Original Research

Screening Genetic Alterations of Biomarkers BRAF, ROS-1, and HER2 by Immunohistochemistry in Ovarian Carcinomas for Targeted Therapy

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Ovarian neoplasms are group of aggressive and lethal diseases. Surgical and radiochemical therapies on ovarian neoplasm are conventional approaches but with limited progress in years. Targeted therapy with drugs targeting specific genetic alterations and/or protein molecules have brought new hope fighting against ovarian neoplasms. Targeted therapy on genetic alterations of BRAF, ROS-1 and HER2 have been carried out in melanoma, lung cancer and breast cancer with promising results. However, knowledge of these genetic alterations in ovarian neoplasms is limited and deserves further exploration. In this study, we screened genetic alterations of BRAF, ROS-1 and HER2 by immunohistochemistry (IHC) on a variety of ovarian neoplasm, including 13 mucinous carcinomas, 12 clear cell carcinomas, 9 endometrioid carcinomas, 9 serous borderline tumors and 10 high grade serous tumor of fallopian tube. Although ROS-1 and HER2 abnormalities were not identified, BRAF V600E mutations were identified in 2 of 9 (22%) in borderline serous tumors. This finding has provided a knowledge base to further study the possibility of targeted therapy using BRAF inhibitor, such as vemurafeni, on borderline serous tumor of the ovary.
[N A J Med Sci. 2017;10(2):53-55. **DOI:** *10.7156/najms.2017.1002053]*

Key Words: *ovarian cancer, targeted therapy, BRAF, ROS-1, HER2*

INTRODUCTION

Based on recent statistics from American Cancer Society, ovarian cancer ranks fifth in cancer deaths among women, accounting for more deaths than any other cancer of the female reproductive system (https://cancerstatisticscenter.cancer.org). A woman's risk of getting ovarian cancer during her lifetime is about 1 in 75. Her lifetime chance of dying from ovarian cancer is about 1 in 100. It is estimated that, in 2016, about 22,280 women will receive a new diagnosis of ovarian cancer, and about 14,240 women will die from ovarian cancer.[1] The standard treatment of the high-grade serous ovarian cancers includes surgery and combination of platinum-and taxane-based chemotherapy. Although initial treatment usually has high response rate of about 70%, the cancer typically will become resistant to the drug, and the outcome of the patients is poor. It demands alternate therapies, such as targeted therapy, to improve treatment effects and deal with resistance to chemotherapy.[2] Targeted therapies with Herceptin (antibody against HER2 receptor) for HER2 amplified breast cancer, vemurafenib (BRAF inhibitor) for metastatic melanomas harboring BRAF V600E mutation, and crizotinib (receptor tyrosine kinase inhibitor) for non-small cell lung cancer have been approved by FDA for clinical use with promising results.[3-5] However, these genetic alterations in ovarian cancer have not been adequately explored . The purpose of study is to identify types and frequency of genetic alterations of BRAF, ROS-1 and HER2, in ovarian carcinomas to facilitate development of targeted therapy for ovarian carcinoma.

METHODS

Immunohistochemical stains (IHC) for BRAF V600E(VE1, Ventana), HER2 (HercepTest, Dako), and ROS1 (D4D6, Cell Signaling) were performed on tissue microarray (TMA) slides, provided by Dr Jian-Jun Wei of Northwestern University School of Medicine, using Ventana Benchmark LT/XT automated immunostainers (Ventana Medical System, Tucson, AZ) with appropriate antibody dilutions, and positive and negative controls. The TMAs contain 13 samples of mucinous carcinomas, 12 clear cell carcinomas, 9 endometrioid carcinomas, 9 serous borderline tumors and 10 high grade serous tumor of fallopian tube. IHC for BRAFV600E and ROS1 is cytoplasmic stain, while for HER2 is membrane stain. More than 10% tumor cells with cytoplasmic stain, either weak or strong intensity, is considered as positive for BRAFV600E and ROS expression. HER2 expression is evaluated based on the criteria for breast cancer (6), i.e. negative (0 or 1+), equivocal (2+), and positive (3+).

Received: 01/15/2017; Revised: 03/27/2017; Accepted: 04/16/2017
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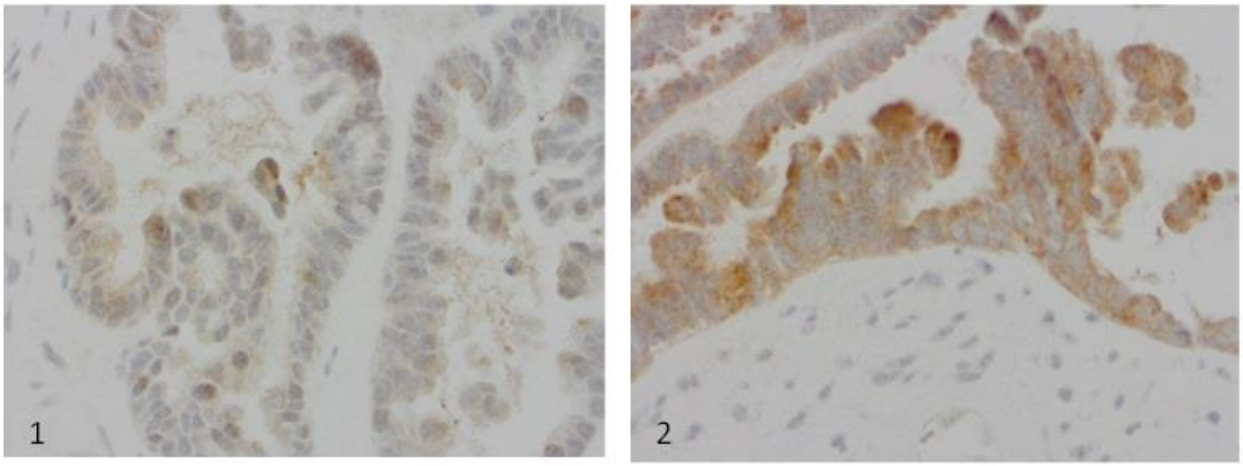

Figure 1. Samples of BRAF V600E expression on 2 borderline serious tumors by immunohistochemistry. Left case (1) show weaker IHC stain than that of right one (2), but both of them are positive.

Table 1. Genetic Alteration of BRAF, HER2 and ROS1 in Ovarian Carcinoma by Immunohistochemistry.

Immunostain	Mucinous Carcinoma (n)		Clear cell Carcinoma (n)		Endometrioid carcinoma (n)		Serous borderline tumor (n)		Fallopian tube high grade serous tumor (n)	
	+	-	+	-	+	-	+	-	+	-
BRAF	0	13	0	12	0	9	2	7	0	10
HER2	0	13	0	12	0	9	0	9	0	10
ROS1	0	13	0	12	0	9	0	9	0	10

RESULTS

The immunostains using antibodies against BRAF (V600E), HER2, and ROS1 were performed on 53 cases of ovarian cancers, including 13 mucinous carcinomas, 12 clear cell carcinomas, 9 endometrioid carcinomas, 9 serous borderline tumors, and 10 fallopian tube high grade serous tumors, using microarray sections with appropriate positive and negative controls.

Positive BRAF (V600E) stain was observed in 2 of 9 (22%) cases of serous borderline tumors. Non-specific background stain with BRAF antibody was seen focally in 1 of 9 cases. All other types of tumors tested showed no BRAF V600E immunostain. See **Figure 1** and **Table 1**.

However, no positive HER2 or ROS1 expression was identified in all types of 53 ovarian carcinomas tested.

DISCUSSION

Low frequency of HER2 and ROS1 genetic alterations in ovarian cancers have been reported before.[2,6,7] In our study, no HER2 or ROS1 genetic alterations was identified by immunostains on ovarian cancers, which is consistent with previous publications. Therefore, targeted therapy to these genes seems implausible.

However, BRAF V600E mutation was identified in 22% (2 of 9) of serous borderline tumors by IHC, which has provided scientific base for treating such tumor with a BRAF inhibitor. Patients with tumor carrying BRAF V600E mutation could potentially get benefit of targeted therapy with BRAF inhibitor, vemurafenib, a FDA-approved medication for melanoma. BRAF V600E in serous ovarian neoplasm has been previously reported mainly by PCR method and rarely by IHC methods.[8-10] Our current study provides additional evidence that BRAFV600E is present in serous borderline tumors, although case number tested is limited. The current treatment of borderline serous ovarian tumors is surgical removal of those with low stage. No consensus has been reached concerning treatment of patients with stage II-IV disease. Platinum-based chemotherapy regimens have been used with varying results (http://emedicine.medscape.com/article/1950573-overview#a11, 4/1/2016 updated). Apparently, targeted therapy with BRAF inhibitor is a potential alterative for treating the patients. Up to now, vemurafenib has not been clinically investigated or used for ovarian cancer yet.

Immunostains is a convenient and cost-effective method with shorter turn-around time for detection of cancer biomarkers. HER2 by IHC is a FDA approved assay for breast cancer and has been used for many years with reproducible results. BRAF V600E by IHC has demonstrated a sensitive and specific assay in detection of BRAF V600E mutation.[11] ROS-1 by IHC, an surrogate test for the gene rearrangement, is new, and its sensitivity and specificity has not been well established.

In summary, BRAF V600E mutation was identified in 22% of ovarian borderline tumors by IHC assay, additional evidence or confirmation of previous observation. Clinical trial with

BRAF inhibitor, vemurafenib, on ovarian borderline tumors with BRAFV600E mutation should be explored as a potential targeted therapy in appropriate clinical settings.

CONFLICT OF INTEREST
None.

ACKNOWLEDGEMENT
This research was supported by Christine Schoeck Blakely Ovarian Cancer Research Fund (2014-2015).

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