Expression of the interleukin-1 receptor antagonist 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 receptor antagonist for the cytokine interleukin-1 (IL-1) (IL-1RN) when comparing tumor transcriptomes based on progression-free survival. IL-1RN 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-1 receptor antagonist, IL-1RN.

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-1RN was among
6 the genes most differentially expressed when comparing tumors based on patient survival. We
7 mined separate published microarray data³ and found that IL-1RN was also significantly
8 differentially expressed when comparing HGSC tumors to the normal ovary. IL-1RN 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-1RN 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-1RN 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-1RN 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-1RN ranked 122 out of 29377 total transcripts. Differential expression of
5 IL-1RN in HGSC tumors based on progression-free survival less than 100 days or greater than
6 1000 days was statistically significant (Table 1; $p=6.73E-05$).

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10 IL-1RN 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-1RN
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 - 1 R N was expressed at
16 significantly lower levels in primary HGSC tumors from patients with PFS greater than 1000
17 days when compared to that of patients with PFS less than 100 days (Figure 1; $p=0.0030$). We
18 calculated a mean fold change of 0.9006 ± 0.0579 in IL-1RN expression when comparing
19 tumors from patients with PFS > 1000 days to that of tumors from patients with PFS < 100 days
20 (Table 1).
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25 IL-1RN is differentially expressed in ovarian tumors from women diagnosed with HGSC.

26 In a separate microarray dataset, we found that IL-1RN was also differentially expressed
27 in primary HGSC tumors when compared to the tissue of origin, the ovary (Table 2). When
28 sorting all of the transcripts measured by microarray based on change in expression between

1 HGSC and the normal ovary, IL-1RN ranked 545 out of 41093 total transcripts (Table 2).
2 Differential expression of IL-1RN in HGSC tumors was statistically significant (Table 2;
3 $p=1.15E-04$).
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6 Thus, we found using published microarray data that IL-1RN 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
9 IL-1RN 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, identifying the IL-1 receptor antagonist (IL-1RN) as among the genes most differentially
21 expressed when comparing tumor transcriptomes of those with the best and worst progression-
22 free survival.
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27 IL-1RN, also known as IL-1RA, functions as a receptor antagonist, binding to the IL-1R
28 but preventing rather the transducing signaling⁷⁻⁹. IL-1RN deficient mice develop

1 spontaneous autoimmune arthritis⁹. This arthritis occurs in BALB/C IL-1RN -/- mice but not
2 C57BL/6 IL-1RN -/- mice, suggesting that other genetic factors collaborate with IL-1 receptor
3 antagonist deficiency to generate this inflammatory phenotype¹⁰. These mice produce large
4 amounts of the pro-inflammatory cytokine IL-1 β . Interestingly, the development of arthritis in
5 these mice is dependent on toll-like receptor (TLR) activation by microbial flora, as germ-free
6 mice do not develop joint damage¹¹, suggesting interactions between the IL-1R antagonist,
7 microbial flora, toll-like receptors TLR2 and TLR4¹¹, and most likely other genetic factors that
8 conspire to cause systematic inflammation resulting in arthritis. Inflammatory damage in
9 IL1RN deficient mice is not restricted to arthritis; IL1rn -/- mice also develop arterial
10 inflammation¹².

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16 Deficiency of IL1RN in humans causes a disorder similar to the neonatal multi-system
17 inflammatory disorder NOMID¹³, caused by mutations in NLRP3; this is the canonical
18 inflammasome component that leads to activation of caspase-1 and subsequent maturation of
19 pro-IL1 β to the active form of IL1 β , demonstrating that in humans, mutations in IL-1RN can
20 result in phenotypes similar to those resulting from mutations in inflammasome machinery.
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23 IL1-RN is implicated in a number of human cancers. In pancreatic cancer, functioning to
24 inhibit growth of pancreatic tumors through impairment of NF-kB signaling¹⁴. An allele of
25 IL-1RN, IL-1RN*2 is associated with the risk of development of gastric cancer in Caucasians
26 but not Asians¹⁵. IL-1RN has been described as part of a inflammatory transcriptional signature
27 in patients with metastatic breast cancer¹⁶.
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IL-1RN is expressed in the ovary¹⁷ where it has described functions. IL-1 β can inhibit accumulation of P induced by FSH (follicle stimulating hormone) in the granulosa cell of the rat ovary, and IL-1RN can impair this inhibition in a dose-dependent manner¹⁸. In addition, IL-1RN can blunt the production of prostaglandin E from whole dispersates of the rat ovary induced by IL-1beta, together demonstrating that antagonistic functions of IL-1RN are conserved in the ovaries¹⁸. A variant allele of IL-1RN (“Allele V”) is associated with the risk of development of polycystic ovary syndrome¹⁹. One function of IL-1 β signaling in the ovary that could be affected by changes in IL-1RN expression in ovarian tumors is suppression of cell death (apoptosis) in response to nitric oxide²⁰. Two polymorphisms in the IL-1RN gene have been associated with the risk of developing epithelial ovarian cancer - an allele known as allele “2” and a variable number tandem repeat (VNTR) in the IL-1RN gene known as allele “V”^{21,22}.

Differential expression of IL-1RN in HGSC should be validated in larger and separate cohorts of women with HGSC. We found that IL-1RN was among the genes whose expression was most significantly different among high-grade serous ovarian cancer patients with enhanced survival outcomes, expressed at 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
122	ILMN_1806249	6.73E-05	-4.372028	1.617809	0.9006 ± 0.0579	IL1RN

Table 1: IL-1RN 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-1RN 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-1RN expression in HGSC tumors associates with patient survival outcomes.

The mRNA expression of IL-1RN 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-1RN expression between the groups, a p-value, listed above.

Rank	ID	p-value	t	B	Gene	Gene name
545	A_23_P209995	1.15E-04	5.6566578	1.47613	IL1RN	interleukin 1 receptor antagonist

Table 2: IL-1RN 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.