a template in the second round of PCR with primers P9 and P10 under reaction conditions: initial denaturation at 95°C for 10 minutes, followed by 45 cycles: (95°C for 15 seconds, 59°C for 30 seconds, and 72°C for 45

seconds), and final elongation at 61°C for 30 minutes. The PCRs were performed with Perpetual OptiTaq DNA Polymerase HOT START (EURx). The products of PCR were electrophoresed in 1% agarose gel stained with ethidium bromide. The products of the second round of PCR were purified with GeneMATRIX PCR/DNA Clean-Up Purification Kit (EURx), cloned into pGEM-T vector (Promega, Madison, WI), and transformed into *Escherichia coli* Fusion-Blue Competent Cells (Clontech, Mountain View, CA). Clones were checked with colony-PCR performed with primers SP6 and T7 specific for the pGEM-T vector, Perpetual OptiTaq DNA Polymerase HOT START (EURx), and under the following conditions: initial denaturation at 95°C for 10 minutes, followed by 40 cycles (denaturation at 95°C for 20 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for 1 minute), and final elongation at 61°C for 30 minutes. The products of PCR were analyzed by agarose gel electrophoresis, and positive clones were used for isolation of plasmids with standard alkaline lysis procedure. Plasmids with cloned splicing variants were sequenced with Big-Dye terminator kit

FIG. 4. Correlation between expression ratios of DIO1 transcripts group D:group C and splicing factors SF2/ASF:hnRNPA1. (**A**) Control samples C. (**B**) ccRCC samples T.
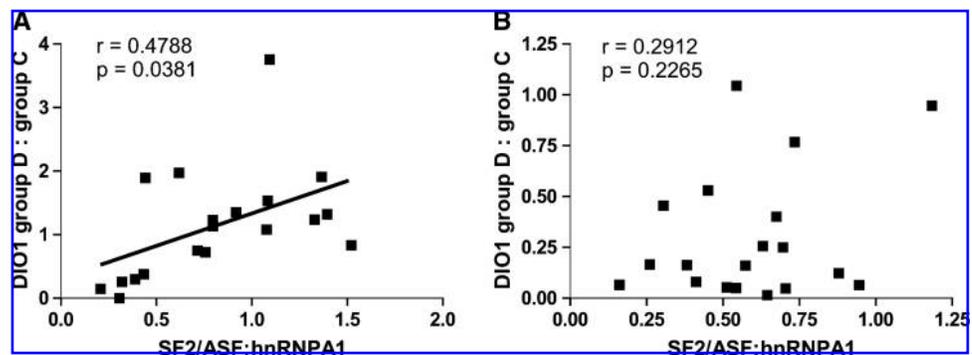
(Applied Biosystems, Foster City, CA) and primers SP6 and T7 on an ABI3100 machine.

In silico analysis

The obtained sequences were compared with GenBank reference sequence NC_000001.9–GI:89161185 using BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Protein sequence predictions were performed with Expasy Translate tool (http://www.expasy.ch/tools/dna.html).

Statistical analysis

Normal distribution was tested using the Shapiro-Wilk test, followed by either Wilcoxon signed rank test or paired t-test; $p < 0.05$ was considered statistically significant. Pearson's coefficient of correlation was used to analyze the correlation between the ratio of DIO1 variants and SF2/ASF:hnRNPA1 mRNAs ratio.

Results

mRNA expression analysis

Four reference sequences of transcript variants of human *DIO1* gene have been deposited in Entrez-Gene database: variant 1 (NM_000792.4), variant 2 (NM_213593.2), variant 3 (NM_001039715.1), and variant 4 (NM_001039716.1). The transcripts differ, among others, by the presence or absence of exon 2 encoding the active center of the enzyme and a fragment of exon 1 encoding amino acids responsible for substrate specificity (Fig. 1). The primers for real-time PCR analysis were designed to detect the following groups of transcripts: (A) all four variants (primers P3 and P4 located in exon 4, common to all transcripts); (B) transcripts with the longer version of exon 1 (i.e., variants 1, 3, and 4; primers P1 and P2); (C) variant 3 (lacking exon 2; primers P7 and P8); (D) transcripts with exon 2 and 3 (i.e., variants 1 and 2; primers P5 and P6).

The expression of all four groups of transcripts was detectable in all samples analyzed (Fig. 2). There were no significant differences in the expression of all four transcript groups between control samples C and N. However, the expression of transcripts was dramatically and statistically significantly decreased in samples T when compared with both types of control samples. The level of expression of mRNA groups A, B, C, and D in tumor samples versus control samples C was ~4%, 9%, 7%, and 1%, respectively ($p \leq 0.0003$) (Fig. 2).

The observed decrease in expression of DIO1 mRNAs in tumor samples was not uniform (i.e., the expression level of each transcript groups was differently altered when compared to control samples). Therefore, as a result of such differences, a change in ratios of transcript variants in tumor samples when compared to relevant ratios in normal tissue must have occurred. Therefore, we calculated the ratio of transcript groups D (variants 1 and 2) and C (splice variant 3) in samples T, C, and N (Fig. 2E). The ratio, calculated as mean of D:C ratios in individual samples, was 0.6 ± 0.2 SE, 1.0 ± 0.1 SE, and 0.3 ± 0.1 SE in N, C, and T samples, respectively.

The imbalanced expression of the two groups of DIO1 transcript variants suggested disturbances in splice site selection. Therefore, we decided to analyze the expression profiles of mRNA of two splicing factors, SF2/ASF and hnRNPA1, known to participate in 5′ splice site selection (Fig. 3). The expression of mRNA for the two factors was similar in both types of control samples. However, in tumor samples we observed a decrease of SF2/ASF mRNA expression to $37 \pm 9\%$ SE and hnRNPA1 mRNA to $44 \pm 11\%$ SE of the respective levels in the control. The ratio, calculated as mean of SF2/ASF:hnRNPA1 mRNA ratios in individual samples, was 0.7 ± 0.1 SE, 0.8 ± 0.1 SE, and 0.6 ± 0.1 SE in N, C, and T samples, respectively (Fig. 3C).

To check if there is any relation between the expression of various transcripts of DIO1 and the ratio of the two splicing factors, we performed a correlation analysis of the ratios of these transcripts in individual samples C and T. We found a positive correlation ($r = 0.4788$; $p = 0.0381$) between the ratio of DIO1 transcripts possessing and lacking the region encoding the active center of the enzyme (group D:group C) and the ratio SF2/ASF:hnRNPA1 mRNAs in control samples (Fig. 4A). No such correlation was observed in samples T (Fig. 4B).

Identification of DIO1 splicing variants

PCRs performed on cDNA from all samples revealed a complex pattern of bands, differing between patients and also different for samples C and T of a given patient (Fig. 5). The strongest bands corresponded to the expected sizes of the four transcript variants present in ENTREZ-GENE database (881 bp for transcript variant 1, 689 bp for variant 2, 737 bp for variant 3, and 681 bp for variant 4). However, we noticed also bands of lower as well as higher molecular weight. Cloning and sequencing revealed that the bands corresponded to known as well as new transcript variants of *DIO1* gene (Fig. 6). Three of the seven not previously reported transcript variants were found exclusively in the tumor samples (numbers 9, 10, and 11 in Fig. 6). All of them lacked the region encoding the active center of the enzyme.

Discussion

This is the first report analyzing the alternative splicing of type 1 deiodinase mRNA in cancer. We identified 11 splicing variants, among them 7 new ones, previously not reported. All of 11 splicing variants were identified in T samples, while only 8 of them occurred in C and N samples. Four transcripts cloned from control and tumor samples correspond to known DIO1 mRNA splicing variants, deposited in Entrez
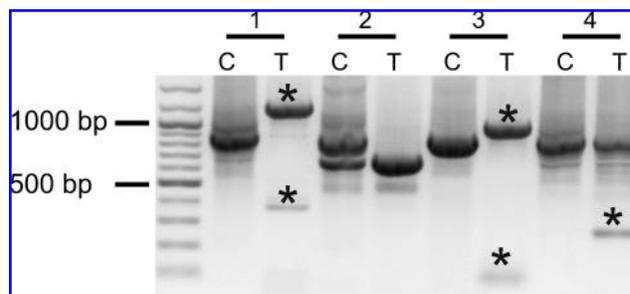

FIG. 5. Various spliced isoformes of DIO1. Profiles from four representative patients showing differences between control samples C and cancer samples T and the presence of canonical as well as novel splicing variants (*).

Gene database as reference sequences: transcript variant 1 (NM_000792.4), variant 2 (NM_213593.2), variant 3 (NM_001039715.1), and variant 4 (NM_001039716.1). Three other transcripts partially correspond to the sequences deposited as "related sequences," cloned from human liver tissue: AY560382.1 (number 3 in Fig. 6), AY560379.1 (number 5 in Fig. 6), and AY560376.1 (number 6 in Fig. 6). However, clones identified in our study are longer in both upstream and downstream directions. We cannot exclude the possibility that this difference can result from different position of primers used in our study.

Three of the new transcript variants presented in this study were cloned exclusively from tumor samples (numbers 9, 10, and 11 in Fig. 6). Recent reports show that splicing variants of different genes can serve as markers of cancerogenesis (25,26). Also, DIO1 expression was proposed as a differentiation marker in cancer cells (11,12). Further analysis is needed to confirm if the transcripts identified in our study are specific

FIG. 6. Splicing variants of DIO1 from control and cancer samples. Variants 1–8 were found in N, C, and T samples, variants 9–11 exclusively in cancer samples. Upper panel shows electrophoretic analysis of the cloned splicing variants. Lower panel shows the exonic structures. Exons are shown as black boxes. Numbers above exons refer to nucleotide positions in the *DIO1* gene (GenBank locus: NC_000001.9, region 54132449–54149347).
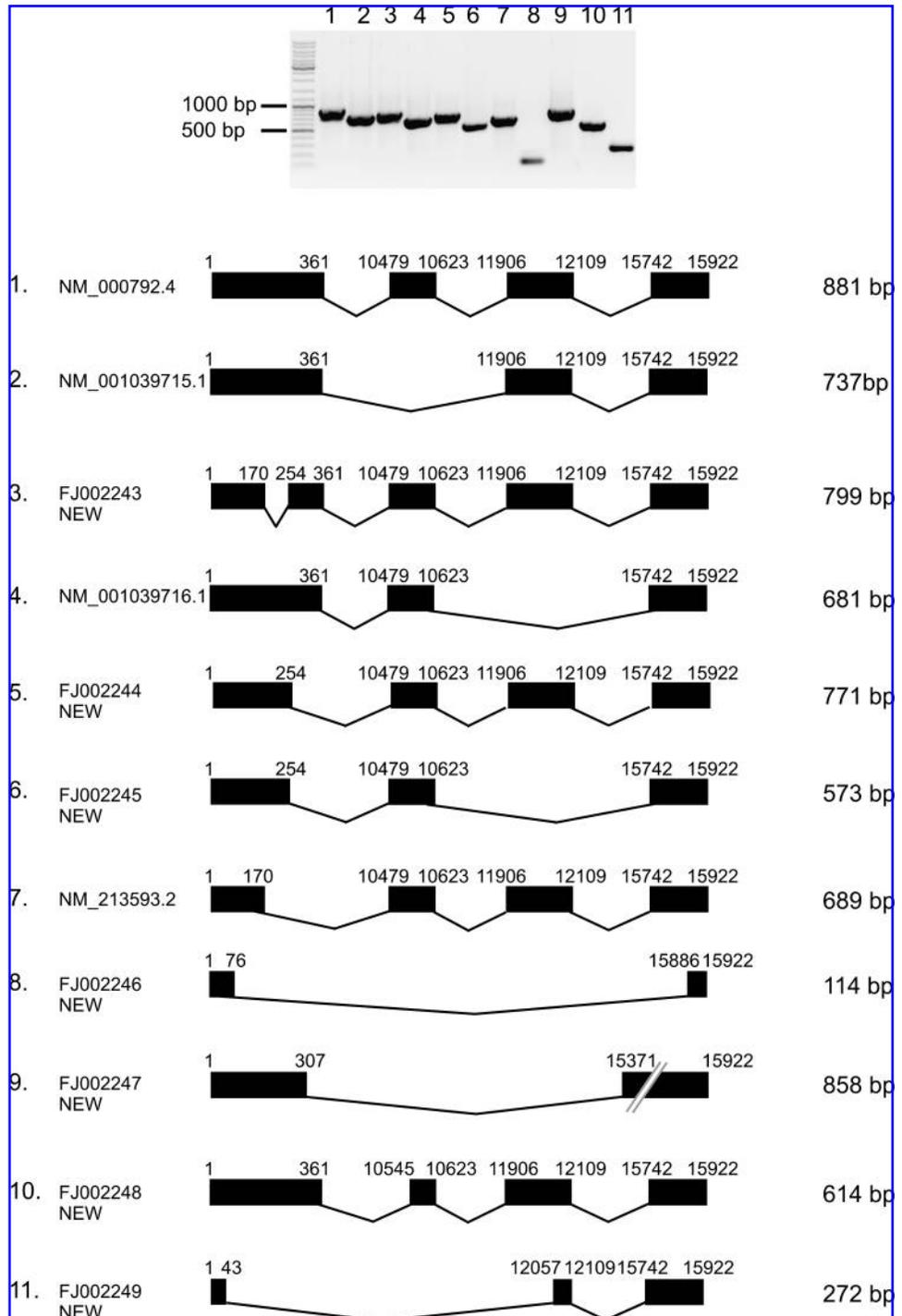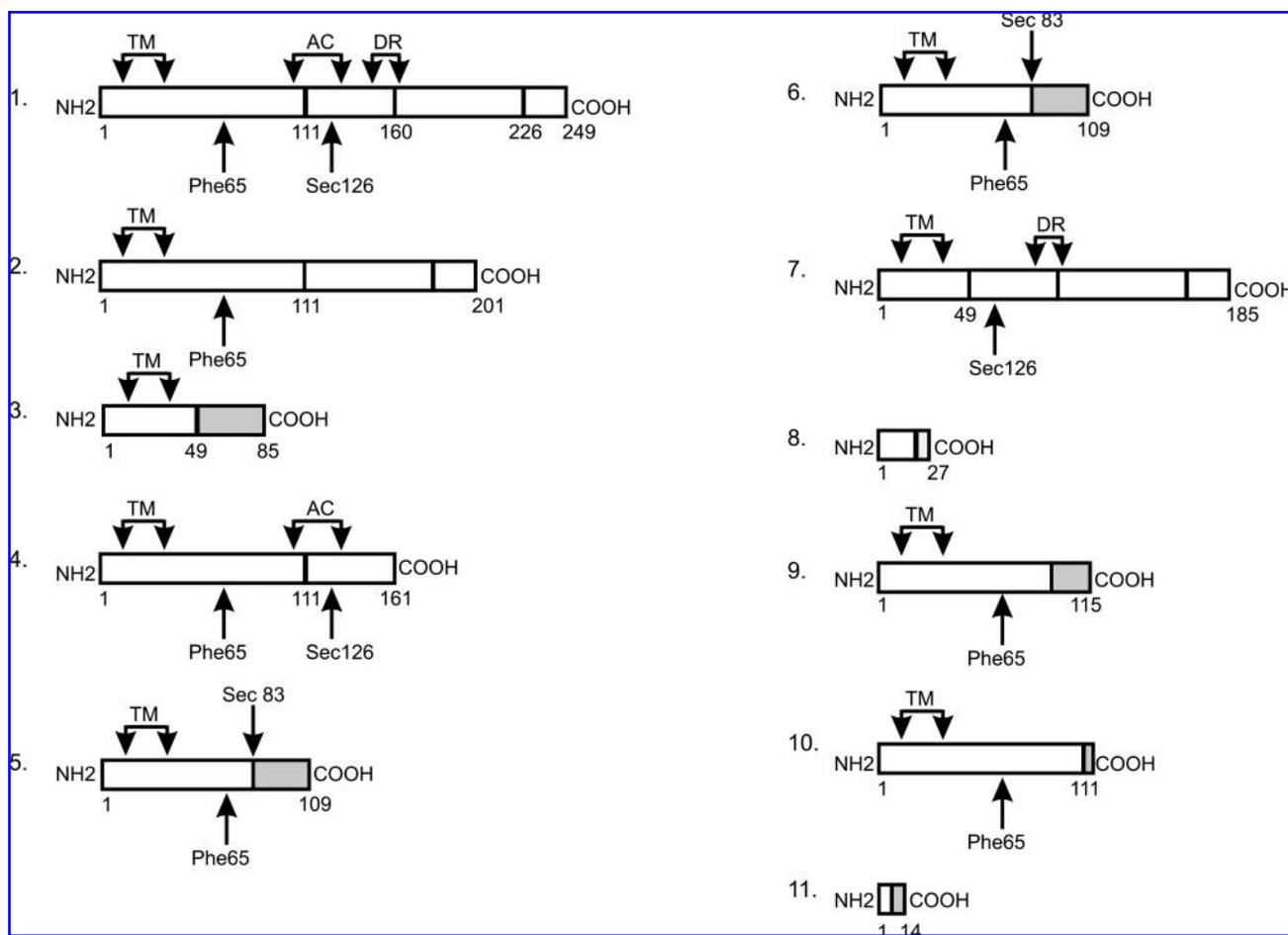
FIG. 7. Translation prediction for the DIO1 splicing variants. The numbering refers to the numbers in Figure 6. Protein regions corresponding to exons are boxed. Regions of frameshift are shaded. Key regions of the enzyme are marked. TM, transmembrane region; AC, active center; DR, dimerization region; Phe65, rT3 substrate specificity; Sec126, catalytically active residue.

for cancerous tissues and if they can suit as possible markers of cancerogenesis.

It is not known whether protein products of the alternative splicing variants of DIO1 exist *in vivo* and we were not able to check it due to the lack of specific antibodies. However, it has been suggested that the protein isoforms resulting from alternative splicing of *DIO1* gene transcript could contribute to DIO1 activity in porcine kidney (13). Translation prediction shows that the cloned splicing variants can give proteins of different properties than those of the wild-type enzyme (Fig. 7). Our previous results (23) have shown that DIO1 activity poorly correlated with mRNA level in healthy renal tissues. This observation suggests significant posttranscriptional regulation of DIO1 expression. Interestingly, seven of the splicing variants cloned in this study possess a premature termination codon (PTC). In four variants (numbered 3, 8, 9, and 11 in Fig. 7) PTC is located more than 50 nucleotides upstream of the final exon–exon junction. Such alternative splice forms are believed to be recognized by nonsense-mediated mRNA decay mechanism (NMD) (27). NMD is generally considered a mechanism of degradation of PTC

containing mRNAs and thus protecting cells from potentially harmful effects of abnormally truncated proteins. It was also suggested that NMD, coupled with alternative splicing, may serve as a regulatory mechanism of gene expression (28). Interestingly, DIO1 belongs to the group of selenoproteins, and it was found that NMD regulates the expression of selenocysteine-codon containing transcripts (29). In our previous work we showed that the activity of DIO1 in ccRCC samples is not detectable (23). Thus, although purely speculative, it is possible that what we observe in ccRCC tissue samples is a possible shift in alternative splicing, leading to production of PTC forms that are degraded by NMD, resulting in lowered DIO1 protein level. Pursuant to the above, the previously observed lowered activity of DIO1 in ccRCC would result from not only lowered transcription rate but also down-regulated translation due to NMD of PTC variants.

An interesting issue is whether the observed changes in DIO1 expression in ccRCC may effect the T3 levels in plasma or kidney tissues. According to previously published results, DIO2 but not DIO1 activity is the major source of serum T3 (30). In 2008, Panicker *et al.*, however, showed that

polymorphism in *DIO1* leads to alteration in the ratio between circulating free T3 and free T4 (31). Unfortunately, the serum of patients is not available for analysis under this study; therefore, this problem remains for further research. Interestingly, analyzes performed recently on another set of tissue samples have shown that ccRCC tissue possess lowered T3 and variable T4 levels compared to nontumoral paired control samples (manuscript in preparation). Whether this coincides with lowered DIO1 activity remains to be clarified.

Results published previously have shown disturbances in expression and functioning of TRs in ccRCC (3,4). These observations, together with results presented in this paper suggest that the pathway of thyroid hormone action is disturbed in ccRCC. Since thyroid hormone is an important regulator of expression of oncogenes and tumor suppressors (5,6), it is possible that T3 pathway disturbance might be one of the origins of neoplastic transformation of ccRCC. This hypothesis, however, needs further investigation.

The analysis of ratios of groups of transcripts showed that the balance between transcripts encoding active (group D) and nonactive (group C) enzyme was disturbed in ccRCC samples. The expression levels of both groups of transcripts were similar in control samples C (Fig. 2E). However, the ratio of group D to group C transcripts in tumor samples was more than threefold lower than in control samples. Such a result suggests disturbed alternative splicing of DIO1 pre-mRNA in cancerous tissues leading to increase in skipping of exon 2. One of the mechanisms of alternative splicing is that of 5′ splice site selection (32) regulated by the ratio of splicing factors SF2/ASF and hnRNPA1 (16). When the level of hnRNPA1 is higher than that of SF2/ASF, distal 5′ splice site is preferred, which in the case of the analyzed DIO1 variants would mean the predominance of variants devoid of exon 2 (group C) over variants with this exon (group D). In our experiments the ratio of SF2/ASF:hnRNPA1 correlated with the ratio of group D:group C in control but not in tumor samples (Fig. 4), in which the expression of both SF2/ASF and hnRNPA1 was significantly, but not proportionally, lowered resulting in a decreased SF2/ASF:hnRNPA1 ratio. This suggests that alternative splicing of DIO1 could be regulated via SF2/ASF and hnRNPA1 and that this regulation could be lost in renal cancer. This hypothesis needs further research.

Previous reports have shown that the alternative splicing of other genes is also disturbed in ccRCC. It was shown that expression of specific splicing variants of survivin (19), CD-44 adhesion molecule (20), and *TCF-4* gene (22) was related to tumor progression in ccRCC. These results, together with present study, suggest that disturbances in alternative splicing in renal cancer can result from common changes in splicing regulatory mechanisms. It was shown that SF2/ASF can function as a proto-oncogen and its overexpression can lead to immortal transformation of rodent fibroblasts (33). The same paper reported also that SF2/ASF is upregulated in various human tumors. The authors observed ≥2-fold overexpression of SF2/ASF mRNA in less than 5% clear cell renal cell carcinoma samples analyzed. In our study, however, all the analyzed samples had at least twofold decrease of expression. This discrepancy could result from primers we used in real-time PCR analysis, which detected solely transcript variant 1 of SF2/ASF, in contrast to Karni *et al*. (33), who detected as well transcript variant 1 as 2. It is known, however, that the active splicing factor SF2/ASF is synthesized

from transcript variant 1, and it is doubtful if transcript variant 2 undergoes translation at all (34).

In conclusion, we show that expression and alternative splicing of type 1 deiodinase is disturbed in ccRCC. These disturbances are accompanied by changes in expression of splicing factors SF2/ASF and hnRNPA1. The new DIO1 splicing variants cloned exclusively from cancer samples suggest their use as potential molecular markers of renal cancer.

Acknowledgments

Disclosure Statement

The authors declare that no competing financial interests exist.

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