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LETTER TO THE EDITOR

Hawaii natural compounds are promising to reduce ovarian cancer deaths

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ABSTRACT

The low survival rate of patients with ovarian cancer largely results from the advanced ovarian tumors as well as tumor resistance to chemotherapy, leading to metastasis and recurrence. However, it is missing as to an effective therapeutic approach that focuses on these aspects to prolong progression-free survival and to decrease mortality in ovarian cancer patients. Here, based on our cancer drug discovery studies, we provide prospective insights into the development of a future line of drugs to effectively reduce ovarian cancer deaths. Pathways that increase the probability of cancer, such as the defective Fanconi anemia (FA) pathway, may render cancer cells more sensitive to new drug targeting.

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Ovarian cancer begins in the ovaries that are ovum-producing reproductive organs.[1] There are more than 30 ovarian cancer types, which are grouped into 3 major categories based on the locations where they grow in the ovary: epithelial tumor, germ cell tumor, and sex cord-stromal tumor.[2] The epithelial ovarian cancer is the most common ovarian cancer type, accounting for roughly 90 percent of all cases.[3] It is the leading cause of death in women with gynecological malignancies.[4] The American Cancer Society estimates that in 2015, about 21,290 new cases of ovarian cancer will be diagnosed and 14,180 women will die of ovarian cancer in the United States.[5] The overall 5-year survival rate of patients with ovarian cancers is only about 30%.[6] Women diagnosed at an early stage have a much higher 5-year survival rate than those diagnosed at an advanced stage.[7] Unfortunately, the majority of ovarian cancers were diagnosed at an advanced stage[4] because of the ovaries anatomic location in the deep part of the abdominopelvic cavity.[1] The high/advanced stage tumors with metastasis are a key cause of the low survival rate for ovarian cancer patients.[8]

As known, treatment options for epithelial ovarian cancer include surgery, radiation therapy, chemotherapy, and immunotherapy. Owing to most tumors being advanced when diagnosed chemotherapy is always applied to the patients after primary surgical cytoreduction of ovarian tumors in order to kill the migrated tumor cells.[9] The most used chemotherapy for ovarian cancer patients is the carboplatin-related therapy, a cisplatin analog with fewer side effects but similar response rates.[10] However, patients develop resistance, which is a major factor causing the high rate of mortality for the patients with ovarian cancer.

In patients with Fanconi anemia (FA), a rare human genetic disease, the cancer incidence is about 50-fold higher than the one in the general population.[11] This cancer-prone phenomenon indicates that FA genes play crucial roles in tumor suppression. Thus, the signaling pathway constituted by at least 17 FA proteins (FANCA /B /C /D1(BRCA2) /D2 /E /F /G /I /J(BRIP1) /L /M /N(PALB2) /O(Rad51C) /P(SLX4) /Q(XRCC4) /S (BRCA1)[12,13] has been termed the FA pathway. Numerous studies have clearly demonstrated a relationship between germline FA gene mutations in cancer patients and the development of a subset of cancers associated with these mutations. Indeed, carriers of mutations in FANCD1 (BRCA2) have a 23% risk of ovarian cancer.[14,15] The FANCF promoter was hypermethylated in the range from 6.7% to 30% of tested tumor cell lines, including ovarian cancer cell lines.[16-18] However, the clear involvement of this FA pathway in human ovarian cancers is barely known compared to other tumor suppressor pathways or oncogenic ones, such as p53 and Ras pathways.[19-23] To this point, 17 FA genes, whose products constitute the FA pathway, were analyzed in DNA sequence data set derived from 316 ovarian cancer cases via c-BioPortal.[24,25] Surprisingly, the mutated status of the FA pathway not only has a considerably high rate (40%) in the 316 ovarian cancer cases examined, but also is significantly associated with the length of patient survival as well as the cancer-free period (Fig. 1). This strongly suggests that 40% ovarian cancer patients have a relatively better prognosis for those carrying altered FA genes, speaking of 17 genes quoted because defective FA genes may decrease DNA repair, make cells more sensitive to DNA disrupters, and reduce resistance to DNA-damaging chemotherapeutic agents.

The FA signaling pathway is involved in the cellular response to DNA damage, especially those damages triggered by DNA cross-linkers, such as chemotherapeutic drugs, Cisplatin and its analogs, and mitomycin C (MMC).[26] Coincidently, after surgery, Cisplatin was used in all of these 316 cases.[6] Therefore, we believe that platinum-chemotherapy is more rational for patients with ovarian tumors carrying an impaired FA pathway, instead of being used as a general procedure to follow surgery. This actually inspires us to think what kind of ovarian cancer cells should be targeted to begin a new

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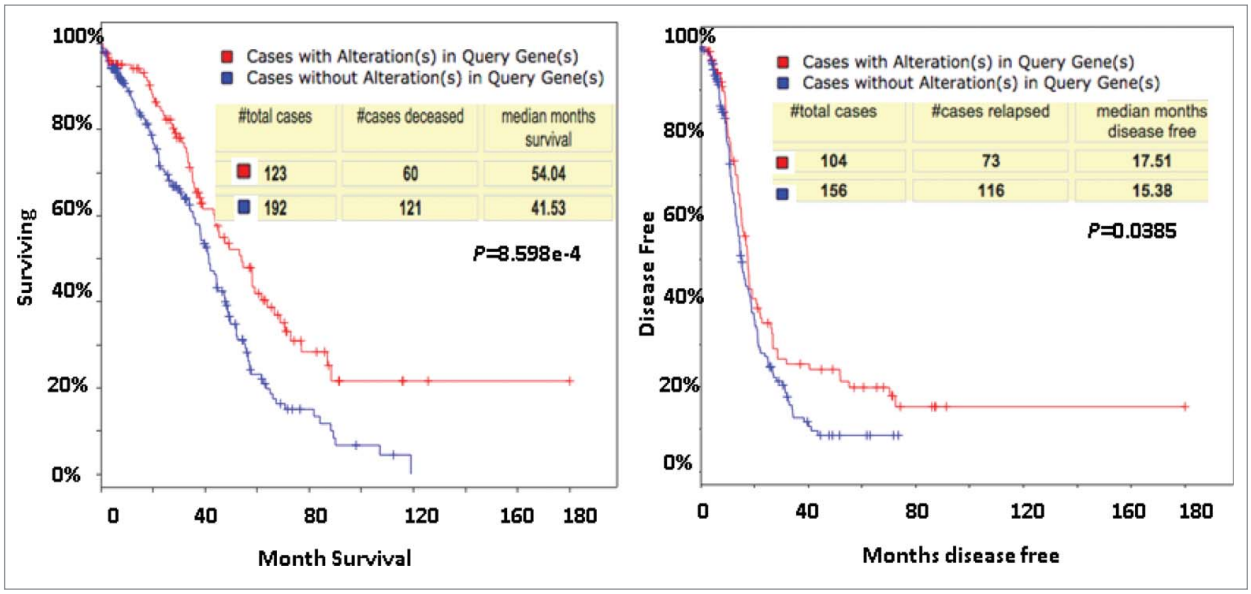

Figure 1. Patients with ovarian tumors harboring an impaired FA pathway show a better prognosis than those patients with tumors carrying an intact pathway. Seventeen FA genes were input into c-Bioportal when choosing TCGA data set containing 316 tumor samples.[6] The output shows that 40% of 316 quarried samples carry a mutated FA pathway, and the mutated status (red lines) is significantly associated with the survival as well as the period of disease free (in months) via Kaplan-Meier Estimate. Those patients were all treated with Cisplatin.[6]

line of anti-ovarian cancer drug discovery. It appears to us that the given new line of drugs, when aiming to fight over the insensitivity of ovarian tumor cells to platinum, should attack the cells chosen based on the cellular context (targeted cells), instead of cancer cells in general, to save time and cost. For instance, we should have asked if the targeted cells carry an impaired or intact FA pathway before we set up a massive high throughput screen.

Fungi have been the sources of many marketed drugs and biologically active agents. The well-known antibiotic penicillin and its analogs are produced from various species of *Penicillium* and *Aspergillus*,[27] long presenting importance of using fungal sources for the drug discovery. The anti-cancer drug Taxol from the Pacific yew tree, targeting mitotic microtubules, was also isolated from the endophytic fungus, *Bartalinia robillardoides*,[28] which is a widely-used anti-cancer drug on the market. However, cytotoxicity occurs given the fact that the drug target is not cancer cell specific. Thus, it is urgent to find new drugs to target cancer cells specifically. Our laboratory has established a local natural product library composed of more than 5 thousand semi-pure fractions from more than 2,000 fungal strains collected all over the Hawaiian islands. This is a unique resource to find new drugs to effectively target ovarian cancer cells and increase the survival rate of patients with ovarian cancer. Therefore, we started a pilot screen by using 2 ovarian cancer cell lines carrying an intact or impaired FA signaling pathway (A2780 and IGR-OV1, respectively) and obtained several novel compounds from Hawaiian fungi. The exact

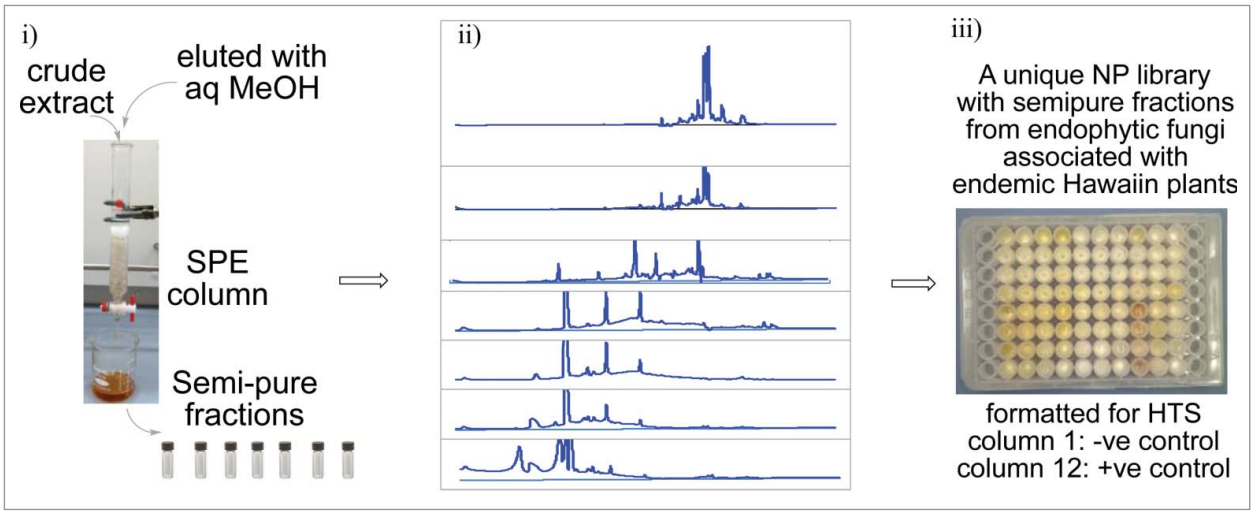

Figure 2. Fractionation and preparation of semi-pure fractions into 96-well plates. Detailed overview of the process for generating pre-fractionated samples. i). HP20 SPE pre-fractionation. ii). HPLC analysis of 7 fractions from strain FT001. As shown in ii, compounds in pre-fractionated sample could be readily separated. iii). The seven fractions and the crude (A-H) from each culture are formatted into a 96-well block for screening, 80 samples per plate.

structures of these compounds are yet to be determined. However, when we started to validate these compounds, we found a compound derived from screening both types of cells indeed shows a similar growth inhibitory effect on both IGR-OV1 and A2780 cells with an IC_{50} of 6–7 μg/mL. While the others, obtained from screening either one of cell lines, carry an IC_{50} varying from 6 to 20 μg/mL when treating IGR-OV1 or A2780 cells. Collectively, our pilot study of potential new drugs for treating ovarian cancer cells indicates that the new lead compounds would be more effective when coupling the updated knowledge into our drug screening system.

The natural product library was built up through a) Environmental sample collection; b) Fungal strain culture, isolation and selection; c) Fermentation; d) Harvest and extraction and fractionation; and e) Sample preparation for screening (Fig. 2). Our specific tropical weather defines the novelty of fungal samples collected in step a. In addition, the other processes further support the novelty of the natural product library. For example, we removed the redundancy by selecting new organisms based on the classic taxonomy, ITS (Internal Transcribed Spacer), and 18S rRNA at step b. We also used well-established biochemical methods including the routine use of LCMS-DAD-ELSD (Liquid Chromatography Mass Spectrometry-Diode Array Detector-Evaporative Light Scattering Detectors), PCA (Principal Component Analysis), and other methods to identify specific compounds. As shown in our lab recent publications,[29,30] the very pure and structurally defined natural products that were characterized are previously unknown natural compounds. In the meantime, what we learned from the pilot screening suggests that these novel compounds will be more effective when choosing representative cells integrated with the previously unknown features of ovarian cancer cells. Therefore, it is very promising for us to develop a new line of drugs to reduce ovarian cancer deaths by large scale of screening using the cell system tested above as well as the natural resources that only Hawaii can offer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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