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CYP24A1 splice variants—Implications for the antitumorigenic actions of 1,25-$(OH)_2D_3$ in colorectal cancer☆

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ABSTRACT

25-Hydroxyvitamin D_3 24-hydroxylase (CYP24A1), the catabolizing enzyme of the active vitamin D_3, is often overexpressed in solid tumors. The unbalanced high levels of CYP24A1 seem to be a determinant of vitamin D resistance in tumors. Splice variants of CYP450 enzymes are common. Existence of CYP24A1 isoforms has been reported recently. We have investigated the presence of CYP24A1 splicing variants (SV) in human colon cancer cell lines and tissue samples. Using a set of primer combination we have screened the entire coding sequence of CYP24A1 and identified three splice variants in colon cancer cell lines. The presence of these SVs in human colon tissue samples showed a correlation with histological type of the tissue and gender of patients. The sequencing of the alternatively spliced fragments showed that two have lost the mitochondrial target domain, while the third lacks the heme-binding domain. All SVs retained their sterol binding domain. Translation of these variants would lead to a dysfunctional enzyme without catalytic activity that still binds its substrates therefore they might compete for substrate with the synthesizing and catabolizing enzymes of vitamin D.

1. Introduction

The most active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1,25-D_3) has pleiotropic effects. Besides controlling calcium and phosphate homeostasis, 1,25-D_3 regulates cell proliferation, differentiation, apoptosis and immunomodulation in normal and pathological conditions. Synthesis of 1,25-D_3 from 25-hydroxyvitamin D_3 (25-D_3) is catalyzed by 25-hydroxyvitamin D_3 1α-hydroxylase (CYP27B1). Both 25-D_3 and 1,25-D_3 are catabolized by 25-hydroxyvitamin D_3 24-hydroxylase (CYP24A1) in a negative feedback manner. Thus, CYP24A1 plays an essential role in attenuating the potentially detrimental hypercalcaemic side effects of 1,25-D_3.

Unbalanced high CYP24A1 levels were found in a variety of human malignancies, e.g. colorectal, ovary, breast, and lung tumors [1–3]. However, the activity of the CYP24A1 is highly variable and does not correlate always with the expression level of the mRNA or protein [4–6].

Recently, new data on post-transcriptional modifications of CYP24A1 mRNA in human myelomonocytic [7] and prostate adenocarcinoma [8] cell lines suggested that alternative splicing might be the cause of the observed differences in CYP24A1 activity and thus, of inconsistent vitamin D-effects.

We investigated whether there are any CYP24A1 splice variants (SV) in colon cancer cell lines and colorectal tumors and if the existence of SVs is associated with clinical parameters.

2. Materials and methods

2.1. Cell cultures

Three colorectal cancer cell lines, COGA-1A und COGA-13 (both from Boehringer Ingelheim, Germany) and Caco-2/15 (American Type Culture Collection) were grown in DMEM (Invitrogen, San Diego, USA) with 10% FCS, 4 mM L-glutamine, 20 mM HEPES, 100 U/ml penicillin and 100 μg/ml streptomycin. After a 2-day incubation in DMEM without FCS, cells were treated with 10^{-8} M 1,25-D_3 for 4 h to induce CYP24A1 expression.

2.2. Tissue specimens

Tissue samples of colorectal tumors and normal mucosa outside the tumor border were obtained from 123 patients. Median age of

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Table 1
Primer pairs used in the RT-PCR reactions (35 cycles). bp = base pair; Fw = forward; Rev = reverse; Ex = exon; In = intron (NCBI Reference Sequence: NG_008334).

Primer	Oligonucleotide sequence (5′ → 3′)	Product (bp)	Position on genome	$T_{annealing}$ (C°)
CYP24A1_Fw_A	CCCACTAGCACCTCGTACCAAC	510	Ex 1 5325–5346	59
CYP24A1_Rev_A	CGTAGCCTTCTTTGCGGTAGTC		Ex 2 6035–6056	
CYP24A1_Fw_B	GAGACTGGTGACATCTACGGCGTACA	488	Ex 1 5484–5498	68
CYP24A1_Rev_B	AACGTGGCCTCTTTCATCAC		Ex 4 9327–9346	
CYP24A1_Fw_C	CTCAAAATCCCTGAACCCAA	428	In 2 7031–7050	58
CYP24A1_Rev_C	AACGTGGCCTCTTTCATCAC		Ex 4 9327–9346	
CYP24A1_Fw_D	GAAACCAGGGGAAGTGATGA	404	Ex 3 7359–7378	58
CYP24A1_Rev_D	GGAAATCTGCACTAGGCTGC		Ex 7 16156–16175	
CYP24A1_Fw_E	GCAGCCTAGTGCAGATTTCC	335	Ex 7 16156–16175	58
CYP24A1_Rev_E	ATTCACCCAGAACTGTTGCC		Ex 9 20856–20875	
CYP24A1_Fw_F	GGACTCTTGACAAGGCAACAGTTC	283	Ex 9 20843–20866	58
CYP24A1_Rev_F	TTGTCTGTGGCCTGGATGTCGTAT	85	Ex 11 21700–21723	

the 54 male and 69 female patients was 67 ± 14.93 years (range 20–91). 90 of the tumors were malignant: 87 adenocarcinomas, 2 carcinomas in polyp and 1 carcinoid tumor. From the adenocarcinomas 6 were well differentiated (G1), 49 moderately differentiated (G2), and 26 poorly differentiated or undifferentiated (G3). In 8 carcinoma cases, no differentiation data were available. 33 lesions were benign: 5 hyperplastic, 17 tubular, 10 tubulovillous and 1 villous polyps. Permission from the Ethics Commission was granted prior to the initiation of the study.

2.3. RNA extraction and purification

Total RNA was extracted either with TRIzol reagent (Invitrogen) (from cell lines) or using RNEasyMini Kit (QIAGEN, Hilden, Germany) (from the tissue samples). The integrity of the RNA was determined by ethidium bromide staining on agarose gel. Possible contamination of the RNA with genomic DNA was removed by DNAse (Invitrogen-Life Technologies, Carlsbad, USA) treatment according to the manufacturer's instructions.

2.4. Reverse transcriptase polymerase chain reaction (RT-PCR)

Two micrograms of total RNA from each sample were reverse transcribed using Moloney Murine leukemia virus reverse transcriptase according to the manufacturer's instructions (Promega, Madison, USA).

Primers for screening were designed either with the NCBI-Primer-BLAST tool (www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi) or were obtained from the literature (A: [9], F: [8]). cDNA was amplified with Taq polymerase (Fermentas, Burlington, Canada) on a MyCycler™ thermal cycler (BIO-RAD, Hercules, USA). The PCR program included initial denaturation at 94 °C for 2 min, 35 amplification cycles and final extension at 72 °C for 15 min. Primers and annealing temperatures are listed in Table 1. The PCR products were visualized with ethidium bromide on a 2% agarose gel (Sigma–Aldrich, St. Louis, USA). To verify the quality of the RT-reaction we amplified cytokeratin 8 (CK8 Fw: 5′-TGGGCAGCAGCAACTTTC-3′, CK8 Rev: 5′-AGGCGAGACTCCAGCTCTAC-3′) as an endogenous control.

2.5. Sequencing

The amplified cDNA band was extracted from the agarose gel and purified with the RNEasyMini Kit (QIAGEN). Sequencing was performed on an ABI Prism 310 Genetic Analyzer using Big Dye RR Terminator Cycle Sequencing Kit V1.1 (Applied Biosystems, Foster City, USA) according to the manufacturer's directions. The sequencing program included 25 cycles of denaturation for 10 s at 96 °C, annealing for 5 s at 50 °C, and extension for 3 min at 60 °C. The extension products were purified with Centri-Sep™ columns and stored in HI DI Formamid (both from Applied Biosystems). Sequences were analyzed with the ABI software.

2.6. Statistical analysis

The association between the expression of SVs and the histological and clinical parameters was analyzed with the Chi-squared test. For correlation analysis, Spearman's rank correlation analysis was used. Significance was defined as $p < 0.05$. Data were analyzed using the SPSS statistics package (SPSS, Chicago, USA; version 16.0 for Windows).

3. Results

3.1. Screening of CYP24A1 splice variants in colon cancer cell lines

We hypothesized that CYP24A1 splice variants are present also in colon cancer cell lines and colorectal tumors. We analyzed three cell lines, each with specific CYP24A1 expression and activity: the differentiated, well-known colon adenocarcinoma cell line Caco-2/15 with undetectable basal CYP24A1 expression, COGA-1A with highly active inducible CYP24A1, and COGA-13 cells expressing high basal CYP24A1 levels and high basal enzymatic activity. In order to test whether we could identify any splice variants in these cells either basally or whether they express any inducible variants, we treated the cells with 10^{-8} M 1,25-D_3 for 4 h. The cell lines were then screened for the presence of CYP24A1 splice variants by RT-PCR with a panel of primer pairs (see Table 1) targeting different exons of CYP24A1, covering the whole mRNA sequence.

Primer pair A amplified the expected 510 bp long fragment (Fig. 1). The primer pairs B, D, and E have not amplified any additional RT-PCR fragments besides the expected bands (data not shown).

Ren et al. showed that macrophage like cells express a CYP24A1 splice variant that misses the first two exons and starts with a sequence from intron 2. Primer pair C (forward primer on intron 2, reverse primer on exon 4) amplified a 428 bp long product, SV1, in all 3 colon cancer cell lines. Sequencing demonstrated that it is the same splice variant as the one published by Ren et al. [7]. This variant contains an alternative translation start codon on intron 2 (NG_008334 position 2285) of the *CYP24A1* gene. In addition, we detected a smaller product but only in COGA-13 cells (SV2) (Fig. 1). The sequencing showed the loss of a 209 bp long fragment from intron 2 (start: position 2099 and end: position 2307), however the remaining part still harbours a translation start codon.

Primer pair F, covering the 3′ region of CYP24A1 mRNA (exons 9–11) amplified besides the expected fragment of 283 bp, an additional 85 bp long fragment SV3. Sequencing this product revealed that it consisted of the 3′ end of exon 9 and the 5′ end of exon 11, lacking exon 10 (Fig. 2). Exon 10 is coding for the heme-binding domain and is therefore crucial for the enzymatic activity. 1,25-D_3

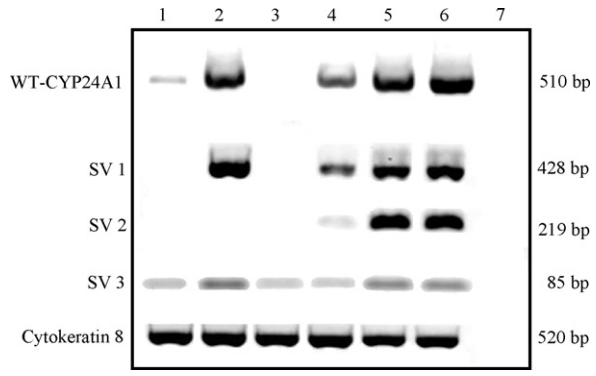

Fig. 1. Expression of CYP24A1 splice variants (SV) in three colon cancer cells. Amplification with primer pair A results in the 510 bp long wild type (WT) CYP24A1 fragment. Primer pair D amplified two SVs (SV1 and SV2) 428 and 219 bp long. Primer pair F amplified the 85 bp fragment SV3. Cytokeratin 8 was used as internal control. 1. COGA-1A, 2. COGA-1A + 1,25-D_3, 3. Caco-2/15, 4. Caco-2/15 + 1,25-D_3, 5. COGA-13, 6. COGA-13 + 1,25-D_3, 7. Negative control.

treatment induced not only the wild type CYP24A1 but also the splice variants.

3.2. Colon tissue samples

Based on the results from the cell lines, we have examined whether any of the splice variants detected in the cell lines were expressed also ex vivo, in colon tissue specimens.

Using primer pair C, the 428 bp long SV1 was amplified in 20 (16.3%) normal, 10 (30.3%) benign and 32 (35.6%) malignant samples. SV2 was detected in 16 normal (13.0%), 8 (24.2%) benign and 22 (24.4%) carcinoma samples. Amplification with primer pair F revealed the presence of 85 bp long SV3 in 19 (15.4%) normal, 1 (3.0%) benign and 20 (22.2%) malignant samples.

Table 2
Correlation of the SV 1 expression with the histological diagnosis. n = number of samples.

Histology	N-terminal SV1 (428 bp)		Total
	Present	Absent	
Normal mucosa (n)	19	104	123
Polyp/adenoma (n)	10	23	33
Carcinoma (n)	32	58	90
Total (n)	61	185	246
Chi-squared test	$p < 0.002$		

We have sequenced the bands obtained with primer C and F from several tumors. The sequences confirmed that these were the splice variants found in the cell lines. Interestingly, in several tumors we have detected a C to T mutation at position 4298 (Fig. 3B). However, this mutation would not cause any changes in the amino acid sequence since both the wild type codon (GCC) and the mutant codon (GCT) code for alanine.

The prevalence of SV1 was significantly different depending on the histological categories ($p < 0.002$) (Table 2). In addition, a significant difference in SV2 expression was detected between male and female patients ($p < 0.002$). No significant difference was found in the prevalence of the SVs among the study groups regarding age of patients, localization, grade, TNM-stage and morphological characteristics (necrosis, perifocal inflammation, vascular infiltration) of tumors.

Spearman's rank analysis revealed significant correlation between the expression of both SV1 and SV2 with the histological type of the sample (normal–benign–malignant): for SV1 $r_s = 0.216$

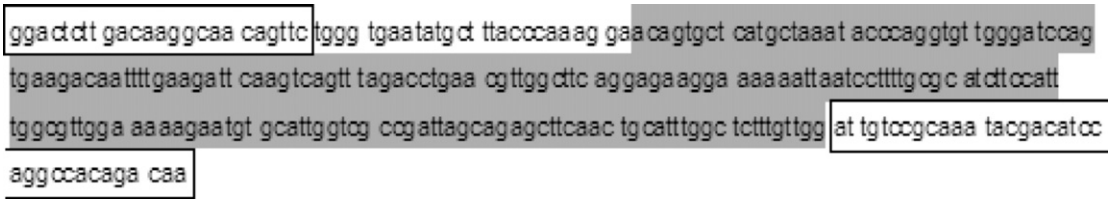

Fig. 2. Sequence of the 283 bp long fragment amplified by primer F. The unshaded sequence is SV3, consisting of the 3′ end of exon 9 and the 5′ end of exon 11 and lacks exon 10 (shaded sequence). The boxes represent the primers.

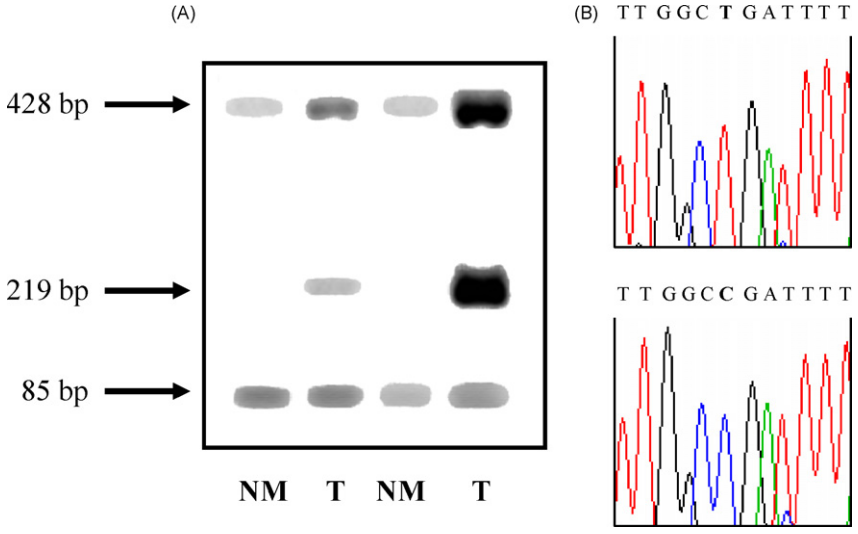

Fig. 3. (A) Presence of SVs in normal colon mucosa (NM) and adenocarcinoma samples (T). (B) Detection of a C to T mutation at position 4298 of the CYP24A1 cDNA in colon tumor tissues (NCBI Reference Sequence: NG_008334).

($p < 0.01$), for SV2 $r_s = 0.146$ ($p < 0.05$). The presence of SV2 positively correlated with female ($r_s = 0.200$, $p < 0.01$) and that of the SV3 with male gender ($r_s = 0.150$, $p < 0.05$). SV1 and SV2 were almost always simultaneously expressed ($r_s = 0.732$, $p = 0.01$).

4. Discussion

Upregulation of CYP24A1 seems to be a common feature of several solid tumors [1–3]. CYP24A1 was suggested to be an oncogene [10]. However, detailed information about the presence and distribution of its splice variants in these tumors was missing. This is the first study to show the presence of alternative SVs of CYP24A1 in normal colon tissue and colorectal tumors. Moreover, we could show that the prevalence of the SVs differs depending on the histological type of the tissue (normal–benign–malignant).

Based upon the studies of Ren et al. [7] we know that SV1 can be translated into a truncated protein retaining substrate binding ability. This variant lacks the mitochondrial targeting sequence, therefore has no access to the mitochondrial electron-transport chain (consisting of NADPH-ferredoxin reductase, ferredoxin and the CYP24A1), which results in the loss of enzymatic activity. Whether also the SV2 will be translated into a functionally similar protein still needs to be proved.

In our patient cohort we have not found the CYP24A1 variant described by Muindi et al. [8] in which intron 9 and intron 10 were not spliced out. Nevertheless, we have proven the presence of another splice variant, SV3. Despite the fact that dearth of exon 10 has been identified previously by the analysis of several thousand human cDNA sequences [11], our study is the first to show that this variant can be found in colonocytes. We have found also that SV3 has a higher prevalence in adenocarcinomas from male colon cancer patients, compared with female patients.

The alternative splicing leading to loss of exon 10 is still in-frame, suggesting that there is a high possibility that it would be translated into a protein. Exon 10 codes for the heme-binding domain of the enzyme. CYP24A1 is a protoporphyrin IX hemoprotein [12]. This isoform would lose its ability to bind molecular oxygen and becomes enzymatically inactive as well.

Although, all SVs found in our study preserve their substrate binding domain, how their affinity to the substrate will be affected by the loss of the first two exons or of exon 10 needs to be examined. Molecular modeling of CYP24A1 suggests that loss of exons 1 and 2 might affect the substrate binding pocket of the enzyme as well.

Ren et al. have shown that the SV1 isoform is still able to bind 25-D_3 and is capable to suppress synthesis of 1,25-D_3 probably by competing with CYP27B1 [7]. This ability of the isoforms, to bind 25-D_3 and thus compete with CYP27B1, might represent an additional explanation for our previous observations in COGA cells, namely, that in spite of the presence of the CYP27B1 protein, no 1α-hydroxylated metabolites were found after treatment with 25-D_3 [13]. Intriguingly, in Caco-2/15 cells, although the SVs are also present, the 1α-hydroxylase activity is high and remains higher than the CYP24A1 activity. Moreover, these cells are highly sensitive to the growth inhibiting effect of 1,25-D_3 [13].

The SV 1 and/or SV 2 in COGA-1A and COGA-13 cells seem to be present in amounts comparable with the wild type CYP24A1 (Fig. 1). In these cell lines that have high CYP24A1 activity, the presence of the SVs apparently does not interfere with the activity of the holoenzyme. We have already shown that these cells are either partially (COGA-1A) or completely (COGA-13) resistant to the antiproliferative effect of 1,25-D_3, suggesting that the splice variants might further reduce the bioavailability of the active compound [13].

Although we do not have any stoichiometric data in colorectal tumors on the expression levels of the various SVs compared to the wild type, the level seems to vary to a great extent, although it seems to be less abundant as in the cell lines (Fig. 3 A). Using laser-capture microdissection technology would increase homogeneity of the samples facilitating a more quantitative approach.

5. Conclusions

Alternative splicing of the CYP24A1 gene was detected in human colon cancer cell lines and tissue samples resulting in isoforms of the 1,25-D_3 catabolizing enzyme. The catalytically dysfunctional isoenzymes may cause alterations in the microenvironmental regulation of vitamin D_3 levels in colorectal tissue. The impact of the splice variants for colorectal tumorigenesis is not yet clear and their possible predictive potential needs to be investigated further.

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References

[1] M.G. Anderson, M. Nakane, X. Ruan, P.E. Kroeger, J.R. Wu-Wong, Expression of VDR and CYP24A1 mRNA in human tumors, Cancer Chemother. Pharmacol. 57 (2006) 234–240.
[2] K. Townsend, C.M. Banwell, M. Guy, K.W. Colston, J.L. Mansi, P.M. Stewart, M.J. Campbell, M. Hewison, Autocrine metabolism of vitamin D in normal and malignant breast tissue, Clin. Cancer Res. 11 (2005) 3579–3586.
[3] R.A. Parise, M.J. Egorin, B. Kanterewicz, M. Taimi, M. Petkovich, A.M. Lew, S.S. Chuang, M. Nichols, T. El-Hefnawy, P.A. Hershberger, CYP24, the enzyme that catabolizes the antiproliferative agent vitamin D, is increased in lung cancer, Int. J. Cancer 119 (2006) 1819–1828.
[4] G.J. Miller, G.E. Stapleton, T.E. Hedlund, K.A. Moffat, Vitamin D receptor expression, 24-hydroxylase activity, and inhibition of growth by 1alpha,25-dihydroxyvitamin D3 in seven human prostatic carcinoma cell lines, Clin. Cancer Res. 1 (1995) 997–1003.
[5] G. Jones, H. Ramshaw, A. Zhang, R. Cook, V. Byford, J. White, M. Petkovich, Expression and activity of vitamin D-metabolizing cytochrome P450s (CYP1alpha and CYP24) in human nonsmall cell lung carcinomas, Endocrinology 140 (1999) 3303–3310.
[6] K. Mimori, Y. Tanaka, K. Yoshinaga, T. Masuda, K. Yamashita, M. Okamoto, H. Inoue, M. Mori, Clinical significance of the overexpression of the candidate oncogene CYP24 in esophageal cancer, Ann. Oncol. 15 (2004) 236–241.
[7] S. Ren, L. Nguyen, S. Wu, C. Encinas, J.S. Adams, M. Hewison, Alternative splicing of vitamin D-24-hydroxylase: a novel mechanism for the regulation of extrarenal 1,25-dihydroxyvitamin D synthesis, J. Biol. Chem. 280 (2005) 20604–20611.
[8] J.R. Muindi, A. Nganga, K.L. Engler, L.J. Coignet, C.S. Johnson, D.L. Trump, CYP24 splicing variants are associated with different patterns of constitutive and calcitriol-inducible CYP24 activity in human prostate cancer cell lines, J. Steroid Biochem. Mol. Biol. 103 (2007) 334–337.
[9] P. Bareis, E. Kallay, M.G. Bischof, G. Bises, H. Hofer, C. Potzi, T. Manhardt, R. Bland, H.S. Cross, Clonal differences in expression of 25-hydroxyvitamin D(3)-1alpha-hydroxylase, of 25-hydroxyvitamin D(3)-24-hydroxylase, and of the vitamin D receptor in human colon carcinoma cells: effects of epidermal growth factor and 1alpha,25-dihydroxyvitamin D(3), Exp. Cell Res. 276 (2002) 320–327.
[10] D.G. Albertson, B. Ylstra, R. Segraves, C. Collins, S.H. Dairkee, D. Kowbel, W.L. Kuo, J.W. Gray, D. Pinkel, Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene, Nat. Genet. 25 (2000) 144–146.
[11] R.L. Strausberg, E.A. Feingold, L.H. Grouse, J.G. Derge, R.D. Klausner, F.S. Collins, L. Wagner, C.M. Shenmen, G.D. Schuler, S.F. Altschul, B. Zeeberg, K.H. Buetow, C.F. Schaefer, N.K. Bhat, R.F. Hopkins, H. Jordan, T. Moore, S.I. Max, J. Wang, F. Hsieh, L. Diatchenko, K. Marusina, A.A. Farmer, G.M. Rubin, L. Hong, M. Stapleton, M.B. Soares, M.F. Bonaldo, T.L. Casavant, T.E. Scheetz, M.J. Brownstein, T.B. Usdin, S. Toshiyuki, P. Carninci, C. Prange, S.S. Raha, N.A. Loquellano, G.J. Peters, R.D. Abramson, S.J. Mullahy, S.A. Bosak, P.J. McEwan, K.J. McKernan, J.A. Malek, P.H. Gunaratne, S. Richards, K.C. Worley, S. Hale, A.M. Garcia, L.J. Gay, S.W. Hulyk, D.K. Villalon, D.M. Muzny, E.J. Sodergren, X. Lu, R.A. Gibbs, J. Fahey, E. Helton, M. Ketteman, A. Madan, S. Rodrigues, A. Sanchez, M. Whiting, A. Madan, A.C. Young, Y. Shevchenko, G.G. Bouffard, R.W. Blakesley, J.W. Touchman, E.D. Green, M.C. Dickson, A.C. Rodriguez, J. Grimwood, J. Schmutz, R.M. Myers, Y.S. Butterfield, M.I. Krzywinski, U. Skalska, D.E. Smailus, A. Schnerch, J.E. Schein, S.J. Jones, M.A. Marra, Generation and initial analysis of more than 15000 full-length human and mouse cDNA sequences, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 16899–16903.
[12] J. Omdahl, The 25-hydroxyvitamin D 24-hydroxylase, in: D. Feldman (Ed.), Vitamin D, Elsevier Academic Press, Los Angeles, 2005, pp. 85–104.
[13] D. Lechner, E. Kallay, H.S. Cross, 1 Alpha,25-dihydroxyvitamin D3 downregulates CYP27B1 and induces CYP24A1 in colon cells, Mol. Cell. Endocrinol. 263 (2007) 55–64.