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The role of IFITM3 in the immune response of BRCA-deficient high grade serous ovarian carcinoma

Han Cun

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The role of IFITM3 in the immune response of *BRCA*-deficient high grade serous ovarian carcinoma

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The role of IFITM3 in the immune response of *BRCA*-deficient high grade serous ovarian carcinoma

A

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Master of Science

By

Han Cun, MD

Houston, Texas

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Dedication

To my husband, Willis Yu, who has been my pillar of unwavering support throughout this long and winding journey. *“You may be gone from my sight, but you are never gone from my heart.”*

The role of IFITM3 in the immune response of *BRCA*-deficient high grade serous ovarian carcinoma

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Background: Prior studies showed that *BRCA*-deficient high grade serous ovarian carcinoma (HGSOC) had increased tumor infiltrating lymphocytes (TILs) compared to *BRCA*-wildtype (WT). To better understand the underlying immune mechanism in these tumors, a preliminary transcriptome analysis was performed on a set of microdissected HGSOC tumor specimens with *BRCA1*-mutation, *BRCA2*-mutation, or WT. This demonstrated an upregulation of *IFITM3*, an essential gene in modulating immune function. Based on these findings, we hypothesized that *BRCA*-deficient HGSOC have increased DNA damage leading to upregulation of *IFITM3* and subsequent increase in antigen presentation and T-cell activation.

Methods: Following IRB approval, preliminary transcriptome analysis was performed followed by validation using immunohistochemistry (IHC) for *IFITM3* expression in a larger cohort of patients with HGSOC. Various HGSOC cell lines, including *BRCA1*-null lines, were used to validate mRNA expression. Immune biomarkers were investigated using multiplex immunofluorescence (IF) and imaging mass cytometry (IMC) on our cohort, and HLA mRNA expression was evaluated in HGSOC cell lines. *IFITM3* expression was manipulated in HGSOC cells using siRNAs to determine the effect of *IFITM3* on the expression of immune-related genes and survival in HGSOC cell lines.

Results: A total of 57 samples were used including 12 with *BRCA1*-mutation, 9 *BRCA2*-mutation, and 36 WT. Tumors with *BRCA* mutations had a significantly higher *IFITM3* protein expression compared to WT ($p=0.0001$). *IFITM3* mRNA expression was also significantly higher in UWB1.289, a *BRCA1*-null cell line, than UWB1.289+*BRCA1*, the same cell line with *BRCA1* gene function restored ($p=0.0001$). CD8⁺GranzymeB⁺ cell density was significantly higher in *BRCA*-deficient tumors compared to the WT

($p=0.0004$). HLA-F mRNA expression in UWB1.289 was significantly higher than in UWB1.289+BRCA1 ($p=0.0001$), and this correlated with IFITM3 mRNA expression ($r=0.617$, $p=0.014$). IFITM3 silencing leads to an increase in survival of *BRCA1*-null HGSOC cell lines ($p<0.0001$; $p<0.0001$).

Conclusion: This study suggests that IFITM3 stimulates the immune response and decreases cancer cell growth in *BRCA*-deficient HGSOC. This novel relationship provides insight into the association of IFITM3 with DNA damage, which may be valid in other malignancies. Further examination of IFITM3 in HGSOC provides many opportunities to better understand the pathogenesis of ovarian carcinoma, potential screening methods, and therapeutic options.

Table of Contents

Approvals.....	i
Title.....	iii
Dedication.....	iv
Abstract.....	v
Table of Contents.....	vii
List of Figures.....	viii
List of Tables.....	ix
Background and Introduction.....	1
Hypothesis and Specific Aims.....	19
Methods and Materials.....	20
Results.....	29
Discussion.....	42
Bibliography.....	47
Vita.....	70

List of Figures

Figure 1. Commonly upregulated genes in <i>BRCA1</i> - and <i>BRCA2</i> -deficient ovarian cancer cells.....	13
Figure 2. Proposed regulation of IFITM3 in <i>BRCA</i> -deficient ovarian carcinoma cells.....	19
Figure 3. Increased IFITM3 protein expression in <i>BRCA</i> -deficient high grade serous ovarian carcinomas.....	31
Figure 4. Relative IFITM3 mRNA expression in <i>BRCA1</i> -null and wildtype ovarian cancer cells.....	32
Figure 5. Multiplex immunofluorescence for activated cytotoxic T cells in <i>BRCA</i> -deficient and <i>BRCA</i> -wildtype ovarian tumors.....	34
Figure 6. Correlation of IFITM3 and activated cytotoxic T cells.....	36
Figure 7. Imaging mass cytometry in <i>BRCA</i> -deficient and <i>BRCA</i> -wildtype ovarian tumors.....	37
Figure 8. Correlation of IFITM3 and HLA expression.....	39
Figure 9. Effects of downregulation of IFITM3 in <i>BRCA1</i> -null ovarian carcinoma cells.....	41

List of Tables

Table 1. Patient demographic for transcriptome analysis.....12

Table 2. Significantly upregulated genes in both *BRCA1*- and *BRCA2*-deficient ovarian tumor cells compared to *BRCA*-wildtype.....14

Table 3. Imaging mass cytometry antibody panel.....28

Table 4. Patient demographic for the immunohistochemistry staining.....30

Background and Introduction

Ovarian Cancer

Epithelial ovarian carcinoma (EOC) is one of the most lethal gynecologic malignancies in the world. For decades now, the current standard of care therapy has included cytoreductive surgery either before or after platinum-based chemotherapy. More recently, the discovery of molecular markers such as *BRCA* gene mutations or homologous recombination deficiencies (HRD) has led to the development of targeted therapies, which have improved progression-free survival (PFS), overall survival (OS), and quality of life for these patients. However, despite these advancements, morbidity from this malignancy has remained high. In the United States, the 5-year relative survival rate for ovarian cancer is 49%, while the 5-year relative survival rate for women with invasive breast cancer is 90% and for uterine cancer is 81% [1, 2]. This is in part due to lack of screening for the disease and the minimal symptoms associated with this cancer, leading to a delay in diagnosis. But further work in understanding the pathogenesis of ovarian carcinoma is needed to better combat the disease with novel approaches to treatment and therapy.

Most ovarian carcinomas are epithelial in origin. Furthermore, there are five major histologic subtypes each with its own distinct presentation and response to therapy: high grade serous, low grade serous, clear cell, endometrioid, and mucinous ovarian cancer. High grade serous ovarian carcinoma (HGSOC) accounts for 70% of all EOC [3].

HGSOC is characterized by mutations in DNA repair mechanisms. In particular, 97% of all HGSOC have a *p53* mutation [4, 5]. As the guardian of the genome, this transcription factor is integral for DNA damage repair to activate genes involved in DNA repair pathways, various cell cycle steps, and apoptosis when DNA damage is irredeemable. Other DNA repair mechanisms are also implicated in the development of HGSOC. Homologous recombination (HR) is a high-fidelity repair

pathway that requires a homologous DNA template to function for repair of double strand breaks. When HR is unable to occur, such as when a *BRCA1* or *BRCA2* gene mutation is present, an error-prone mechanism is then elicited, non-homologous end joining (NHEJ). The lack of DNA repair mechanisms and increased DNA replication stress leads to genomic instability and ultimately an increased risk for cancer development.

Additionally, not only is this deficiency of DNA repair integral to development of cancer, it is believed that *p53* mutations occur early in tumorigenesis. Through the evaluation of specimens from prophylactic risk-reducing salpingo-oophorectomies in high-risk patients, this same *p53* mutation was found in serous tubal intraepithelial carcinomas (STICs), a precursor lesion to HGSOC typically found in the distal end of the fallopian tube [6, 7]. Thus, this mutation is believed to occur early in carcinogenesis, emphasizing its role as a driver mutation for this malignancy [8]. This also suggests a clonal relationship and likely direct evolutionary descent from these cells for HGSOC [9]. Because the majority of HGSOC are believed to initiate here, “ovarian cancer” often refers to all epithelial cancers that originate in the ovary, fallopian tube, or from primary peritoneal cancers.

Hereditary Ovarian Cancer: *BRCA1* and *BRCA2*

The majority of EOCs are sporadic, but roughly 15-20% of women with HGSOC have a hereditary predisposition due to mutations in *BRCA1*, *BRCA2*, or other homologous recombination (HR) genes [4]. HR genes include but are not limited to *RAD51*, *ATM*, *ATR*, *Fanconi anemia*, *BARD1*, *BRIP1*, *PALB2*, *RB1*, and *NF1* [4, 10, 11]. This is compared to 1% of the general population [12, 13]. In fact, *BRCA* mutations account for the majority of mutations in hereditary ovarian cancers, up to 75% [14-16], but somatic *BRCA* mutations also account for 5-7% of ovarian cancers [17]. *BRCA* mutations confer a lifetime risk of developing ovarian cancer, 40-60% for *BRCA1* and 11-27% for *BRCA2*

mutations. Patients with ovarian cancer with a genetic susceptibility tend to be younger, usually 10 years younger than the median age; have a history of other malignancies like breast cancer; have a family history of malignancies such as breast and ovarian cancer in females and prostate in males; and are nearly exclusively associated high-grade serous histology [14, 18, 19].

Both *BRCA1* and *BRCA2* genes encode for tumor suppressor proteins that assist in the repair of damaged DNA and ultimately ensure the stability of the genetic material within a cell. They are unrelated proteins but are both involved in repair of double-strand breaks in DNA. The *BRCA1* gene is located on the long arm of Chromosome 17, at 17q21 and encodes for breast cancer type 1 susceptibility protein [20]. Together with other tumor suppressors, DNA damage sensors, and signal transducers, it forms a large protein complex known as the BRCA1-associated genome surveillance complex, or BASC [21]. This complex assists in the repair of double-strand breaks via homology-directed repair, but it is also involved in DNA mismatch repair via interaction with MSH2. There are over 1600 mutations that may cause a nonfunctional BRCA1 protein, but hypermethylation of the *BRCA1* promoter may also inactivate its expression. The *BRCA2* gene is located on the long arm of chromosome 13 at 13q12.3 and encodes for breast cancer type 2 susceptibility protein. This protein binds to single strand DNA and recombinase RAD51 to assist in strand invasion for homologous recombination [22]. A defective BRCA1 or BRCA2 protein causes the inability to fix DNA damage, which subsequently leads to an increase in genetic mutations. For instance, the alternative NHEJ will be used, leading to the accumulation of mutations, neoantigens, and increased susceptibility to develop cancer.

Prior studies have demonstrated that HGSOC patients with *BRCA1*- or *BRCA2*-deficiency have improved survival [23, 24]. This is believed to be due to their sensitivity to platinum-based chemotherapy because of their inherent loss of DNA repair. Interestingly, some studies have shown a longer survival in *BRCA2*-mutation carriers compared to patients with *BRCA1*-mutations or *BRCA*-

wildtype (WT) [14, 25, 26]. However, more recent studies suggest that this favorable prognosis may be lost after 10 years. A study by Patch et al. found DNA repair deficiency was predictive of a higher 5-year survival probability, but at 10 years, this benefit was lost [5]. Other studies suggest that *BRCA*-deficiency may actually have a negative impact on disease-specific and all-cause survival beyond 10 years [27, 28]. Thus, the survival advantage related to *BRCA*-deficiency may be attributed to other mechanisms aside from DNA repair deficiency and chemosensitivity.

Treatment of Ovarian Cancer

The current standard treatment of HGSOc includes cytoreductive surgery and platinum-based chemotherapy. Disease diagnosed in the early stage is curable in 90% of women [29, 30], but most women are diagnosed at an advanced stage disease and moreover, 75% of these women will die of their disease [31]. Also, most women will relapse within 2 years and develop chemoresistance, leading to an overall poor prognosis [32].

The goal of surgery is for complete resection of gross disease. This includes full exploration of abdomen and pelvis, and at least a hysterectomy, bilateral salpingo-oophorectomy, omentectomy, with or without pelvic and para-aortic lymphadenectomy [33]. Any remaining visible residual disease is associated with a worse OS [34].

For some patients, surgery is deferred until after chemotherapy is commenced possibly due to extensive disease. To achieve complete resection, this may more readily be achieved with neoadjuvant chemotherapy, followed by interval cytoreductive surgery. Multiple phase 3 trials have demonstrated that neoadjuvant chemotherapy followed by interval debulking does not have inferior survival outcomes compared to primary cytoreductive surgery followed by adjuvant chemotherapy including EORTC [35] and CHORUS [36].

Standard chemotherapy regimens consist of platinum-based chemotherapy, either cisplatin or carboplatin, together with paclitaxel. Carboplatin has reduced toxicity and improved tolerability with maintaining therapy efficacy [37], and thus is more often utilized. Patients are typically treated with six cycles of adjuvant chemotherapy, even those with early stage disease [38]. Another consideration for this chemotherapy regimen include frequency of dosing, such as dose-dense paclitaxel accompanied by carboplatin, which has been found to be well-tolerated and effective. For instance, in a Japanese study, weekly dose-dense chemotherapy with carboplatin improved PFS and OS [39, 40]. However, other studies with a predominately Caucasian cohort of patients (MITO-7 and ICON8) demonstrated that dose-dense administration did not show the same benefit [41, 42]. This raises the possibility that there is a pharmacologic difference between ethnic groups and how they may respond to treatments.

Another consideration is the route of administration, such as use of intraperitoneal (IP) chemotherapy. Because ovarian cancer typically spreads via peritoneal metastasis, the use of IP chemotherapy was hypothesized to provide direct diffusion to cancer cells. Its ideal use is following optimal cytoreduction with only microscopic disease remaining. Randomized studies have demonstrated that this route of administration has improved PFS and OS [43, 44], but may be associated with significant toxicity [45] depending on the dose. Moreover, these improvements may be nullified when other therapies are incorporated such as bevacizumab [46].

As mentioned above, most patients will experience recurrence within 2 years of treatment. Some of these patients will have recurrence after 6 months, suggesting sensitivity to platinum-based therapy, while others may recur in a shorter interval and demonstrate platinum-resistance. Thus, these patients may incorporate non-platinum based therapeutic agents including novel targeted therapies to potentially treat ovarian carcinoma.

In HGSOC, angiogenesis plays a role in tumor growth, metastasis, and ascites formation. There is frequent activation of hypoxia-driven proangiogenic pathways in carcinogenesis, triggering increased expression of vascular endothelial growth factor (VEGF). Studies have demonstrated a differential expression of VEGF in malignant specimens compared to benign ovarian tumors [47, 48], and additional studies have demonstrated that VEGF-driven angiogenesis is a component of an early, critical event in HGSOC development [49] and growth [50]. Aside from hypoxia, VEGF is also known to be induced by a variety of other factors. In ovarian carcinoma, VEGF may be induced through gonadotropins [51], which may be autocrine or paracrine through ovarian cancer cells upregulating angiopoietin 2 in host endothelial cells [52]. Other regulators of VEGF include insulin-like growth factor 1, EGF, platelet-derived growth factor (PDGF), transforming growth factor- β , tumor necrosis factor- α (TNF- α), TNF-like weak inducer of apoptosis, IL-1 β , IL-6, endothelin-1, prostaglandin E2, 4-hydroxyl-estradiol, matrix metalloproteinases (MMPs), reactive oxygen species (ROS), and cyclooxygenase [53].

VEGF also contributes to metastasis and cancer dissemination. For instance, it stimulates the migration of monocytes and macrophages, both providing support to the tumor stromal microenvironment [54]. Preclinical studies demonstrated that increased expression of VEGF can convert normal ovarian epithelium tissue into neoplastic tissue with ascites production [55, 56]. Furthermore, VEGF also causes the breakdown of the endothelial barrier that subsequently leads to tumor cell extravasation at metastatic sites [57]. Activation of these pathways ultimately causes capillary leaks resulting in interstitial pressure, ascites, and pleural effusions [58, 59]. Thus, the use of VEGF inhibitors have been widely investigated.

Bevacizumab is the first and only FDA approved anti-VEGF therapy in ovarian cancer use. It is a recombinant humanized VEGF monoclonal antibody that reacts to all active isoforms of VEGF, inhibiting its binding with VEGF-receptors (VEGFR) [60]. Frontline use has conflicting results. ICON7

and GOG 0218 showed improved PFS with concurrent and maintenance use, but GOG 0218 showed no OS advantage, even though ICON7 did show minimal improvement in OS within their population [61, 62]. Its use in the recurrent setting has demonstrated improvement in PFS [63, 64].

There are other methods of targeting angiogenesis, although their efficacy in treating EOC has been mixed, and they are not yet approved for treatment. For example, Aflibercept, a VEGF trap, is a soluble decoy receptor that blocks members of the VEGF family including VEGF, VEGF-B, and PlGF [65]. Although its phase II study in advanced EOC showed improvement in malignant ascites [66], there is a significant risk of bowel perforation [67]. Another method of targeting the angiogenesis pathway is by blocking VEGFR. Pazopanib is an inhibitor of VEGFR, platelet-derived growth factor receptor, and c-kit. For maintenance therapy in patients following first line chemotherapy use, it demonstrated a 6-month PFS advantage but no OS improvement [68]. Nintedanib, an inhibitor of VEGFR, platelet-derived growth factor receptor, and fibroblast growth factor receptor was found to have only 0.6 month improvement in PFS for first line therapy and maintenance use [69].

Because of the high rate of HRD and replication stress in ovarian cancer, poly (ADP-ribose) polymerase (PARP) inhibitors have emerged as a treatment option for many patients with HGSOC. PARPs are a group of enzymes that perform PARylation and are essential to DNA repair of single-strand breaks. They are able to sense DNA damage and bind DNA for posttranslational modification to assist in homologous recombination, nucleotide excision repair, and base excision repair [70-72]. Once the DNA is repaired, PARP1 performs autoPARylation which results in the release of the DNA [70].

The effectiveness of PARP inhibitors are based on the concept of synthetic lethality. This describes how two genetic lesions, which are not lethal individually alone, but become lethal if both occur in the same cell. The use of PARP inhibitors leads to an accumulation of double-strand DNA

breaks due to replication fork collapse when the fork encounters persistent single-strand breaks, which cannot be repaired in a high-fidelity mechanism if homologous recombination is impaired [73]. Thus, cells that have impaired homologous recombination are more sensitive to PARP inhibitors. Additional studies have also shown that PARP inhibitors may also lead to the trapping of PARP molecules at the site of DNA damage, which prevents autoPARylation [74, 75].

The current FDA-approved PARP inhibitors for ovarian cancer treatment include: olaparib, rucaparib, niraparib for various indications including treatment of recurrent disease, maintenance following first-line treatment, and maintenance following treatment of recurrent disease in both *BRCA*-deficient and *BRCA*-WT patients. The most pronounced effect on PFS by PARP inhibition for maintenance use is in patients with *BRCA*-deficiency but also affects patients with mutations in HR genes [76-78]. PARP inhibitors have also been FDA-approved in other malignancies including prostate, breast, and pancreatic cancers with either *BRCA1*-mutation, *BRCA2*-mutation, or HRD status.

The role of immunotherapy in ovarian cancer is left to be defined. These therapies include immune checkpoint inhibitors, cancer vaccines, and chimeric antigen receptor-modified T cells (CAR-T) therapy, which have emerged as an innovative and effective treatment for various solid and hematologic malignancies, including melanoma, head and neck cancers, and leukemia [79-83]. However, their use in ovarian cancer has been limited. Multiple monotherapy clinical trials have demonstrated modest or no effect [84-92], including pembrolizumab, the first drug that received site-agnostic FDA approval. The results from the clinical trial KEYNOTE-100 demonstrated that single drug therapy with pembrolizumab, a PD1 immune checkpoint inhibitor, in recurrent ovarian cancer patients provided an objective response rate (ORR) of only 7.4-9.9% [91]. Currently, there are no FDA approved immunotherapies directed against ovarian carcinoma.

The Immune System and Ovarian Cancer

Although clinical trials have not yet supported the use of immunotherapy in ovarian cancer treatment, there is evidence that demonstrates the presence of immune modulation in this disease site. Prior studies have demonstrated that the immune environment of ovarian cancer is linked to clinical survival and outcomes. For instance, within the tumor, the presence of tumor infiltrating lymphocytes (TILs), such as CD3⁺ T cells and CD8⁺ T cells, are associated with improved survival and decreased progression of disease in ovarian cancer patients [93-98]. On the other hand, immune evasion mechanisms such as the presence of T regulatory cells or myeloid-derived suppressor cells (MDSCs) correlate with poor survival [95, 99, 100]. Additionally, current standard of care treatment with platinum-based chemotherapy produces immune modulation and inflammation in ovarian cancer mouse models [101]. A study investigating long term survivors of HGSOC found that they had increased somatic mutation burden; enrichment of activated CD4⁺ T cells, CD8⁺ T cells; and effector memory CD4⁺ T cells [102]. These findings suggest a function of the immune system within the tumor microenvironment in the pathogenesis, survival, and treatment response in these tumors, warranting further investigation.

As previously mentioned, HGSOC is strongly associated with defects in DNA repair pathways. As suspected, *BRCA*-deficient tumors have a higher mutational load [25], translating to higher neoantigen load and possibly increased immunogenicity. But knowledge of tumor microenvironment of *BRCA*-deficient ovarian tumors is limited. In general, there is a higher neoantigen load in *BRCA*-deficient ovarian cancers compared to HR proficient ovarian tumors [103]. In this same study, this translated to significantly higher number of CD3⁺ TILs in *BRCA*-deficient tumors compared to levels found HR proficient tumors. Overall, increased neoantigen burden in pre-treatment tumors is associated with improved survival [104]. Additionally, Clarke et al. demonstrated that *BRCA1* loss had significant correlation with the presence of intraepithelial CD8⁺ T

cells, and furthermore, this correlated with improved clinical outcomes for all stages of HGSOC, which was an independent prognostic factor by multivariate analysis [93]. Thus, there seems to not only be a survival benefit in *BRCA*-deficient HGSOC, but there is also evidence of immune modulation and stimulation in these tumors, which has been associated with survival.

The underlying biological mechanisms for this survival advantage in *BRCA*-deficient tumors is poorly understood. Although traditionally, *BRCA* status was thought to have a favorable prognosis due to its enhanced responsiveness to platinum-based therapy, alternative intrinsic biologic properties, such as immunologic differences, may also contribute to improved outcomes. A comprehensive understanding of the immune response and tumor immune microenvironment of *BRCA*-deficient tumors could allow us to further exploit the immune system for therapeutic targeting and use of immunotherapies.

Identification of differential expression of *IFITM3* in *BRCA*-deficient and *BRCA* wildtype ovarian tumors

To delineate the underlying mechanism by which *BRCA*-deficiency result in increased intraepithelial CD8⁺T cells in HGSOC, we sought to identify differentially expressed genes that are associated with immune cell activity and can potentially be regulated by homologous recombination defect in *BRCA*-deficient HGSOC compared with *BRCA*-proficient HGSOC. Transcriptome analysis was performed on microdissected epithelial compartment of ovarian carcinoma specimens obtained from a cohort of treatment-naïve advanced HGSOC patients who either had a *BRCA1*-mutation (n=5), had a *BRCA2*-mutation (n=5), or were *BRCA*-WT (n=5) (Table 1). This analysis demonstrated a number of up- or down-regulated genes in both the *BRCA1*- and *BRCA2*-deficient ovarian carcinoma patients compared to *BRCA*-WT group (Figure 1). In particular, the gene *IFITM3* was statistically

significantly upregulated in both the *BRCA1* and *BRCA2* cohorts (Table 2) and selected for further validation studies. This was also because it has been shown to be down-regulated by transcription factor KLF4 [105], which can be inactivated by increased DNA damage. In addition, IFITM3 has been shown to up-regulate MHC class I and II expression that may modulate immune function [106, 107].

Table 1. Patient demographic for transcriptome analysis			
	BRCA 1 (n=5)	BRCA 2 (n=5)	Wildtype (n=5)
Race/Ethnicity			
Asian	2 (40%)	3 (60%)	0 (0%)
Black	1 (20%)	0 (0%)	1 (20%)
Hispanic	0 (0%)	1 (20%)	1 (20%)
Caucasian	2 (40%)	1 (20%)	3 (60%)
Histology			
Serous	5 (100%)	5 (100%)	5 (100%)
Age at Diagnosis			
Median (IQR)	55 (3)	58 (7)	53 (5)
Progression Free Survival			
Range	1-88 mo	10-72 mo	4-61 mo

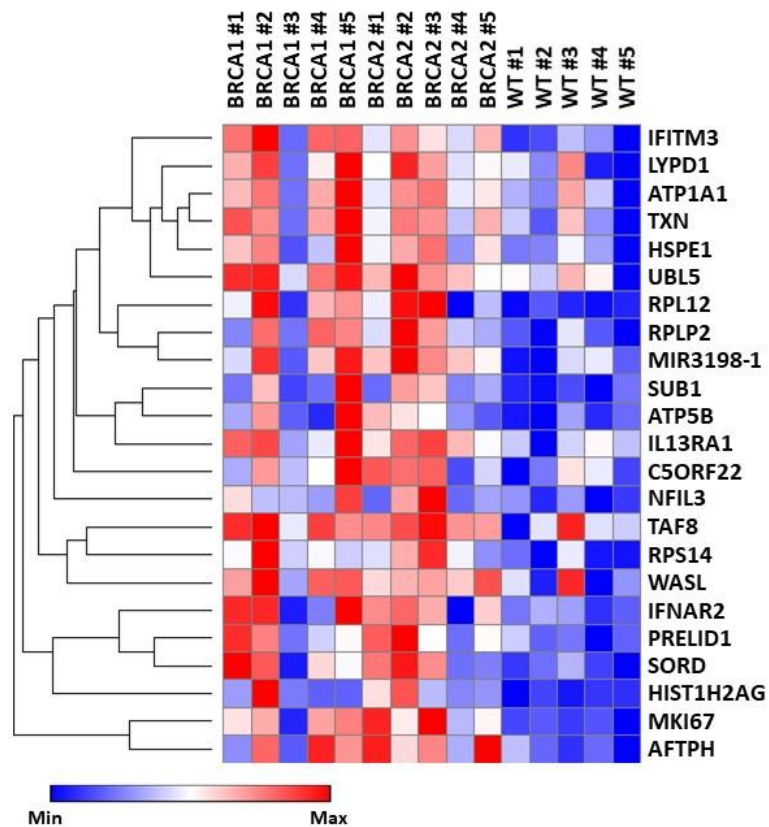


Figure 1. Commonly upregulated genes in *BRCA1*- and *BRCA2*-deficient ovarian cancer cells

Heatmap of the commonly upregulated genes in the *BRCA1*- and *BRCA2*-deficient ovarian carcinoma cells constructed from the transcriptome analysis of the microdissected tumor specimens (n=15). Genes represented here have at least a 2-fold increase in gene expression compared to the *BRCA*-WT cohort and a $p < 0.05$.

Table 2. Significantly upregulated genes in both *BRCA1*- and *BRCA2*-deficient ovarian tumor cells compared to *BRCA*-wildtype

Gene	<i>BRCA1</i> Fold Change	P-Value	<i>BRCA2</i> Fold Change	P-Value
HSPE1	5.11949	0.037219	3.635112	0.030764
SUB1	3.944931	0.001114	2.624423	0.000312
TXN	3.879848	0.045627	3.285243	0.03734
SORD	3.853048	0.033333	3.837056	0.019576
LYPD1	3.716643	0.008907	4.425989	0.006657
MIR3198-1	3.685857	0.001501	3.506423	0.008935
IFITM3	3.103723	0.037071	2.524504	0.023283
NFIL3	2.907945	0.029913	2.743467	0.003153
RPL12	2.85997	0.00901	2.131693	0.032192
ATP1A1	2.698205	0.012386	2.661058	0.007904
IL13RA1	2.675855	0.009266	2.720742	0.006158
TAF8	2.613531	0.049107	2.401607	0.004907
RPS14	2.591882	0.020127	2.462289	0.004806
UBL5	2.500125	0.016014	2.011121	0.01583
HIST1H2AG	2.404938	0.039673	2.096525	0.041596
RPLP2	2.34242	0.036243	2.185555	0.00908
PRELID1	2.32624	0.006017	2.222217	0.037624
ATP5B	2.310172	0.002274	2.952629	0.00031
WASL	2.284692	0.020558	2.728296	0.000841
IFNAR2	2.15248	0.041627	2.231479	0.042255

MKI67	2.125791	0.005985	2.111107	0.005201
C5ORF22	2.082043	0.01298	2.683284	0.000546
AFTPH	2.027919	0.036922	2.421666	0.001301

IFITM3 and Malignancy

Interferon induced transmembrane protein 3 (IFITM3), also known as fragilis or I-8U, is a 15-kDa type 2 double transmembrane protein encoded by a gene on chromosome 11. It belongs to a family of *IFITM* genes that are upregulated by interferon. They are expressed in most tissue and involved in a variety of functions such as early development, cell adhesion, cell growth control, and immune response. Specifically, IFITM3 is constitutively expressed in healthy tissue [108, 109] but its altered expression has been linked to various pathologies and immune-related diseases. It has been extensively studied in viral infections for its antiviral role in RNA viruses like influenza, dengue, West Nile, HIV, and HCV [110], but also found to play a role in inflammatory bowel disease and cancer [106].

The role of IFITM3 in malignancy is still uncertain and may be cancer-specific. For instance, IFITM3 overexpression has been found in various cancer types including colonic, gastric, breast, prostate, lung, and liver [105, 110-113] suggesting a role as an oncogene. It is also found to have a higher expression in metastatic sites compared in the primary tumor in colon [105] and prostate cancer [113], which also supports its potential role as an oncogene. Additionally, Its expression has been associated with poor prognosis in colonic cancer and acute myeloid leukemia, head and neck squamous cell carcinoma [110]. It may even play a role in promoting cancer metastasis. On the other hand, some studies have demonstrated the tumor suppressor effects of IFITM3. For instance, one study found that the increased IFITM3 inhibits melanoma cell proliferation [114].

These varying, and at times opposing, roles of IFITM3 may be possible due to the variety of regulatory mechanisms responsible for aberrant IFITM3 expression. Although it is not yet clearly understood. Some regulatory factors associated with IFITM3 regulation include interferons via IFN receptors in the JAK/STAT pathway, transforming growth factor- β with canonical signaling that includes SMAD proteins and non-canonical signaling that includes MAPK and PI3 pathways, and the

Wnt/ β -catenin signaling. Downstream of these pathways, upregulation of IFITM3 has been demonstrated to increase MHC class I and II transcription [106, 107], ultimately permitting its role in immunomodulation.

IFITM3 interacts with other immune-related molecules including osteopontin, an extracellular glycoprotein implicated in carcinogenesis and the immune response. IFITM3 reduces osteopontin mRNA expression which has subsequent effects on cell adhesion, invasion, and metastasis [115]. In ovarian malignancy, osteopontin has been found to be overexpressed [116], leading to the promotion of cancer cell survival and progression via the activation of PI3-K/Akt pathway and induction of HIF-1 α expression [117]. Osteopontin can be produced by a number of immune cells including T-cells, B-cells, NK cells, and NKT cells and regulates the immune response in a number of ways, as demonstrated in its overexpression in auto-immune disease such as systemic lupus erythematosus, multiple sclerosis, and rheumatoid arthritis [118]. Furthermore, some evidence suggests that osteopontin may act as an immune checkpoint inhibitor by inhibiting the activation of cytotoxic T cells [119].

Another molecule that interacts with IFITM3 and is closely related to the Wnt/ β -catenin pathway is KLF4, or Kruppel Like Factor 4. It is a transcription factor that regulates a number of various pathways and biological factors, including IFITM3. In colon cancer, KLF4 has been found to negatively regulate IFITM3 by binding to its promoter, thus decreasing the expression of IFITM3 [105]. KLF4 is itself, tightly regulated by a number of pathways. One study found that in response to DNA damage, KLF4 will undergo PARylation, or addition of a ADP-ribosylation chain, by PARP1 [120]. This allows KLF4 to migrate from the nucleus to chromatin to assistance in transcription, by binding to various promoters. Thus, this suggests a possible link between DNA damage, KLF4, and IFITM3. Further investigation of this pathway and its downstream effects may provide insight into the role of

IFITM3 in immune function and the surrounding tumor immune microenvironment *BRCA*-deficient ovarian cancer.

Hypothesis and Specific Aims:

We hypothesize that *BRCA*-deficient ovarian cancer cells have increased IFITM3 expression which will lead to downstream signaling to increase MHC class I and II antigen presentation and subsequent T-cell activation (Figure 2).

Specific Aim 1. Validate the differential expression of IFITM3 in *BRCA*-deficient ovarian tumors compared to WT.

Specific Aim 2. Determine the functional role of IFITM3 on cell growth and the immune response in ovarian cancer.

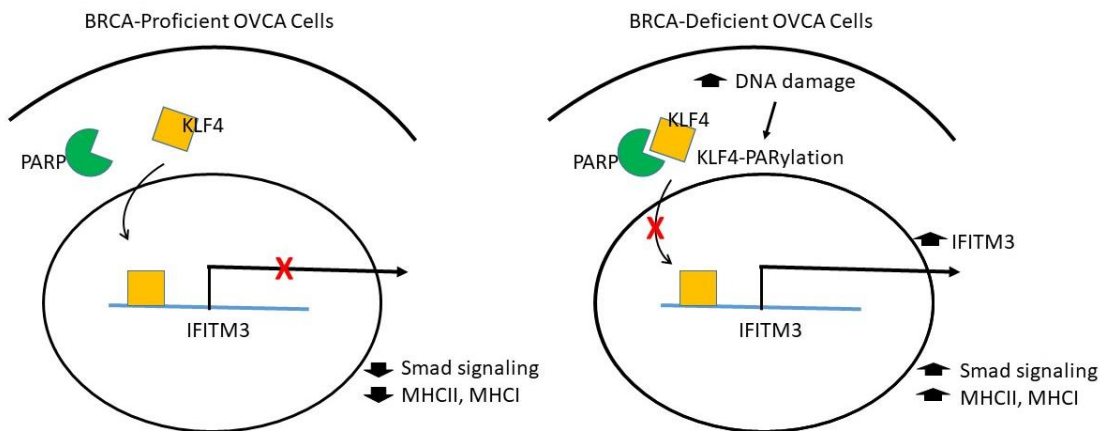


Figure 2: Proposed regulation of IFITM3 in *BRCA*-deficient ovarian carcinoma cells

Based on our hypothesis, we propose the following mechanism for IFITM3 regulation, leading to subsequent MHC class I and II antigen presentation and T-cell activation.

Methods and Materials

Patient Selection

A total of 57 formalin-fixed paraffin-embedded (FFPE) ovarian carcinoma samples were obtained from an ovarian cancer repository at The University of Texas MD Anderson Cancer Center in Houston, Texas, as well as from Gangnam Severance Hospital, Yonsei University College of Medicine in Seoul, South Korea. These samples were collected from patients who underwent primary cytoreductive surgery from 2011 to 2019 and were otherwise treatment-naïve for their ovarian carcinoma. Patients were identified with either a germline *BRCA1*-mutation, *BRCA2*-mutation, or *BRCA*-WT. For the exploratory cohort used for transcriptome analysis, 5 patients with a *BRCA1*-mutation and 5 patients with *BRCA2*-mutation all with advanced stage disease at extremes of progression free survival were utilized. Additionally, 5 *BRCA*-WT patients that were age-matched were also chosen as controls. Demographic and clinical information including race, age, treatment, progression free survival, and overall survival were collected.

All human tissue samples and clinical information used in this study were obtained in accordance approved protocols by the Institutional Review Boards at University of Texas MD Anderson Cancer Center and Yonsei University College of Medicine.

Microdissection and RNA extraction

To isolate individual cells or cell clusters, such as tumor cells or stromal cells, for gene expression analysis, laser microdissection was utilized. Paraffin-embedded tumor tissue samples were sectioned by a microtome and 10 µm sections were mounted onto PEN membrane slides. These tissue sections were then deparaffinized with xylene and then rehydrated with graded alcohol

series. Staining with 1% methyl green was used to visualize histologic features. Microdissection was then performed using a Leica LMD6 Laser Microdissection Microscope (Leica Microsystems Inc.) to separate epithelial tumor component and stromal component. During dissection, the areas of interest were carefully outlined. Areas with immune cells and blood vessels were excluded to minimize contamination. These respective enriched tissue specimens were captured into the lid of the RNase-free Safe-Lock Eppendorf tube and used for subsequent total RNA extraction.

The microdissected enriched tumor specimens were then processed for total RNA extraction using the Qiagen RNeasy FFPE Kit (Cat No. 73504) according to the manufacturer's instructions.

Microarray

Total RNA from 15 microdissected FFPE ovarian tumors were then used for transcriptome analysis using Affymetrix GeneChip Human Clariom D WT Pico microarray performed by the Advanced Technology Genomics Core (ATGC) at the University of Texas MD Anderson Cancer Center. The sample concentration was assessed using the Qubit RNA High Sensitivity Assay (Invitrogen, Life Technologies). The integrity of the total RNA was then assessed using the Agilent 2100 Bioanalyzer Picochip assay (Agilent Technologies). Samples with a concentration under 33ng/ul, degraded or FFPE were selected for target amplification with the GeneChip Whole Transcript (WT) Pico Reagent assay (ThermoFisher Scientific). Following RNA quality control, 3-10ng of total RNA input was used to process the samples for Whole Transcriptome Expression Analysis with the GeneChip WT Pico Reagent assay (ThermoFisher Scientific). The samples were reverse transcribed to generate amplified, fragmented, and biotinylated sense-strand cDNA, according to the manufacturer's standard protocol. The fragmented and labeled sscDNA was then hybridized to the respective human GeneChip Clariom D array at 45°C for 16 hours and subsequently washed and

stained using the ThermoFisher proprietary reagents in the GeneChip Fluidics Station 450 (FS450) and scanned in the GeneChip Scanner 3000 7G (ThermoFisher Scientific). CEL files generated after the GeneChip Microarray scanning were uploaded onto the Expression Console software for analysis with the Robust Multi-array Average (RMA) algorithm (ThermoFisher Scientific). Heatmaps were generated using Broad Institute Software (<https://software.broadinstitute.org/morpheus/>).

Microarray gene expression data analysis

The CEL files were processed by using Transcriptome Analysis Console (TAC) software, and the expression value for each gene was normalized across samples by RAM procedure, which was primarily designed for analyzing gene expression data from Affymetrix arrays. The differential expression analysis between two subgroups was performed by using Limma: Linear Models for Microarray Data from Bioconductor. The final p-values were adjusted using the Benjamini & Hochberg method [121]. Differential expression analysis was further evaluated utilizing the pathway enrichment tool GSEA [122].

Cell lines and culture conditions

BRCA1-deficient (*BRCA1*-null) ovarian cancer cell lines SNU251 (a gift from Dr. Jae-hoon Kim's laboratory at the Yonsei University College of Medicine) and UWB1.289 (American Type Culture Collection, Manassas, VA, USA) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2mM glutamine and penicillin/streptomycin (Life Technologies Corp., Grand Island, NY, USA), and 50% RPMI 1640 medium and 50% Mammary Epithelial Cell Growth Medium (Lonza, Basel, Switzerland) with 5% FBS and penicillin/streptomycin, respectively. Ovarian cancer cell lines with intact *BRCA1* function were also utilized. OVCA432, OVCA433, OVCA420 (gifts from Dr.

Robert Bast's laboratory at MD Anderson Cancer Center), Caov3, OV90 and OVCAR3 (American Type Culture Collection) were maintained in RPMI 1640 medium supplemented with 10% FBS, 2mM glutamine and penicillin/streptomycin (Life Technologies Corp.). PEA1 and PEO1 (European Collection of Authenticated Cell Cultures) were maintained in RPMI 1640 medium supplemented with 10% FBS, 2mM glutamine, penicillin/streptomycin and 1% sodium pyruvate (Sigma-Aldrich Co.). UWB1.289+BRCA1 (American Type Culture Collection), for which UWB1.289 cells were transduced with *BRCA1* gene to restore function, was maintained in 50% RPMI 1640 medium and 50% Mammary Epithelial Cell Growth Medium with 5% FBS, penicillin/streptomycin and 200µg/mL G418. All cell lines were tested negative for mycoplasma contamination.

Real-time quantitative reverse transcription-polymerase chain reaction

Total RNA was extracted from cultured cells using TRI reagent (Molecular Research Center) per the manufacturer's instructions. Total RNA was used to synthesize the single-stranded cDNA using the ImProm-II Reverse Transcription System (Promega Corp., Madison, WI, USA). Real-time PCR was then performed using TaqMan universal master mix, No AmpErase, UNG (Applied Biosystems). Pre-designed, FAM-labeled human IFITM3 (Hs03057129_s1), HLA-DRA (Hs00219575_m1), HLA-F (Hs01587840_m1), and VIC-labeled human GAPDH TaqMan gene expression assays (Life Technologies Corp.) were used. The relative standard curve method ($2^{-\Delta\Delta Ct}$) was used to determine the relative mRNA expression while using GAPDH as a reference.

Immunohistochemistry

Immunolocalization of IFITM3 was performed using 57 FFPE ovarian tumor sections (5 µm) from the patients described above. Slides with the sections were stained using commercially

available anti-IFITM3 antibody (1:100, HPA004337-100UL, Sigma Aldrich). The tissue sections were deparaffinized and rehydrated using a series of graded alcohols. Antigen retrieval was performed in sodium citrate buffer (pH 6.0) in a microwave at 95°C for 15 minutes. Slides were then stained with primary and secondary antibodies using the Lab Vision Autostainer 360 (Thermo Fisher Scientific, Waltham, MA, USA). Digital photomicrographs of representative areas were taken for each slide at ×20 magnification using a light microscope. The staining intensity for each slide was then quantified at least 3 times using the Fiji-Image J software (Version 1.53c, National Institute of Health, USA) by drawing around the stained tumor tissue and obtaining an intensity score from 0 (pure black) to 255 (pure white) with background correction. An average value was calculated for each slide and transformed linearly onto a scale of 0 to 1 for further analyses, such that 0 is pure white and 1 is pure black.

Multiplex Immunofluorescence and Analysis

Multiplex immunofluorescence (IF) was performed for immunolocalization of multiple markers. Fifty-seven FFPE ovarian tumor sections (5 µm) on slides were first deparaffinized and rehydrated using a series of graded alcohols. Antigen retrieval was then performed using retrieval buffer (pH 9.0) (Biogenex) in a microwave at 95°C for 15 minutes. Slides were then blocked and stained with commercially available anti-CD8 (1:50, CD8-4B11-L-CE-H, Leica) and anti-Granzyme B (1:125, 46890S, Cell Signaling Technology) primary antibodies at room temperature for 1 hour. Slides were then washed with PBS-tween, and incubated with secondary antibodies Alexa Fluor 647 goat anti-mouse (1:100, A-21236, Thermo Fisher Scientific) and Alexa Fluor 488 goat anti-rabbit (1:100, A-11034, Thermo Fisher Scientific). Slides were then finished with DAPI counterstain. Stained slides were first scanned using the Vectra Polaris automated quantitative pathology imaging system

(Akoya Biosciences, Marlborough, MA, USA) for the immunofluorescent signals. After that, the slides were stained with hematoxylin and eosin (H&E) and scanned again.

IF images were aligned to their corresponding H&E images with Visiopharm software. After tissue detection, tumor load was calculated by segmenting stroma and tumor areas. Artificial-intelligence (AI)-based pipeline was then run on IF images to detect CD8⁺, Granzyme B⁺, CD8⁺Granzyme B⁺ cells in the tumor or stroma compartments. Cell densities are represented as cells/ μm^2 by normalizing cell counts to the corresponding area (tumor, stroma, or entire tissue). Two samples were excluded from the analysis due to insufficient number of cells or poor morphology.

IFITM3 Silencing

BRCA1-null cell lines were transiently transfected with control small-interfering RNA (siRNA) (SIC001, Sigma Aldrich) or IFITM3 siRNAs (SASI_Hs02_00354510 and SASI_Hs01_00038803, NM_021034, Sigma Aldrich) duplexed with Lipofectamine RNAiMAX (Invitrogen) at a final concentration of 20 nM for 24 h to silence IFITM3 gene expression.

MTT assay

Cells were incubated with 1 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich Co.) in PBS for 3 h. The formazan that formed was then solubilized by adding dimethyl sulfoxide. The absorbance was read at 570 nm using a FLUOstar Galaxy plate reader (BMG Labtech, Offenburg, Germany).

Imaging Mass Cytometry and Analysis

Imaging mass cytometry using metal-tagged antibodies were used to further examine the immune microenvironment of our specimens. Formalin-fixed, paraffin-embedded ovarian tumor sections (10 μm) on slides were first deparaffinized with xylene and rehydrated with a graded alcohol series. Antigen retrieval was then performed using citrate buffer (pH 6) at 95°C in a decloaking chamber for 25 minutes. Slides were then blocked and stained with 21 metal-tagged antibodies (Table 3) for 2 hours at room temperature. Slides were then washed and counterstained with 0.5 μM Cell-ID Intercalator-Ir (Fluidigm).

Imaging mass cytometry data was then obtained by the Fluidigm Helios CyTOF instrument utilizing the Hyperion Imaging System laser ablation module in the Flow Cytometry and Cellular Imaging Facility at University of Texas MD Anderson Cancer Center. For each section, a 1mm³ was acquired and used for analysis. This region of interest was selected based on representative tumor sections with surrounding stroma, as observed on a corresponding hematoxylin and eosin-stained slide.

These data images were processed and converted to TIFF files using MCD Viewer (Fluidigm). Tumor cells was identified by positive pan-Keratin and cytokeratin 8/18 activity. Stromal cells were identified by positive smooth muscle actin activity. Images were then analyzed for various immune marker activity. Samples were excluded from the analysis if there were insufficient number of cells or poor morphology.

Statistical Analysis

Statistical analysis was performed using SPSS software version 24 (IBM Corp, Armonk, NY, USA) and GraphPad Prism Version 8 (GraphPad Software, Inc.). Data was pooled to represent the mean with standard deviation, unless otherwise specified. For normally distributed data, a two-tailed Student t test was used to test differences in the same means. For non-parametric data, the Mann-Whitney U test was performed. A Spearman's correlation coefficient was used to test for linear associations. A p-value of <0.05 was considered to be statistically significant.

Table 3. Imaging mass cytometry antibody panel				
Metal Tag	Antigen	Antibody Clone	Catalog No.	Vendor
141Pr	SMA	1A4	3141017D	Fluidigm
144Nd	CD14	EPR3653	3144025D	Fluidigm
147Sm	CD163	EDHu-1	3147021D	Fluidigm
149Sm	CD11b	EPR1344	3149028D	Fluidigm
151Eu	CD31	EPR3094	3151025D	Fluidigm
152Sm	CD45	CD45-2B11	3152016D	Fluidigm
153Eu	CD44	IM7	3153029D	Fluidigm
156Gd	CD4	EPR6855	3156033D	Fluidigm
158Gd	CD73	EPR6115	3158031D	Fluidigm
159Tb	CD68	KP1	3159035D	Fluidigm
160Gd	Vista	D1L2G	3160025D	Fluidigm
161Dy	CD20	H1	3161029D	Fluidigm
162Dy	CD8a	D8A8Y	3162035D	Fluidigm
163Dy	CD196/ CCR6	Polyclonal	3163029D	Fluidigm
166Er	B7-H4	H74	3166030D	Fluidigm
167Er	Granzyme B	EPR20129-217	3167021D	Fluidigm
168Er	Ki-67	B56	3168022D	Fluidigm
169Tm	Collagen Type I	Polyclonal	3169023D	Fluidigm
171Yb	Histone 3	D1H2	3171022D	Fluidigm
173Yb	CD45RO	UCHL1	3173016D	Fluidigm
174Yb	Keratin 8/18	C51	3174022D	Fluidigm

Results

Upregulated IFITM3 in *BRCA*-deficient ovarian carcinoma cells

To validate the differential expression of IFITM3 in *BRCA*-deficient ovarian tumors compared to WT tumors, treatment-naïve primary tumors from patients with high-grade serous ovarian carcinoma were evaluated for IFITM3 protein expression using immunohistochemistry staining. A total of 57 samples were used, including samples from patients with *BRCA1*-mutation (n=12), *BRCA2*-mutation (n=9), and *BRCA*-WT (n=36). Patients ranged in race or ethnicity and also stage of disease (Table 4). These stains demonstrated that ovarian tumors with *BRCA1* or *BRCA2*- deficiency had a significantly higher amount of IFITM3 protein expression compared to WT tumors ($p=0.0001$, $p=0.0001$) (Figure 3).

Additionally, IFITM3 mRNA expression was also evaluated in various ovarian cancer cell lines, including *BRCA1*-null cell lines, to further assess for differential expression (Figure 4A). IFITM3 mRNA expression varied amongst these cell lines. However, a comparison of UWB1.289, a *BRCA1*-null cell line, and UWB1.289+*BRCA1*, which is the UWB1.289 cell line which has been transduced with *BRCA1* gene to restore function, demonstrates that when *BRCA1* function is lost, IFITM3 mRNA expression is increased ($p=0.0001$) (Figure 4B).

From these studies, we are able to demonstrate that there is an upregulation of IFITM3 expression in *BRCA*-deficient ovarian carcinoma tumors.

Table 4. Patient demographic for the immunohistochemistry staining			
	BRCA 1 (n=12)	BRCA 2 (n=9)	WT (n=36)
Race/Ethnicity			
Asian	8 (66.7%)	7 (77.8%)	1 (2.8%)
Black	1 (8.3%)	0 (0%)	5 (13.9%)
Hispanic	0 (0%)	1 (11.1%)	5 (13.9%)
Caucasian	3 (25%)	1 (11.1%)	24 (66.7%)
Unknown	0 (0%)	0 (0%)	1 (2.8%)
Histology			
Serous	12 (100%)	9 (100%)	36 (100%)
Stage			
I	1 (8.3%)	0 (0%)	2 (5.6%)
II	0 (0%)	0 (0%)	4 (11.1%)
III	10 (83.3%)	7 (77.8%)	28 (77.8%)
IV	1 (8.3%)	2 (22.2%)	2 (5.6%)
Age at Diagnosis			
Median (IQR)	55 (6)	58 (17.5)	61 (15.75)
Progression Free Survival			
Median (IQR)	21.5 mo (46)	23 mo (36)	18 mo (30)

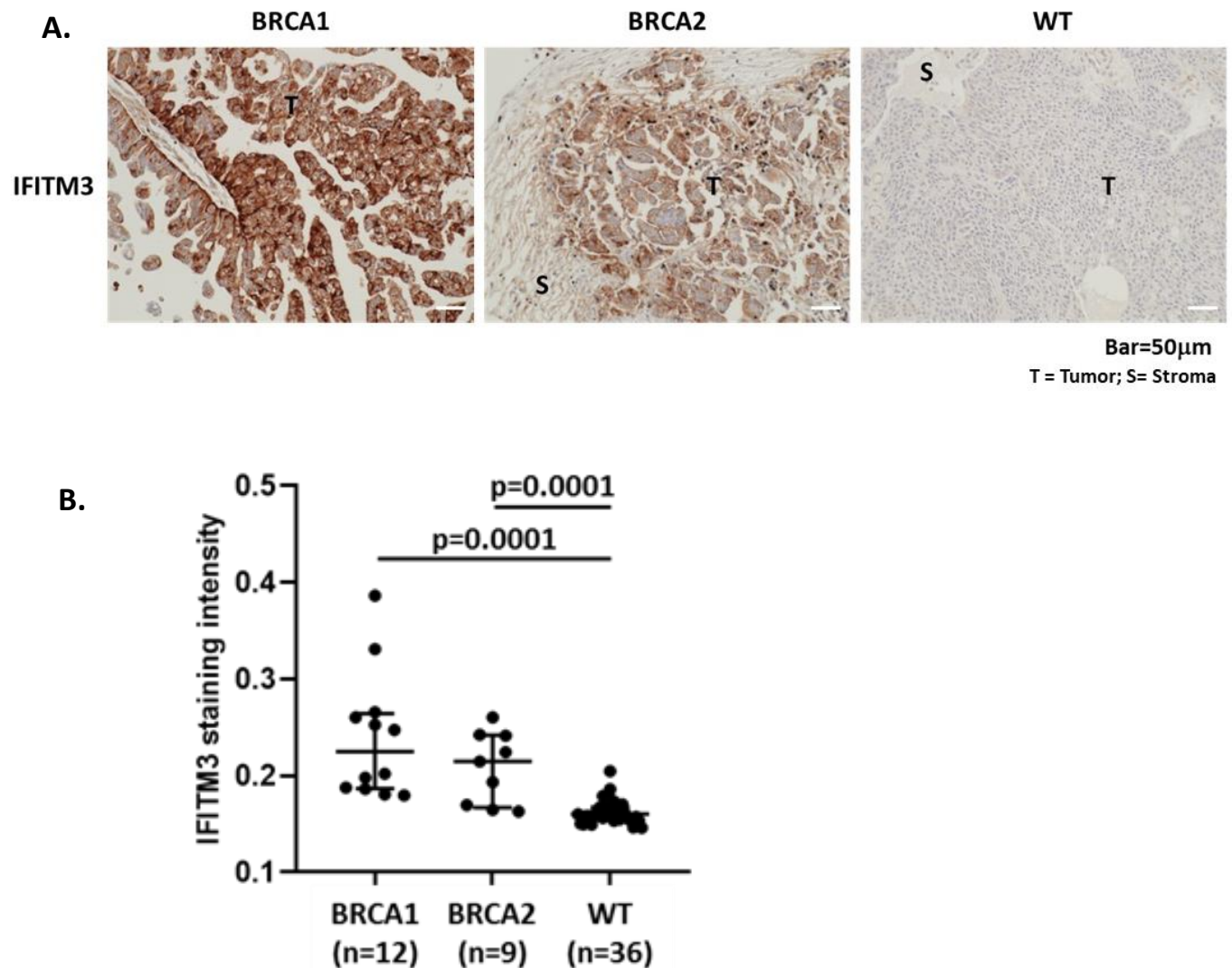


Figure 3. Increased IFITM3 protein expression in *BRCA*-deficient high grade serous ovarian carcinomas

A. Representative images from immunohistochemistry staining demonstrating a higher IFITM3 expression level in HGSOC with *BRCA1*- or *BRCA2*-mutations compared to *BRCA*-WT tumors. T tumor, S stroma; Bar=50μm. B. Staining intensity of IFITM3 was quantified for each comparison group. Total 57 samples, *BRCA1* n=12, *BRCA2* n=9, WT n=36.

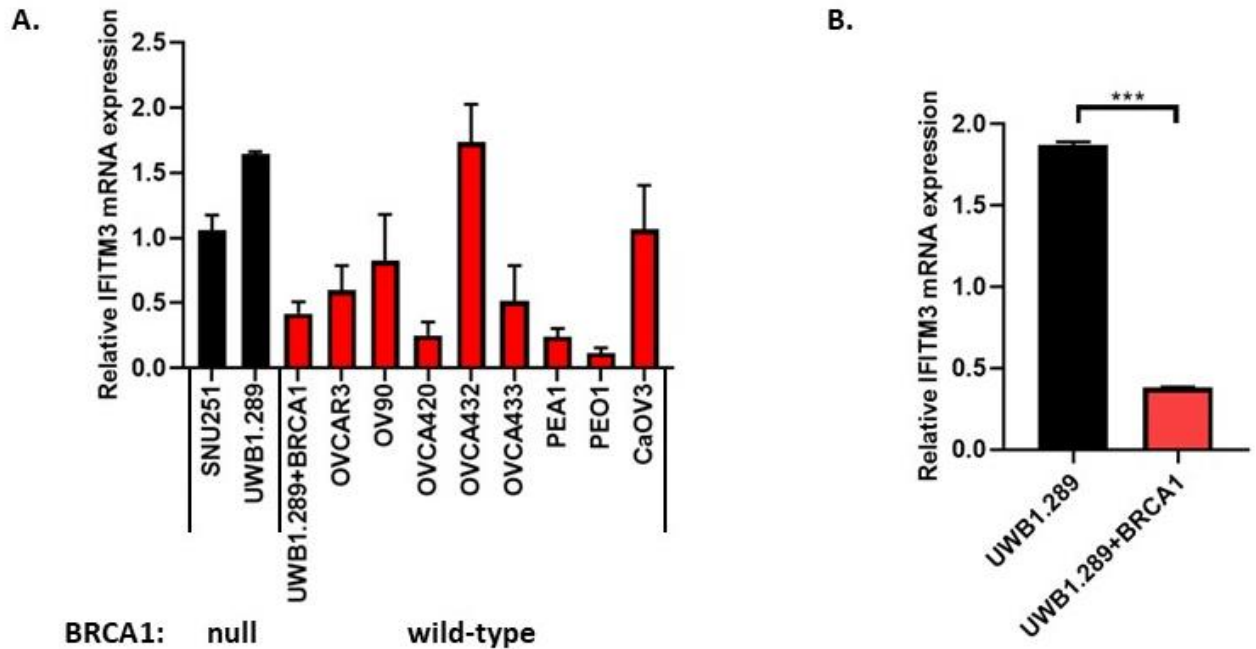


Figure 4. Relative IFITM3 mRNA expression in *BRCA1*-null and wildtype ovarian cancer cells

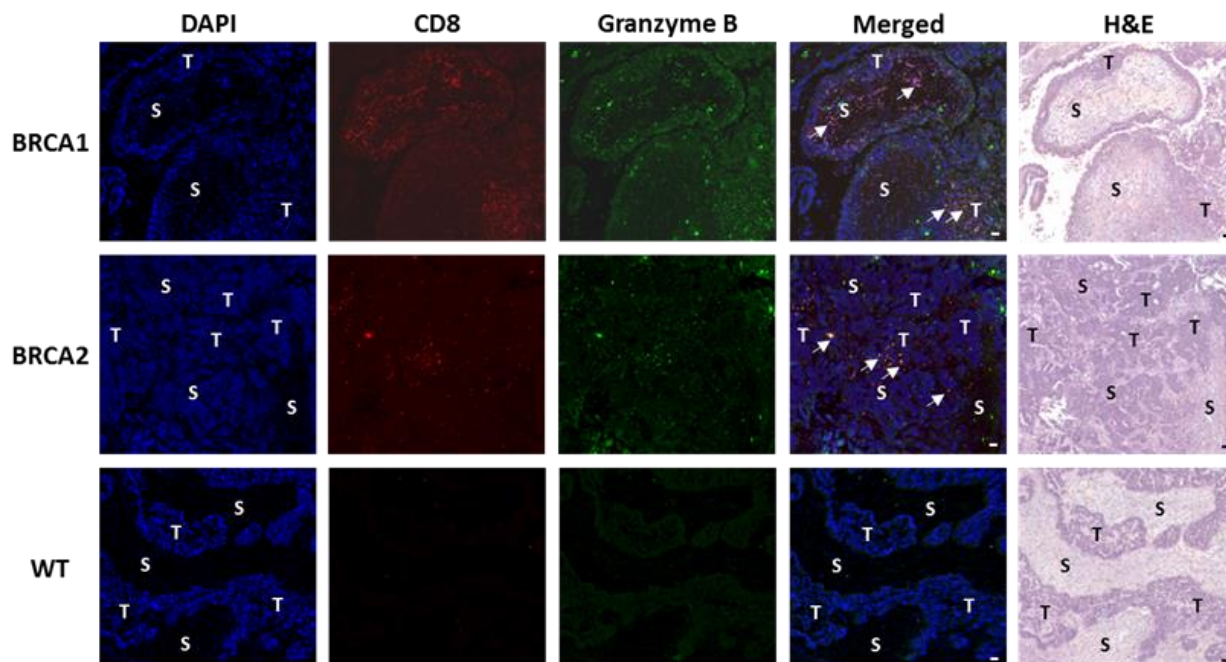
A. QRT-PCR analysis was performed to evaluate the relative IFITM3 mRNA expression in various ovarian cancer cell lines including *BRCA1*-null ovarian cancer cells. B. When *BRCA1* is restored in the UWB1.289 *BRCA1*-null ovarian cancer cell line, IFITM3 mRNA expression is significantly decreased ($p=0.0001$; $n=3$).

Increased IFITM3 expression is correlated with the presence of activated cytotoxic T cells in *BRCA*-deficient ovarian carcinoma

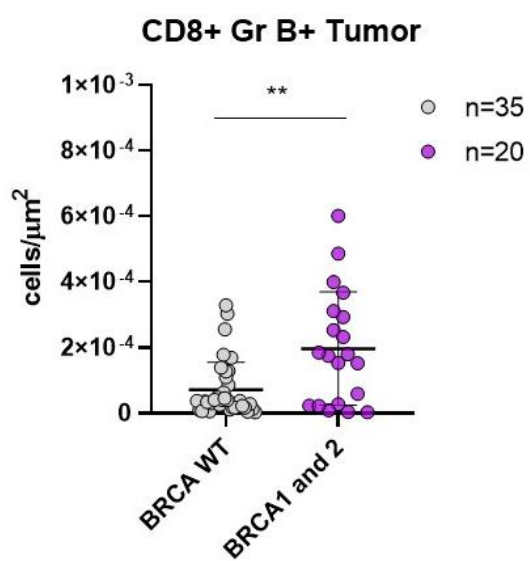
Based on these findings, we sought to determine if there is a correlation between IFITM3 expression and immune function in *BRCA*-deficient ovarian tumors. First, immunolocalization of CD8 and Granzyme B was performed using multiplex immunofluorescence in the previously mentioned 57 ovarian tumor samples to determine the presence of activated cytotoxic T cells (CD8⁺Granzyme B⁺) (Figure 5). The density of CD8⁺GranzymeB⁺ cells was assessed in the tumor region, stromal region, and total tissue of the ovarian tumor sections. In all regions, CD8⁺GranzymeB⁺ cells had a significantly higher density in *BRCA*-deficient ovarian tumors compared to the *BRCA*-WT tumors (tumor $p=0.0007$, stroma $p=0.0030$, total tissue $p=0.0004$). Figure 6 demonstrates that there is a significant correlation between IFITM3 expression and CD8⁺GranzymeB⁺ cells in the tumor region ($r=0.298$, $p=0.034$), stromal region ($r=0.347$, $p=0.013$), and total tissue ($r=0.309$, $p=0.027$) of the ovarian tumor sections ($n=51$).

Imaging mass cytometry (IMC) was also used to evaluate multiple simultaneous protein markers including B cell marker CD20 and helper T cell marker CD4. This analysis included a smaller cohort of patients due to limitations of morphology ($n=23$). Presence of CD20⁺ cells was significantly higher in *BRCA*-deficient group compared to WT group within the tumor region ($p=0.035$) and in the total region ($p=0.02$) (Figure 7). Additionally, the presence of CD4⁺ cells was significantly higher in the *BRCA*-deficient cohort compared to the WT cohort in the total tissue ($p=0.04$). These findings along with the multiplex immunofluorescence suggest an increased immune response of activated T cells, helper T cells, and B cells in the *BRCA*-deficient ovarian tumors.

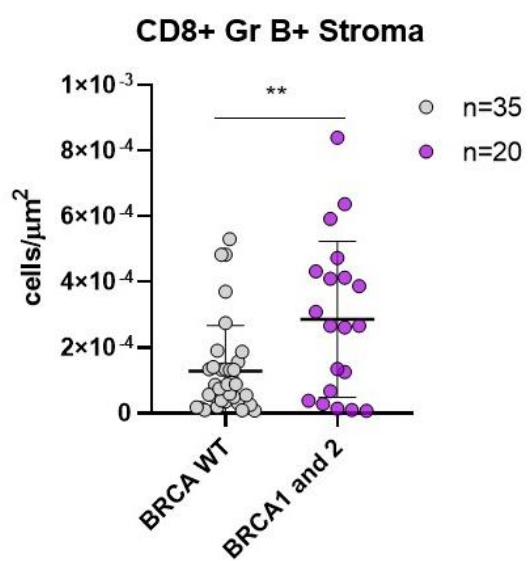
A.



B.



C.



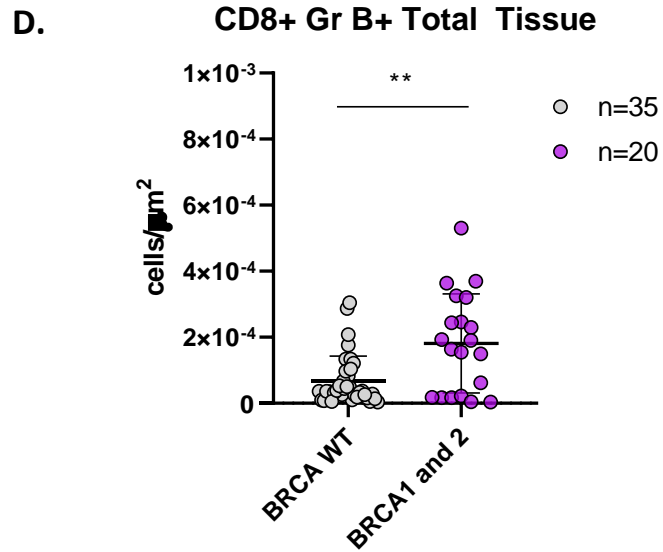
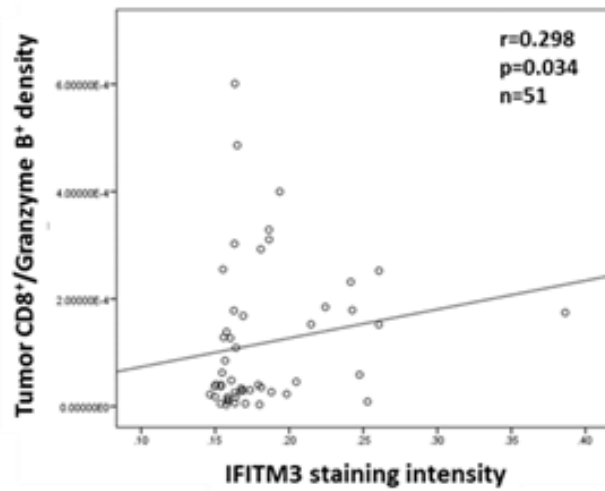


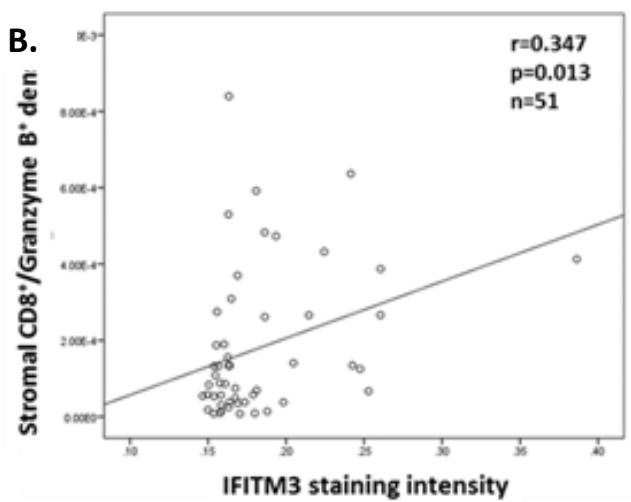
Figure 5. Multiplex immunofluorescence for activated cytotoxic T cells in *BRCA*-deficient and *BRCA*-wildtype ovarian tumors

A. Representative images from immunolocalization of CD8 and Granzyme B using multiplex immunofluorescence demonstrating a significantly higher CD8⁺GranzymeB⁺ expression level in HGSOC with *BRCA1*- or *BRCA2*-mutations compared to *BRCA*-WT tumors. DAPI stained in blue. CD8 stained in red. Granzyme B stained in green. Arrow directed at CD8⁺GranzymeB⁺ Cells. T: tumor, S: stroma; Bar=50 μm . B. Cell density of CD8⁺GranzymeB⁺ cell within the tumor region was quantified for each comparison group. C. Cell density of CD8⁺GranzymeB⁺ cell within the stromal region was quantified for each comparison group. D. Cell density of CD8⁺GranzymeB⁺ cell within total tissue section was quantified for each comparison group. Total 55 samples, *BRCA1* and *BRCA2* n=20, *BRCA* WT n=35.

A.



B.



C.

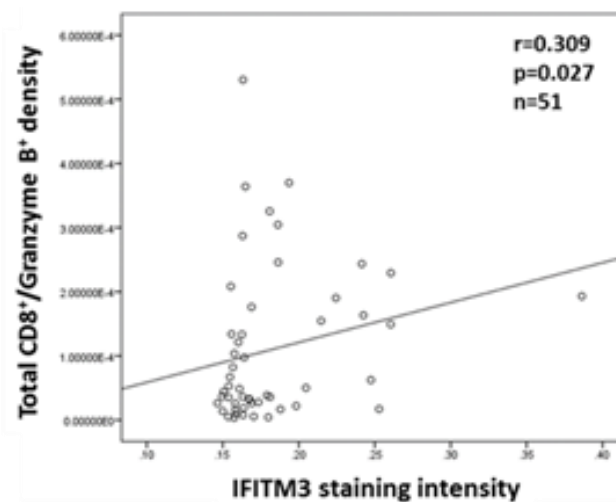


Figure 6. Correlation of IFITM3 and activated cytotoxic T cells

The correlation between IFITM3 protein expression and CD8⁺Granzyme B⁺ cells is potted here for both the tumor region (A), stromal region (B), and total tissue section (C) ($n=51$)

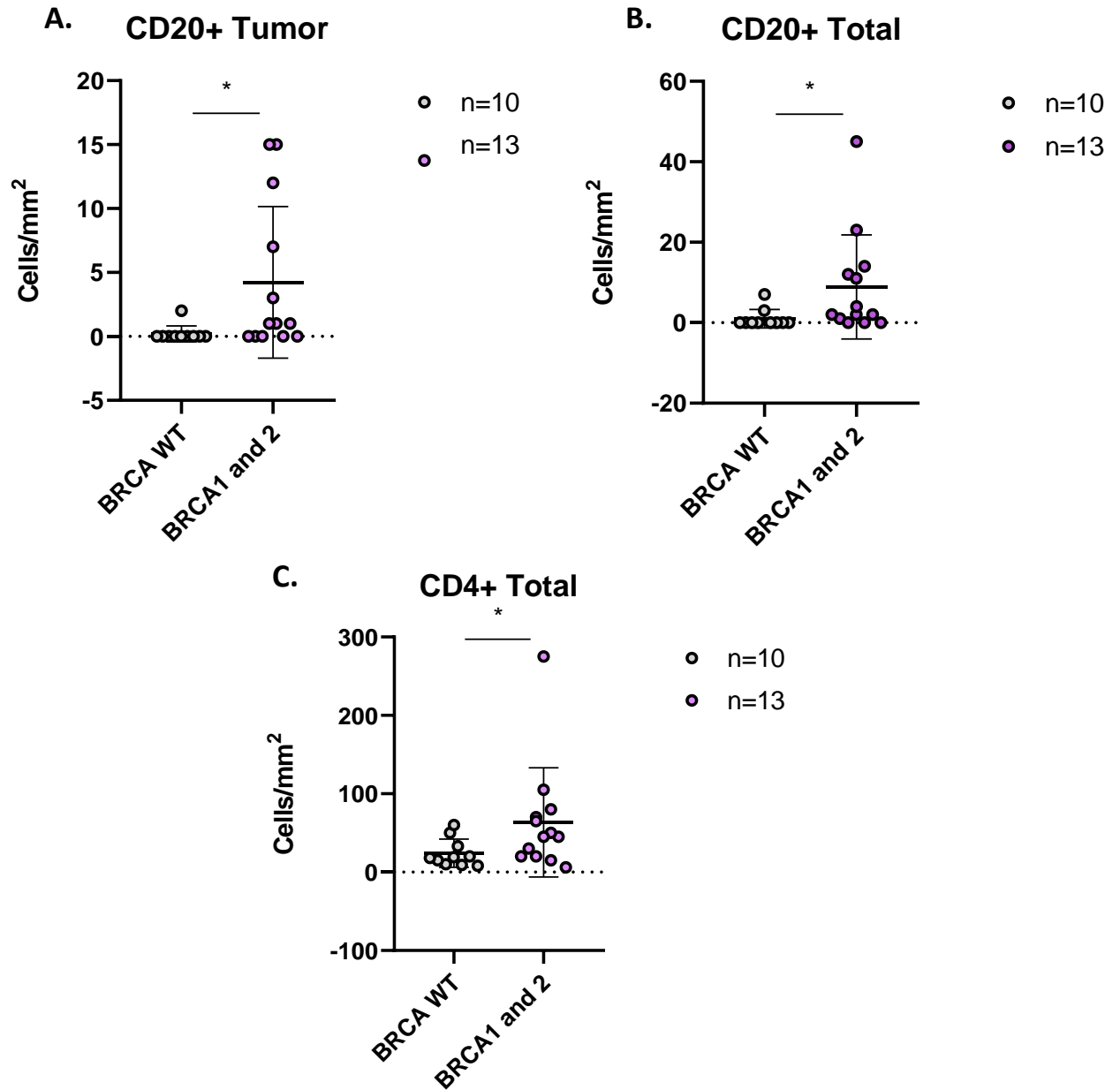


Figure 7. Imaging mass cytometry in *BRCA*-deficient and *BRCA* wildtype ovarian tumors

Imaging mass cytometry (IMC) was performed to evaluate multiple simultaneous protein markers.

A. Cell density of CD20⁺ cells within the tumor region. B. Cell density of CD20⁺ cells within total tissue

section. C. Cell density of CD4⁺ cells within total tissue section. (*BRCA* WT n=10, *BRCA1* and 2 n= 13)

Increased IFITM3 expression is correlated with HLA –F and HLA-DRA expression in *BRCA*-deficient ovarian carcinoma

Next, a correlation between IFITM3 expression and HLA expression was examined. Using the transcriptome data performed on our exploratory cohort of 15 patients, we examined the expression of various HLA molecules. In particular, 2 HLA molecules were correlated with IFITM3 expression: HLA-F, which encodes for MHC class I molecule, and HLA-DRA, which encodes for a MHC class II molecule. In this cohort, there is a strong and statistically significant correlation between their expression and IFITM mRNA expression ($r=0.718$, $p=0.03$; $r=0.617$, $p=0.014$) (Figure 8A and 8B).

This was further explored using the ovarian cancer cell lines. mRNA expression of HLA-F from *BRCA1*-null ovarian cell line, UWB1.289, was compared to UWB1.289+*BRCA1* and was significantly higher in the *BRCA1*-null group ($p=0.0001$) (Figure 8C). HLA-DRA could not be examined in this cell line because it is not expression.

Overall, these findings suggests a positive relationship between IFITM3 and HLA-F and HLA-DRA in *BRCA*-deficient ovarian carcinoma cells.

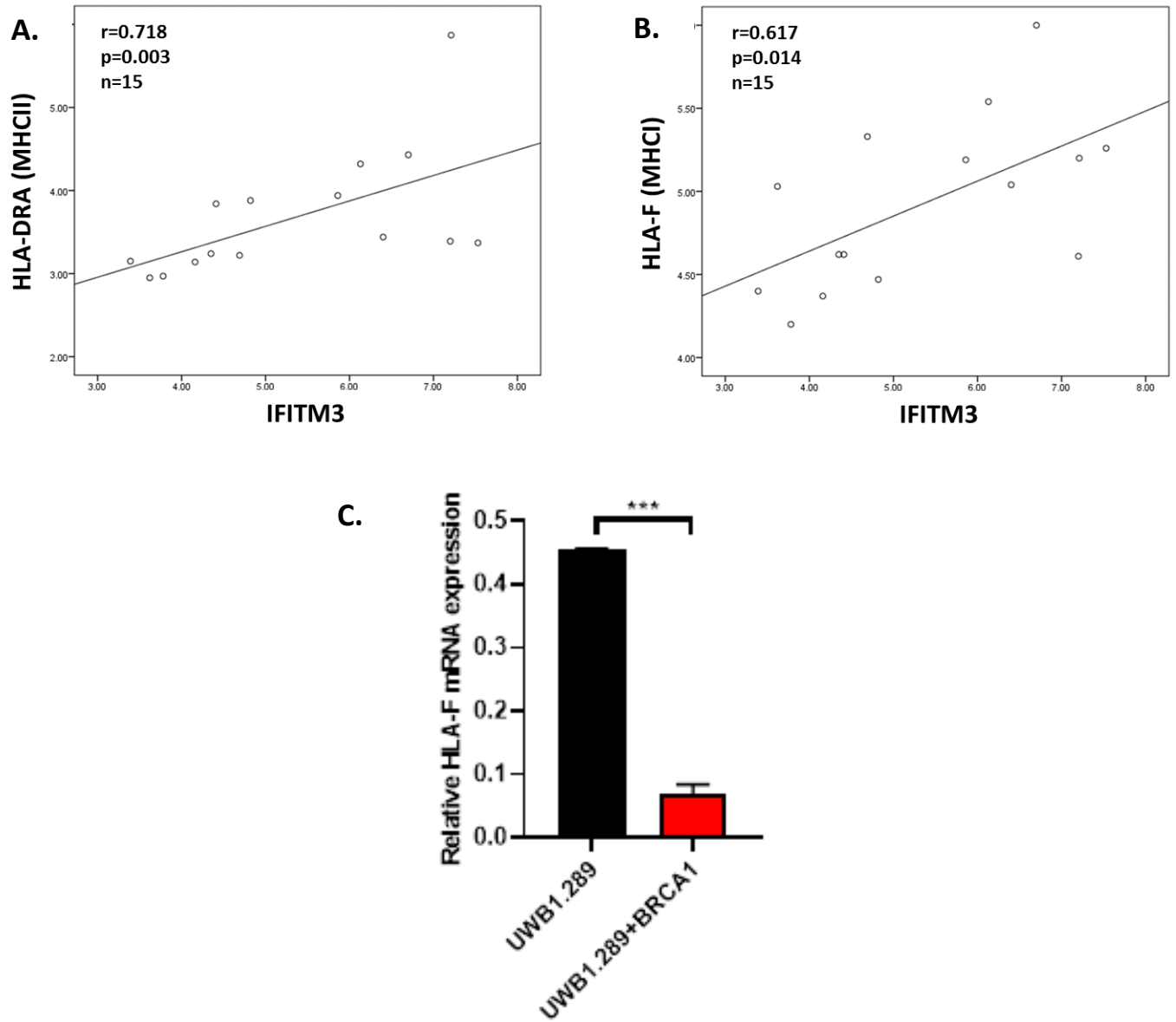


Figure 8. Correlation of IFITM3 and HLA expression

A. The correlation between mRNA expression of HLA-DRA and IFITM3 from microdissected tumor cells ($n=15$). B. The correlation between mRNA expression of HLA-F and IFITM3 from microdissected tumor cells ($n=15$). C. QRT-PCR analysis was performed to evaluate the relative mRNA expression of HLA-F in *BRCA1*-null ovarian cancer cell line, UWB1.289, and *BRCA1* restored cell line, UWB1.289+*BRCA1* ($n=3$).

Decreased IFITM3 expression affects the immune response and cell growth in *BRCA*-deficient ovarian carcinoma cells

Based on these findings demonstrating a correlation between the immune response and IFITM3 in *BRCA*-deficient ovarian carcinoma, we sought to further elucidate the role of IFITM3 on the immune response. In vitro studies were conducted using *BRCA1*-null ovarian cancer cell lines. To downregulate the expression of IFITM3, siRNA directed against IFITM3 were used (Figure 9A). A decrease in IFITM3 expression subsequently decreased HLA-F mRNA expression in both *BRCA1*-null cell lines SNU251 ($p < 0.0001$, $p < 0.0001$) and UWB1.289 ($p < 0.0001$, $p < 0.001$) (Figure 9B) and decreased HLA-DRA mRNA expression in SNU251 ($p < 0.0001$, $p < 0.001$) (Figure 9C). HLA-DRA could not be tested in UWB1.289 because it is not expressed in this cell line.

The effect of silencing IFITM3 on ovarian cancer cell growth was also evaluated in SNU251 and UWB1.289 cells (Figure 9D). The downregulation of IFITM3 leads to a subsequent increase in relative proliferation of these respective *BRCA1*-null ovarian cancer cell lines ($p < 0.0001$, $p < 0.0001$). Thus, this suggests that IFITM3 has an inhibitory effect on tumor cell growth in *BRCA*-deficient ovarian cancer cells.

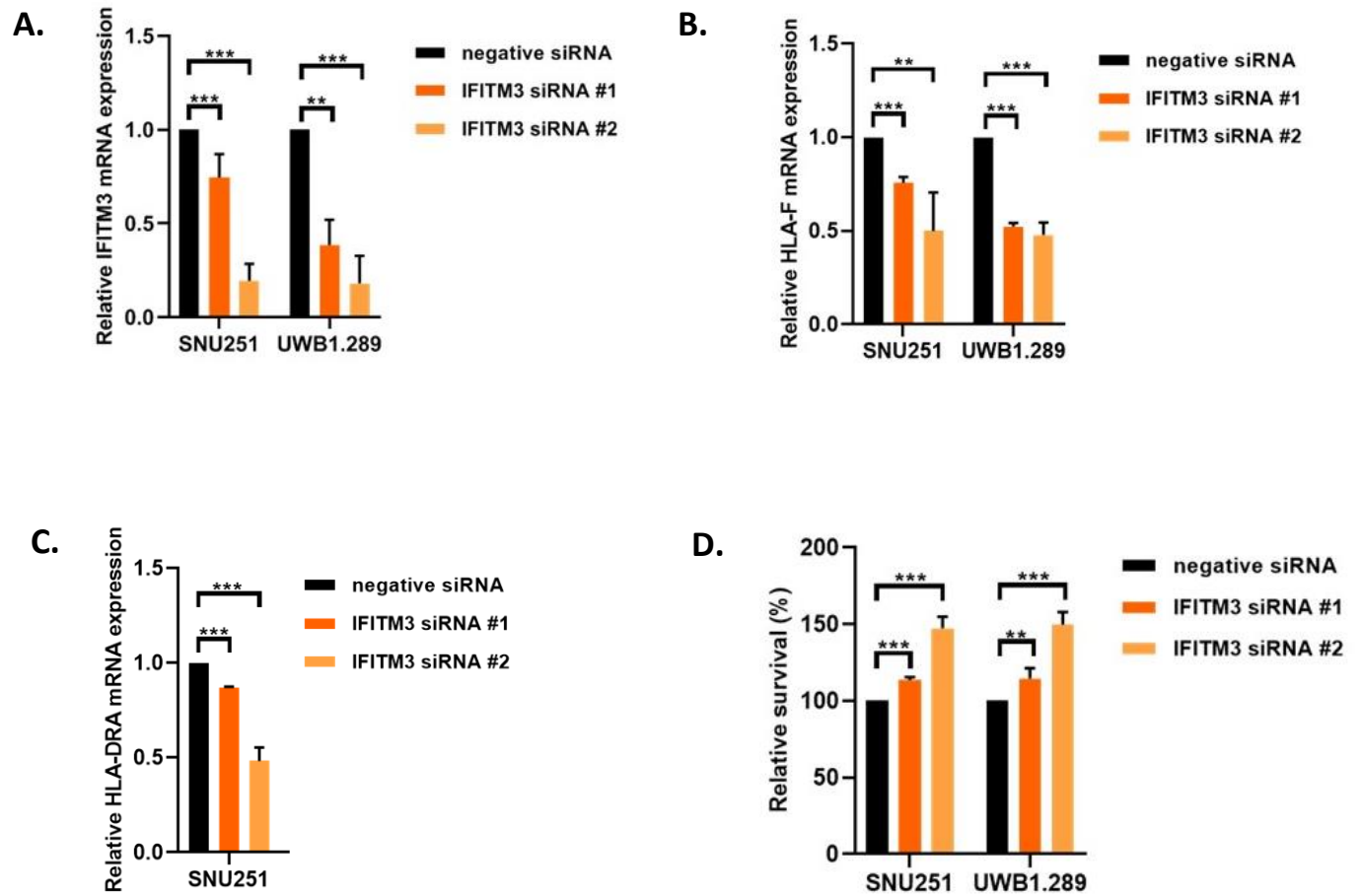


Figure 9. Effects of downregulation of IFITM3 in *BRCA1*-null ovarian carcinoma cells

BRCA1-null ovarian carcinoma cell lines were transiently transfected with IFITM3 siRNA (n=3). A. QRT-PCR was then used to confirm the downregulation of IFITM3 mRNA. B. QRT-PCR was used to measure the relative mRNA expression of HLA-F following downregulation of IFITM3. C. QRT-PCR was used to measure the relative mRNA expression of HLA-DRA following downregulation of IFITM3. D. MTT assay was used to determine relative proliferation of cells following downregulation of IFITM3.

Discussion

Key Findings

The goal of our study was to determine if increased DNA damage in *BRCA*-deficient ovarian cancer cells leads to upregulation of IFITM3 expression and subsequent MHC class I and class II expression as well as T cell activation. This study validated the increased expression of IFITM3 in *BRCA*-deficient ovarian carcinoma cells. It also demonstrated a positive relationship between IFITM3 and activated cytotoxic T cells and a stimulatory effect on HLA expression in these tumors. Moreover, it showed that IFITM3 has a negative effect on *BRCA*-deficient HGSOC cell growth. Overall, this study supports a role for IFITM3 in the immune response and cancer cell growth in *BRCA*-deficient HGSOC.

IFITM3 is known to play a critical role in the immune response to various pathogens and cancer. Thus, its immune-stimulatory effect in *BRCA*-deficient HGSOC is consistent with other studies and its broader role in other disease entities. Prior studies have demonstrated that IFITM3 upregulates MHC class I and II molecules [106, 107], similar to the results of our study. Additionally, its immunostimulatory role is demonstrated in mice with viral infections [123], as well as in its ability to promote Th2 differentiation [124]. Although our study was able to demonstrate a correlation between IFITM3 and various lymphocyte populations as well as show an effect of IFITM3 on HLA expression, its exact role in the immune response in ovarian cancer is yet to be elicited. However, our study provides initial novel insight into its involvement in the immunomodulatory response in *BRCA*-deficient HGSOC, and how this may be related to cancer progression.

IFITM3 has never before been examined in ovarian carcinoma. Then again, prior studies have investigated its expression in ovarian tissue. It has been identified in female germline stem cells and utilized as a germ cell marker [125, 126]. Additionally, it has been identified in human

ovarian endometriosis [127]. However, no previous studies have demonstrated an association with IFITM3 with ovarian carcinoma, despite its association with other various malignancies. Also, no prior studies have identified a relationship between IFITM3 and *BRCA1*- or *BRCA2*-mutation.

Although a recent study did demonstrate the overexpression of IFITM3 in prostate cancer in African American men, which was also found to have lower DNA repair [128]. Thus, the relationship of IFITM3 and impaired DNA damage repair may be applicable in other cancer types, not solely the ovary.

Prior studies have yet to demonstrate a uniform role of IFITM3 throughout various malignancies, possibly due to different regulation pathways. In some malignancies, it functions as a tumor suppressor protein, such as in melanoma [114], while in others its overexpression appears to be related to oncogenesis [105, 111, 112]. Our study demonstrates that in *BRCA*-deficient ovarian carcinoma, IFITM3 displays a tumor suppressor role. Its upregulation has a negative effect on ovarian cancer cell growth. However, the full understanding of this mechanism, including its regulation system, is yet to be determined. However, this data may provide an alternative mechanism for improved survival in *BRCA*-deficient ovarian carcinomas.

Limitations

This study has a number of limitations which may be addressed with future investigations. First, the cohort of patients included are small. As a translational study, we were limited by the samples available which met inclusion criteria, including treatment-naïve tumor specimens. To avoid compounding effects of chemotherapy, patients who received neoadjuvant chemotherapy followed by interval cytoreductive surgery were excluded from the study. In addition, for the analysis of the IMC data, many samples were excluded due to poor morphology or difficulty with staining, which

further decreased our cohort size. Because our patient cohort is small, we are unable to control for confounding factors, such as race or ethnicity in our study. In the future, we may consider expanding our patient population by including other collaborators or by including patients with HRD. In this way, we may be able to incorporate additional patients, but also would evaluate the function of IFITM3 in cells lacking additional DNA damage repair mechanisms, not solely from *BRCA1*-or *BRCA2*-deficiency, and determine if its function remains the same in a broader population.

Another limitation of this study was the minimal use of markers to generate our IMC data. Optimizing the panel of metal-tagged antibodies will allow for a more robust assessment of the tumor immune microenvironment. Our laboratory has developed a panel of 36 metal-tagged antibodies for which we will plan to use for our samples in the future to better identify more immune populations such as T regulatory cells, M1 and M2 macrophages, and B cells. This will provide better correlation between immune biomarkers and IFITM3 in these tumors.

Lastly, this study provides initial evidence for the role of IFITM3 in *BRCA*-deficient ovarian tumors, but many of the data only demonstrate correlative results. Further work must be done with additional functional studies to validate the effect of IFITM3 in this pathway and its mechanism.

Future Directions

Despite the insight provided from this study, additional questions are left to be explored regarding IFITM3 and *BRCA*-deficient ovarian carcinoma. First, we will plan to perform more function studies to determine the direct effect of IFITM3 in this model and its mechanism of action. We will determine the regulatory role of *BRCA* in IFITM3-mediated effects in ovarian carcinoma by performing promoter studies with WT and mutated KLF4 binding sites, as well as examining immunoprecipitation of KLF4 and IFITM3 promoter. A more comprehensive understanding of the

pathway that regulates IFITM3 and its downstream effects can guide us on the utility of IFITM3 or KLF4 as potential therapeutic targets to exploit in the future.

Based on our hypothesis, PARP participates in the regulation of IFITM3 through the PARylation of KLF4, a known regulator of IFITM3. We can better test this interaction in *BRCA*-deficient cells by utilizing a proximity ligation assay. Furthermore, we will also plan to evaluate the effect of PARP inhibitors on IFITM3 expression and cancer cell growth. The clinical implications of PARP inhibition in this pathway will be relevant as PARP inhibitors have become a standard in the treatment of *BRCA*-deficient ovarian tumors.

Other immune pathways implicated in IFITM3 regulation may further be assessed as well including the interaction of IFITM3 and osteopontin in *BRCA*-deficient ovarian carcinoma. Osteopontin has previously been demonstrated to have immune regulatory effects and also been shown to be regulated by IFITM. Thus, the role of IFITM3 in this pathway may be elucidated, particularly in *BRCA*-deficient ovarian carcinoma. A prior study in breast cancer cells has demonstrated the metastatic role of osteopontin in *BRCA1*-deficient cells [129].

Last, because of the upregulation of IFITM3 noted in *BRCA*-deficient cells, its use as a novel biomarker should be explored. Currently, there is no consensus screening for ovarian carcinoma in high risk women because no effective biomarker or screening method is available. An effective, reliable, and non-invasive method of screening for early detection of ovarian carcinoma is critically needed. Because of the demonstrated overexpression of IFITM3 in *BRCA*-deficient ovarian carcinoma cells, IFITM3 would be an excellent candidate to evaluate for this role.

Conclusion

The effect of IFITM3 on the immune response and tumor cell growth in *BRCA*-deficient ovarian carcinoma cells is a novel relationship that provides insight into the association of IFITM3 with DNA damage, the tumor immune microenvironment of HGSOC, and its immune response. In our study, the function of IFITM3 is specific to *BRCA*-deficient ovarian tumor cells, which suggests that the role of IFITM3 in ovarian cancer may only be relevant in cells lacking DNA damage repair mechanisms. Furthermore, this relationship of IFITM3 with impaired DNA damage repair may transcend cancer types and be a common mechanism that is applicable in other cancers that also lack intrinsic DNA repair capacity.

Additionally, although the favorable prognosis of *BRCA*-deficient HGSOC has previously been linked to chemosensitivity, the role of IFITM3 in response to DNA damage may provide an alternative mechanism for improved survival through the immune response. Immunomodulation is known to occur in *BRCA*-deficient HGSOC and is associated with survival, but the exact mechanism or relationship has never been fully elucidated. This study suggests a distinct pathway linking DNA damage, immune function, and survival. Further investigation will be needed to better comprehend this pathway; however, it may provide us with an alternative approach to therapy and allow us to exploit immunotherapy use in ovarian carcinoma.

Bibliography

1. Society, A.C., *Cancer Facts & Figures 2021*. 2021.
2. *Cancer Stat Facts: Ovarian Cancer*. 2020 [cited 2020 June 11, 2020]; Available from: <https://seer.cancer.gov/statfacts/html/ovary.html>.
3. Prat, J., *Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features*. *Virchows Arch*, 2012. **460**(3): p. 237-49.
4. Network, C.G.A.R., *Integrated genomic analyses of ovarian carcinoma*. *Nature*, 2011. **474**(7353): p. 609-15.
5. Patch, A.M., E.L. Christie, D. Etemadmoghadam, D.W. Garsed, J. George, S. Fereday, K. Nones, P. Cowin, K. Alsop, P.J. Bailey, K.S. Kassahn, F. Newell, M.C. Quinn, S. Kazakoff, K. Quek, C. Wilhelm-Benartzi, E. Curry, H.S. Leong, A. Hamilton, L. Mileschkin, G. Au-Yeung, C. Kennedy, J. Hung, Y.E. Chiew, P. Harnett, M. Friedlander, M. Quinn, J. Pyman, S. Cordner, P. O'Brien, J. Leditschke, G. Young, K. Strachan, P. Waring, W. Azar, C. Mitchell, N. Traficante, J. Hendley, H. Thorne, M. Shackleton, D.K. Miller, G.M. Arnau, R.W. Tothill, T.P. Holloway, T. Semple, I. Harliwong, C. Nourse, E. Nourbakhsh, S. Manning, S. Idrisoglu, T.J. Bruxner, A.N. Christ, B. Poudel, O. Holmes, M. Anderson, C. Leonard, A. Lonie, N. Hall, S. Wood, D.F. Taylor, Q. Xu, J.L. Fink, N. Waddell, R. Drapkin, E. Stronach, H. Gabra, R. Brown, A. Jewell, S.H. Nagaraj, E. Markham, P.J. Wilson, J. Ellul, O. McNally, M.A. Doyle, R. Vedururu, C. Stewart, E. Lengyel, J.V. Pearson, A. deFazio, S.M. Grimmond, D.D. Bowtell, and A.O.C.S. Group, *Whole-genome characterization of chemoresistant ovarian cancer*. *Nature*, 2015. **521**(7553): p. 489-94.
6. Levanon, K., C. Crum, and R. Drapkin, *New insights into the pathogenesis of serous ovarian cancer and its clinical impact*. *J Clin Oncol*, 2008. **26**(32): p. 5284-93.

7. Erickson, B.K., M.G. Conner, and C.N. Landen, *The role of the fallopian tube in the origin of ovarian cancer*. Am J Obstet Gynecol, 2013. **209**(5): p. 409-14.
8. Carlson, J.W., A. Miron, E.A. Jarboe, M.M. Parast, M.S. Hirsch, Y. Lee, M.G. Muto, D. Kindelberger, and C.P. Crum, *Serous tubal intraepithelial carcinoma: its potential role in primary peritoneal serous carcinoma and serous cancer prevention*. J Clin Oncol, 2008. **26**(25): p. 4160-5.
9. Labidi-Galy, S.I., E. Papp, D. Hallberg, N. Niknafs, V. Adleff, M. Noe, R. Bhattacharya, M. Novak, S. Jones, J. Phallen, C.A. Hruban, M.S. Hirsch, D.I. Lin, L. Schwartz, C.L. Maire, J.C. Tille, M. Bowden, A. Ayhan, L.D. Wood, R.B. Scharpf, R. Kurman, T.L. Wang, I.M. Shih, R. Karchin, R. Drapkin, and V.E. Velculescu, *High grade serous ovarian carcinomas originate in the fallopian tube*. Nat Commun, 2017. **8**(1): p. 1093.
10. Bowtell, D.D., S. Böhm, A.A. Ahmed, P.J. Aspuria, R.C. Bast, V. Beral, J.S. Berek, M.J. Birrer, S. Blagden, M.A. Bookman, J.D. Brenton, K.B. Chiappinelli, F.C. Martins, G. Coukos, R. Drapkin, R. Edmondson, C. Fotopoulou, H. Gabra, J. Galon, C. Gourley, V. Heong, D.G. Huntsman, M. Iwanicki, B.Y. Karlan, A. Kaye, E. Lengyel, D.A. Levine, K.H. Lu, I.A. McNeish, U. Menon, S.A. Narod, B.H. Nelson, K.P. Nephew, P. Pharoah, D.J. Powell, P. Ramos, I.L. Romero, C.L. Scott, A.K. Sood, E.A. Stronach, and F.R. Balkwill, *Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer*. Nat Rev Cancer, 2015. **15**(11): p. 668-79.
11. Kroeger, P.T. and R. Drapkin, *Pathogenesis and heterogeneity of ovarian cancer*. Curr Opin Obstet Gynecol, 2017. **29**(1): p. 26-34.
12. Alsop, K., S. Fereday, C. Meldrum, A. deFazio, C. Emmanuel, J. George, A. Dobrovic, M.J. Birrer, P.M. Webb, C. Stewart, M. Friedlander, S. Fox, D. Bowtell, and G. Mitchell, *BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women*

- with ovarian cancer: a report from the Australian Ovarian Cancer Study Group.* J Clin Oncol, 2012. **30**(21): p. 2654-63.
13. Torre, L.A., B. Trabert, C.E. DeSantis, K.D. Miller, G. Samimi, C.D. Runowicz, M.M. Gaudet, A. Jemal, and R.L. Siegel, *Ovarian cancer statistics, 2018.* CA Cancer J Clin, 2018. **68**(4): p. 284-296.
 14. Boyd, J., Y. Sonoda, M.G. Federici, F. Bogomolnii, E. Rhei, D.L. Maresco, P.E. Saigo, L.A. Almadrones, R.R. Barakat, C.L. Brown, D.S. Chi, J.P. Curtin, E.A. Poynor, and W.J. Hoskins, *Clinicopathologic features of BRCA-linked and sporadic ovarian cancer.* JAMA, 2000. **283**(17): p. 2260-5.
 15. Walsh, T., S. Casadei, M.K. Lee, C.C. Pennil, A.S. Nord, A.M. Thornton, W. Roeb, K.J. Agnew, S.M. Stray, A. Wickramanayake, B. Norquist, K.P. Pennington, R.L. Garcia, M.C. King, and E.M. Swisher, *Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing.* Proc Natl Acad Sci U S A, 2011. **108**(44): p. 18032-7.
 16. Toss, A., C. Tomasello, E. Razzaboni, G. Contu, G. Grandi, A. Cagnacci, R.J. Schilder, and L. Cortesi, *Hereditary ovarian cancer: not only BRCA 1 and 2 genes.* Biomed Res Int, 2015. **2015**: p. 341723.
 17. Moschetta, M., A. George, S.B. Kaye, and S. Banerjee, *BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer.* Ann Oncol, 2016. **27**(8): p. 1449-55.
 18. Berchuck, A., K.A. Heron, M.E. Carney, J.M. Lancaster, E.G. Fraser, V.L. Vinson, A.M. Deffenbaugh, A. Miron, J.R. Marks, P.A. Futreal, and T.S. Frank, *Frequency of germline and somatic BRCA1 mutations in ovarian cancer.* Clin Cancer Res, 1998. **4**(10): p. 2433-7.
 19. Lakhani, S.R., S. Manek, F. Penault-Llorca, A. Flanagan, L. Arnout, S. Merrett, L. McGuffog, D. Steele, P. Devilee, J.G. Klijn, H. Meijers-Heijboer, P. Radice, S. Pilotti, H. Nevanlinna, R.

- Butzow, H. Sobol, J. Jacquemier, D.S. Lyonet, S.L. Neuhausen, B. Weber, T. Wagner, R. Winqvist, Y.J. Bignon, F. Monti, F. Schmitt, G. Lenoir, S. Seitz, U. Hamman, P. Pharoah, G. Lane, B. Ponder, D.T. Bishop, and D.F. Easton, *Pathology of ovarian cancers in BRCA1 and BRCA2 carriers*. Clin Cancer Res, 2004. **10**(7): p. 2473-81.
20. Miki, Y., J. Swensen, D. Shattuck-Eidens, P.A. Futreal, K. Harshman, S. Tavtigian, Q. Liu, C. Cochran, L.M. Bennett, and W. Ding, *A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1*. Science, 1994. **266**(5182): p. 66-71.
 21. Wang, Y., D. Cortez, P. Yazdi, N. Neff, S.J. Elledge, and J. Qin, *BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures*. Genes Dev, 2000. **14**(8): p. 927-39.
 22. Zhang, H., G. Tomblin, and B.L. Weber, *BRCA1, BRCA2, and DNA damage response: collision or collusion?* Cell, 1998. **92**(4): p. 433-6.
 23. Rubin, S.C., I. Benjamin, K. Behbakht, H. Takahashi, M.A. Morgan, V.A. LiVolsi, A. Berchuck, M.G. Muto, J.E. Garber, B.L. Weber, H.T. Lynch, and J. Boyd, *Clinical and pathological features of ovarian cancer in women with germ-line mutations of BRCA1*. N Engl J Med, 1996. **335**(19): p. 1413-6.
 24. Bolton, K.L., G. Chenevix-Trench, C. Goh, S. Sadetzki, S.J. Ramus, B.Y. Karlan, D. Lambrechts, E. Despiere, D. Barrowdale, L. McGuffog, S. Healey, D.F. Easton, O. Sinilnikova, J. Benítez, M.J. García, S. Neuhausen, M.H. Gail, P. Hartge, S. Peock, D. Frost, D.G. Evans, R. Eeles, A.K. Godwin, M.B. Daly, A. Kwong, E.S. Ma, C. Lázaro, I. Blanco, M. Montagna, E. D'Andrea, M.O. Nicoletto, S.E. Johnatty, S.K. Kjær, A. Jensen, E. Høgdall, E.L. Goode, B.L. Fridley, J.T. Loud, M.H. Greene, P.L. Mai, A. Chetrit, F. Lubin, G. Hirsh-Yechezkel, G. Glendon, I.L. Andrulis, A.E. Toland, L. Senter, M.E. Gore, C. Gourley, C.O. Michie, H. Song, J. Tyrer, A.S. Whittemore, V. McGuire, W. Sieh, U. Kristoffersson, H. Olsson, Å. Borg, D.A. Levine, L. Steele, M.S. Beattie, S.

- Chan, R.L. Nussbaum, K.B. Moysich, J. Gross, I. Cass, C. Walsh, A.J. Li, R. Leuchter, O. Gordon, M. Garcia-Closas, S.A. Gayther, S.J. Chanock, A.C. Antoniou, P.D. Pharoah, EMBRACE, k. Investigators, and C.G.A.R. Network, *Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer*. JAMA, 2012. **307**(4): p. 382-90.
25. Birkbak, N.J., B. Kochupurakkal, J.M. Izarzugaza, A.C. Eklund, Y. Li, J. Liu, Z. Szallasi, U.A. Matulonis, A.L. Richardson, J.D. Iglehart, and Z.C. Wang, *Tumor mutation burden forecasts outcome in ovarian cancer with BRCA1 or BRCA2 mutations*. PLoS One, 2013. **8**(11): p. e80023.
26. Foulkes, W.D., *BRCA1 and BRCA2: chemosensitivity, treatment outcomes and prognosis*. Fam Cancer, 2006. **5**(2): p. 135-42.
27. Candido-dos-Reis, F.J., H. Song, E.L. Goode, J.M. Cunningham, B.L. Fridley, M.C. Larson, K. Alsop, E. Dicks, P. Harrington, S.J. Ramus, A. de Fazio, G. Mitchell, S. Fereday, K.L. Bolton, C. Gourley, C. Michie, B. Karlan, J. Lester, C. Walsh, I. Cass, H. Olsson, M. Gore, J.J. Benitez, M.J. Garcia, I. Andrulis, A.M. Mulligan, G. Glendon, I. Blanco, C. Lazaro, A.S. Whittemore, V. McGuire, W. Sieh, M. Montagna, E. Alducci, S. Sadetzki, A. Chetrit, A. Kwong, S.K. Kjaer, A. Jensen, E. Høgdall, S. Neuhausen, R. Nussbaum, M. Daly, M.H. Greene, P.L. Mai, J.T. Loud, K. Moysich, A.E. Toland, D. Lambrechts, S. Ellis, D. Frost, J.D. Brenton, M. Tischkowitz, D.F. Easton, A. Antoniou, G. Chenevix-Trench, S.A. Gayther, D. Bowtell, P.D. Pharoah, f. EMBRACE, k. Investigators, and A.O.C.S. Group, *Germline mutation in BRCA1 or BRCA2 and ten-year survival for women diagnosed with epithelial ovarian cancer*. Clin Cancer Res, 2015. **21**(3): p. 652-7.
28. Kotsopoulos, J., B. Rosen, I. Fan, J. Moody, J.R. McLaughlin, H. Risch, T. May, P. Sun, and S.A. Narod, *Ten-year survival after epithelial ovarian cancer is not associated with BRCA mutation status*. Gynecol Oncol, 2016. **140**(1): p. 42-7.

29. Rosendahl, M., C.K. Høgdall, and B.J. Mosgaard, *Restaging and Survival Analysis of 4036 Ovarian Cancer Patients According to the 2013 FIGO Classification for Ovarian, Fallopian Tube, and Primary Peritoneal Cancer*. Int J Gynecol Cancer, 2016. **26**(4): p. 680-7.
30. Peres, L.C., K.L. Cushing-Haugen, M. Köbel, H.R. Harris, A. Berchuck, M.A. Rossing, J.M. Schildkraut, and J.A. Doherty, *Invasive Epithelial Ovarian Cancer Survival by Histotype and Disease Stage*. J Natl Cancer Inst, 2019. **111**(1): p. 60-68.
31. Banerjee, S. and S.B. Kaye, *New strategies in the treatment of ovarian cancer: current clinical perspectives and future potential*. Clin Cancer Res, 2013. **19**(5): p. 961-8.
32. Beesley, V.L., A.C. Green, D.K. Wyld, P. O'Rourke, L.F. Wockner, A. deFazio, P.N. Butow, M.A. Price, K.R. Horwood, A.M. Clavarino, Australian Ovarian Cancer Study Group, Australian Ovarian Cancer Study-Quality Of Life Study Investigators, and P.M. Webb, *Quality of life and treatment response among women with platinum-resistant versus platinum-sensitive ovarian cancer treated for progression: a prospective analysis*. Gynecol Oncol, 2014. **132**(1): p. 130-6.
33. Harter, P., J. Sehouli, D. Lorusso, A. Reuss, I. Vergote, C. Marth, J.W. Kim, F. Raspagliesi, B. Lampe, G. Aletti, W. Meier, D. Cibula, A. Mustea, S. Mahner, I.B. Runnebaum, B. Schmalfeldt, A. Burges, R. Kimmig, G. Scambia, S. Greggi, F. Hilpert, A. Hasenburg, P. Hillemanns, G. Giorda, I. von Leffern, C. Schade-Brittinger, U. Wagner, and A. du Bois, *A Randomized Trial of Lymphadenectomy in Patients with Advanced Ovarian Neoplasms*. N Engl J Med, 2019. **380**(9): p. 822-832.
34. du Bois, A., A. Reuss, E. Pujade-Lauraine, P. Harter, I. Ray-Coquard, and J. Pfisterer, *Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-*

- OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). *Cancer*, 2009. **115**(6): p. 1234-44.
35. Vergote, I., C.G. Tropé, F. Amant, G.B. Kristensen, T. Ehlen, N. Johnson, R.H. Verheijen, M.E. van der Burg, A.J. Lacave, P.B. Panici, G.G. Kenter, A. Casado, C. Mendiola, C. Coens, L. Verleye, G.C. Stuart, S. Pecorelli, N.S. Reed, E.O.f.R.a.T.o.C.-G.C. Group, and N.C.T. Group, *Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer*. *N Engl J Med*, 2010. **363**(10): p. 943-53.
 36. Kehoe, S., J. Hook, M. Nankivell, G.C. Jayson, H. Kitchener, T. Lopes, D. Luesley, T. Perren, S. Bannoo, M. Mascarenhas, S. Dobbs, S. Essapen, J. Twigg, J. Herod, G. McCluggage, M. Parmar, and A.M. Swart, *Primary chemotherapy versus primary surgery for newly diagnosed advanced ovarian cancer (CHORUS): an open-label, randomised, controlled, non-inferiority trial*. *Lancet*, 2015. **386**(9990): p. 249-57.
 37. Ozols, R.F., B.N. Bundy, B.E. Greer, J.M. Fowler, D. Clarke-Pearson, R.A. Burger, R.S. Mannel, K. DeGeest, E.M. Hartenbach, R. Baergen, and G.O. Group, *Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study*. *J Clin Oncol*, 2003. **21**(17): p. 3194-200.
 38. Bell, J., M.F. Brady, R.C. Young, J. Lage, J.L. Walker, K.Y. Look, G.S. Rose, N.M. Spirtos, and G.O. Group, *Randomized phase III trial of three versus six cycles of adjuvant carboplatin and paclitaxel in early stage epithelial ovarian carcinoma: a Gynecologic Oncology Group study*. *Gynecol Oncol*, 2006. **102**(3): p. 432-9.
 39. Katsumata, N., M. Yasuda, F. Takahashi, S. Isonishi, T. Jobo, D. Aoki, H. Tsuda, T. Sugiyama, S. Kodama, E. Kimura, K. Ochiai, K. Noda, and J.G.O. Group, *Dose-dense paclitaxel once a week*

- in combination with carboplatin every 3 weeks for advanced ovarian cancer: a phase 3, open-label, randomised controlled trial. Lancet, 2009. 374(9698): p. 1331-8.*
40. Katsumata, N., M. Yasuda, S. Isonishi, F. Takahashi, H. Michimae, E. Kimura, D. Aoki, T. Jobo, S. Kodama, F. Terauchi, T. Sugiyama, K. Ochiai, and J.G.O. Group, *Long-term results of dose-dense paclitaxel and carboplatin versus conventional paclitaxel and carboplatin for treatment of advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer (JGOG 3016): a randomised, controlled, open-label trial. Lancet Oncol, 2013. 14(10): p. 1020-6.*
 41. Pignata, S., G. Scambia, D. Katsaros, C. Gallo, E. Pujade-Lauraine, S. De Placido, A. Bologna, B. Weber, F. Raspagliesi, P.B. Panici, G. Cormio, R. Sorio, M.G. Cavazzini, G. Ferrandina, E. Breda, V. Murgia, C. Sacco, S. Cinieri, V. Salutari, C. Ricci, C. Pisano, S. Greggi, R. Lauria, D. Lorusso, C. Marchetti, L. Selvaggi, S. Signoriello, M.C. Piccirillo, M. Di Maio, F. Perrone, M.I.T.i.O.c. (MITO-7), G.d.I.N.p.I.E.d.C.O.e.d.s. (GINECO), M.N.G.O. (MaNGO), E.N.o.G.O.T.G. (ENGOT-OV-10), and G.C.I.G. Investigators, *Carboplatin plus paclitaxel once a week versus every 3 weeks in patients with advanced ovarian cancer (MITO-7): a randomised, multicentre, open-label, phase 3 trial. Lancet Oncol, 2014. 15(4): p. 396-405.*
 42. Clamp, A.R., E.C. James, I.A. McNeish, A. Dean, J.W. Kim, D.M. O'Donnell, J. Hook, C. Coyle, S. Blagden, J.D. Brenton, R. Naik, T. Perren, S. Sundar, A.D. Cook, G.S. Gopalakrishnan, H. Gabra, R. Lord, G. Dark, H.M. Earl, M. Hall, S. Banerjee, R.M. Glasspool, R. Jones, S. Williams, A.M. Swart, S. Stenning, M. Parmar, R. Kaplan, and J.A. Ledermann, *Weekly dose-dense chemotherapy in first-line epithelial ovarian, fallopian tube, or primary peritoneal carcinoma treatment (ICON8): primary progression free survival analysis results from a GCIg phase 3 randomised controlled trial. Lancet, 2019. 394(10214): p. 2084-2095.*
 43. Provencher, D.M., C.J. Gallagher, W.R. Parulekar, J.A. Ledermann, D.K. Armstrong, M. Brundage, C. Gourley, I. Romero, A. Gonzalez-Martin, M. Feeney, P. Bessette, M. Hall, J.I.

- Weberpals, G. Hall, S.K. Lau, P. Gauthier, M. Fung-Kee-Fung, E.A. Eisenhauer, C. Winch, D. Tu, and H.J. MacKay, *OV21/PETROC: a randomized Gynecologic Cancer Intergroup phase II study of intraperitoneal versus intravenous chemotherapy following neoadjuvant chemotherapy and optimal debulking surgery in epithelial ovarian cancer*. *Ann Oncol*, 2018. **29**(2): p. 431-438.
44. Armstrong, D.K., B. Bundy, L. Wenzel, H.Q. Huang, R. Baergen, S. Lele, L.J. Copeland, J.L. Walker, R.A. Burger, and G.O. Group, *Intraperitoneal cisplatin and paclitaxel in ovarian cancer*. *N Engl J Med*, 2006. **354**(1): p. 34-43.
 45. Markman, M., B.N. Bundy, D.S. Alberts, J.M. Fowler, D.L. Clark-Pearson, L.F. Carson, S. Wadler, and J. Sickel, *Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group*. *J Clin Oncol*, 2001. **19**(4): p. 1001-7.
 46. Walker, J.L., M.F. Brady, L. Wenzel, G.F. Fleming, H.Q. Huang, P.A. DiSilvestro, K. Fujiwara, D.S. Alberts, W. Zheng, K.S. Tewari, D.E. Cohn, M.A. Powell, L. Van Le, S.A. Davidson, H.J. Gray, P.G. Rose, C. Aghajanian, T. Myers, A. Alvarez Secord, S.C. Rubin, and R.S. Mannel, *Randomized Trial of Intravenous Versus Intraperitoneal Chemotherapy Plus Bevacizumab in Advanced Ovarian Carcinoma: An NRG Oncology/Gynecologic Oncology Group Study*. *J Clin Oncol*, 2019. **37**(16): p. 1380-1390.
 47. Abu-Jawdeh, G.M., J.D. Faix, J. Niloff, K. Tognazzi, E. Manseau, H.F. Dvorak, and L.F. Brown, *Strong expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in ovarian borderline and malignant neoplasms*. *Lab Invest*, 1996. **74**(6): p. 1105-15.

48. Hazelton, D., R.F. Nicosia, and S.V. Nicosia, *Vascular endothelial growth factor levels in ovarian cyst fluid correlate with malignancy*. Clin Cancer Res, 1999. **5**(4): p. 823-9.
49. Nakanishi, Y., J. Kodama, M. Yoshinouchi, K. Tokumo, S. Kamimura, H. Okuda, and T. Kudo, *The expression of vascular endothelial growth factor and transforming growth factor-beta associates with angiogenesis in epithelial ovarian cancer*. Int J Gynecol Pathol, 1997. **16**(3): p. 256-62.
50. Mu, J., Y. Abe, T. Tsutsui, N. Yamamoto, X.G. Tai, O. Niwa, T. Tsujimura, B. Sato, H. Terano, H. Fujiwara, and T. Hamaoka, *Inhibition of growth and metastasis of ovarian carcinoma by administering a drug capable of interfering with vascular endothelial growth factor activity*. Jpn J Cancer Res, 1996. **87**(9): p. 963-71.
51. Schiffenbauer, Y.S., R. Abramovitch, G. Meir, N. Nevo, M. Holzinger, A. Itin, E. Keshet, and M. Neeman, *Loss of ovarian function promotes angiogenesis in human ovarian carcinoma*. Proc Natl Acad Sci U S A, 1997. **94**(24): p. 13203-8.
52. Zhang, L., N. Yang, J.W. Park, D. Katsaros, S. Fracchioli, G. Cao, A. O'Brien-Jenkins, T.C. Randall, S.C. Rubin, and G. Coukos, *Tumor-derived vascular endothelial growth factor up-regulates angiopoietin-2 in host endothelium and destabilizes host vasculature, supporting angiogenesis in ovarian cancer*. Cancer Res, 2003. **63**(12): p. 3403-12.
53. Masoumi Moghaddam, S., A. Amini, D.L. Morris, and M.H. Pourgholami, *Significance of vascular endothelial growth factor in growth and peritoneal dissemination of ovarian cancer*. Cancer Metastasis Rev, 2012. **31**(1-2): p. 143-62.
54. Sawano, A., S. Iwai, Y. Sakurai, M. Ito, K. Shitara, T. Nakahata, and M. Shibuya, *Flt-1, vascular endothelial growth factor receptor 1, is a novel cell surface marker for the lineage of monocyte-macrophages in humans*. Blood, 2001. **97**(3): p. 785-91.

55. Ramakrishnan, S., I.V. Subramanian, Y. Yokoyama, and M. Geller, *Angiogenesis in normal and neoplastic ovaries*. *Angiogenesis*, 2005. **8**(2): p. 169-82.
56. Schumacher, J.J., R.P. Dings, J. Cosin, I.V. Subramanian, N. Auersperg, and S. Ramakrishnan, *Modulation of angiogenic phenotype alters tumorigenicity in rat ovarian epithelial cells*. *Cancer Res*, 2007. **67**(8): p. 3683-90.
57. Weis, S., J. Cui, L. Barnes, and D. Cheresh, *Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis*. *J Cell Biol*, 2004. **167**(2): p. 223-9.
58. Alvarez, A.A., H.R. Krigman, R.S. Whitaker, R.K. Dodge, and G.C. Rodriguez, *The prognostic significance of angiogenesis in epithelial ovarian carcinoma*. *Clin Cancer Res*, 1999. **5**(3): p. 587-91.
59. Kumaran, G.C., G.C. Jayson, and A.R. Clamp, *Antiangiogenic drugs in ovarian cancer*. *Br J Cancer*, 2009. **100**(1): p. 1-7.
60. Ferrara, N., K.J. Hillan, and W. Novotny, *Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy*. *Biochem Biophys Res Commun*, 2005. **333**(2): p. 328-35.
61. Perren, T.J., A.M. Swart, J. Pfisterer, J.A. Ledermann, E. Pujade-Lauraine, G. Kristensen, M.S. Carey, P. Beale, A. Cervantes, C. Kurzeder, A. du Bois, J. Sehouli, R. Kimmig, A. Stähle, F. Collinson, S. Essapen, C. Gourley, A. Lortholary, F. Selle, M.R. Mirza, A. Leminen, M. Plante, D. Stark, W. Qian, M.K. Parmar, A.M. Oza, and I. Investigators, *A phase 3 trial of bevacizumab in ovarian cancer*. *N Engl J Med*, 2011. **365**(26): p. 2484-96.
62. Burger, R.A., M.F. Brady, M.A. Bookman, G.F. Fleming, B.J. Monk, H. Huang, R.S. Mannel, H.D. Homesley, J. Fowler, B.E. Greer, M. Boente, M.J. Birrer, S.X. Liang, and G.O. Group,

- Incorporation of bevacizumab in the primary treatment of ovarian cancer.* N Engl J Med, 2011. **365**(26): p. 2473-83.
63. Aghajanian, C., S.V. Blank, B.A. Goff, P.L. Judson, M.G. Teneriello, A. Husain, M.A. Sovak, J. Yi, and L.R. Nycum, *OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer.* J Clin Oncol, 2012. **30**(17): p. 2039-45.
 64. Pujade-Lauraine, E., F. Hilpert, B. Weber, A. Reuss, A. Poveda, G. Kristensen, R. Sorio, I. Vergote, P. Witteveen, A. Bamias, D. Pereira, P. Wimberger, A. Oaknin, M.R. Mirza, P. Follana, D. Bollag, and I. Ray-Coquard, *Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial.* J Clin Oncol, 2014. **32**(13): p. 1302-8.
 65. Wulff, C., H. Wilson, J.S. Rudge, S.J. Wiegand, S.F. Lunn, and H.M. Fraser, *Luteal angiogenesis: prevention and intervention by treatment with vascular endothelial growth factor trap(A40).* J Clin Endocrinol Metab, 2001. **86**(7): p. 3377-86.
 66. Colombo, N., G. Mangili, S. Mammoliti, M. Kalling, B. Tholander, L. Sternas, G. Buzenet, and D. Chamberlain, *A phase II study of aflibercept in patients with advanced epithelial ovarian cancer and symptomatic malignant ascites.* Gynecol Oncol, 2012. **125**(1): p. 42-7.
 67. Gotlieb, W.H., F. Amant, S. Advani, C. Goswami, H. Hirte, D. Provencher, N. Somani, S.D. Yamada, J.F. Tamby, and I. Vergote, *Intravenous aflibercept for treatment of recurrent symptomatic malignant ascites in patients with advanced ovarian cancer: a phase 2, randomised, double-blind, placebo-controlled study.* Lancet Oncol, 2012. **13**(2): p. 154-62.
 68. du Bois, A., A. Floquet, J.W. Kim, J. Rau, J.M. del Campo, M. Friedlander, S. Pignata, K. Fujiwara, I. Vergote, N. Colombo, M.R. Mirza, B.J. Monk, R. Kimmig, I. Ray-Coquard, R. Zang,

- I. Diaz-Padilla, K.H. Baumann, M.A. Mouret-Reynier, J.H. Kim, C. Kurzeder, A. Lesoin, P. Vasey, C. Marth, U. Canzler, G. Scambia, M. Shimada, P. Calvert, E. Pujade-Lauraine, B.G. Kim, T.J. Herzog, I. Mitrica, C. Schade-Brittinger, Q. Wang, R. Crescenzo, and P. Harter, *Incorporation of pazopanib in maintenance therapy of ovarian cancer*. J Clin Oncol, 2014. **32**(30): p. 3374-82.
69. du Bois, A., G. Kristensen, I. Ray-Coquard, A. Reuss, S. Pignata, N. Colombo, U. Denison, I. Vergote, J.M. Del Campo, P. Ottevanger, M. Heubner, T. Minarik, E. Sevin, N. de Gregorio, M. Bidziński, J. Pfisterer, S. Malander, F. Hilpert, M.R. Mirza, G. Scambia, W. Meier, M.O. Nicoletto, L. Bjørge, A. Lortholary, M.O. Sailer, M. Merger, P. Harter, and A.S.G.I.G.C.I.E.N.o.G.O.T.G.I. Consortium, *Standard first-line chemotherapy with or without nintedanib for advanced ovarian cancer (AGO-OVAR 12): a randomised, double-blind, placebo-controlled phase 3 trial*. Lancet Oncol, 2016. **17**(1): p. 78-89.
70. Lord, C.J. and A. Ashworth, *PARP inhibitors: Synthetic lethality in the clinic*. Science, 2017. **355**(6330): p. 1152-1158.
71. Pines, A., M.G. Vrouwe, J.A. Marteijn, D. Typas, M.S. Luijsterburg, M. Cansoy, P. Hensbergen, A. Deelder, A. de Groot, S. Matsumoto, K. Sugawara, N. Thoma, W. Vermeulen, H. Vrieling, and L. Mullenders, *PARP1 promotes nucleotide excision repair through DDB2 stabilization and recruitment of ALC1*. J Cell Biol, 2012. **199**(2): p. 235-49.
72. Horton, J.K., D.F. Stefanick, R. Prasad, N.R. Gassman, P.S. Kedar, and S.H. Wilson, *Base excision repair defects invoke hypersensitivity to PARP inhibition*. Mol Cancer Res, 2014. **12**(8): p. 1128-39.
73. Farmer, H., N. McCabe, C.J. Lord, A.N. Tutt, D.A. Johnson, T.B. Richardson, M. Santarosa, K.J. Dillon, I. Hickson, C. Knights, N.M. Martin, S.P. Jackson, G.C. Smith, and A. Ashworth,

- Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy.* Nature, 2005. **434**(7035): p. 917-21.
74. Murai, J., S.Y. Huang, B.B. Das, A. Renaud, Y. Zhang, J.H. Doroshow, J. Ji, S. Takeda, and Y. Pommier, *Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors.* Cancer Res, 2012. **72**(21): p. 5588-99.
 75. Pommier, Y., M.J. O'Connor, and J. de Bono, *Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action.* Sci Transl Med, 2016. **8**(362): p. 362ps17.
 76. Ledermann, J., P. Harter, C. Gourley, M. Friedlander, I. Vergote, G. Rustin, C. Scott, W. Meier, R. Shapira-Frommer, T. Safra, D. Matei, E. Macpherson, C. Watkins, J. Carmichael, and U. Matulonis, *Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer.* N Engl J Med, 2012. **366**(15): p. 1382-92.
 77. Coleman, R.L., A.M. Oza, D. Lorusso, C. Aghajanian, A. Oaknin, A. Dean, N. Colombo, J.I. Weberpals, A. Clamp, G. Scambia, A. Leary, R.W. Holloway, M.A. Gancedo, P.C. Fong, J.C. Goh, D.M. O'Malley, D.K. Armstrong, J. Garcia-Donas, E.M. Swisher, A. Floquet, G.E. Konecny, I.A. McNeish, C.L. Scott, T. Cameron, L. Maloney, J. Isaacson, S. Goble, C. Grace, T.C. Harding, M. Raponi, J. Sun, K.K. Lin, H. Giordano, J.A. Ledermann, and A. investigators, *Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial.* Lancet, 2017. **390**(10106): p. 1949-1961.
 78. Mirza, M.R., B.J. Monk, J. Herrstedt, A.M. Oza, S. Mahner, A. Redondo, M. Fabbro, J.A. Ledermann, D. Lorusso, I. Vergote, N.E. Ben-Baruch, C. Marth, R. Mądry, R.D. Christensen, J.S. Berek, A. Dørum, A.V. Tinker, A. du Bois, A. González-Martín, P. Follana, B. Benigno, P. Rosenberg, L. Gilbert, B.J. Rimel, J. Buscema, J.P. Balser, S. Agarwal, U.A. Matulonis, and E.-

- O.N. Investigators, *Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer*. N Engl J Med, 2016. **375**(22): p. 2154-2164.
79. Hodi, F.S., S.J. O'Day, D.F. McDermott, R.W. Weber, J.A. Sosman, J.B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J.C. Hassel, W. Akerley, A.J. van den Eertwegh, J. Lutzky, P. Lorigan, J.M. Vaubel, G.P. Linette, D. Hogg, C.H. Ottensmeier, C. Lebbé, C. Peschel, I. Quirt, J.I. Clark, J.D. Wolchok, J.S. Weber, J. Tian, M.J. Yellin, G.M. Nichol, A. Hoos, and W.J. Urba, *Improved survival with ipilimumab in patients with metastatic melanoma*. N Engl J Med, 2010. **363**(8): p. 711-23.
 80. Seiwert, T.Y., B. Burtness, R. Mehra, J. Weiss, R. Berger, J.P. Eder, K. Heath, T. McClanahan, J. Lunceford, C. Gause, J.D. Cheng, and L.Q. Chow, *Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial*. Lancet Oncol, 2016. **17**(7): p. 956-965.
 81. Kruger, S., M. Ilmer, S. Kobold, B.L. Cadilha, S. Endres, S. Ormanns, G. Schuebbe, B.W. Renz, J.G. D'Haese, H. Schloesser, V. Heinemann, M. Subklewe, S. Boeck, J. Werner, and M. von Bergwelt-Baildon, *Advances in cancer immunotherapy 2019 - latest trends*. J Exp Clin Cancer Res, 2019. **38**(1): p. 268.
 82. Grupp, S.A., M. Kalos, D. Barrett, R. Aplenc, D.L. Porter, S.R. Rheingold, D.T. Teachey, A. Chew, B. Hauck, J.F. Wright, M.C. Milone, B.L. Levine, and C.H. June, *Chimeric antigen receptor-modified T cells for acute lymphoid leukemia*. N Engl J Med, 2013. **368**(16): p. 1509-1518.
 83. Topalian, S.L., F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D.F. McDermott, J.D. Powderly, R.D. Carvajal, J.A. Sosman, M.B. Atkins, P.D. Leming, D.R. Spigel, S.J. Antonia, L. Horn, C.G. Drake, D.M. Pardoll, L. Chen, W.H. Sharfman, R.A. Anders, J.M. Taube, T.L.

- McMiller, H. Xu, A.J. Korman, M. Jure-Kunkel, S. Agrawal, D. McDonald, G.D. Kolia, A. Gupta, J.M. Wigginton, and M. Sznol, *Safety, activity, and immune correlates of anti-PD-1 antibody in cancer*. N Engl J Med, 2012. **366**(26): p. 2443-54.
84. Hamanishi, J., M. Mandai, T. Ikeda, M. Minami, A. Kawaguchi, T. Murayama, M. Kanai, Y. Mori, S. Matsumoto, S. Chikuma, N. Matsumura, K. Abiko, T. Baba, K. Yamaguchi, A. Ueda, Y. Hosoe, S. Morita, M. Yokode, A. Shimizu, T. Honjo, and I. Konishi, *Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer*. J Clin Oncol, 2015. **33**(34): p. 4015-22.
85. Alberts, D.S., C. Marth, R.D. Alvarez, G. Johnson, M. Bidzinski, D.R. Kardatzke, W.Z. Bradford, J. Loutit, D.H. Kirn, M.C. Clouser, M. Markman, and G.C.T. Consortium, *Randomized phase 3 trial of interferon gamma-1b plus standard carboplatin/paclitaxel versus carboplatin/paclitaxel alone for first-line treatment of advanced ovarian and primary peritoneal carcinomas: results from a prospectively designed analysis of progression-free survival*. Gynecol Oncol, 2008. **109**(2): p. 174-81.
86. Aoki, Y., K. Takakuwa, S. Kodama, K. Tanaka, M. Takahashi, A. Tokunaga, and T. Takahashi, *Use of adoptive transfer of tumor-infiltrating lymphocytes alone or in combination with cisplatin-containing chemotherapy in patients with epithelial ovarian cancer*. Cancer Res, 1991. **51**(7): p. 1934-9.
87. Disis, M.L., M.H. Taylor, K. Kelly, J.T. Beck, M. Gordon, K.M. Moore, M.R. Patel, J. Chaves, H. Park, A.C. Mita, E.P. Hamilton, C.M. Annunziata, H.J. Grote, A. von Heydebreck, J. Grewal, V. Chand, and J.L. Gulley, *Efficacy and Safety of Avelumab for Patients With Recurrent or Refractory Ovarian Cancer: Phase 1b Results From the JAVELIN Solid Tumor Trial*. JAMA Oncol, 2019. **5**(3): p. 393-401.

88. Gray, H.J., B. Benigno, J. Berek, J. Chang, J. Mason, L. Mileskin, P. Mitchell, M. Moradi, F.O. Recio, C.M. Michener, A.A. Secord, N.E. Tchabo, J.K. Chan, J. Young, H. Kohrt, S.E. Gargosky, and J.C. Goh, *Progression-free and overall survival in ovarian cancer patients treated with CVac, a mucin 1 dendritic cell therapy in a randomized phase 2 trial*. J Immunother Cancer, 2016. **4**: p. 34.
89. Hodi, F.S., M. Butler, D.A. Oble, M.V. Seiden, F.G. Haluska, A. Kruse, S. Macrae, M. Nelson, C. Canning, I. Lowy, A. Korman, D. Lautz, S. Russell, M.T. Jaklitsch, N. Ramaiya, T.C. Chen, D. Neuberger, J.P. Allison, M.C. Mihm, and G. Dranoff, *Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients*. Proc Natl Acad Sci U S A, 2008. **105**(8): p. 3005-10.
90. Kandalaft, L.E., D.J. Powell, and G. Coukos, *A phase I clinical trial of adoptive transfer of folate receptor-alpha redirected autologous T cells for recurrent ovarian cancer*. J Transl Med, 2012. **10**: p. 157.
91. Matulonis, U.A., R. Shapira-Frommer, A.D. Santin, A.S. Lisysanskaya, S. Pignata, I. Vergote, F. Raspagliesi, G.S. Sonke, M. Birrer, D.M. Provencher, J. Sehouli, N. Colombo, A. González-Martín, A. Oaknin, P.B. Ottevanger, V. Rudaitis, K. Katchar, H. Wu, S. Keefe, J. Ruman, and J.A. Ledermann, *Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study*. Ann Oncol, 2019. **30**(7): p. 1080-1087.
92. Nham, T., S.M. Poznanski, I.Y. Fan, M.M. Shenouda, M.V. Chew, A.J. Lee, F. Vahedi, Y. Karimi, M. Butcher, D.A. Lee, H. Hirte, and A.A. Ashkar, *Ex vivo-expanded NK cells from blood and ascites of ovarian cancer patients are cytotoxic against autologous primary ovarian cancer cells*. Cancer Immunol Immunother, 2018. **67**(4): p. 575-587.

93. Clarke, B., A.V. Tinker, C.H. Lee, S. Subramanian, M. van de Rijn, D. Turbin, S. Kalloger, G. Han, K. Ceballos, M.G. Cadungog, D.G. Huntsman, G. Coukos, and C.B. Gilks, *Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss*. Mod Pathol, 2009. **22**(3): p. 393-402.
94. Zhang, L., J.R. Conejo-Garcia, D. Katsaros, P.A. Gimotty, M. Massobrio, G. Regnani, A. Makrigiannakis, H. Gray, K. Schlienger, M.N. Liebman, S.C. Rubin, and G. Coukos, *Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer*. N Engl J Med, 2003. **348**(3): p. 203-13.
95. Sato, E., S.H. Olson, J. Ahn, B. Bundy, H. Nishikawa, F. Qian, A.A. Jungbluth, D. Frosina, S. Gnjjatic, C. Ambrosone, J. Kepner, T. Odunsi, G. Ritter, S. Lele, Y.T. Chen, H. Ohtani, L.J. Old, and K. Odunsi, *Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer*. Proc Natl Acad Sci U S A, 2005. **102**(51): p. 18538-43.
96. Hwang, W.T., S.F. Adams, E. Tahirovic, I.S. Hagemann, and G. Coukos, *Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis*. Gynecol Oncol, 2012. **124**(2): p. 192-8.
97. Adams, S.F., D.A. Levine, M.G. Cadungog, R. Hammond, A. Facciabene, N. Olvera, S.C. Rubin, J. Boyd, P.A. Gimotty, and G. Coukos, *Intraepithelial T cells and tumor proliferation: impact on the benefit from surgical cytoreduction in advanced serous ovarian cancer*. Cancer, 2009. **115**(13): p. 2891-902.
98. Bachmayr-Heyda, A., S. Aust, G. Heinze, S. Polterauer, C. Grimm, E.I. Braicu, J. Sehouli, S. Lambrechts, I. Vergote, S. Mahner, D. Pils, E. Schuster, T. Thalhammer, R. Horvat, C. Denkert, R. Zeillinger, and D.C. Castillo-Tong, *Prognostic impact of tumor infiltrating CD8+ T cells in*

- association with cell proliferation in ovarian cancer patients--a study of the OVCAD consortium.* BMC Cancer, 2013. **13**: p. 422.
99. Curiel, T.J., G. Coukos, L. Zou, X. Alvarez, P. Cheng, P. Mottram, M. Evdemon-Hogan, J.R. Conejo-Garcia, L. Zhang, M. Burow, Y. Zhu, S. Wei, I. Kryczek, B. Daniel, A. Gordon, L. Myers, A. Lackner, M.L. Disis, K.L. Knutson, L. Chen, and W. Zou, *Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival.* Nat Med, 2004. **10**(9): p. 942-9.
 100. Gabrilovich, D.I. and S. Nagaraj, *Myeloid-derived suppressor cells as regulators of the immune system.* Nat Rev Immunol, 2009. **9**(3): p. 162-74.
 101. Grabosch, S., M. Bulatovic, F. Zeng, T. Ma, L. Zhang, M. Ross, J. Brozick, Y. Fang, G. Tseng, E. Kim, A. Gambotto, E. Elishaev, R. P Edwards, and A.M. Vlad, *Cisplatin-induced immune modulation in ovarian cancer mouse models with distinct inflammation profiles.* Oncogene, 2019. **38**(13): p. 2380-2393.
 102. Yang, S.Y.C., S. Lheureux, K. Karakasis, J.V. Burnier, J.P. Bruce, D.L. Clouthier, A. Danesh, R. Quevedo, M. Dowar, Y. Hanna, T. Li, L. Lu, W. Xu, B.A. Clarke, P.S. Ohashi, P.A. Shaw, T.J. Pugh, and A.M. Oza, *Landscape of genomic alterations in high-grade serous ovarian cancer from exceptional long- and short-term survivors.* Genome Med, 2018. **10**(1): p. 81.
 103. Strickland, K.C., B.E. Howitt, S.A. Shukla, S. Rodig, L.L. Ritterhouse, J.F. Liu, J.E. Garber, D. Chowdhury, C.J. Wu, A.D. D'Andrea, U.A. Matulonis, and P.A. Konstantinopoulos, *Association and prognostic significance of BRCA1/2-mutation status with neoantigen load, number of tumor-infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer.* Oncotarget, 2016. **7**(12): p. 13587-98.

104. O'Donnell, T., E.L. Christie, A. Ahuja, J. Buros, B.A. Aksoy, D.D.L. Bowtell, A. Snyder, and J. Hammerbacher, *Chemotherapy weakly contributes to predicted neoantigen expression in ovarian cancer*. BMC Cancer, 2018. **18**(1): p. 87.
105. Li, D., Z. Peng, H. Tang, P. Wei, X. Kong, D. Yan, F. Huang, Q. Li, X. Le, and K. Xie, *KLF4-mediated negative regulation of IFITM3 expression plays a critical role in colon cancer pathogenesis*. Clin Cancer Res, 2011. **17**(11): p. 3558-68.
106. Shen, C., Y.J. Li, Q.Q. Yin, W.W. Jiao, Q.J. Li, J. Xiao, L. Sun, F. Xu, J.Q. Li, H. Qi, and A.D. Shen, *Identification of differentially expressed transcripts targeted by the knockdown of endogenous IFITM3*. Mol Med Rep, 2016. **14**(5): p. 4367-4373.
107. Gómez-Herranz, M., M. Nekulova, J. Faktor, L. Hernychova, S. Kote, E.H. Sinclair, R. Nenutil, B. Vojtesek, K.L. Ball, and T.R. Hupp, *The effects of IFITM1 and IFITM3 gene deletion on IFN γ stimulated protein synthesis*. Cell Signal, 2019. **60**: p. 39-56.
108. Ling, S., C. Zhang, W. Wang, X. Cai, L. Yu, F. Wu, L. Zhang, and C. Tian, *Combined approaches of EPR and NMR illustrate only one transmembrane helix in the human IFITM3*. Sci Rep, 2016. **6**: p. 24029.
109. Uhlén, M., L. Fagerberg, B.M. Hallström, C. Lindskog, P. Oksvold, A. Mardinoglu, Å. Sivertsson, C. Kampf, E. Sjöstedt, A. Asplund, I. Olsson, K. Edlund, E. Lundberg, S. Navani, C.A. Szigartyo, J. Odeberg, D. Djureinovic, J.O. Takanen, S. Hober, T. Alm, P.H. Edqvist, H. Berling, H. Tegel, J. Mulder, J. Rockberg, P. Nilsson, J.M. Schwenk, M. Hamsten, K. von Feilitzen, M. Forsberg, L. Persson, F. Johansson, M. Zwahlen, G. von Heijne, J. Nielsen, and F. Pontén, *Proteomics. Tissue-based map of the human proteome*. Science, 2015. **347**(6220): p. 1260419.
110. Rajapaksa, U.S., C. Jin, and T. Dong, *Malignancy and IFITM3: Friend or Foe?* Front Oncol, 2020. **10**: p. 593245.

111. Hu, J., S. Wang, Y. Zhao, Q. Guo, D. Zhang, J. Chen, J. Li, Q. Fei, and Y. Sun, *Mechanism and biological significance of the overexpression of IFITM3 in gastric cancer*. *Oncol Rep*, 2014. **32**(6): p. 2648-56.
112. Yang, M., H. Gao, P. Chen, J. Jia, and S. Wu, *Knockdown of interferon-induced transmembrane protein 3 expression suppresses breast cancer cell growth and colony formation and affects the cell cycle*. *Oncol Rep*, 2013. **30**(1): p. 171-8.
113. Liu, X., L. Chen, Y. Fan, Y. Hong, X. Yang, Y. Li, J. Lu, J. Lv, X. Pan, F. Qu, X. Cui, Y. Gao, and D. Xu, *IFITM3 promotes bone metastasis of prostate cancer cells by mediating activation of the TGF- β signaling pathway*. *Cell Death Dis*, 2019. **10**(7): p. 517.
114. Brem, R., K. Oraszlan-Szovik, S. Foser, B. Bohrmann, and U. Certa, *Inhibition of proliferation by 1-8U in interferon-alpha-responsive and non-responsive cell lines*. *Cell Mol Life Sci*, 2003. **60**(6): p. 1235-48.
115. El-Tanani, M.K., D. Jin, F.C. Campbell, and P.G. Johnston, *Interferon-induced transmembrane 3 binds osteopontin in vitro: expressed in vivo IFITM3 reduced OPN expression*. *Oncogene*, 2010. **29**(5): p. 752-62.
116. Kim, J.H., S.J. Skates, T. Uede, K.K. Wong, J.O. Schorge, C.M. Feltmate, R.S. Berkowitz, D.W. Cramer, and S.C. Mok, *Osteopontin as a potential diagnostic biomarker for ovarian cancer*. *JAMA*, 2002. **287**(13): p. 1671-9.
117. Song, G., Q.F. Cai, Y.B. Mao, Y.L. Ming, S.D. Bao, and G.L. Ouyang, *Osteopontin promotes ovarian cancer progression and cell survival and increases HIF-1 α expression through the PI3-K/Akt pathway*. *Cancer Sci*, 2008. **99**(10): p. 1901-7.
118. Clemente, N., D. Raineri, G. Cappellano, E. Boggio, F. Favero, M.F. Soluri, C. Dianzani, C. Comi, U. Dianzani, and A. Chiocchetti, *Osteopontin Bridging Innate and Adaptive Immunity in Autoimmune Diseases*. *J Immunol Res*, 2016. **2016**: p. 7675437.

119. Klement, J.D., A.V. Paschall, P.S. Redd, M.L. Ibrahim, C. Lu, D. Yang, E. Celis, S.I. Abrams, K. Ozato, and K. Liu, *An osteopontin/CD44 immune checkpoint controls CD8+ T cell activation and tumor immune evasion*. J Clin Invest, 2018. **128**(12): p. 5549-5560.
120. Zhou, Z., F. Huang, I. Shrivastava, R. Zhu, A. Luo, M. Hottiger, I. Bahar, Z. Liu, M. Cristofanilli, and Y. Wan, *New insight into the significance of KLF4 PARylation in genome stability, carcinogenesis, and therapy*. EMBO Mol Med, 2020. **12**(12): p. e12391.
121. Benjamini, Y. and Y. Hochberg, *Controlling the false discovery rate: A practical and powerful approach to multiple testing*. Journal of Royal Statistical Society, 1995. **57**: p. 289-300.
122. Subramanian, A., P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, and J.P. Mesirov, *Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles*. Proc Natl Acad Sci U S A, 2005. **102**(43): p. 15545-50.
123. Infusini, G., J.M. Smith, H. Yuan, A. Pizzolla, W.C. Ng, S.L. Londrigan, A. Haque, P.C. Reading, J.A. Villadangos, and L.M. Wakim, *Respiratory DC Use IFITM3 to Avoid Direct Viral Infection and Safeguard Virus-Specific CD8+ T Cell Priming*. PLoS One, 2015. **10**(11): p. e0143539.
124. Yáñez, D.C., H. Sahni, S. Ross, A. Solanki, C.I. Lau, E. Papaioannou, A. Barbarulo, R. Powell, U.C. Lange, D.J. Adams, M. Barenco, M. Ono, F. D'Acquisto, A.L. Furmanski, and T. Crompton, *IFITM proteins drive type 2 T helper cell differentiation and exacerbate allergic airway inflammation*. Eur J Immunol, 2019. **49**(1): p. 66-78.
125. Parte, S.C., A. Smolenkov, S.K. Batra, M.Z. Ratajczak, and S.S. Kakar, *Ovarian Cancer Stem Cells: Unraveling a Germline Connection*. Stem Cells Dev, 2017. **26**(24): p. 1781-1803.
126. Hu, X., H. Lu, S. Cao, Y.L. Deng, Q.J. Li, Q. Wan, and S.M. Yie, *Stem cells derived from human first-trimester umbilical cord have the potential to differentiate into oocyte-like cells in vitro*. Int J Mol Med, 2015. **35**(5): p. 1219-29.

127. Fraunhofer, N.A., A. Meilerman Abuelafia, I. Stella, S. Galliano, M. Barrios, and A.D. Vitullo, *Identification of germ cell-specific VASA and IFITM3 proteins in human ovarian endometriosis*. J Ovarian Res, 2015. **8**: p. 66.
128. Awasthi, S., A. Berglund, J. Abraham-Miranda, R.J. Rounbehler, K. Kensler, A. Serna, A. Vidal, S. You, M.R. Freeman, E. Davicioni, Y. Liu, R.J. Karnes, E.A. Klein, R.B. Den, B.J. Trock, J.D. Campbell, D.J. Einstein, R. Gupta, S. Balk, P. Lal, J.Y. Park, J.L. Cleveland, T.R. Rebbeck, S.J. Freedland, and K. Yamoah, *Comparative Genomics Reveals Distinct Immune-oncologic Pathways in African American Men with Prostate Cancer*. Clin Cancer Res, 2021. **27**(1): p. 320-329.
129. El-Tanani, M.K., H.F. Yuen, Z. Shi, A. Platt-Higgins, N.E. Buckley, P.B. Mullan, D.P. Harkin, P.G. Johnston, and P.S. Rudland, *Osteopontin can act as an effector for a germline mutation of BRCA1 in malignant transformation of breast cancer-related cells*. Cancer Sci, 2010. **101**(6): p. 1354-60.

Vita

Han Cun was born in 1988 in Ho Chi Minh City, Vietnam, the daughter of Chieng Cun and Mui Vong. Her family moved to the United States in the Fall of 1988, where they settled in Evansville, Indiana. After graduating from William Harrison High School in 2006, she enrolled at Brown University, Providence, RI. There, she received her Bachelor of Science degree in Biology with Honors and a focus in Immunology in 2010. After a year of conducting research at the University of Indiana School of Medicine – Evansville, she was accepted into the University of Indiana School of Medicine, and received her Doctor of Medicine in 2015. She then went on to enter the Obstetrics and Gynecology residency program at the University of Wisconsin, Madison, Wisconsin, and completed her training in 2019. Upon graduation in the summer of 2019, she began a fellowship in Gynecologic Oncology at the University of Texas MD Anderson Cancer Center, Houston, Texas. At that time, she also enrolled in the Master's Program at the University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences. She is mentored by Dr. Samuel C. Mok with a focus on tumor immune microenvironment in *BRCA*-deficient ovarian carcinomas. During her research time, she has investigated the role of IFITM3 on the immune response of *BRCA*-deficient ovarian carcinomas, assessed exosomal miRNAs as a novel biomarker in high-risk ovarian cancer patients, and utilized innovative technologies such as imaging mass cytometry and spatial transcriptomics.

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