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Loss of heterozygosity of selected tumor suppressor genes in human testicular germ cell tumors

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Loss of heterozygosity of tumor suppressors in testicular tumors

***Keywords*:** loss of heterozygosity; tumor suppressor genes; testicular germ cell tumors

Abstract

Human testicular germ cell tumors (TGCTs) are histologically heterogenous neoplasms with variable malignant potential. Two main groups of germ cell tumors occur in men: seminomas and nonseminomas. In the present study a set of four tumor suppressor genes was investigated in testicular cancers. *CDH1*, *APC*, *p53* and *nm23-H1* genes were tested for the loss of heterozygosity (LOH). Thirtyeight testicular germ cell tumors (17 seminomas and 21 nonseminomas) were analyzed by PCR using restriction fragment length polymorphism or dinucleotide/tetranucleotide repeat polymorphism method. An allelic loss of *p53* at exon 4 was detected in five nonseminomas, whereas LOH of *p53* at intron 6 occured in one of the seminoma and two of the nonseminoma samples. Allelic losses of the *APC* gene were present in three seminomas and one nonseminoma, whereas one seminoma and three nonseminomas showed LOH of *CDH1*. The analysis of allelic losses has shown no common structural genetic alterations in tumor tissues, although a different pattern of LOH has been observed between the two main histological groups of TGCTs.

Introduction

Testicular germ cell tumors (TGCTs) are the most common tumors in young men. It is generally accepted that the TGCTs originate from intratubular germ cell neoplasia (ITGCN). Histologically and clinically, TGCTs are classified into seminomas and nonseminomas. About 50% of TGCTs are pure seminomas, and 40% are pure or mixed nonseminomas. The remaining 10% containing both seminoma and nonseminoma

components are classified as being nonseminomas according to the WHO 2004 classification system [4].

Seminomas develop from the sperm-producing germ cells of the testicle. The two main types of seminomatous tumors are classic seminoma and spermatocytic seminoma. Seminoma is the most common testicular germ cell neoplasm and usually occurs in patients in the fourth decade of life.

There are four main types of nonseminomatous tumors: embryonal carcinoma, yolk sac tumor, immature or mature teratoma and choriocarcinoma. These tumor types are often seen together in various combinations, referred to as mixed TGCTs, which may also include a seminoma component [1]. The undifferentiated stem cells of nonseminomas are termed embryonal carcinoma cells and can differentiate into a broad spectrum of somatic tissues (teratomas) and the extraembryonal derivates: yolk sac tumors and choriocarcinomas [15]. Nonseminoma occurs most frequently between 20 and 40 years of age.

Molecular genetic studies of TGCTs have reported allele loss on chromosomes 1, 3, 5, 9, 11, 12, 13, 17 and 18. However, mutation analyses of candidate tumor suppressor genes such as *RB*, *TP53*, *WT1*, *BRCA1*, *APC*, *CDH1*, *MCC*, *NF2* and *DCC* have demonstrated only rare alterations, so major TGCT suppressor genes are yet to be identified [10]. To date the exact cellular and molecular mechanisms leading to the neoplastic progression of ITGCN are still not fully understood [13]. Increasing evidence suggests that carcinogenesis must be understood in terms of an accumulation of mutations in regulatory genes, including activation of oncogenes and inactivation or loss of tumor suppressor genes.

APC and E-cadherin proteins are components of the adherens junction, where E-cadherin is bound to β-catenin, which in turn binds to the central part of the APC. Besides their roles in cellular architecture, the APC and β-catenin play a signaling role as components of the Wnt signal transduction pathway [17]. APC acts as a negative regulator of the Wnt signaling pathway, being a critical component of the β-catenin destruction machinery heading to the proteasome. E-cadherin has a role in Wnt signaling, too; where it acts as an indirect modulator of Wnt signaling. Since it binds to and sequesters cytoplasmic β-catenin, it is involved in the modulation of the signal [16]. These genes are also implicated in cancer, especially in neoplasms of epithelial origin.

Several studies have suggested that TGCTs express high levels of wild type p53 protein but apparently do not contain *p53* mutations [6], [7]. The p53 pathway responds to a variety of intrinsic and extrinsic stress signals and when activated maintains cellular integrity by inducing cell arrest, senescence or apoptosis. It has been shown that β-catenin and p53 form a negative feedback loop that regulates Wnt signaling. β-catenin induces p53 activation by inducing p14ARF expression, p14ARF inhibits Mdm2 and consequently leads to an accumulation of active p53 [3].

Nucleoside diphosphate (NDP) kinases, responsible for the synthesis of nucleoside triphosphates and produced by the *nm23* genes, are involved in numerous regulatory processes associated with cell growth, differentiation, tumor progression, metastasis and development. Nm23-H1 is overexpressed in solid tumors as compared to normal tissues with a reduced expression correlated with the metastatic potential of some tumors [5]. The immunohistochemically detected expression of both the *nm23-H1* and -

H2 gene products is not associated with the metastatic status or the invasive status of testicular seminoma [11].

Our objective was to examine the loss of heterozygosity (LOH) of *APC*, *CDH1 (E-cadherin)*, *p53* and *nm23-H1* tumor suppressor genes in 38 TGCTs (17 seminomas and 21 nonseminomas). Gene products of *APC*, *CDH1* and *p53* are involved in the Wnt signaling pathway implicated in embryogenesis and cancerogenesis, whereas the role of nm23-H1 protein in the development of TGCTs has not been elucidated.

Materials and Methods

Patients and tumor material. Thirtyeight TGCTs (17 seminomas and 21 nonseminomas) were collected from the Sisters of Mercy University Hospital and University Hospital Center, Zagreb, Croatia. The samples were formalin-fixed and paraffin-embedded. Clinical and pathological data for 38 TGCTs according to the WHO 2004 classification are shown in Table 1.

DNA extraction. For each specimen, a 20 μm paraffin-embedded section was prepared for DNA extraction. In addition, a 4 μm section was stained with hemalaun-eosin to identify the tumor and normal tissue areas which were removed separately from the microscopic slide, transferred to microtubes and extracted using QIAamp DNA Mini Kit (Qiagen, Germany).

LOH analysis of APC, p53, CDH1 and nm23-H1 genes. LOH of *APC* and *p53* genes were detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Table 2). PCR of *APC* gene exon 11 amplified a 133-bp target sequence containing a polymorphic *RsaI* restriction site, with restriction 48-

and 85-bp fragments (heterozygous alleles). The reaction mixture for *APC* exon 11 amplification was: 2 μmol of each primer (Operon Biotechnologies, Germany), 0.2 mM of each dNTP, 100 ng DNA, 0.4 U *Taq polymerase* (Fermentas, Lithuania), 2 mM $MgCl_2$, 1x reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.8). PCR was performed for 45 cycles under the following conditions: initial denaturation, 3 min/96ºC; denaturation, 30 sec/96ºC; annealing, 30 sec/58ºC; extension, 30+1 sec/72ºC; final extension, 10 min/72ºC. PCR aliquots (10-20 μl) were digested with 5 U *RsaI* (Fermentas, Lithuania) for 12 hours. A *Bsh1236I* restriction site in exon 4 and *MspI* restriction site in intron 6 of the *p53* were examined as previously mentioned [12].

Analyses of the D16S752 tetranucleotide microsatellite marker linked to *CDH1* and M2 dinucleotide microsatellite marker linked to *nm23-H1* were carried out by PCR using 5 pmol of each primer (Table 2), under the *APC* PCR conditions. Obtained fragments were analyzed on silver-stained 15% polyacrylamide gel. Loss of heterozygosity of *CDH1* and *nm23-H1* was considered to occur if one out of two alleles (heterozygous samples) of gene markers was missing or significantly reduced in comparison to alleles from adjacent normal tissue.

Results

Genetic studies were performed for the presence of possible allelic losses of *APC*, *p53*, *CDH1* and *nm23-H1* tumor suppressor genes.

First, the *RsaI* polymorphic site in *APC*'s exon 11 was analyzed. From 38 TGCTs, 29 samples (76%, 13 seminomas and 16 nonseminomas) were informative for this polymorphism. Four (14%) samples: three seminomas (23%) and one nonseminoma (6%)

demonstrated LOH (Tables 3 and 4). These tumor samples showed either only one band, a 133-bp fragment (Fig. 1), or two bands (48-bp and 85-bp fragments).

Two polymorphic sites of *p53* gene, a *Bsh123GI* restriction site in exon 4 and a *MspI* restriction site in intron 6, were used. Analysis of these two markers showed that 30 tumors (79%, 12 seminomas and 18 nonseminomas) were heterozygous for the first polymorphism. Loss of heterozygosity was observed in five (28%) nonseminoma cases as one of the alleles missing in comparison to bands from the adjacent normal testis tissue (Fig. 2, Tables 3 and 4). The polymorphic marker in intron 6 of the *p53* was less informative than the first one. Twenty out of 38 samples (53%, 12 seminomas and 8 nonseminomas) were heterozygous. Allelic loss was found in three (15%) tumors: one seminoma (8%) and two nonseminomas (25%); Fig. 3, Tables 3 and 4.

The D16S752 polymorphic marker linked to the *CDH1* gene was analyzed. From 38 TGCTs, 20 tumors (53%, 9 seminomas and 11 nonseminomas) were informative. Our analysis revealed that four (20%) samples: one seminoma (11%) and three nonseminomas (27%), had LOH of the *CDH1* when tumor DNA was compared to autologous normal testis tissue DNA (Fig. 4, Tables 3 and 4).

The result of *nm23-H1* repeat polymorphism analysis demonstrated no changes of the gene among 34 (89%) informative TGCTs (15 seminomas and 19 nonseminomas).

Although we observed moderate levels of allelic losses of *p53* and *CDH1* genes in nonseminomas, and moderate level of LOH of *APC* gene in seminomas (Table 4), there are no statistically significant differences in LOHs between seminomas and nonseminomas found using Fisher's exact test due to the small number of samples.

Discussion

Human tumorigenesis is a multistep process. For some cancers there is considerable knowledge of the molecular events associated with progression from early precursor lesions to metastatic cancer. Although knowledge of the pathogenesis of TGCTs is more limited, a multistep model with the progression from ITGCN to seminoma and then to nonseminoma has been widely proposed. However, the specific gene alteration events that might determine the progression from ITGCN to nonseminoma are ill-defined [10].

The majority of infantile testis tumors, seminomas and nonseminomas, show a biallelic expression of imprinted genes. These data support the hypothesis that these germ cell tumors originate from an early erased germ cell [15]. If a loss of genomic imprinting affects a tumor suppressor gene, one allele is already inactivated due to the passage through either a male or a female germ line.

Recently, a novel model for examing testicular teratomas has been identified [19]. This model indicates that Wnt signaling is able to induce teratomas in p53 mutant cells and suggests that a single copy of the *p53* gene is sufficient for blocking tumorigenesis in male germ cells.

In the present study, we investigated allelic losses of *APC*, *CDH1*, *p53* and *nm23-H1* genes to elucidate whether LOHs of these genes are linked to a particular phenomenon of the signaling pathway disruption or are a consequence of whole genome instability in view of the high number and variety of chromosome abnormalities, including deletions and rearrangements present in TGCTs [2].

It is important to mention that we tested more nonseminoma than seminoma samples (17 seminomas and 21 nonseminomas), but only informative (heterozygous) samples were calculated. The number of informative samples for single genetic marker does not differ significantly between two main histological groups of TGCTs (Table 4).

Exon 4 of the *p53* gene is involved in induction of apoptosis, whereas changes in intron 6 are implicated in its expression by protein stabilization. A moderate level (28%) of allelic loss of the *p53* in nonseminomas suggests that loss of this gene may contribute to their development. Previously, Peng *et al*. [18] have observed a LOH of *p53* gene in 22% of TGCTs, despite the fact that mutations within the coding region of *p53* rarely occur in testis cancer. However, they have not observed the discrepancies between two different markers in the same gene in terms of LOH scoring in seminomas and nonseminomas (20% versus 26%, respectively).

In self-renewing tissues such as colon and testis, the APC protein is thought to modulate the stem cell differentiation by controlling the β-catenin /Tcf-Lef pathway in which APC targets β-catenin for degradation. To date, the function of APC in human testes is unclear [14]. Most mutations of the *APC* gene are frameshift mutations, however, it is recognised that a number of tumors demonstrated LOH of this gene.

Peng *et al*. [18] observed that LOH occured in 28% of informative cases at *APC* gene. However, they did not detect statistically significant differences in LOH of *APC*, by using two different markers in the same gene, between the seminomas and nonseminomas (31% versus 25%, respectively).

To increase both informativity and sensitivity, we examined the *MspI* restriction site polymorphism in *APC*'s exon 15, a 550 bp target sequence containing a polymorphic

MspI restriction site with restriction to two 250 bp fragments (heterozygous alleles). However, the PCR amplification of *APC*'s exon 15 was unsuccessful. In general, it is particulary difficult to amplify DNA from paraffin-embedded tissue when the amplified fragment is larger than 300 bp, perhaps due to degradation of the DNA. Therefore, we have not searched for two different markers in the same gene (with the exception of *p53*, those markers are presented separately), to avoid any influence on the outcome of the results.

E-cadherin has been viewed as a tumor suppressor, mainly by maintaining cell-cell adhesion and anchoring β-catenin in a submembranous location [16]. Mutations of *CDH1* gene have been found in a number of tumors, but not in testicular cancers. Although two groups of authors [21], [9] reported increased expression of *CDH1* in nonseminomas compared to seminomas, and Sonne *et al*. [23] observed increased expression of *CDH1* in both seminomas and nonseminomas from pateints with stage II/III disease, Heidenreich *et al*. [8] proposed a correlation between loss of E-cadherin and lymph node metastases in stage I nonseminomas. However, Honecker *et al*. [9] have found that neither vascular invasion nor the frequency of metastatic spread correlate with E-cadherin expression.

Interestingly, in our study one seminoma with *CDH1* allelic deletion also demonstrated allelic loss of *APC*'s exon 11. Furthermore, one nonseminoma with *p53* exon 4 allelic deletion demonstrated LOH of the *CDH1* gene (Table 3). Allelic losses of both *CDH1* and *p53* genes are more prominent in nonseminomas, which may contribute to their increased genomic instability and invasivness. All LOHs of the *p53*, except for one, were found in tumors with an embryonal carcinoma component, and all cases of

CDH1 LOHs in nonseminoma were found in tumors with a yolk sac tumor component. Both are highly invasive types of nonseminomas. The majority of *CDH1* LOHs were found in T2 and T3 stage tumors, which might be because it is either a late occurence in the tumorigenesis, or it has a role in the invasiveness of these tumors.

Although nm23 proteins are expressed in testis germinal cells, studies on mutations and expression of the *nm23-H1* and *nm23-H2* genes in TGCTs have failed to establish a significant role in the development of these tumors [22]. In accordance with these findings, we did not find aberrations of *nm23-H1* in TGCTs.

In the present study, analysis of allelic losses has shown no common structural genetic alterations in tumor tissues. Different pattern of LOH has been observed between two main histological groups of TGCTs, in agreement with the data published by Rothe *et al*. [20]. Occurrence of LOHs within two main groups of TGCTs turned out to be either 6–11% or 23-28% for examined gene markers. These LOH rates correspond to cytogenetically normal and abnormal chromosomes, respectively [18]. Although we have not performed cytogenetic analysis, our results suggest that different chromosomal aberrations are present in two main groups of TGCTs. Since we observed neither LOHs of *nm23-H1* nor *BRCA1* (unpublished results), both located on chromosome 17q, we believe the short arm of chromosome 17 is involved in cytogenetic changes of TGCTs.

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References

[1] A. Bahrami, J.Y. Ro, A.G. Ayala, An overview of testicular germ cell tumors. Arch Pathol Lab Med 131 (2007) 1267-1280.

[2] J.T. Bergthorsson, B.A. Agnarsson, T. Gudbjartsson, K. Magnusson, A. Thoroddsen, B. Palsson, J. Bjornsson, K. Stefansson, J. Gulcher, G.V. Einarsson, L.T. Amundadottir, R.B. Barkardottir, A genome-wide study of allelic imbalance in human testicular germ cell tumors using microsatellite markers. Cancer Genet Cytogenet 164 (2005) 1-9.

[3] A. Damalas, S. Kahan, M. Shtutman, A. Ben-Ze'ev, M. Oren, Deregulated β-catenin induces a p53- and ARF-dependent growth arrest and cooperates with Ras in transformation. EMBO J 20 (2001) 4912-4922.

[4] J.N. Eble, G. Sauter, J.I. Epstein, I.A. Sesterhenn (Eds.), Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs, In: P. Kleihues, L.H. Sobin (Eds.), World Health Organization Classification of Tumour. IARC Press, Lyon (2004).

[5] M. Engel, M. Seifert, B. Theisinger, U. Seyfert, C. Welter, Glyceraldehide-3-phosphate dehydrogenase and Nm23-H1/nucleoside diphosphate kinase A. Two old enzymes combine for the novel Nm23 protein phosphotransferase function. J Biol Chem 273 (1998) 20058-20065.

[6] M. Fleischhecker, T. Strohmeyer, Y. Imai, D.J. Slamon, H.P. Koeffler, Mutations of the p53 gene are not detectable in human testicular tumors. Mod Pathol 7 (1994) 435-439.

[7] L. Guillou, A. Estreicher, P. Chaubert, J. Hurlimann, A.-M. Kurt, G. Metthez, R. Iggo, A.C. Gray, P. Jichlinski, H.-J. Leisinger, J. Benhattar, Germ cell tumors of the testis overexpress wild-type p53. Am J Pathol 149 (1996) 1221-1228.

[8] A. Heidenreich, I.A. Sesterhenn, F.K. Mostofi, J.W. Moul, Prognostic risk factors that identify patients with clinical stage I nonseminomatous germ cell tumors at low risk and high risk for metastasis. Cancer 83 (1998) 1002-1011.

[9] F. Honecker, A.-M.F. Kersemaekers, M. Molier, P.C. van Weeren, H. Stoop, R.R. de Krijger, K.P. Wolffenbuttel, W. Oosterhuis, C. Bokemeyer, L.H.J. Looijenga, Involvement of E-cadherin and β-catenin in germ cell tumours and in normal male fetal germ cell development. J Pathol 204 (2004) 167-174.

[10] S. Honorio, A. Agathanggelou, N. Wernert, M. Rothe, E.R. Maher, F. Latif, Frequent epigenetic inactivation of the *RASSF1A* tumour suppressor gene in testicular tumours and distinct methylation profiles of seminoma and nonseminoma testicular germ cell tumours. Oncogene 22 (2003) 461-466.

[11] K. Hori, K. Uematsu, H. Yasoshima, K. Sakurai, A. Yamada, M. Ohya, Immunohictochemical analysis of the nm23 gene products in testicular seminoma. Pathol Int 47 (1997) 288-292.

[12] R. Hrašćan, N. Pećina-Šlaus, T. Nikuševa Martić, J. Franekić Čolić, K. Gall-Trošelj, K. Pavelić, N. Karapandža, Analysis of selected genes in neuroendocrine tumours: insulinomas and phaeochromocytomas. J Neuroendocrinol 20 (2008) 1015-1022.

[13] R.H. Jones, P.A. Vasey, New directions in testicular cancer: molecular determinants of oncogenesis and treatment success. Eur J Cancer 39 (2003) 147-156.

[14] N. Kato, H. Shibuya, M. Fukase, G. Tamura, T. Motoyama, Involment of adenomatous polyposis coli (*APC*) gene in testicular yolk sac tumor of infants. Human Pathol 37 (2006) 48-53.

[15] L.H.J. Looijenga, J.W. Oosterhuis, Pathogenesis of testicular germ cell tumours. Rev Reprod 4 (1999) 90-100.

[16] N. Pećina-Šlaus, Tumor suppressor gene E-cadherin and its role in normal and malignant cells. Cancer Cell Int 3 (2003) 17.

[17] M. Peifer, P. Polakis, Wnt signaling in oncogenesis and embriogenesis-a look outside the nucleus. Science 287 (2000) 1606-1609.

[18] H.-Q. Peng, D. Bailey, D. Bronson, P.E. Goss, D. Hogg, Loss of heterozygosity of tumor suppressor genes in testis cancer. Cancer Res 55 (1995) 2871-2875.

[19] A.G. Ridgeway, J. McMenamin, P. Leder, P53 levels determine outcome during β-catenin tumor initiation and metastasis in the mammary gland and male germ cells. Oncogene 25 (2006) 3518-3527.

[20] M. Rothe, P. Albers, N. Wernert, Loss of heterozygosity, differentiation, and clonality in microdissected male germ cell tumours. J Pathol 188 (1999) 389-394.

[21] T. Saito, A. Katagiri, R. Watanabe, T. Tanikawa, T. Kawasaki, Y. Tomita, K. Takahashi, Expression of E-cadherin and catenins on testis tumor. Urol Int 65 (2000) 140-143.

[22] B. Schmitd, R. Ackermann, M. Hartmann, T. Strohmeyer, Alterations of the metastasis suppressor gene nm23 and the proto-oncogene c-myc in human testicular germ cell tumors. J Urol 158 (1997) 2000-2005.

[23] S.B. Sonne, C.E. Hoei-Hansen, J.E. Nielsen, A.S. Herlihy, A.-M. Andersson, K. Almstrup, G. Daugaard, N.E. Skakkebaek, H. Leffers, E. Rajpert-De Meyts, CDH1 (E-cadherin) in testicular germ cell neoplasia: suppressed translation of mRNA in pre-

invasive carcinoma *in situ* but increased protein levels in advanced tumours. APMIS 114 (2006) 549-558.

Fig. 1. Loss of heterozygosity of the *APC* gene at exon 11. Silver-stained 15% polyacrylamide gel. Lane 1: ΦX174 DNA/*BsuRI* (Fermentas, Lithuania); lane 2: heterozygous normal testis tissue (48-, 85- and 133-bp fragments); lane 3: allelic loss in the corresponding testicular germ cell tumor (patient no. 15; 133-bp fragment).

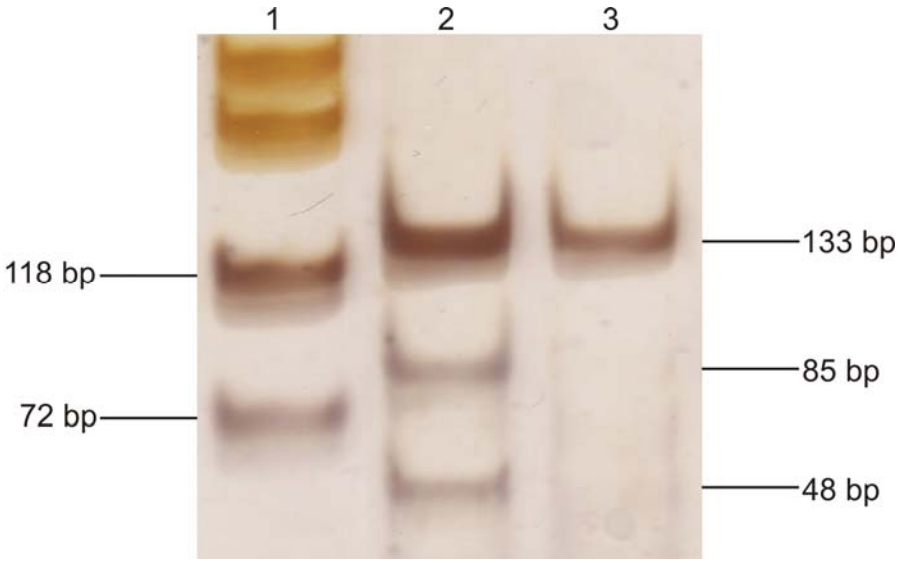

Fig. 2. Loss of heterozygosity of the *p53* gene at exon 4. Silver-stained 15% polyacrylamide gel. Lane 1: 50-bp DNA ladder (Fermentas, Lithuania); lane 2: heterozygous normal testis tissue (87-, 160- and 247-bp fragments); lane 3: allelic loss in the corresponding testicular germ cell tumor (patient no. 37; 87- and 160-bp fragments).

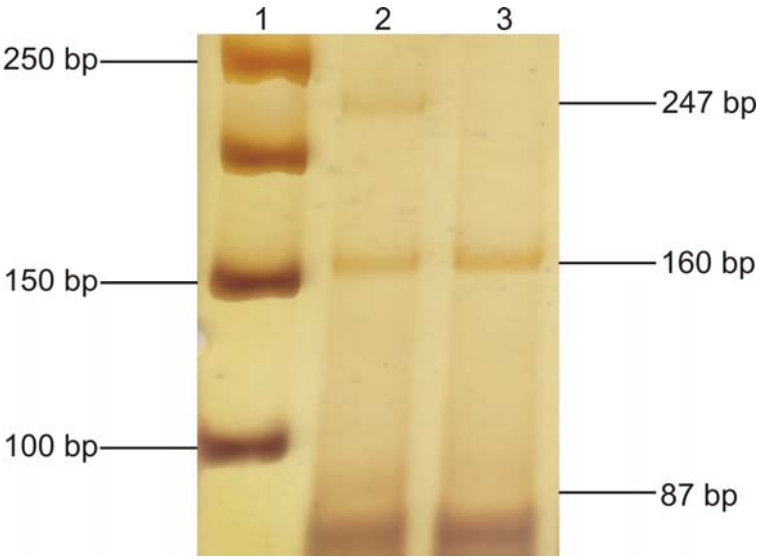

Fig. 3. Loss of heterozygosity of the *p53* gene at intron 6. Silver-stained 15% polyacrylamide gel. Lane 1: ΦX174 DNA/*BsuRI* (Fermentas, Lithuania); lane 2: heterozygous normal testis tissue (44-, 63- and 107-bp fragments); lane 3: allelic loss in the corresponding testicular germ cell tumor (patient no. 30; 44- and 63-bp fragments).

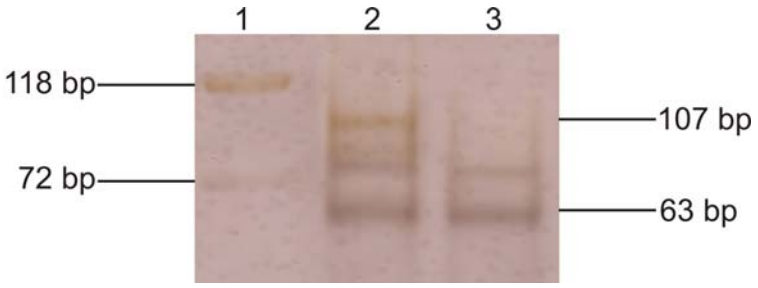

Fig. 4. Loss of heterozygosity of the *CDH1* gene at D16S752 polymorphic marker. Silver-stained 15% polyacrylamide gel. Lane 1: 50-bp DNA ladder (Fermentas, Lithuania); lane 2: heterozygous normal testis tissue; lane 3: allelic loss in the corresponding testicular germ cell tumor (patient no. 27).

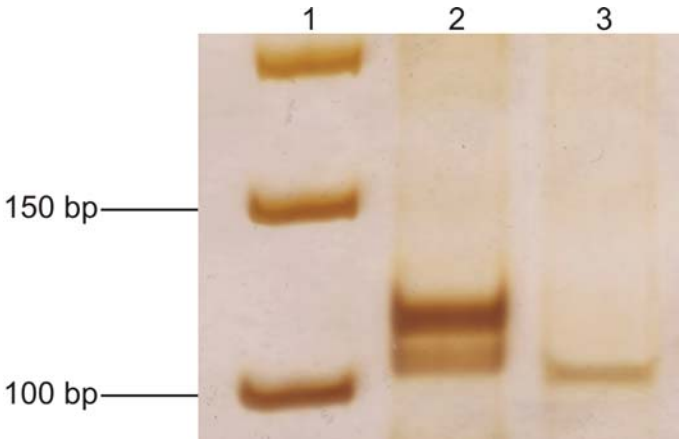

Table 1. Clinical and pathological data for 38 testicular germ cell tumor cases.

Patient no.	Age	pTNM	Histology
1	26	pT1NXMX	ITGCN, S
2	26	pT1NXMX	ITGCN, S
3	37	pT1NXMX	S
4	33	pT1NXMX	ITGCN, S
5	31	pT1NXMX	ITGCN, S
6	29	pT1NXMX	ITGCN, S
7	39	pT1NXMX	ITGCN, S
8	27	pT3NXMX	S
9	41	pT1NXMX	ITGCN, S
10	48	pT1NXMX	S
11	48	pT2NXMX	S
12	34	pT1NXMX	ITGCN, S
13	60	pT1NXMX	ITGCN, S
14	29	pT1NXMX	ITGCN, S
15	60	pT1NXMX	S
16	28	pT1NXMX	ITGCN, S
17	32	pT1NXMX	ITGCN, S
18	37	pT1NXMX	EC
19	18	pT2NXMX	EC, IT, MT, S
20	24	pT1NXMX	EC, ITGCN, S
21	37	pT1NXMX	EC, ITGCN, S
22	28	pT2NXMX	C, EC, IT, MT
23	17	pT2NXMX	EC, MT
24	34	pT2NXMX	EC
25	19	pT1NXMX	EC, ITGCN, MT, YST
26	39	pT1NXMX	MT, YST
27	21	pT2NXMX	EC, MT, YST
28	23	pT2NXMX	EC, IT, MT
29	22	pT1NXMX	MT, YST
30	25	pT3NXMX	EC
31	45	pT2NXMX	EC, ITGCN, S, YST
32	NK	pT2NXMX	C, EC, ITGCN, S, YST
33	23	pT2NXMX	EC, IT, ITGCN, MT, YST
34	39	pT1NXMX	EC, ITGCN, S, YST
35	24	pT2NXMX	EC, ITGCN, YST
36	30	pT1NXMX	EC, ITGCN, YST
37	36	pT1NXMX	EC, ITGCN, MT, YST
38	58	pT2NXMX	EC, ITGCN, YST

C-choriocarcinoma; EC-embryonal carcinoma; IT-immature teratoma; ITGCN-intratubular germ cell neoplasia; MT-mature teratoma; S-seminoma; YST-yolk sac tumor; NK-not known

Table 2. The sequences of pairs of primers used in the PCR reaction followed by LOH analysis of *CDH1*, *APC*, *p53* and *nm23-H1* genes.

Gene	Amplimer size (bp)	Tandem repeat	Restriction enzyme; Incubation temperature	Nucleotide sequence recognized by enzyme	Heterozygous alleles (bp)/ Homozygous alleles (bp)	Primer sequence (5'-3')
CDH1 D16S752	102-126	GATA				AATTGACGGTATATCTATCTGTCTG GATTGGAGGAGGGTGATTCT
nm23-H1 M2	110-120	CA				TATGAGTTCAACTACGCACG CTCGAGCACAGGAGCAGGTT
APC exon 11	133		*RsaI*; 37°C	5'-GT↓AC-3' 3'-CA↑TG-5'	48, 85, 133/ 48, 85; 133	GGACTACAGGCCATTGCAGAA GGCTACATCTCCAAAAGTCAA
p53 exon 4	247		*Bsh1236I*; 37°C	5'-CG↓CG-3' 3'-GC↑GC-5'	87, 160, 247/ 87, 160; 247	GATGCTGTCCGCGGACGATAT CGTGCAAGTCACAGACTTGGC
p53 intron 6	107		*MspI*; 37°C	5'-C↓CGG-3' 3'-GGC↑C-5'	44, 63, 107/ 44, 63; 107	AGGTCTGGTTTGCAACTGGG GAGGTCAAATAAGCAGCAGG

Table 3. Loss of heterozygosity (LOH) of *APC*, *p53* and *CDH1* genes in testicular germ cell tumors.

Patient no., tumor group	*APC* exon 11	*p53* exon 4	*p53* intron 6	*CDH1* D16S752
1, S	LOH	NI	I	I
8, S	LOH	I	I	LOH
12, S	I	NI	LOH	NI
15, S	LOH	I	I	NI
18, NS	NI	LOH	I	I
19, NS	I	LOH	NI	NI
23, NS	NI	LOH	I	I
27, NS	I	LOH	NI	LOH
28, NS	LOH	I	NI	NI
29, NS	I	I	NI	LOH
30, NS	I	NI	LOH	I
32, NS	I	I	LOH	I
35, NS	I	I	NI	LOH
37, NS	I	LOH	I	NI

S, seminoma; NS, nonseminoma; I, informative (heterozygous); NI, not informative (homozygous)

Table 4. The percentage of LOH of *CDH1*, *APC*, *p53* and *nm23-H1* tumor suppressor genes in testicular germ cell tumors.

Tumor	*CDH1* D16S752	*APC* exon 11	*p53* exon 4	*p53* intron 6	*nm23-H1* M2
Seminoma, Σ 17	11% (1/9)	23% (3/13)	0% (0/12)	8% (1/12)	0% (0/15)
Nonseminoma, Σ 21	27% (3/11)	6% (1/16)	28% (5/18)	25% (2/8)	0% (0/19)

Numbers in parentheses: the number of tumors demonstrating LOH over the number of informative tumors