1 **HLF is differentially expressed in high-grade serous ovarian cancers.**

2 Shahan Mamoor¹

3 ¹shahanmamoor@gmail.com

4 East Islip, NY 11730

5 Ovarian cancer is the most lethal gynecologic cancer¹. We sought to identify genes associated
6 with high-grade serous ovarian cancer (HGSC) by comparing global gene expression profiles of
7 normal ovary with that of primary tumors from women diagnosed with HGSC using published
8 microarray data^{2,3}. We found significant differential expression of the transcription factor hepatic
9 leukemia factor^{4,5} (HLF) in high-grade serous ovarian tumors.

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24 Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in
25 ovarian cancer, systems biology of ovarian cancer, HLF.

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^{6,7}. 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. We mined
5 published microarray data^{2,3} to compare global gene expression profiles between HGSC ovarian
6 tumors and that of normal ovarian tissue. We identified the gene encoding hepatic leukemia
7 factor, HLF, as among the most differentially expressed in HGSC tumors of the ovary. HLF may
8 be a gene of interest when prioritizing the study of target genes and pathways for the
9 development of novel therapeutic interventions in high-grade serous ovarian cancers.
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11 12 **Methods**

13 We used microarray data from datasets GSE146556² and GSE124766³ for this
14 differential gene expression analysis of high-grade serous carcinomas. The Benjamini and
15 Hochberg method of p-value adjustment was used for ranking of differential expression but raw
16 p-values were used for assessment of statistical significance of global differential expression.
17 Log-transformation of data was auto-detected, and the NCBI generated category of platform
18 annotation was used. GSE146556² was generated using Affymetrix Human Gene 1.0 ST Array
19 with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade serous
20 ovarian cancer. GSE124766³ was generated using Agilent-014850 Whole Human Genome
21 Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from
22 patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only
23 for the top 250 most differentially expressed genes.
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25 A statistical test was performed to evaluate whether HLF expression was significantly
26 different when comparing normal ovarian tissue from control subjects and primary tumors from
27 women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only
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p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray data^{2,3} to identify differentially expressed genes in high-grade serous ovarian cancer (HGSC), the type of ovarian cancer responsible for 70-80% of deaths resulting from the most lethal gynecologic malignancy.

HLF is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified hepatic leukemia factor (HLF) as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, HLF ranked 15 out of 29088 total transcripts (Table 1). Differential expression of HLF in HGSC tumors was statistically significant (Table 1; $p=5.43E-14$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of HLF could be observed in tumors from a different group of patients. We again found that HLF was differentially expressed in primary HGSC tumors (Table 2). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, in this data set, HLF ranked 265 out of 41093 total transcripts (Table 2). Differential expression of HLF in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=2.3E-05$).

HLF is expressed at significantly lower levels in HGSC when compared to the normal ovary.

We then obtained exact mRNA expression levels for the differentially expressed HLF transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. HLF was expressed at significantly lower levels in high-grade serous ovarian cancers when

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1 compared to the normal ovary (Figure 1; $p < 0.0001$). We calculated a mean fold change of
2 0.1232 ± 0.0515 in HLF expression when comparing HGSC tumors to normal ovarian tissue
3 (Table 1).

4 Thus, we found using published microarray data that HLF was among the genes most
5 differentially expressed in tumors from women with high-grade serous carcinomas and that HLF
6 expression was significantly lower in HGSC tumors when compared to the normal ovary.
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8 **Discussion**

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10 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
11 classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated,
12 proliferative, and mesenchymal⁸. We sought to continue to describe the transcriptional
13 landscape of high-grade serous ovarian cancers⁸ and identify genes whose differential
14 expression was associated with HGSC by using published microarray data from primary tumors
15 of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}.
16 In both datasets analyzed, HLF was among the genes whose expression changed most
17 significantly when comparing the normal ovary to primary HGSC tumors
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19 Hepatic leukemia factor (HLF) was first identified as a gene fusion to the E2A protein in
20 acute lymphoblastic leukemias^{4,5}, wherein the carboxy-terminal leucine zipper domain of HLF
21 was fused to the amino-terminal transactivation domain of E2A, resulting from the t(17;19)
22 chromosomal translocation.
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24 By comparing transcriptional profiles of HSC and granulocyte/monocyte/lymphoid
25 progenitors (GMLP) with that of more differentiated progenitor cells to identify genes most
26 differentially expressed between stem and progenitor cells in the blood, HLF was shown to be a
27 regulator of early hematopoiesis⁹. HLF is expressed at significantly higher levels in HSC and
28 GMLP than in granulocyte-monocyte progenitors (GMP) or the common lymphoid progenitor.
Inducible expression of HLF *in vivo* leads to shift in HSC differentiation to myeloipoiesis, with a

1 significant increase in the GMP population concomitant with a decrease in the lymphoid
2 compartment⁹. Within three days of HLF induction *in vivo*, thymic atrophy is observable⁹.

3 The hematopoietic system of HLF-deficient mice is phenotypically unremarkable until
4 challenge with transplantation, where upon serial transplantation the pool of HSC is gradually
5 depleted¹⁰. Treatment of HLF-deficient mice with 5-fluorouracil to target actively cycling cells
6 lead to complete loss of the LSK population¹⁰. Together with data showing that HLF-deficient
7 mice possessed a significant reduction in hematopoietic progenitors after sub-lethal irradiation,
8 the findings indicated that HLF is important for maintaining the quiescent state of HSC¹⁰.

9 One study found a high frequency of leukemia stem cells in triple-mutated acute myeloid
10 leukemia patients containing mutations in Flt3, Npm1 and Dnmt3a, characterized by a
11 Gpr56^{high}CD34^{low} phenotype and specific up-regulation of HLF gene expression¹¹. In patients
12 possessing a mutation at position R882 in the DNMT3a gene, high expression of HLF correlated
13 with poor survival; high expression of HLF also correlated with CpG hypomethylation at the
14 transcription start site of HLF¹¹. Deletion of HLF using CRISPR in primary human AML cells
15 lead to a significant decrease in Gpr56^{high}CD34^{low} leukemia stem cells upon serial
16 transplantation. Thus, in triple-mutant AML, increased expression of HLF is associated with the
17 leukemia stem phenotype and poor survival¹¹.

18 Differential expression of HLF in HGSC should be validated in larger and separate
19 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
20 cancers¹²⁻¹⁴ can be utilized to assess the effect of conditional expression of HLF in HGSC
21 tumors post-development and on tumor cell proliferation and cancer progression *in vivo*.
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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
15	8008588	5.43E-14	-10.7914123	21.4814661	0.1232 ± 0.0515	HLF	HLF, PAR bZIP transcription factor

Table 1: HLF 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 fold change of HLF in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
265	A_23_P356585	2.3E-05	-6.7390985	3.05737	HLF	HLF, PAR bZIP transcription factor

Table 2: HLF is among the most differentially expressed genes in a tumor dataset from patients with 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 fold change of HLF in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: HLF is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of HLF in normal ovarian tissues from control subjects (left) and in the primary tumors of patients with HGSC (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in HLF expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.