Pathology – Research and Practice 206 (2010) 163–167

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.de/prp

Original Article

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

Tomislav Vladušić [a,*,1], Reno Hrašćan [a,1], Ivana Vrhovac [a], Božo Krušlin [b], Marija Gamulin [c], Mislav Grgić [c], Nives Pećina-Šlaus [d,e], Jasna Franekić Čolić [a]

[a] Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia
[b] Ljudevit Jurak Department of Pathology, Sisters of Mercy University Hospital, Zagreb, Croatia
[c] Department of Oncology, University Hospital Center, Zagreb, Croatia
[d] Laboratory of Neurooncology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia
[e] Department of Biology, School of Medicine, University of Zagreb, Zagreb, Croatia

ARTICLE INFO

Article history:
Received 6 July 2009
Received in revised form
5 October 2009
Accepted 22 October 2009

Keywords:
Loss of heterozygosity
Tumor suppressor genes
Testicular germ cell tumors

ABSTRACT

Human testicular germ cell tumors (TGCTs) are histologically heterogenous neoplasms with a 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 *nm*23-*H1* genes were tested for loss of heterozygosity (LOH). Thirty-eight testicular germ cell tumors (17 seminomas and 21 nonseminomas) were analyzed by PCR using restriction fragment length polymorphism or the 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 occurred 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 showed no common structural genetic alterations in tumor tissues, although a different pattern of LOH was 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 allelic 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; therefore, major TGCT suppressor genes are yet to be identified [10]. At present, 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.

* Corresponding author at: Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia.
E-mail address: tvladusic@pbf.hr (T. Vladušić).
[1] These two authors contributed to this work equally.

0344-0338/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.prp.2009.10.007

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, 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 may 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. Compared to normal tissues with a reduced expression, nm23-H1 is overexpressed in solid tumors, which correlates 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.

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.

Materials and methods

Patients and tumor material

Thirty-eight 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. Table 1 shows the clinical and pathological data for 38 TGCTs according to the WHO 2004 classification.

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 hematoxylin–eosin to identify the tumor and normal tissue areas 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 the 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 $MgCl_2$, 1 × 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/96 °C; denaturation, 30 s/96 °C; annealing, 30 s/58 °C; extension, 30+1 s/72 °C; final extension, 10 min/72 °C. PCR aliquots (10–20 μl) were digested with 5 U *RsaI* (Fermentas, Lithuania) for 12 h. A *Bsh1236I* restriction site in exon 4 and an *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.