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ORIGINAL ARTICLE

Testicular persistence of Parvovirus B19: Evidence for preferential infection of germ cell tumors

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Abstract

Human Parvovirus B19 has previously been implicated in the pathogenesis of testicular germ cell tumors, but this could not have been confirmed. This study was designed to investigate the testicular persistence of Parvovirus B19 and possible associations with germ cell tumors. Paraffin-embedded or fresh tissues from 36 germ cell tumors, 20 germ cell aplasias, 26 normal testicular tissues, 20 liver tissues, and 20 spleen tissues were evaluated by two different molecular assays: a nested PCR for Parvovirus B19 capsid genes and a commercial quantitative real-time PCR. Positive results were further confirmed by another commercial real-time PCR assay. Viral DNA was detected in 3 of 36 (8.3%) germ cell tumors, but not in other groups. Viral loads observed in all positive samples were less than 20 IU/reaction, suggesting very low levels of viral replication or latency. These results either directly or indirectly imply the involvement of Parvovirus B19 with testicular germ cell tumors. Viral persistence in normal testis, germ cell aplasia tissues, or hepatic/splenic tissues was not observed in this study.

Keywords: Parvovirus B19; Testicular tumors; Germ cell tumors; Viral persistence

Introduction

Human Parvovirus B19, an un-enveloped single-strand DNA virus, is a widespread human pathogen associated with a variety of clinical syndromes [12]. Although the majority of the Parvovirus B19 infections are asymptomatic, Erythema Infectiosum (Fifth Disease) and arthropathy/arthralgia are the most commonly observed manifestations in children and adults, respectively [3,12]. Most Parvovirus B19 infections are acute and self-limiting unless certain underlying factors, such as immunosuppression, that permit continuous viral replication are present [12]. Persistent viral infections have recently been demonstrated in patients without any predisposing conditions, and viral DNA has been detected in various tissues by nucleic acid-based tests [4,16]. The importance and clinical relevance of these findings still need to be determined.

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The presence of Parvovirus B19 DNA has also been observed in testicular tissues of patients with testicular germ cell tumors [11]. Although early results suggest an etiologic role, the association of Parvovirus B19 with testicular carcinogenesis could not be confirmed in the following studies [7,17]. This study is designed to detect the presence of Parvovirus B19 DNA in testicular germ cell tumors, germ cell aplasias, and normal testicular tissues using quantitative real-time PCR and nested PCR to reveal tissue persistence of the virus and possible associations with testicular germ cell tumors.

Materials and methods

Samples

Formalin-fixed, paraffin-embedded tissues from 33 germ cell tumors, 20 germ cell aplasias (Sertoli-cell only syndrome), and 2 normal testicular tissues, along with fresh, unfixed tissues from 3 germ cell tumors and 4 normal testes, were obtained from Hacettepe University, Faculty of Medicine, Department of Pathology. Fresh unfixed testicular, liver, and spleen tissues from 20 autopsies were obtained from National Institute of Forensic Medicine. Informed consent and approval of the relevant ethics committees were given previously. No testicular trauma, tortion, or previous operation was identified in autopsy cases. All samples were re-evaluated by an expert pathologist after hematoxylin/eosin staining to confirm diagnosis and histologic properties. For testicular tumors, tissues devoid of any visible vascular structure and/or necrosis were obtained after microscopic dissection. Type and distribution of tissues included in the study are given in Table 1.

Table 1. Histological types and distribution of tissue samples evaluated in the study

Germ cell tumors	
Seminoma	10
Embryonal carcinoma	5
Yolk sac tumor	2
Teratoma	3
Choriocarcinoma	1
Mixed germ cell tumor	15
Total	36
Germ cell aplasias	
Total	20
Normal tissues	
Testis	26
Liver	20
Spleen	20
Total	66

Mean age of the study groups was 30.54 years (median: 30, standard deviation: $\pm$6.49) for germ cell tumors, 30.95 years (median: 31, standard deviation: $\pm$6.63) for germ cell aplasias, and 30.34 years (median: 30, standard deviation: $\pm$6.65) for controls. Serological data on previous exposure to Parvovirus B19 were not available.

Nucleic acid purification

Sections (50 μm) obtained from paraffin-embedded tissues and 1–2 mm^3 samples obtained from fresh tissues were used for nucleic acid purification. All tissues were deparaffinized and/or extracted by the phenol-chloroform method using standard protocols as described previously [5]. Microtome blades were cleaned or changed after each paraffin-embedded sample processing. Internal control DNAs were added to each sample before nucleic acid extraction according to the manufacturer's instructions (see commercial quantitative real-time PCR assays). All purified nucleic acids were stored at –80 °C until amplification.

Detection of Parvovirus B19 DNA

In-house nested PCR assay

All samples were subjected to amplification by an in-house nested PCR assay targeting VP1/VP2 region of the viral genome as described previously [15,18]. Ten microliters of the purified nucleic acids were used in a 50 μl PCR mix. Oligonucleotide primers used in the first round were 5′-CTT TAG GTA TAG CCA ACT GG-3′ (sense primer, nucleotides 2905-2931) and 5′-ACA CTG AGT TTA CTA GTG GG-3′ (anti-sense primer, nucleotides 4016-3997), yielding a 1112 base pair product. A second round PCR was performed using primers 5′-CAA AAG CAT GTG GAG TGA GG-3′ (sense primer, nucleotides 3187-3206) and 5′-CCT TAT AAT GGT GCT CTG GG-3′ (anti-sense primer, nucleotides 3290-3271) for a product of 104 base pairs. Preparation of PCR mix and thermocycling conditions were performed as described previously [18]. Sample suitability for PCR was confirmed by β-globin sequence amplification as described elsewhere [19]. All PCR reactions were performed on a MJ Research PTC-200 Peltier Thermal Cycler (MJ Research Inc., USA). PCR products were subjected to electrophoresis in 1.5–2% agarose gels, and expected amplicons were observed under ultraviolet light after staining with ethidium bromide. Analytical sensitivity of the assay, investigated using serial dilutions of a positive serum sample with known viral load, was determined as 10 IU/reaction.