1 **NFE2L3 is differentially expressed in high-grade serous ovarian cancers.**

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7 Ovarian cancer is the most lethal gynecologic cancer¹. We sought to identify genes associated
8 with high-grade serous ovarian cancer (HGSC) by comparing global gene expression profiles of
9 normal ovary with that of primary tumors from women diagnosed with HGSC using published
10 microarray data^{2,3}. We found significant differential expression of the gene encoding the nuclear
11 factor, erythroid 2 like 3 transcription factor NFE2L3 in high-grade serous ovarian tumors.
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26 Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in
27 ovarian cancer, systems biology of ovarian cancer, NFE2L3.
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1 The five-year survival rate for women diagnosed with high-grade serous ovarian cancer is
2 between 30-40% and has not changed significantly in decades^{4,5}. 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 nuclear factor, erythroid 2 like 3
7 transcription factor NFE2L3 as among the most differentially expressed genes in HGSC tumors
8 of the ovary. NFE2L3 may be a gene of interest when prioritizing the study of target genes and
9 pathways for the development of novel therapeutic interventions in high-grade serous ovarian
10 cancers.
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15 **Methods**

16 We used microarray data from datasets GSE124766² and GSE146556³ for this
17 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
18 and Hochberg method of p-value adjustment was used for ranking of differential expression but
19 raw p-values were used for assessment of statistical significance of global differential expression.
20 Log-transformation of data was auto-detected, and the NCBI generated category of platform
21 annotation was used. GSE124766 was generated using Agilent-014850 Whole Human Genome
22 Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from
23 patients with high-grade serous ovarian cancer. GSE146556 was generated using Affymetrix
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Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only for the top 250 most differentially expressed genes.

A statistical test was performed to evaluate whether NFE2L3 expression was significantly different when comparing normal ovarian tissue from control subjects and primary tumors from women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only 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.

NFE2L3 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified the gene encoding the nuclear factor, erythroid 2 like 3 (NFE2L3) as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that NFE2L3 was differentially expressed in primary HGSC tumors (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, NFE2L3 ranked 157 out of

41093 total transcripts (Table 1). Differential expression of NFE2L3 in HGSC tumors was statistically significant (Table 1; $p=7.07E-06$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of NFE2L3 could be observed in tumors from a separate group of patients. We again found that NFE2L3 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, NFE2L3 ranked 1428 out of 29088 total transcripts (Table 2). Differential expression of NFE2L3 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=3.07E-04$).

NFE2L3 is expressed at higher levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed NFE2L3 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. NFE2L3 was expressed at higher levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference was statistically significant (Figure 1; $p=0.0055$). We calculated a mean fold change of 1.3847 ± 0.0741 in NFE2L3 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that NFE2L3 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that NFE2L3 expression was higher in HGSC tumors when compared to the normal ovary.

Discussion

Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated, proliferative, and mesenchymal⁶. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers⁶ and identify genes whose differential expression was associated with HGSC by using published microarray data from primary tumors of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}. In both datasets analyzed, NFE2L3 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

NFE2L3 is a member of Cap 'n' Collar (CNC) family of basic-region leucine zipper (bZIP) transcription factors⁷. Human NFE2L3 is 694 amino acids in length and possesses at its amino-terminus two N-terminal homology boxes (NHBs), a PEST degradation domain, a transactivation domain at its middle region and at its carboxy-terminus a CNC domain, basic region and leucine zipper^{7,8}. Highest expression of NFE2L3 is detected in the placenta but lower levels of expression are found in the brain, lung, kidney, pancreas, leukocytes, thymus, heart and spleen⁸. On gel electrophoresis, three forms of NFE2L3 protein can be detected; these correspond to a glycosylated form ("A"), a non-glycosylated form of NFE2L3 A ("B"), as well as a third, non-glycosylated form ("C") whose identity is not completely clear but may represent an amino-terminally truncated form of NFE2L3⁹. Mice deficient in NFE2L3 are apparently normal, born in classical mendelian ratios with no gross phenotypic features, and are similar to

1 wild-type mice with respect to red and white blood cell count and immunity to acute
2 lymphocytic choriomeningitis virus infection when measuring B and T-lymphocyte responses¹⁰.
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4 NFE2L3 can function both as an activator and repressor of gene expression; specific target genes
5 of NFE2L3 have remained elusive but some proposed candidates include Prdx6, Nqo1, calponin
6 and Nox4^{11,12}. Transactivation of genes in the nucleus by NFE2L3 is considered to require
7 heterodimerization of NFE2L3 “C” with small Maf proteins to Maf recognition elements
8 (MARE) and antioxidant-response elements (ARE)⁷⁻⁹.
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11 NFE2L3 is implicated is described in the literature in relation to a number of cancers.
12 Mice deficient in NFE2L3, while phenotypically normal in non-challenged conditions, develop
13 T-cell lymphoblastic lymphoma at high incidence when exposed to cigarette smoke carcinogen
14 benzo[a]pyrene¹³. NFE2L3 expression is elevated in human colon adenocarcinomas. NFE2L3
15 expression could be regulated by the NF-kB transcription factor RELA, and depletion of
16 NFE2L3 resulted in increased expression of double homeobox 4 (DUX4), an inhibitor of the
17 cyclin dependent kinase CDK1¹⁴. Depletion of NFE2L3 *in vivo* resulted in decreased tumor
18 growth in the HCT116 colon cancer xenograft mouse model¹⁴. Thus, in colon cancer, NFE2L3
19 controlled expression of DUX4 to regulate cell cycle activity through CDK1 and promote cancer
20 cell proliferation¹⁴. A separate study demonstrated that in DLD-1 colon cancer cells, NFE2L3
21 was rapidly degraded through association with the ER-associated degradation ubiquitin ligase
22 HRD1 and the valosin-containing protein (VCP), as well as by beta-TRCP¹⁵. Translocation of
23 NFE2L3 from the ER to the nucleus required DNA-damage inducible 1 homolog 2 (DDI2), and
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1 in the nucleus, NFE2L3 activated gene expression of U2AF homology motif kinase 1 (UHMK1)
2 to promote cell proliferation in DLD-1 colon cancer cells¹⁵. NFE2L3 also controls the
3 expression of the pro-inflammatory cytokine tumor necrosis factor alpha in colon cancers¹⁶.
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6 NFE2L3 is over-expressed in human thyroid cancers as well as in distant metastases¹⁷,
7 and was shown to be a key effector of the regulator of calcineurin 1, isoform 4 (RCAN1-4), a
8 factor whose depletion *in vivo* results in enhanced tumor growth in FTC236 human follicular
9 thyroid cancer and in HTh74 anaplastic thyroid carcinoma xenograft models¹⁷. NFE2L3 over-
10 expression is also reported in pancreatic cancer; pancreatic cancer patients expressing higher
11 levels of NFE2L3 possessed significantly worse outcomes, and increased NFE2L3 expression
12 was associated with lymph node metastasis. Depletion of NFE2L3 in PANC-1 and SW1990
13 pancreatic cancer cells resulted in impaired invasion in vitro while not affecting cell
14 proliferation. Expression of NFE2L3 was correlated with that of vascular endothelial growth
15 factor A¹⁸. Over-expression of NFE2L3 has also been reported in Hodgkin's lymphoma,
16 anaplastic lymphoma¹⁹⁻²⁰ as well as Mantle cell lymphoma²¹.
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21 Differential expression of NFE2L3 in HGSC should be validated in larger and separate
22 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
23 cancers²²⁻²⁴ can be utilized to assess the effect of conditional expression of NFE2L3 in HGSC
24 tumor growth post-development and on survival *in vivo*.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
157	A_23_P42718	7.07E-06	7.608627	4.20245	1.3847 ± 0.0741	NFE2L3	nuclear factor, erythroid 2 like 3

Table 1: NFE2L3 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 NFE2L3 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
1428	8131944	3.07E-04	3.9154	0.0359315	NFE2L3	nuclear factor, erythroid 2 like 3

Table 2: NFE2L3 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 NFE2L3 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: NFE2L3 is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of NFE2L3 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 NFE2L3 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.