Interleukin-16 expression associates with patient survival in high-grade serous ovarian cancer.

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Ovarian cancer is the most lethal gynecologic cancer¹. We sought to identify genes associated with enhanced survival in high-grade serous ovarian cancer (HGSC) by comparing global gene expression profiles of tumors from women diagnosed with HGSC with the best and worst progression-free survival². We found significant differential expression of the gene encoding the cytokine interleukin-16 (IL-16) when comparing tumor transcriptomes based on progression-free survival. IL-16 was expressed at significantly lower levels in high-grade serous ovarian tumors of women with the longest progression-free survival.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, IL-16.

1 The five-year survival rate for women diagnosed with high-grade serous ovarian cancer
2 is between 30-40% and has not changed significantly in decades^{5,6}. The development of novel,
3 targeted therapeutics to treat HGSC can be facilitated by an enhanced understanding of the
4 transcriptional behavior of ovarian tumors relative to that of the normal ovary. In a microarray
5 dataset generated with tumors from patients enrolled in the ICON7 trial⁴, IL-16 was among the
6 genes most differentially expressed when comparing tumors based on patient survival. We
7 mined separate published microarray data³ and found that IL-16 was also significantly
8 differentially expressed when comparing HGSC tumors to the normal ovary. IL-16 may be a
9 gene of interest when prioritizing the study of target genes and pathways for the development of
10 novel therapeutic interventions in high-grade serous ovarian cancers.

11 **Methods**

12 We used microarray data from datasets GSE140082² and GSE124766³ for this
13 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
14 and Hochberg method of p-value adjustment was used for ranking of differential expression but
15 raw p-values were used for assessment of statistical significance of global differential
16 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
17 platform annotation was used. GSE140082 was generated using Illumina HumanHT-12 WG-
18 DASL V4.0 R2 expression beadchip technology with $n=11$ for tumors from patients with
19 progression-free survival less than 100 days and $n=36$ for tumors from patients with
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1 progression-free survival greater than 1000 days. GSE124766 was generated using
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3 Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=3$ of for normal
4 ovarian tissue and $n=8$ for tumors from patients with high-grade serous ovarian cancer.
5 GEO2R provides mRNA expression levels only for the top 250 most differentially expressed
6 genes.
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9 A statistical test was used to compare whether expression of IL-16 was significantly
10 different between HGSC patients with progression-free survival (PFS) less than 100 days or
11 HGSC patients with PFS greater than 1000 days using a two-tailed, unpaired t-test with Welch's
12 correction. We used PRISM for all statistical analyses (Version 8.4.0)(455).
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14 15 **Results**

16 We mined published microarray data^{2,3} to identify differentially expressed genes in high-
17 grade serous ovarian cancer (HGSC) based on patient survival and when compared to the
18 normal ovary in the type of ovarian cancer responsible for 70-80% of deaths resulting from the
19 most lethal gynecologic malignancy.
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22 IL-16 expression in HGSC associates with survival outcomes.

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24 To identify genes associated with greater patient survival in ovarian cancer, we
25 compared tumors from patients with progression-free survival (PFS) less than 100 days to that
26 of patients with PFS greater than 1000 days, using microarray data obtained from tumors of
27 patients enrolled in the ICON7 trial (2). We found that IL-16 was among the genes most
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1 differentially expressed in HGSC based on survival outcomes (Table 1 and Figure 1). When
2 sorting all genes based on degree of change in expression between tumors of patients with PFS
3 less than 100 days and tumors of patients with PFS greater than 1000 days (maximum PFS:
4 1326 days), IL-16 ranked 217 out of 29377 total transcripts. Differential expression of IL-16 in
5 HGSC tumors based on progression-free survival less than 100 days or greater than 1000 days
6 was statistically significant (Table 1; $p=0.00222298$).
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10 IL-16 is expressed at lower levels in HGSC tumors from women the best progression-free
11 survival.
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13 We obtained exact mRNA expression levels for the differentially expressed IL-16
14 transcript from high-grade serous ovarian tumors when comparing tumors based on progression-
15 free survival less than 100 days or greater than 1000 days. IL-16 was expressed at significantly
16 lower levels in primary HGSC tumors from patients with PFS greater than 1000 days when
17 compared to that of patients with PFS less than 100 days (Figure 1; $p=0.0019$). We calculated a
18 mean fold change of 0.9208 ± 0.0485 in IL-16 expression when comparing tumors from patients
19 with PFS > 1000 days to that of tumors from patients with PFS < 100 days (Table 1).
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23 IL-16 is differentially expressed in ovarian tumors from women diagnosed with HGSC.
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25 In a separate microarray dataset, we found that IL-16 was also differentially expressed
26 in primary HGSC tumors when compared to the tissue of origin, the ovary (Table 2). When
27 sorting all of the transcripts measured by microarray based on change in expression between
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1 HGSC and the normal ovary, IL-16 ranked 8031 out of 41093 total transcripts (Table 2).
2 Differential expression of IL-16 in HGSC tumors was statistically significant (Table 2;
3 $p=4.24E-02$).
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6 Thus, we found using published microarray data that IL-16 was among the genes most
7 differentially expressed in HGSC tumors from women with progression-free survival greater
8 than 1000 days; HGSC tumors from these women expressed significantly lower levels of IL-16
9 than tumors from women with progression-free survival less than 100 days.
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14 Discussion

15 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic
16 analysis classified HGSC into four subtypes based on gene clustering: immunoreactive,
17 differentiated, proliferative, and mesenchymal⁶. We sought to continue to describe the
18 transcriptional landscape of high-grade serous ovarian cancers⁶ and identify genes whose
19 differential expression was associated with survival in HGSC by using published microarray
20 data.
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24 Interleukin-16 is also known as the lymphocyte chemoattractant factor⁷. IL-16 is a
25 soluble ligand for the CD4 molecule and possesses the ability to inhibit mixed lymphocyte
26 reactions *in vitro*⁸. IL-16 can be produced by eosinophils and mast cells^{9,10}. Activation
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of CD4⁺ T-lymphocytes by IL-2 can be enhanced by IL-16¹¹ but inhibit lymphocyte proliferation following CD3 engagement; IL-2 and IL-16 treatment results in expansion of CD4⁺ T-cells without affecting natural killer cells and monocytes¹². Interaction of CD4 and IL-16 results in activation of the tyrosine kinase p56lck and IL-16 can promote T-cell migration dependent on interaction between CD4 and p56lck; Th1 migration through CD4-IL-16 is dependent on CCR5^{13,14}. IL-16 produced by B-lymphocytes can also promote CD4⁺ lymphocyte chemotaxis¹⁵. IL-16 is present in greater than 70% of CD4⁺ and CD8⁺ T-lymphocytes in circulation and a majority of this is immature, pro-IL-16. CD3-ligation and activation of T-lymphocytes results in processing of pro-IL-16 into mature, active IL-16 cytokine¹⁶. While IL-16 is expressed at the mRNA level under basal conditions in fibroblasts from endogenous tissues, IL-16 protein is undetectable; exposure of fibroblasts to interleukin-1 β (IL-1 β) results in emergence of IL-16 protein. IL-1 β treatment results in induction of caspase-3 expression in fibroblasts, and IL-16 protein activity is dependent on caspase-3 function¹⁷.

IL-16 is implicated in some fashion in a number of human cancers. Polymorphisms in the IL-16 gene are associated with the risk of developing colorectal and gastric cancer¹⁸. IL-16 serum levels are increased in patients with stage III and IV tumors among breast, gastrointestinal, uterine, ovarian, renal and bladder cancers, suggesting tumor progression could be accompanied by IL-16 elevation¹⁹. In the laying hen model of spontaneously arising ovarian cancer, serum IL-16 was significantly higher in hens with ovarian cancer than in normal hens;

1 IL-16 was expressed in the ovarian stroma as well as in epithelial tumor cells in this model²⁰.
2 The number of IL-16+ cells and the number of CD8+ T-lymphocytes is significantly higher in
3 hens with microscopic ovarian cancer as compared to normal hens²¹. In humans, serum IL-16
4 levels were significantly elevated in patients with benign tumors as compared to healthy
5 controls, and serum IL-16 was even higher in patients with early- and late-stage ovarian
6 cancers²¹. Mechanistically, IL-16 could induce the expression of CD9 on ovarian surface
7 epithelial cells²². Thus, IL-16 genetic variants are associated with the risk of developing cancer
8 and studies have documented increased IL-16 expression in the serum of patients with ovarian
9 cancers.
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14 Differential expression of IL-16 in HGSC should be validated in larger and separate
15 cohorts of women with HGSC. We found that IL-16, a cytokine with the ability to induce T-cell
16 migration, was among the genes whose expression was most significantly different among high-
17 grade serous ovarian cancer patients with enhanced survival outcomes, expressed at
18 significantly lower levels in patients with progression-free survival greater than 1000 days.
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Rank	ID	p-value	t	B	FC	Gene
217	ILMN_1813572	0.00222298	-3.4382565	-1.38023	0.9208 ± 0.0485	IL16

Table 1: IL-16 is differentially expressed in high-grade serous ovarian carcinomas when comparing tumors based on patient progression-free survival.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of IL-16 in HGSC tumors when comparing patients with progression-free survival (PFS) less than 100 days with patients with PFS greater than 1000 days, the gene and gene name are listed in this chart.

Figure 1: IL-16 expression in HGSC tumors associates with patient survival outcomes.

The mRNA expression of IL-16 in tumors from patients with progression-free survival (PFS) < 100 days (left) and in the HGSC tumors from patients with PFS > 1000 days (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in IL-16 expression between the groups, a p-value, listed above.

Rank	ID	p-value	t	B	Gene
8031	A_24_P73599	4.24E-02	-2.2756384	-4.32125	IL16

Table 2: IL-16 is differentially expressed in high-grade serous ovarian carcinomas.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the gene and gene name are listed in this chart.