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Genomic profile in gestational and non-gestational choriocarcinomas

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ABSTRACT

Introduction: Gestational (GC) (derived from the placenta) and non-gestational (NGC) choriocarcinomas are trophoblastic diseases originated from abnormal proliferation of trophoblastic cells. These rare tumors share similar morphology and pathological features and differ on chemotherapy response, genetic origin and prognosis. In this study, the genomic profile of choriocarcinomas was performed according to their origin (GC or NGC) aiming to better understand these poorly characterized diseases.
Methods: Thirteen patients were included in this study; 10 presented previous history of hydatidiform mole and six developed metastasis. Twelve polymorphic microsatellite markers (D15S659, APOC2, D5S816, BAT25, D3S1614, D3S1311, D1S1656, APC-D5S346, D3S1601, D18S70, D8S1110 and D11S1999) were investigated to distinguish GC from NGC. All choriocarcinomas were evaluated by copy number alterations using array CGH.
Results: Eight cases were classified as GC and five as NGC. Although potentially polymorphic, NGC exhibited significant gain of 21p11. Rare copy number alterations (CNA) were detected as a frequent event in GC including gains of 1p36.33-p36.32 (3 cases), 17q25.3 (4 cases), and losses of 9q33.1 (5 cases), 17q21.3 (3 cases) and 18q22.1 (4 cases) (varying from 724 to 3,053 Kb).
Discussion: Two tumor suppressor genes are candidates to be involved in GC: *TRIM32* (9q33.1) and *CDH19* (18q22.1). Gains of *CBX2*, *CBX4* and *CBX8* were frequently found in high risk prognostic score in GC. The *in silico* functional interaction analysis revealed the involvement of PTEN and PI3K-Akt signaling pathways. These data pointed out significant genomic alterations in GC, opening new avenues to better characterize the pathobiology of this disease.

1. Introduction

Trophoblastic diseases originate from abnormal proliferation of trophoblastic cells and encompass different lesions including the choriocarcinoma, which is classified in gestational (GC) and non-gestational (NGC) [1]. Gestational choriocarcinoma usually affects women at reproductive age and is frequently derived from pregnancies, molar pregnancy, induced and spontaneous abortion, ectopic pregnancy and term or pre-term deliveries [2]. The incidence of GC has been estimated in 1:40.000–50.000 pregnancies, and 1:40 hydatidiform mole cases. The incidence of NGC is unknown, being reported as a very rare tumor [2,3]. GC is characterized by the presence of paternal genetic material. These conditions are highly invasive, vascularized and metastatic [4]. Non-gestational choriocarcinoma is independent of pregnancies and less responsive to chemotherapy than gestational choriocarcinoma [2].

The pathogenesis of these rare trophoblastic lesions is poorly

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understood. Although differences have been reported in the chemotherapy response, genetic origin and prognosis, GC and NGC exhibit similar morphological pattern, histopathological classification and biochemical markers [2,5,6]. In patients with NGC, surgical tumor resection and treatment with multiple chemotherapy agents are indicated. Even with strategies of multidrug therapy, a large number (~84%) of patients with NGC died from the disease [6]. Therefore, the difference in therapeutic response and prognosis is clinically relevant and highlight the importance of distinguishing these two entities [2].

Polymorphic microsatellite markers have been used to identify the parental origin of these trophoblastic diseases contributing to therapeutic decision and management of the patients [6–8]. Microsatellites are highly repeated DNA sequences distributed throughout the genome, composed by short sequences of DNA repeated in tandem [9]. Due to the high level of heterozygosis, these markers can differentiate parental alleles with high precision, being considered an efficient molecular strategy to distinguish GC from NGC [7,8,10].

Array Comparative Genomic Hybridization (aCGH) is a screening procedure of genomic imbalances that have the potential to identify recurrent copy-number alterations and its potential as prognostic biomarkers and molecular targets for therapy [11]. To our knowledge, only one study reported genomic alterations in 10 primary GC by aCGH [12]. The authors reported normal genomic profiles in six cases; four of them showed copy number alterations on chromosomes 1, 11, 14, 17, 18, 19, 20 and X.

In this study, we used aCGH to characterize and compare the genomic profile of gestational and non-gestational choriocarcinomas, aiming to identify potential molecular markers useful for clinical practice.

2. Methods

2.1. Patients

Thirteen primary choriocarcinoma samples were enrolled between 1995 and 2009 at the Department of Gynecology and Obstetrics, Botucatu Medical School, UNESP - São Paulo State University, Botucatu, SP, Brazil. The Institutional Human Research Ethics Committee approved this study (Protocol #3543–2010). All patients were advised of the procedures and provided written consent. Five mL of peripheral blood samples were collected from 13 patients and her partner/husband (available in 10 cases).

2.2. Clinical and histopathological characterization

The clinical data, demographic information, pregnancy history, clinical presentation, management and response to therapy were collected for each patient (Table 1). The prognostic score was based on the International Federation of Gynecology and Obstetrics (FIGO) [13].

Histopathological analysis confirmed epithelial neoplastic biphasic cells composed by mononuclear atypical cells with scant, clear to granular cytoplasm (cytotrophoblast), and multinucleated cells with pleomorphic nucleus (syncytiotrophoblast) (Fig. 1). Extensive area of necrosis and the presence of hemorrhage were frequently observed. Tumor cells were positively stained for AE1/AE3 (Dako, Glostrup, DK), hCG (Dako, Glostrup, DK), hPL (Dako, Glostrup, DK) and negatively for PLAP (Dako, Glostrup, DK) (detailed information are available in Supplementary Table 1). Cell proliferation rate was evaluated by Ki67 (Dako, Glostrup, DK) immunostaining, varying from 40 to 95% of nuclei positivity (Table 1).

2.3. Samples and DNA extraction

Histological slides stained with hematoxylin-eosin containing the tumor samples were used as reference to guide the tumor microdissection. Non-tumor cells, necrotic cells and hemorrhagic areas were excluded. The tumor tissues were obtained from 10 to 15 unstained slides (6–10 μ) per case. Genomic DNA from six fresh frozen tumor samples and seven formalin fixed paraffin embedded (FFPE) tumor samples were isolated using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, USA), according to the manufacturer's instructions. Genomic DNA was isolated from peripheral blood leukocytes using a standard phenol-chloroform (Invitrogen, Life Technologies, CA, USA) extraction and ethanol precipitation procedure.

2.4. Microsatellite analysis

A panel of 12 highly polymorphic microsatellite markers (D1S1656, D3S1311, D3S1601, D3S1614, D5S346, D5S816, D8S1110, D11S1999, D15S659, D18S70, APOC2 and BAT25) was selected to evaluate the parental origin of the cases. Supplemental Methods summarizes the PCR conditions.

The fluorescent PCR products were analyzed using ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, USA). Each fluorescent peak was quantified by its size (in base pairs), peak height, and peak area as previously reported [14]. The results were analyzed by GeneMapper 4.1 software (Applied Biosystems, Foster City, USA).

2.5. Array comparative genomic hybridization and data analysis

Genomic DNA from 13 choriocarcinomas and a male commercial genomic DNA (Promega, Madison, USA) were hybridized on Agilent Human 4 × 44 K CGH Microarray (Agilent Technologies, Santa Clara, USA) according to the manufacturer's instructions. Supplemental Methods describes in details the data extraction and the statistical analysis. The genomic alterations obtained in our study were compared with the Database of Genomic Variants (DGV, http://dgv.tcag.ca/dgv/app/home, updated in July 2015). For this comparison, the same type of alteration (gains or losses), same size or overlapping for >80% were considered. Rare copy number alterations (observed in less than 10 individuals from the DGV database) detected in at least 50% of samples were evaluated in more details. The CNAs were also compared with those described in normal placenta [15]. Sexual chromosomes were excluded from the analysis. Chi Square, Mann-Whitney and Fisher exact test P value ≤ 0.05, (SPSS 18.0, Chicago, USA) was applied to compare the clinical data and genomic alterations.

2.6. In silico analysis

The molecular processes, functions and networks were evaluated by the analyses of genes mapped in regions affected by genomic imbalances in gestational choriocarcinoma samples using the Ingenuity Pathways Analysis (IPA) (Ingenuity Systems, http://www.ingenuity.com) and KOBAS software (v. 2.0; http://kobas.cbi.pku.edu.cn/home.do). Protein-protein interaction (PPI) networks were annotated, visualized and analyzed using NAViGaTOR v2.03 (http://ophid.utoronto.ca/navigator/).

3. Results

3.1. Clinical data

Ten of 13 presented the tumor located in the uterus (10 cases)