NLRP7 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 NLR family pyrin domain containing 7 (NLRP 7) when comparing tumor transcriptomes based on progression-free survival. NLRP7 was expressed at significantly higher 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, NLRP7.

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, NLRP7 was among
6 the genes most differentially expressed when comparing tumors based on patient survival. We
7 mined separate published microarray data³ to compare global gene expression profiles between
8 HGSC ovarian tumors and that of normal ovarian tissue; NLRP7 was also significantly
9 differentially expressed genes in HGSC tumors of the ovary in one dataset. NLRP7 may be a
10 gene of interest when prioritizing the study of target genes and pathways for the development of
11 novel therapeutic interventions in high-grade serous ovarian cancers.
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17 **Methods**

18 We used microarray data from datasets GSE140082² and GSE124766³ for this
19 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
20 and Hochberg method of p-value adjustment was used for ranking of differential expression but
21 raw p-values were used for assessment of statistical significance of global differential
22 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
23 platform annotation was used. GSE140082 was generated using Illumina HumanHT-12 WG-
24 DASL V4.0 R2 expression beadchip technology with $n=11$ for tumors from patients with
25 progression-free survival less than 100 days and $n=12$ for tumors from patients with
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1 progression-free survival greater than 1100 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 NLRP7 was significantly
10 different between HGSC patients with progression-free survival (PFS) less than 100 days or
11 HGSC patients with PFS greater than 1100 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 NLRP7 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 1100 days, using microarray data obtained from tumors of
27 patients enrolled in the ICON7 trial (2). We found that NLRP7 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 1100 days (maximum PFS:
4 1326 days), NLRP7 ranked 245 out of 29377 total transcripts. Differential expression of
5 NLRP7 in HGSC tumors based on progression-free survival less than 100 days or greater than
6 1100 days was statistically significant (Table 1; $p=0.00258874$).

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10 NLRP7 is expressed at higher 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 NLRP7
14 transcript from high-grade serous ovarian tumors when comparing tumors based on progression-
15 free survival less than 100 days or greater than 1100 days. N L R P 7 w a s e x p r e s s e d a t
16 significantly higher levels in primary HGSC tumors from patients with PFS greater than 1100
17 days when compared to that of patients with PFS less than 100 days (Figure 1; $p=0.0057$). We
18 calculated a mean fold change of 1.3141 ± 0.3179 in NLRP7 expression when comparing
19 tumors from patients with PFS > 1100 days to that of tumors from patients with PFS < 100 days
20 (Table 1).
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25 NLRP7 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

26 In a separate microarray dataset, we found that NLRP7 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, NLRP7 ranked 6981 out of 41093 total transcripts (Table 2).
2 Differential expression of NLRP7 in HGSC tumors was statistically significant (Table 2;
3 $p=3.16E-02$).
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6 Thus, we found using published microarray data that NLRP7 was among the genes most
7 differentially expressed in HGSC tumors from women with progression-free survival greater
8 than 1100 days; HGSC tumors from these women expressed significantly higher levels of
9 NLRP7 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 Nod-like receptor proteins, NLRPs, are pattern-recognition receptors that can activate
25 but also attenuate inflammatory gene expression such as NF- κ B and IL-1 signaling in response
26 to detection of a variety of pathogen-derived or synthetic ligands. NLRP7 responds to microbial
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1 lipopeptides similar to canonical inflammasomes by activation of caspase-1 for cleavage and
2 maturation of IL-1beta and IL-18. ATP binding is a necessary step in activation of the NLRP7
3 inflammasome^{7,8}. NLRP7 influence on cytokine secretion has been reported to involve
4 localization at the Golgi apparatus and microtubule-organizing center⁹.

7 NLRP7 has important roles in the female reproductive tract. NLRP7 expression can be
8 detected in the ovine ovary¹⁰. Mutations, including deletions and missense mutations in NLRP7
9 are recurrently found in biparentally inherited hydatiform mole, a genetic condition resulting in
10 abnormal pregnancy with no embryo^{11,12}. NLRP7 can influence trophoblast differentiation, and
11 is able to interact with transcriptional molecule YY1¹³. Depletion of NLRP7 in hES cells
12 promoted trophoblast differentiation and resulted in alteration of CpG methylation in genes
13 including SUCLG2 and FBXO4¹³. Consistent with an important role for NLRP7 in maintaining
14 pregnancy in women, women with mutations in NLRP7 are at increased risk for development of
15 reproductive wastage¹⁴. Thus, NLRP7 has important roles in innate immunity and in
16 reproductive functions in females. We could not identify any literature regarding NLRP7 in
17 high-grade serous ovarian cancer.

22 Differential expression of NLRP7 in HGSC should be validated in larger and separate
23 cohorts of women with HGSC. We found that NLRP7 was among the genes whose expression
24 was most significantly different among high-grade serous ovarian cancer patients with enhanced
25 survival outcomes, expressed at significantly higher levels in patients with progression-free
26 survival greater than 1100 days.

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Rank	ID	p-value	t	B	FC	Gene
245	ILMN_1658632	0.00258874	3.3755865	-1.50484	1.3141 ± 0.3179	NLRP7

Table 1: NLRP7 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 NLRP7 in HGSC tumors when comparing patients with progression-free survival (PFS) less than 100 days with patients with PFS greater than 1100 days, the gene and gene name are listed in this chart.

Figure 1: NLRP7 expression in HGSC tumors associates with patient survival outcomes.

The mRNA expression of NLRP7 in tumors from patients with progression-free survival (PFS) < 100 days (left) and in the HGSC tumors from patients with PFS > 1100 days (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in NLRP7 expression between the groups, a p-value, listed above.

Rank	ID	p-value	t	B	Gene	Gene name
6981	A_23_P16384	3.16E-02	2.4394138	-4.04439	NLRP7	NLR family pyrin domain containing 7

Table 2: NLRP7 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.