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UROLOGIC ONCOLOGY

Urologic Oncology: Seminars and Original Investigations 32 (2014) 727–734

Original article

Expression of human chorionic gonadotropin in testicular germ cell tumors

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Received 20 September 2013; received in revised form 11 November 2013; accepted 14 November 2013

Abstract

Background: We have shown that most patients with seminomas have elevated serum concentrations of the free β subunit of human chorionic gonadotropin (hCGβ) and that in nonseminomatous testicular cancer, most of the hCG in the serum is hyperglycosylated (hCG-h). However, the tissue expression of hCG-h or hCGβ in germ cell tumors (GCTs) has not been reported. Our objective was to study the expression and diagnostic value of hCG-h and hCGβ in testicular GCTs.

Methods: We studied the immunohistochemical expression of hCG, hCG-h, hCGβ, and the free α subunit of hCG (hCGα) in GCTs from 154 patients. We compared the tissue expression with serum concentrations and evaluated the correlation between staining intensity, established prognostic variables, and outcome.

Results: The expression varied between tumor types. All forms of hCG, including hCG-h, were detected in embryonal carcinomas (22%) and mixed GCTs (48%). Polyclonal hCG and monoclonal hCGβ antibodies detected immunoreactivity in some seminomas (7%). No form of hCG was found in spermatocytic seminomas, pure teratomas, or a yolk sac tumor. The serum concentrations correlated with the corresponding tumor expression. The staining intensities of hCG, hCGβ, hCG-h, and hCGα correlated with disease stage but not significantly with relapse, disease-related mortality, or progression-free survival.

Conclusion: Trophoblastic tissue expresses hCG, hCG-h, and free subunits together whereas seminoma tissue occasionally expresses hCGβ. This difference might aid in differential diagnosis of some difficult-to-classify cases. © 2014 Elsevier Inc. All rights reserved.

Keywords: hCG; Hyperglycosylated hCG; Glycosylation; Free β subunit of hCG; Testicular cancer; Germ cell tumor

1. Introduction

In men aged 15 to 35 years, testicular cancer is the most common malignant tumor. Approximately 95% of these are germ cell tumors (GCTs). For clinical purposes, GCTs are divided into seminomas and nonseminomatous GCTs (NSGCTs) because their management differs. Nearly half of the tumors are seminomatous and the other half NSGCTs [1]. The latter are further classified as embryonal carcinomas, choriocarcinomas, yolk sac tumors, and mature or immature teratomas. Most NSGCTs are mixed, i.e., they contain several histological components.

Testicular cancer is highly sensitive to platinum-based chemotherapy, and the overall cure rate is more than 95%. However, chemotherapy may have serious long-term side effects and therefore treatment of low-stage disease with good prognosis is often limited to surgery [2]. Reliable prognostic markers are needed to aid in the identification of high-risk patients requiring first-line chemotherapy.

Human chorionic gonadotropin (hCG) is an established marker for testicular cancer. hCG is a heterodimeric glycoprotein hormone composed of an α subunit (hCGα) and a β subunit (hCGβ). The α subunit is common to the other glycoprotein hormones, whereas the β subunit is

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1078-1439/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.urolonc.2013.11.007

specific for hCG and conveys the biological function. hCG is produced by trophoblasts, and tissue expression of hCG is usually found in tumors containing trophoblastic components and in some seminomas [3–6].

hCG is extensively glycosylated, approximately 30% of its molecular weight consists of carbohydrates. In early pregnancy, most of hCG is hyperglycosylated (hCG-h), i.e., it contains more complex carbohydrate chains than later in pregnancy [7]. hCG-h is also produced by trophoblastic tumors [8–12] and has been suggested to be a marker of aggressive disease and a key to invasion [9,13]. Many nontrophoblastic cancers express hCGβ, and this is associated with adverse prognosis [14–16]. In the normal testis, peritubular cells, presumably Leydig cells, express hCGβ [17]. Both forms, hCG-h and hCGβ, stimulate invasion of trophoblast cells independent of the classical luteinizing hormone/hCG receptor [18]. hCG-h also stimulates angiogenesis and this effect is mediated by the transforming growth factor-β receptor activation [19].

We have previously shown that most (57%) patients with seminomas have elevated serum concentrations of hCGβ, whereas hCG is elevated in only 15%, indicating that most of the hCG immunoreactivity expressed by seminomas consists of hCGβ [20]. We have also shown that the immunoreactive hCG in the serum of patients with NSGCTs consists of both hCG and hCGβ and that most of the hCG in the serum is hyperglycosylated [21]. To our knowledge, there are no studies on the expression of hCGβ or hCG-h in GCT tissue. Our aim was to study the expression of hCG, hCG-h, hCGβ, and hCGα in various testicular GCTs by immunohistochemistry and to evaluate their value as prognostic markers.

2. Material and methods

2.1. Patients and samples

We analyzed histological samples from 164 patients with testicular cancer treated between 1990 and 2003 at Helsinki University Central Hospital. Preoperative serum samples were available from 125 patients. Clinical characteristics (Table 1) were retrieved from patient charts in 2006 and reevaluated by A.L. and C.B. so as to unify staging based on the International Union Against Cancer classification [22]. GCTs with an extratesticular primary site were not staged ($n = 1$). Histological diagnosis was reevaluated by an experienced uropathologist (A.S.) based on hematoxylin-eosin (HE) staining and immunohistochemical profile (PLAP, OCT-3/4, D2-40, α-fetoprotein [AFP], CD30, and c-KIT). Routine follow-up consisted of radiographic imaging, serum tumor marker determinations (AFP, hCG, and hCGβ), and clinical examination.

The local ethics committee approved the study. Exemption for obtaining informed consent was provided by the National Supervisory Authority for Welfare and Health.

2.2. Antibodies

The primary antibody for hCG (IR508/IS508, DakoCytomation) is a polyclonal antibody (PAb) against the β chain of hCG, and thus, it detects both intact hCG and hCGβ. A monoclonal antibody (MAb) against hCG-h (B152) recognizes the core-2 O-glycan on Ser-132 and surrounding peptide structures on the carboxy-terminal peptide of hCG-h and hCGβ-h [11,23]. We used in-house MAbs specific for different forms of hCG. The epitopes of these have been characterized by comparison of their reactivity with reference antibodies with known specificity [24]. MAb 9C11 detects a β chain epitope and MAb 7E10 an α chain epitope hidden on intact hCG. MAb11C5 detects an epitope in the α-β junction, which is present only on intact hCG.

2.3. Immunohistochemistry

Tissue samples obtained at orchiectomy were fixed in 10% buffered formalin, processed, embedded in paraffin, and stored at the Department of Pathology of Helsinki University Central Hospital Laboratory Division. A.S. reviewed HE-stained slides of each specimen to verify the presence of tumor and to select the most representative areas for tissue microarray (TMA) construction. A total of 4 cores, measuring 1 mm each, of malignant tissue from each tumor representing different tissues types, if present, were sampled using a manual tissue arrayer (MTA-1, Beecher Instruments) and placed coordinately into 12 TMA blocks. Tissue cores from early pregnancy placenta were incorporated in each TMA block and served as positive controls. Additionally, separate samples from early placenta were used as controls in each staining series. After arraying, 4-μm sections were prepared and a single section was stained using HE to verify the presence of tumor tissue.

Immunostaining of hCG, hCGβ, and hCG-h was performed using an automated immunostainer (Lab Vision, Thermo Fisher) and visualized with a polymer-based detection system (EnVision, K5007, DakoCytomation) using diaminobenzidine as chromogen. Tissue sections were pretreated with tris-EDTA buffer (pH 9.0) and heated at 98°C for 20 minutes before staining for hCG and hCG-h. Before assay of hCGβ, the sections were treated with 0.5% trypsin (Difco Trypsin 250, Becton Dickinson) solution for 30 minutes at +37°C. Immunostaining of hCGα and intact hCG was performed manually using a polymer-conjugated alkaline phosphatase kit (MACH4 Universal AP Polymer KIT, Biocare Medical) using fuchsin as chromogen. Tissue sections were pretreated with citrate buffer (pH 6.0) and heated in a microwave oven at 1,050 W for 2 minutes before assay of intact hCG. Negative controls were stained by replacing the primary antibody with mouse IgG (10 μg/ml, Vector Laboratories). Details on antigen retrieval methods, primary antibodies, and reaction times are given in Table 2.

Staining intensity was interpreted by 2 reviewers (A.S. and A.L.) blinded to the clinical data. The intensity of

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