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The genetic landscape of 87 ovarian germ cell tumors

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HIGHLIGHTS

- Ovarian germ cell tumors represent a group of histologically different phenotypes that affect children and young adults.
- They are characterized by a low mutation rate and very few recurrent somatic mutations.
- 12p gain is the most frequent copy number aberration, except in immature teratomas.
- PI3K/AKT/PTEN pathway seems enriched in yolk sac tumors.

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ABSTRACT

Background. Ovarian germ cell tumors (OGCT) are rare gynecological neoplasms, mostly affecting children and young women. The underlying molecular genetic background of these tumors is poorly characterized.

Methods. We analyzed somatic copy number aberration (CNA) profiles in 87 OGCT tumors and performed whole exome sequencing (WES) on 24 OGCT tumor and matched germline samples to further elucidate their molecular genetic landscape.

Results. The overall mutation rate was very low in OGCT compared to other human cancers, with an average of 0.05 mutations per Mb, consistent with their embryological origin. We identified recurrent mutations in *KIT* and *KRAS*, while CNA profiling revealed frequent focal amplifications affecting *PIK3CA* and *AKT1* in yolk sac

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Copy number
Mutation rate
KIT
PI3K-AKT-PTEN pathway

tumors, recurrent focal deletions affecting chromosomal regions 1p36.32, 2q11.1, 4q28.1, 5p15.33, 5q11.1 and 6q27, as well as gains in chromosome 12p that were present in all tumors, except for pure immature teratomas.

Conclusion. We here present the first whole exome sequencing data and to our knowledge the largest CNA study in OGCT. We confirmed that earlier reported *KIT* mutations were frequent in dysgerminomas and mixed forms with a dysgerminoma component, whereas chromosome 12p gains were present in all histological subtypes except pure immature teratomas. We detected recurrent *KRAS* mutations, recurrent focal deletions and an enrichment in the *PI3K/AKT/PTEN* pathway in yolk sac tumors. Several of these aberrations involve targetable pathways, offering novel treatment modalities for OGCT.

1. Introduction

Malignant OGCTs are rare gynecological neoplasms, accounting for an estimated 3% of all ovarian cancers [1]. They consist of several histological different tumor types, all derived from primordial germ cells (PGCs): yolk sac tumor (YST), immature teratoma (IT), embryonal carcinoma (EC), polyembryoma, non-gestational choriocarcinoma (CC) and dysgerminoma (DG), the latter being the most frequent subtype, accounting for 30–40% of all malignant OGCTs [2]. They usually affect children and young women with the median age depending on the specific subtype and cure rates are high due to their high chemosensitivity, mainly for platinum salts. In contrast, there are limited therapeutic options for platinum-resistant disease and the outcome is detrimental [2,3].

The pathogenesis of malignant OGCT remains poorly understood. Much more is known about their male counterpart, i.e. testicular germ cell tumors (TGCTs). There are similarities in both histological and immunophenotypical features between both diseases, certainly between seminomas and dysgerminomas [4]. Mutations in *KIT* have been observed in dysgerminomas, gonadoblastomas and yolk sac tumors, most frequently in exon 17 codon 816, leading to an increased survival and proliferation of undifferentiated oogonia [4].

Germ cell tumors, ovarian and testicular, are markedly aneuploid. This is consistent with the hypothesis of GCTs emerging due to an abnormal segregation of chromosomes through a dysregulation of the mitosis/meiosis switch [5,6]. TGCTs are further characterized by low mutation rates [6–8], activating KIT mutations limited to seminomas [7,8] and recurrent KRAS mutations [6,7]. Genome-wide association studies have reported 59 germline susceptibility loci in TGCTs, most notably in KITLG, BAK1 and TERT [9].

Mutations in *KIT* have not been reported in immature teratomas and are most commonly seen in unilateral dysgerminomas, suggesting that mutations in *KIT* occur after migration to the gonadal ridges [4].

Ichikawa et al. found a *KRAS* mutation in an immature teratoma profiled in a series of 6 malignant OGCTs (2 dysgerminomas, 4 immature teratomas) [10]. Dysgerminomas, such as seminomas, have an increase in chromosome 12p abnormalities (gain of 12p, isochrome 12p) [4,11]. In a series of 25 OGCTs, Kraggerud et al. also demonstrated gain of genetic material in chromosome 1p, 6p, 12q, 15q, 20q, 21q and 22q and whole chromosome gains of chromosome 7, 8, 17 and 19. Loss of genetic material is less frequently observed, although loss of chromosome 13q has already been reported [12].

In summary, there is a paucity of information about the molecular genetic make-up of OGCTs. Here we report whole exome sequencing on 24 OGCTs and whole genome CNA on 87 tumors to detect possible driver genes and provide novel insights into tumor biology.

2. Material and methods

2.1. Patients and tumors

Samples were collected from patients treated at the University Hospital of Leuven (Belgium), Vall d'Hebron Institute of Oncology (Barcelona, Spain), Maria Sklodowska-Curie Memorial Cancer Centre, Institute of oncology (Warsaw, Poland), Haukeland University Hospital (Bergen, Norway), Medical University of Vienna (Austria), Alexandra Hospital (Athens, Greece), Rigshospitalet (Copenhagen, Denmark), Medical University of Innsbruck (Austria), Kliniken Essen-Mitte (Essen, Germany), Universitäts-Klinikum Hamburg Eppendorf (Germany), Charite Hospital Berlin (Germany) and General University Hospital Prague (Czech Republic). Written informed consent was obtained from all patients by each center and the study was approved by the local ethics committee of the University of Leuven (s55308).

We included 24 patients with ovarian germ cell tumors for the whole-exome sequencing. Histology and patient characteristics are listed in Table 1. All biopsies were obtained from primary surgery. They were either snap frozen at time of surgery or archival FPPE blocks. Germline DNA from the 24 patients in the WES experiment was obtained from a whole-blood sample. A further 63 tumor samples, either fresh frozen or FFPE blocks were included for copy number variation profiling (Table 1). Staging and grading were performed according to International Federation of Gynecology and Obstetrics (FIGO) 2014 standards. All tumor samples were revised by a pathologist, expert in the field of gynecological tumors (PM).

2.2. DNA isolation

Fresh frozen tumor sections were obtained by cryosectioning biopsies at 10 μm. Three to five 20 μm-thick sections were prepared from

Table 1
Patients' characteristics in the exome (WES) and copy number (CNA) experiments.

Characteristics	WES ($n = 24$)	CNA ($n = 87$)
Histology		
Dysgerminoma	6 (25%)	28 (32%)
Yolk sac tumor	4 (17%)	19 (22%)
Immature teratoma	5 (21%)	15 (17%)
Mixed	8 (33%)	23 (26%)
Embryonal carcinoma	1 (4%)	1 (1%)
Gonadoblastoma	–	1 (1%)
FIGO stage		
I	12 (50%)	40 (46%)
II	1 (4%)	9 (10%)
III	9 (38%)	22 (25%)
IV	–	1 (1%)
Unknown	2 (8%)	15 (17%)
Age		
<18 years	6 (25%)	17 (20%)
>18 years	17 (71%)	56 (64%)
Unknown	1 (4%)	14 (16%)
Recurrence		
No	17 (71%)	55 (63%)
Yes	4 (17%)	10 (12%)
Unknown	3 (12%)	22 (25%)
Death of disease		
No	21 (88%)	80 (92%)
Yes	2 (8%)	6 (7%)
Unknown	1 (4%)	1 (1%)

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