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Research Paper

Premalignant SOX2 overexpression in the fallopian tubes of ovarian cancer patients: Discovery and validation studies

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ABSTRACT

Current screening methods for ovarian cancer can only detect advanced disease. Earlier detection has proved difficult because the molecular precursors involved in the natural history of the disease are unknown. To identify early driver mutations in ovarian cancer cells, we used dense whole genome sequencing of micrometastases and microscopic residual disease collected at three time points over three years from a single patient during treatment for high-grade serous ovarian cancer (HGSOC). The functional and clinical significance of the identified mutations was examined using a combination of population-based whole genome sequencing, targeted deep sequencing, multi-center analysis of protein expression, loss of function experiments in an in-vivo reporter assay and mammalian models, and gain of function experiments in primary cultured fallopian tube epithelial (FTE) cells. We identified frequent mutations involving a 40 kb distal repressor region for the key stem cell differentiation gene *SOX2*. In the apparently normal FTE, the region was also mutated. This was associated with a profound increase in SOX2 expression ($p < 2^{-16}$), which was not found in patients without cancer ($n = 108$). Importantly, we show that SOX2 overexpression in FTE is nearly ubiquitous in patients with HGSOCs ($n = 100$), and common in BRCA1-BRCA2 mutation carriers ($n = 71$) who underwent prophylactic salpingo-oophorectomy. We propose

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that the finding of SOX2 overexpression in FTE could be exploited to develop biomarkers for detecting disease at a premalignant stage, which would reduce mortality from this devastating disease.

1. Introduction

Because of late presentation and chemotherapy resistance ovarian cancer is the deadliest amongst all gynecological malignancies. Over 80% of ovarian tumors are high-grade serous ovarian cancers (HGSOCs), which is a particularly fatal type due to its initial asymptomatic but aggressive growth behavior. Current methods of detection have been successfully implemented for detection and possible reduction of mortality from ovarian cancer (Menon et al., 2015; Drescher et al., 2013). However, such methods are only capable of the detection of established invasive cancers. Understanding the natural history of the disease and the discovery of novel markers for detection at a premalignant stage will enable the effective control of ovarian cancer.

In this work, we prospectively analyzed the genomic composition of a single tumor over a three-year period to identify driver mutations that may have contributed to the initiation of the tumor. We identified non-coding mutations that cluster near genes involved in stem cell regulation. We established that one mutation is located in a previously unrecognized repressor element of *SOX2*, an important stem cell gene, and is associated with induction of SOX2 expression. We demonstrate that the expansion of SOX2-expressing cells within the fallopian tube epithelium is a common feature of HGSOCs, a crucial finding that opens new avenues for early disease detection prior to clinical presentation.

2. Materials and Methods

2.1. Overall Description of the Study Design

The clinical samples for this translational study were obtained from patients recruited to the Gynecological Oncology Targeted Therapy Study 01 (GO-Target-01) and the Oxford Ovarian Cancer Predict Chemotherapy Response Trial (OXO-PCR-01) under research ethics approval number 11-SC-0014 and 12-SC-0404, respectively. We performed intraoperative video recording to document sampling sites (Supplementary video). Strict standard operating procedures were used to diminish the risk of DNA cross-contamination during sample collection and processing. Whole genome sequencing (WGS) of laser capture microdissected tumor islets ($n = 30$, Supplementary Fig. S1A) and bulk tissue samples of a single HGSOC (patient study ID: 11152). We obtained WGS data from 39 samples from three independent data sets and a tumor recurrence set (Supplementary Fig. S1B). Samples were obtained from different locations before chemotherapy, after neoadjuvant chemotherapy and approximately two and half years later at the time of first recurrence (Fig. 1, Supplementary Table 1). Complete macroscopic clearance at all sites (Supplementary video) as well as microscopic clearance of the peritoneal implants at sites A and B were documented following chemotherapy. Microscopic residual chemoresistant disease

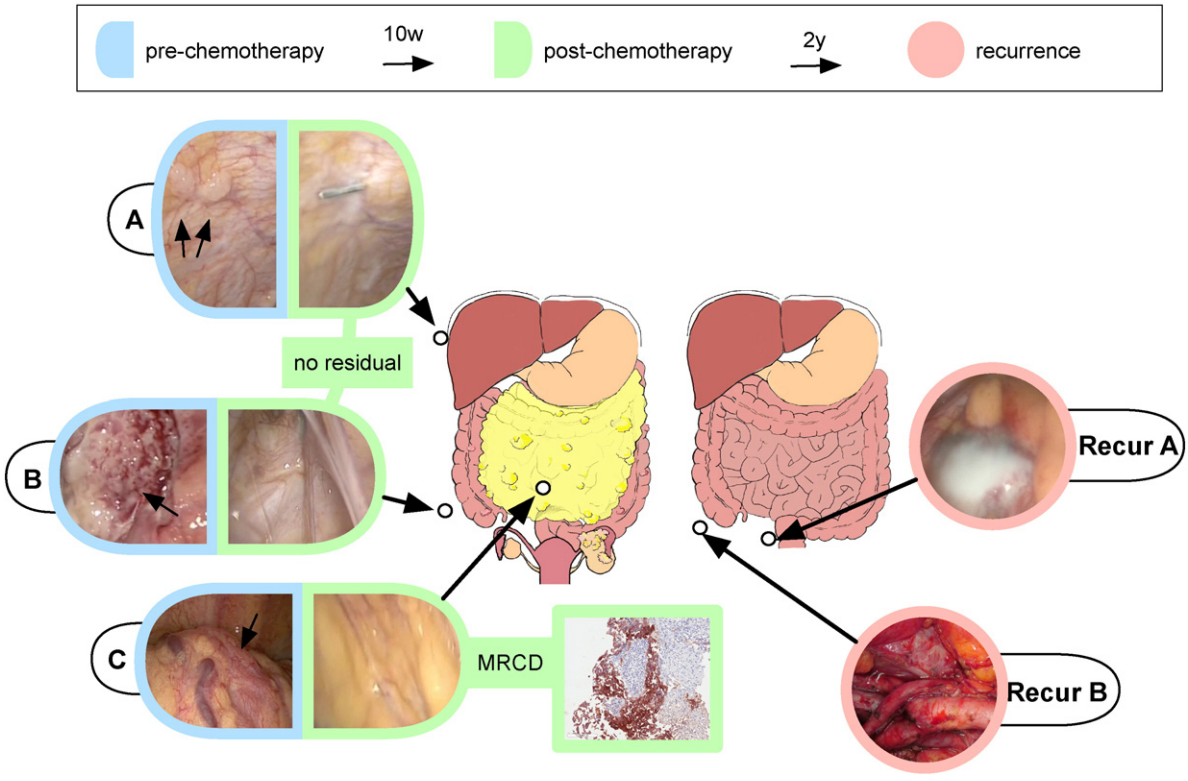

Fig. 1. An ovarian cancer model for investigating primary chemotherapy resistance. A diagram illustrating the sites from which the biopsies were obtained in patient 11152 and the corresponding intra-operative images of the biopsy sites. The sub-diaphragmatic peritoneum (site A), the para-cecal peritoneum (site B) and the omentum (site C) were sampled in the primary tumor. A para-rectal mass (Recur A) and a pelvic node (Recur B) were sampled at presentation of disease recurrence. Note the complete macroscopic resolution of the tumor following chemotherapy (also see Supplementary video). TP53 immunohistochemical staining of a tumor islet from MRCD is also presented.