

DNMT1 and HDAC1 gene expression in impaired spermatogenesis and testicular cancer

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Accepted: 14 August 2006 / Published online: 8 September 2006
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Abstract DNA methylation catalyzed by DNA methyltransferases (DNMTs) and histone deacetylation catalyzed by histone deacetylases (HDACs) play an important role for the regulation of gene expression during carcinogenesis and spermatogenesis. We therefore studied the cell-specific expression of DNMT1 and HDAC1 for the first time in human testicular cancer and impaired human spermatogenesis. During normal spermatogenesis, DNMT1 and HDAC1 were colocalized in nuclei of spermatogonia. While HDAC1 was additionally present in nuclei of Sertoli cells, DNMT1 was restricted to germ cells exhibiting a different expression pattern of mRNA (in pachytene spermatocytes and round spermatids) and protein (in round

spermatids). Interestingly, in infertile patients revealing round spermatid maturation arrest, round spermatids lack DNMT1 protein, while pachytene spermatocytes became immunopositive for DNMT1. In contrast, no changes in the expression pattern could be observed for HDAC1. This holds true also in testicular tumors, where HDAC1 has been demonstrated in embryonal carcinoma, seminoma and teratoma. Interestingly, DNMT1 was not expressed in seminoma, but upregulated in embryonal carcinoma.

Keywords DNMT1 · HDAC1 · Spermatogenesis · Testis · Tumor

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Introduction

DNA methylation catalyzed by DNA methyltransferases (DNMTs) and histone deacetylation catalyzed by histone deacetylases (HDACs) are known to play an important role for the regulation of gene expression during embryogenesis and carcinogenesis (Klose and Bird 2006).

DNA methylation of deoxycytosine residues in CpG dinucleotides is carried out by either the maintenance DNMT1 (Bestor et al. 1988) or the de-novo DNMTs 3a and 3b (Okano et al. 1998) catalyzing the transfer of a methyl group from the S-adenosyl-methionine to the 5' position of cytosine in DNA. Hypermethylation of CpG dinucleotides within gene promoters is known to be associated with gene silencing causing loss of tumor suppressor gene function in cancer (Turek-Plewa and Jagodzinski 2005).

Within the testis, Trasler et al. (1992) demonstrated a 5.2 kb DNMT mRNA in spermatogonia and round

spermatids and a 6.2 kb DNMT mRNA in pachytene spermatocytes. Applying northern and western blot analyses, both the 5.2 kb transcript and the corresponding protein have been reported to be most abundant in testis from mice aged 6 days (approximately twofold the 70-day values) (Benoit and Trasler 1994). Both transcript and protein could be demonstrated in spermatogonia, spermatocytes (Numata et al. 1994) and round spermatids (Benoit and Trasler 1994). Similar results were obtained in rat testis (Jue et al. 1995). Sakai et al. (2001) reported DNMT1 protein in primordial germ cells and Sertoli cells in 12.5 days post coitum mouse embryos. Real time RT-PCR demonstrated a peak of DNMT1 mRNA expression levels around birth (La Salle et al. 2004).

Acetylation and deacetylation of lysine residues, clustered at the amino-terminal end of core histones, involves histone acetyl transferases (HATs) and histone deacetylases (HDACs), respectively (Davie 1998). It has been demonstrated that HATs may act as transcriptional coactivators (Spencer and Davie 1999), while HDACs may induce transcriptional repression (De Ruijter et al. 2003). Histone hyperacetylation, which has been reported to play an important role for the production of fertile spermatozoa (Gatewood et al. 1990; Meistrich et al. 1992; Hazzouri et al. 2000; Sonnack et al. 2002; Fenic et al. 2004), therefore, may be caused by either activation of HATs or repression of HDACs.

Inhibitors of either HDAC or DNMT activity offer exciting opportunities for novel anti-cancer drugs, as an increase in histone acetylation may enhance apoptosis, while DNA hypomethylation may result in reactivation of tumor suppressor genes (Zhou and Otterson 2003). HDAC inhibitors, in addition, have been demonstrated to cause male infertility and, therefore, may represent possible contraceptives (Fenic et al. 2004).

To improve our knowledge on the regulation of DNA methylation and histone acetylation, we analyzed for the first time the cell-specific expression of DNMT1, known to represent the major enzyme that is responsible for the maintenance of the DNA methylation pattern (Turek-Plewa and Jagodzinski 2005), and HDAC1, known to represent the predominant HDAC within the testis (Hazzouri et al. 2000), in both impaired human spermatogenesis and human testicular cancer.

Materials and methods

Tissue

After written informed consent, testicular biopsies from the following patients were analyzed: men with

obstructive azoospermia after vasectomy ($n = 8$). These biopsies revealed normal spermatogenesis and served as controls. Patients with spermatogenic arrest at the level of round spermatids ($n = 2$), spermatocytes ($n = 2$) and spermatogonia ($n = 2$), as well as Sertoli cell only (SCO) syndrome ($n = 2$). In addition, samples from patients exhibiting embryonal carcinoma ($n = 8$), seminoma ($n = 16$) and teratoma ($n = 8$) were analyzed. All tumors were obtained from adults. No germ cell tumors of infants have been included in the study. Material was fixed in either Bouin's fixative (biopsy material) or 4% phosphate buffered formalin (tumor material) and embedded in paraffin using standard techniques. For histological evaluation, 5 μ m sections were stained with hematoxylin and eosin (H&E) and scored, according to Bergmann and Kliesch (1998).

Real time quantitative PCR (QPCR)

Prior to RNA extraction, serial sections were taken to ensure sample purity and amount of target tissue estimated in the first and last section by standard H&E stain. Total RNA from patients mentioned in the "Tissue" section revealing normal spermatogenesis ($n = 6$), embryonal carcinoma ($n = 6$), seminoma ($n = 4$) and teratoma ($n = 2$) was extracted using RNeasy Extraction Kit (Qiagen, Hilden, Germany). QPCR was performed on cDNA synthesized from 1 μ g of RNA using nonamer primers and Omniscript Synthesis Kit (Qiagen, Hilden, Germany). The intron-spanning primer pairs were designed by Applied Biosystems (Assay ID Hs00154749_m1 for DNMT1). Quantitative PCR (QPCR) was performed using 5 μ l PCR supermix from Abgene (Abgene, Hamburg, Germany) on the AB7900 Detection System. Primers were added to the reaction mixture at a final concentration of 200 nM. Thermal cycle parameters were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min. All reactions were performed in duplicate with β -actin as an internal control.

Digoxigenin-labeled cRNA probes for DNMT1 mRNA

Total RNA was extracted from testis homogenates using Trizol (Invitrogen, Karlsruhe, Germany). First strand cDNA-synthesis was performed using Omniscript synthesis kit (Qiagen, Hilden, Germany). The cDNA clone for human DNMT1 (GenBank accession number NM_001379.1) was generated using RT-PCR (1 \times 3 min for 95°C, 30 \times 45 s for 95°C, 45 s for 64°C, 45 s for 72°C, 1 \times 5 min for 72°C) with 5'-CAAGCAAGAAGTGAAGCC3' as forward primer

(bp 569–588) and 5'CAGGTTCTTCTGCAGGAA GC3' as reverse primer (bp 885–905). A 336 bp PCR product of the human DNMT1 cDNA was subcloned in pGEM-T (Promega, Mannheim, Germany). The plasmid was transformed in the XL1-Blue *Escherichia coli* strain (Stratagene, Amsterdam, The Netherlands) and extracted by column purification (Qiagen, Hilden, Germany). In vitro transcription of the DIG-labeled cRNA was performed using the RNA-DIG Labeling Mix (Boehringer Mannheim, Mannheim, Germany) and RNA-polymerases T7 and SP6. Prior to cRNA synthesis, the vector containing the DNMT1 insert had been digested with SacI or SacII (New England Biolabs, Frankfurt, Germany) for the production of sense cRNA (SacI/T7) and antisense cRNA (SacII/SP6).

In situ hybridization

In situ hybridization was performed as previously reported (Steger et al. 1998b). Briefly, 5 µm sections were mounted on slides coated with aminopropyltriethoxysilane (Sigma, Munich, Germany). After deparaffinization, sections were digested with proteinase K (20 µg/ml) for 30 min at 37°C, postfixed in 4% paraformaldehyde for 10 min and prehybridized in 20% glycerol for 30 min. Sections were then incubated with the DIG-labeled sense and antisense cRNA probes. Both probes were used at a dilution of 1:100 in hybridization-buffer containing 50% deionized formamide, 10% dextran sulfate, 2× SSC, 1× Denhardt's solution, 10 µg/ml salmon sperm DNA and 10 µg/ml yeast t-RNA. Hybridization was performed overnight at 37°C in a humid chamber containing 50% formamide in 2× SSC. Following posthybridization washes, tissue samples were incubated overnight at 4°C with an anti-DIG Fab-antibody conjugated to alkaline phosphatase (Boehringer Mannheim, Mannheim, Germany). Staining was visualized with NBT/BCIP (KPL, MD, USA) in a humid chamber protected from light.

Immunohistochemistry

The polyclonal antibodies goat anti-human DNMT1 (Santa Cruz, Heidelberg, Germany) and rabbit anti-human HDAC1 (Sigma, Munich, Germany) were used. After deparaffinization, antigen retrieval was performed in 0.01 mol/l sodium citrate buffer (pH 6) for 20 min at 100°C in a microwave oven. Subsequently, sections were treated with 3% H₂O₂ in methanol for 20 min followed by Tris-buffered saline (TBS, pH 7) containing 5% bovine serum albumin for 1 h. Sections were then incubated with the primary antibody (DNMT1 1:500 in TBS, HDAC1 1:1,000 in TBS)

overnight at 4°C. This was followed by a biotinylated secondary antibody (rabbit anti-goat or goat anti-rabbit, 1:200 in TBS, Dako, Hamburg, Germany) for 1 h and an avidin biotin complex (ABC, Vector, USA) also for 1 h. For color development, sections were incubated with DAB/H₂O₂.

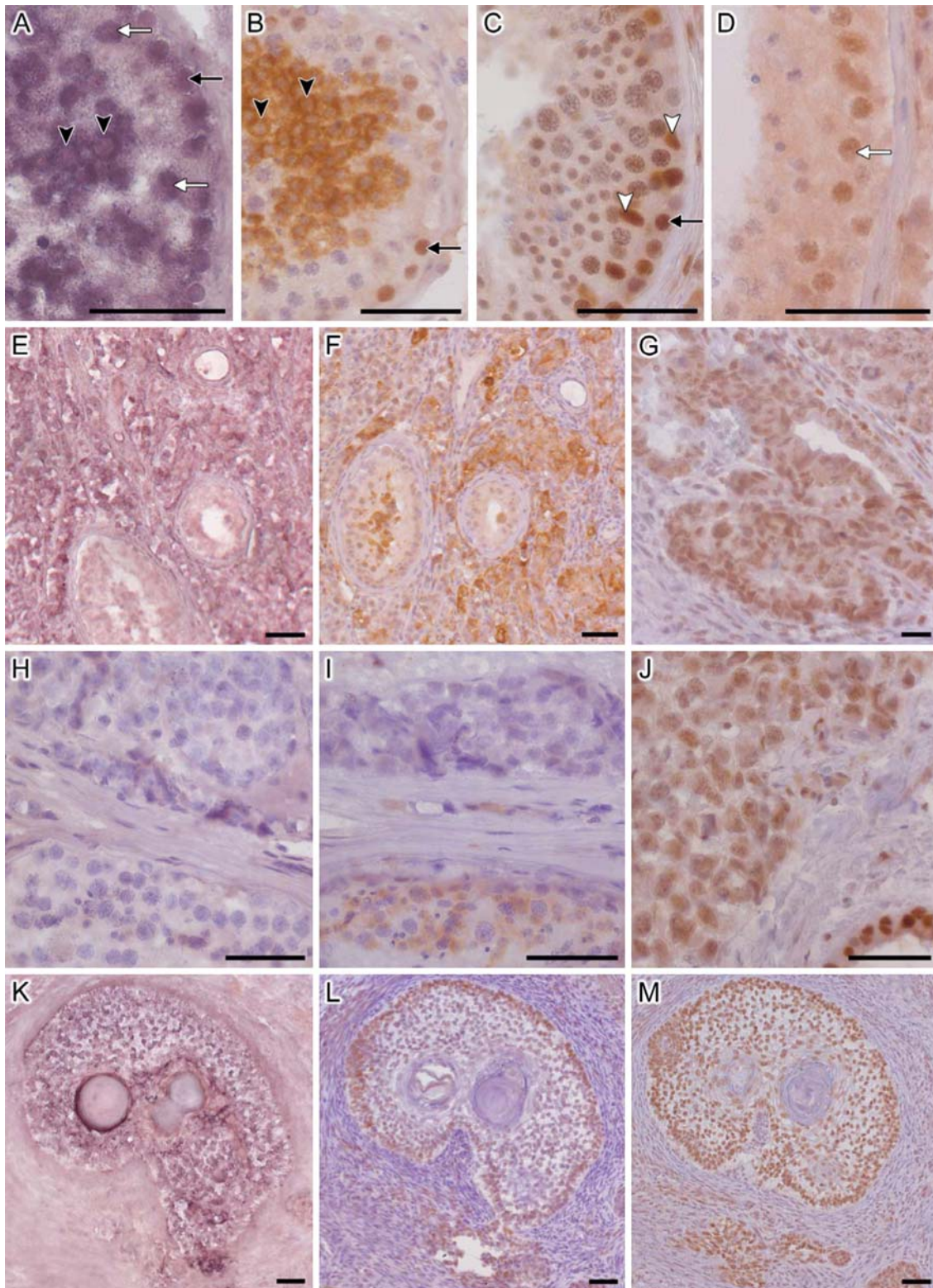
Results

During normal spermatogenesis, DNMT1 mRNA was expressed in spermatogonia, pachytene spermatocytes and round spermatids (Fig. 1a). Interestingly, the corresponding protein could be observed in the nuclei of spermatogonia and in the cytoplasm of round spermatids, but was absent in pachytene spermatocytes (Fig. 1b). The HDAC1 protein was present in nuclei of spermatogonia and Sertoli cells (Fig. 1c).

A similar staining pattern could be demonstrated in seminiferous tubules exhibiting impaired spermatogenesis. Interestingly, in seminiferous tubules revealing round spermatid maturation arrest, solely pachytene spermatocytes exhibited positive signals for DNMT1 protein (Fig. 1d). Tubules with spermatogenic arrest at the level of spermatocytes exhibited positive signals for both mRNA and protein in spermatogonia and pachytene spermatocytes, while tubules with spermatogenic arrest at the level of spermatogonia displayed positive signals solely in spermatogonia. Tubules with Sertoli cell only (SCO) characteristics were completely negative for both mRNA and protein. For HDAC1, spermatogonia and Sertoli cell nuclei remain positive even in seminiferous tubules exhibiting spermatogenic arrest at the level of spermatogonia or SCO characteristics (data not shown).

In embryonal carcinoma, positive signals could be observed for DNMT1 mRNA and protein, as well as HDAC1 protein (Fig. 1e–g). Although signal intensity for DNMT1 mRNA and protein was to a certain extent heterogeneous between samples analyzed, all cases revealed stronger signals in embryonal carcinoma when compared with teratoma. In seminoma, both mRNA and protein of DNMT1 were completely absent in all specimens analyzed, while HDAC1 protein permanently exhibited positive signals (Fig. 1h–j). In teratoma, DNMT1 mRNA and protein, as well as HDAC1 protein revealed weak positive signals (Fig. 1k–m).

In addition to in situ hybridization demonstrating the cell-specific localization of DNMT1 transcripts, we studied the quantitative expression of DNMT1 mRNA in a panel of testicular tumors applying real time quantitative PCR. In accordance with our data obtained



by in situ hybridization and immunohistochemistry (Table 1), we found that DNMT1 gene expression is significantly upregulated in embryonal carcinoma when compared with seminoma and teratoma (Fig. 2).

Discussion

We studied the gene expression of the major enzyme responsible for the maintenance of DNA methylation

◀ **Fig. 1** DNMT1 in situ hybridization (**a, e, h, k**), DNMT1 immunohistochemistry (**b, d, f, i, l**) and HDAC1 immunohistochemistry (**c, g, j, m**) in seminiferous tubules exhibiting normal spermatogenesis (**a, b, c**) and round spermatid maturation arrest (**d**), as well as in embryonal carcinoma (**e–g**), seminoma (**h–j**) and teratoma (**k–m**). During normal spermatogenesis, DNMT1-mRNA is expressed in spermatogonia (**a, black arrow**), pachytene spermatocytes (**a, white arrows**) and round spermatids (**a, black arrowheads**), while DNMT1 protein is present in spermatogonia (**b, black arrow**) and round spermatids (**b, black arrowheads**). HDAC1 protein could be observed in nuclei of spermatogonia (**c, black arrow**) and Sertoli cells (**c, white arrowheads**). Interestingly, in round spermatid maturation arrest, DNMT1 could only be demonstrated in nuclei of pachytene spermatocytes (**d, white arrow**). Embryonal carcinoma exhibit positive cells for DNMT1-mRNA (**e**), DNMT1 protein (**f**) and HDAC1 protein (**g**). Note seminiferous tubules with spermatogenic arrest at various stages of spermatogenesis. **h** (DNMT1-mRNA) and **i** (DNMT1 protein) show seminoma (upper part) in direct vicinity to seminiferous tubule with at least qualitative normal spermatogenesis (lower part). While still some weak signals could be observed in the seminiferous tubule, seminoma remains completely negative. By contrast, HDAC1 reveals a strong signal in seminoma (**j, lower right**, seminiferous tubule with spermatogenic arrest at the level of spermatogonia displaying positive Sertoli cell nuclei). Teratoma displays positive cells for DNMT1-mRNA (**k**), DNMT1 protein (**l**) and HDAC1 protein (**m**). Bars 50 μ m

(DNMT1) and the predominant HDAC within the testis (HDAC1) in impaired spermatogenesis and testicular cancer. DNMT1 has been demonstrated to bind HDAC1 (Fuks et al. 2000) and coexpression of DNMT1 and HDAC1 has been reported in prostate cancer (Patra et al. 2001) and in nuclei of spermatogonia (this study). As spermatogonia are mitotically active cells (Steger et al. 1998a), expression of DNMT1 may be due to the maintenance of the DNA methylation pattern in the course of DNA synthesis. In addition, data from Mortusewicz et al. (2005) point to a direct role of DNMT1 in the restoration of epigenetic information during DNA repair processes mediated by binding to the proliferation cell nuclear antigen (PCNA) binding domain. This is corroborated by the coexpression of PCNA (Steger et al. 1998a) and DNMT1 (this study) in the nuclei of spermatogonia.

While HDAC1 revealed an additional signal in the nuclei of somatic Sertoli cells, DNMT1 gene expression was restricted to germ cells exhibiting positive signals for DNMT1 mRNA in pachytene spermatocytes and round spermatids and for DNMT1 protein in the cytoplasm of round spermatids. The different expression pattern between transcript and corresponding protein may be similar to the mouse model, where it has been demonstrated that three transcript isoforms may be expressed by the DNMT1 locus due to alternative usage of multiple first exons (Mertineit et al. 1998; La Salle et al. 2004). While DNMT1s is expressed in somatic cells, DNMT1p and DNMT1o are expressed in

pachytene spermatocytes and oocytes, respectively. DNMT1p mRNA does not produce active protein, because short open reading frames in the first exon likely interfere with translation of the authentic open reading frame (Ko et al. 2005). It has been suggested that the first exon of DNMT1 may be involved in the localization of DNMT1 during various stages of gametogenesis. Interestingly, a tissue-dependent differentially methylated region (DMR) has been demonstrated in the 5' region of DNMT1o, but not in that of DNMT1s and DNMT1p (Ko et al. 2005).

Although the cytoplasmic localization of DNMT1 in round spermatids is in line with data obtained from mouse (Benoit and Trasler 1994), the functional significance of this finding remains unclear, but may be associated with the structural chromatin reorganization due to histone-to-protamine exchange. It may play a role in the exact timing of protamine expression, which is of great importance, as premature protamine expression

Table 1 Summary of results from in situ hybridization (ISH) and immunohistochemistry (IHC)

	DNMT-1		HDAC-1
	mRNA (ISH)	Protein (IHC)	Protein (IHC)
Normal spermatogenesis			
Sertoli cells	–	–	+ (nuclei)
Spermatogonia	+ (cytoplasm)	+ (nuclei)	+ (nuclei)
Pachytene spermatocytes	+ (cytoplasm)	–	–
Round spermatids	+ (cytoplasm)	+ (cytoplasm)	–
Impaired spermatogenesis			
SCO			
Sertoli cells	–	–	+
SGA			
Sertoli cells	–	–	+
Spermatogonia	+	+	+
SCA			
Sertoli cells	–	–	+
Spermatogonia	+	+	+
Spermatocytes	+	+	–
RSMA			
Sertoli cells	–	–	+
Spermatogonia	–	–	+
Spermatocytes	+	+	–
Round spermatids	–	–	–
Tumor			
Embryonal carcinoma	+	+	+
Seminoma	–	–	+
Teratoma	+	+	+

SCO Sertoli-cell-only syndrome, SGA Spermatogenic arrest at the level of spermatogonia, SCA Spermatogenic arrest at the level of pachytene spermatocytes, RSMA Round spermatid maturation arrest. Positive and negative signals are indicated as + and –, respectively

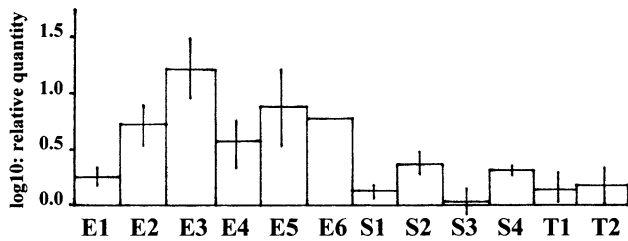


Fig. 2 Real time quantitative PCR shows high expression levels for DNMT1 in embryonal carcinoma (*E1–E6*) when compared with seminoma (*S1–S4*) and teratoma (*T1–T2*). Gene expression levels were determined using the $2^{-\Delta\Delta C_t}$ method and are expressed relatively to the β -actin transcript selecting *E6* as a reference sample

is known to result in precocious chromatin condensation followed by male infertility (Lee et al. 1995). Replacement of DNA-binding histones by protamines in elongating spermatids is associated with histone hyperacetylation, which reduces the number of positive charges and, as a consequence, decreases the affinity of histones for DNA (Turner 1991). Histone H4 hyperacetylation has been demonstrated in murine (Hazzouri et al. 2000) and human (Sonnack et al. 2002) spermatogonia (due to mitosis) and elongating spermatids (due to histone-to-protamine exchange). In the present study, we demonstrated a precocious appearance of DNMT1 protein in the cytoplasm of pachytene spermatocytes within seminiferous tubules revealing round spermatid maturation arrest. Thus, precocious expression of DNMT1 protein in spermatocytes might result in aberrant protamine expression, which in turn is followed by the failure of spermatocytes to differentiate into spermatids.

Smiraglia et al. (2002) reported distinct epigenetic phenotypes in seminomatous and non-seminomatous testicular germ cell tumors, namely an average CpG islands methylation of 0.08 and 1.11%, respectively. Increased genome methylation in non-seminomatous germ cell tumors is likely to be caused by DNMTs in this type of testicular malignancy (Almstrup et al. 2005; Skotheim et al. 2005). In the present study, both DNMT1 mRNA and DNMT1 protein exhibited strong signals in embryonal carcinoma and weak signals in teratoma, but were completely absent in seminoma. The differential gene expression of DNMT1 between various forms of testicular cancer was corroborated by data from microarray analysis (Biermann et al. 2006) and real time quantitative PCR. Both methods displayed low DNMT1 mRNA levels in seminoma and teratoma in combination with significantly upregulated transcript levels in embryonal carcinoma. These results are in line with the fact that an increased DNMT1 gene expression is found in carcinoma from a variety of

tissues (Jones and Baylin 2002). An increase in DNA methyltransferase activity is associated with neoplastic transformation, though the precise molecular mechanism of DNMT1 function remains unclear. Specific deletion of DNMT1 markedly potentiated the ability of 5-aza-2'-deoxycytidine to reactivate silenced tumor suppressor genes suggesting that DNMT1 is both necessary and sufficient to maintain global methylation and aberrant CpG island methylation in cancer cells (Robert et al. 2003). In addition, human cancer cells lacking DNMT1 expression have been reported to reveal an altered pattern of histone H3 modification resulting in an increase in acetylation and a decrease in methylation of lysine 9 (Espada et al. 2004).

In conclusion, our study clearly demonstrated that expression of DNMT1 is clearly regulated in both impaired spermatogenesis and development of embryonal carcinoma, while HDAC1 expression is not regulated during aberrant germ cell differentiation. DNMT1 expression in aggressive forms of testicular cancer including embryonal carcinoma and teratoma, as demonstrated in the present study, might be of clinical importance, as DNA methyltransferase inhibitors represent promising new drugs for cancer therapies. The first of these compounds (5-azacytidine, Vidaza) has recently been approved as an antitumor agent and others are presently in various stages of preclinical and clinical development. In contrast to the differentially regulated DNMT1, weak immunopositive signals for HDAC1 have been demonstrated in embryonal carcinoma, seminoma and teratoma suggesting that HDAC1 gene expression during testicular carcinogenesis is regulated, if at all, at a very low level.

Acknowledgments Funding of this research program was provided by the German Research Foundation (DFG) Research Training Group 533 Cell–Cell-Interaction in Reproduction and the German Academic Exchange Service (DAAD).

References

- Almstrup K, Hoei-Hansen CE, Nielsen JE, Wirkner U, Ansorge W, Skakkebaek NE, Rajpert-DE Mayts E, Leffers H (2005) Genome-wide gene expression profiling of testicular carcinoma in situ progression into overt tumours. *Br J Cancer* 92:1934–1941
- Benoit G, Trasler JM (1994) Developmental expression of DNA methyltransferase messenger ribonucleic acid, protein, and enzyme activity in the mouse testis. *Biol Reprod* 50:1312–1319
- Bergmann M, Kliesch S (1998) Hodenbiopsie. In: Krause W, Weidner W (eds) *Andrologie*. Ferdinand Enke, Stuttgart, pp 66–71
- Bestor T, Laudano A, Mattaliano R, Ingram V (1988) Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. The carboxy-terminal domain of the

- mammalian enzyme is related to bacterial restriction methyltransferases. *J Mol Biol* 203:971–983
- Biermann K, Heukamp LC, Steger K, Zhou H, Franke FE, Sonnack V, Brehm R, Berg J, Bastian PJ, Müller SC, Wang-Eckert L, Büttner R (2006) Early molecular events in precursor lesions and testicular germ cell tumors. *J Pathol* (in press)
- Davie JR (1998) Covalent modifications of histones: expression from chromatin templates. *Curr Opin Genet Dev* 8:173–178
- De Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB (2003) Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J* 370:737–749
- Espada J, Ballestar F, Fraga MF, Villar-Garea A, Juarranz A, Stockert JC, Robertson KD, Fuks F, Esteller M (2004) Human DNA methyltransferase 1 is required for maintenance of the histone H3 modification pattern. *J Biol Chem* 279:37175–37184
- Fenic I, Sonnack V, Failing K, Bergmann M, Steger K (2004) In vivo effects of histone-deacetylase inhibitor trichostatin-A on murine spermatogenesis. *J Androl* 25:811–818
- Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T (2000) DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat Genet* 24:88–91
- Gatewood JM, Cook GR, Balhorn R, Schmid CW, Bradbury EM (1990) Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. *J Biol Chem* 25:20662–20666
- Hazzouri M, Pivot-Pajot C, Faure AK, Usson Y, Pelletier R, Sele B, Khochbin S, Rousseaux S (2000) Regulated hyperacetylation of core histones during mouse spermatogenesis: involvement of histone deacetylases. *Eur J Cell Biol* 79:950–960
- Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 3:415–428
- Jue K, Benoit G, Alcivar-Warren AA, Trasler JM (1995) Developmental and hormonal regulation of DNA methyltransferase in the rat testis. *Biol Reprod* 52:1394–1371
- Klose RJ, Bird AP (2006) Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci* 31:89–97
- Ko YG, Nishino K, Hattori N, Arai Y, Tanaka S, Shiota K (2005) Stage-by-stage change in DNA methylation status of DNA methyltransferase 1 (Dnmt1) locus during mouse early development. *J Biol Chem* 280:9627–9634
- La Salle S, Mertineit C, Taketo T, Moens PB, Bestor TH, Trasler JM (2004) Windows for sex-specific methylation marked by DNA methyltransferase expression profiles in mouse germ cells. *Dev Biol* 268:403–415
- Lee K, Haugen HS, Clegg CH, Braun RE (1995) Premature translation of protamine 1 mRNA causes precocious nuclear condensation and arrests spermatid differentiation in mice. *Proc Natl Acad Sci USA* 92:12451–12455
- Meistrich ML, Trostle-Weige PK, Lin R, Bhatnagar YM, Allis CD (1992) Highly acetylated H4 is associated with histone displacement in rat spermatids. *Mol Reprod Dev* 31:170–181
- Mertineit C, Yoder JA, Taketo T, Laird DW, Trasler JM, Bestor TH (1998) Sex-specific exons control DNA methyltransferase in mammalian germ cells. *Development* 125:889–897
- Mortusewicz O, Schermelleh L, Walter J, Cardoso MC, Leonhardt H (2005) Recruitment of DNA methyltransferase I to DNA repair sites. *Proc Natl Acad Sci USA* 102:8905–8909
- Numata M, Ono T, Iseki S (1994) Expression and localization of the mRNA for DNA (cytosine-5)-methyltransferase in mouse seminiferous tubules. *J Histochem Cytochem* 42:1271–1276
- Okano M, Xie S, Li E (1998) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat Genet* 19:219–220
- Patra SK, Patra A, Dahiya R (2001) Histone deacetylase and DNA methyltransferase in human prostate cancer. *Biochem Biophys Res Commun* 287:705–713
- Robert MF, Morin S, Beaulieu N, Gauthier F, Chute IC, Barsalou A, MacLeod AR (2003) DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells. *Nat Genet* 33:61–65
- Sakai Y, Suetake I, Itoh K, Mizugaki M, Tajima S, Yamashima S (2001) Expression of DNA methyltransferase (Dnmt1) in testicular germ cells during development of mouse embryo. *Cell Struct Funct* 26:685–691
- Skotheim RI, Lind GE, Monni O, Nesland JM, Abeler VM, Fossa SD, Duale N, Brunborg G, Kallioniemi O, Andrews PW, Lothe RA (2005) Differentiation of human embryonal carcinomas in vitro and in vivo reveals expression profiles relevant to normal development. *Cancer Res* 65:5588–5598
- Smiraglia DJ, Szymanska J, Kagerud SM, Lothe RA, Peltomaki P, Plass C (2002) Distinct epigenetic phenotypes in seminomatous and nonseminomatous testicular germ cell tumors. *Oncogene* 21:3909–3916
- Sonnack V, Failing K, Bergmann M, Steger K (2002) Expression of hyperacetylated histone H4 during normal and impaired human spermatogenesis. *Andrologia* 34:384–390
- Spencer VA, Davie JR (1999) Role of covalent modifications of histones in regulating gene expression. *Gene* 15:1–12
- Steger K, Aleithe I, Behre H, Bergmann M (1998a) The proliferation of spermatogonia in normal and pathological human seminiferous epithelium: an immunohistochemical study using monoclonal antibodies against Ki-67 protein and proliferating cell nuclear antigen. *Hum Reprod* 4:227–233
- Steger K, Klönisch T, Gavenis K, Drabent B, Doenecke D, Bergmann M (1998b) Expression of mRNA and protein of nucleoproteins during human spermiogenesis. *Mol Hum Reprod* 4:939–945
- Turner BM (1991) Histone acetylation and control of gene expression. *J Cell Sci* 99:13–20
- Turek-Plewa J, Jagodzinski PP (2005) The role of mammalian DNA methyltransferases in the regulation of gene expression. *Cell Mol Biol Lett* 10:631–647
- Trasler JM, Alcivar AA, Hake LE, Bestor T, Hecht NB (1992) DNA methyltransferase is developmentally expressed in replicating and non-replicating male germ cells. *Nucleic Acid Res* 20:2541–2545
- Zhou WG, Otterson GA (2003) The interaction of histone deacetylase inhibitors and DNA methyltransferase inhibitors in the treatment of human cancer cells. *Curr Med Chem Anticancer Agents* 3:187–199