1 **Over-expression of the retinoid-inducible nuclear factor, RINF (CXXC5) in high-grade**
2 **serous ovarian cancers.**

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6 Ovarian cancer is the most lethal gynecologic malignancy and 70-80% of ovarian cancers are of
7 the high-grade serous type¹⁻³. To identify the most significant changes in gene expression in
8 high-grade serous ovarian cancer (HGSC), we compared global gene expression profiles of
9 tumors from patients with HGSC to that of normal ovary using published microarray datasets^{4,5}.
10 We found that gene encoding the retinoid-inducible nuclear factor, RINF (also known as CXXC
11 finger protein 5, CXXC5)⁶ was among the genes whose expression changed most significantly
12 when comparing HSGC tumors to the ovary. RINF/CXXC5 may be relevant to the biology of
13 high-grade serous ovarian tumors.
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28 **Keywords:** CXXC5, ovarian cancer, HGSC, high-grade serous ovarian cancer, systems biology
of ovarian cancer, targeted therapeutics in ovarian cancer.

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 decades¹⁻³. 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^{4,5} to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the gene encoding the CXXC
7 finger protein 5, CXXC5, as among the genes most differentially expressed in HGSC tumors of
8 the ovary. RINF/CXXC5 may be a gene of interest when prioritizing the study of target genes
9 and pathways for the development of novel therapeutic interventions in high-grade serous
10 ovarian cancers.
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12 **Methods**

13 We used microarray data from datasets GSE146556⁴ and GSE124766⁵ 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. GSE146556⁴ 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. GSE124766⁵ 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 CXXC5 expression was
26 significantly different when comparing normal ovarian tissue from control subjects and primary
27 tumors from women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's
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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^{4,5} 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.

CXXC5 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified the gene encoding CXXC5 as among the genes whose expression was quantitatively most different in a published microarray dataset⁴ when comparing primary HGSC tumors to normal ovarian tissue (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC tumors and the normal ovary, CXXC5 ranked 189 out of 29088 total transcripts (Table 1). Differential expression of CXXC5 in HGSC tumors was statistically significant (Table 1; $p=1.38E-07$).

We analyzed a second microarray dataset⁵ generated using normal ovarian tissues and tumors from women diagnosed with HGSC (5) to determine whether differential expression of CXXC5 could be observed in a separate group of patients. We again found that CXXC5 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, CXXC5 ranked 163 out of 41093 total transcripts (Table 2). Differential expression of CXXC5 in HGSC tumors was statistically significant (Table 2; $p=7.77E-06$).

CXXC5 is expressed at significantly higher levels in HGSC when compared to the normal ovary.

We then obtained exact mRNA expression levels for the differentially expressed CXXC5 transcript from normal ovarian tissue and from high-grade serous ovarian tumors. CXXC5 was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to

the normal ovary (Figure 1; $p=0.0003$). We calculated a mean fold change of 3.6337 ± 1.1410 in CXXC5 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Analysis of CXXC5 differential expression in a separate dataset demonstrated that CXXC5 was again expressed at significantly lower levels in primary tumors from women diagnosed with high-grade serous ovarian cancer when compared to normal ovarian tissue (Figure 2; $p<0.0001$). We calculated a mean fold change of 1.2870 ± 0.0586 in CXXC5 expression when comparing HGSC tumors to normal ovarian tissue (Table 2) in this second dataset (5).

Thus, we found using published microarray data^{4,5} that CXXC5/RINF was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that CXXC5 expression was significantly higher in HGSC tumors when compared to the normal ovary.

Discussion

We sought to continue to describe the transcriptional landscape of ovarian cancer⁷ by mining published microarray data^{4,5} comparing the normal ovary to tumors from patients with the most common type of epithelial ovarian cancer, high-grade serous ovarian cancer (HGSC). We identified CXXC5 as among the genes most differentially expressed in HGSC tumors.

CXXC5/RINF is a member of a family of zinc finger (ZF)-CXXC domain-containing proteins that includes KDM2A (CXXC8), KDM2B (CXXC2), FBXL19, DNMT1 (CXXC9), MLL1 (CXXC7), MBD1, TET1 (CXXC6) and TET3. CXXC-ZF domain-containing proteins are broadly involved in epigenetic regulation of gene expression and have the ability to interact with non-methylated CpG nucleotides⁸. CXXC5 protein displays nuclear localization and can interact with the Ten eleven translocation (Tet) family of dioxygenases that catalyze conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) to facilitate DNA demethylation in embryonic stem cells⁶. CXXC5/Rinf formed a complex with Nanog, Oct4, Tet1 and Tet2 to

1 promote expression of pluripotency genes⁶. In telencephalic neural stem cells, CXXC5 was
2 required for induction of Wnt pathway targets (e.g., Axin) following signals transduced by bone
3 morphogenetic protein 4 (BMP4)⁹. In plasmacytoid dendritic cells, CXXC5 was responsible for
4 recruiting Tet2 to interferon pathway transcriptional targets following by signaling by Toll-like
5 receptors TLR7/9¹⁰. CXXC5-deficient mice manifest increased bone mineral density, and
6 CXXC5 was found to cooperate with Dishevelled (Dvl) to inhibit Wnt/ β -catenin signaling during
7 differentiation of osteoblasts, indicating that CXXC5 was a negative regulator of osteoblast
8 differentiation and bone formation¹¹. Thus, CXXC5 has roles in maintenance of ES cell
9 pluripotency, in development and in immunity across tissues.

11 CXXC5 is implicated in a variety of cancers, of the blood and in solid tumors. A study
12 searching for transcriptional targets of all-trans retinoid acid (ATRA) using the NB4 cell culture
13 model of acute promyelocytic leukemia (APML) identified 9 ATRA-induced genes; one of these
14 was named retinoid acid-inducible factor, or RINF¹². Genetic depletion of RINF in NB4 cells
15 blocked ATRA-induced differentiation of APML cells¹². Acute myeloid leukemia (AML) patients
16 with 5q deletion lose expression of CXXC5, encoded on 5q31¹³. Higher expression of CXXC5 is
17 associated with decreased survival in newly-diagnosed AML patients receiving cytarabine and
18 anthracycline chemotherapy¹⁴. In the HOVON/SAKK cohort (498 patients), AML patients with
19 lower expression of CXXC5 trended towards higher complete remission rates, while overall
20 survival and event-free survival were significantly greater in patients with low CXXC5
21 expression¹⁴. In KG1a and K562 cells, expression of CXXC5 limited induction of Wnt pathway
22 transcriptional targets Axin2 and Survivin by Wnt3a, indicating that CXXC5 functioned to restrict
23 canonical Wnt signaling in AML¹⁵. 17- β estradiol induced expression of CXXC5 through
24 estrogen receptor alpha (ER α) in MCF-7 breast cancer cells¹⁶. Expression of CXXC5 correlated
25 with levels of ER α mRNA and protein in tumors from patients with breast cancer, and was
26 associated with worse prognosis in patients with ER-positive breast cancer¹⁶. In malignant

1 peripheral nerve sheath (MPNST) tumors, CXXC5 was found to be a transcriptional target of the
2 KANK1 gene¹⁷. Lower levels of KANK1 in MPNSTs resulted in decreased CXXC5 expression,
3 leading to decreases in KANK1-induced apoptosis¹⁷. In the hepatocellular carcinoma cell line
4 Hep3B, CXXC5 was identified as a transcriptional target of TGF- β signaling in a study searching
5 for mechanisms by which TGF- β exerted inhibitory effects on tumor growth in the liver¹⁸. Thus,
6 in both MPNST and HCC cancer models, CXXC5 functions in a tumor suppressive role, with
7 increased levels inhibiting tumor growth.
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10 We found here that the gene encoding CXXC finger protein 5 (CXXC5) was expressed
11 at significantly higher levels in tumors from a group of patients with high-grade serous ovarian
12 cancer when compared to the normal ovary, using published microarray data^{4,5}. This change in
13 CXXC5 expression was among the highest transcriptome-wide when comparing HGSC tumors
14 to benign ovarian tissue. A specific requirement for CXXC5 in high-grade serous ovarian cancer
15 can be assessed by measuring tumor cell proliferation in HGSC cell lines *in vitro*¹⁹ and on
16 survival outcomes and tumor progression *in vivo* using HGSC xenografts and genetically
17 engineered mouse models of HGSC²⁰⁻²².
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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
189	8108447	1.38E-07	6.2550693	7.3980455	3.6337 ± 1.1410	CXXC5	CXXC finger protein 5

Table 1: CXXC5 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 CXXC5 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	Fold change	Gene	Gene name
163	A_24_P930062	7.77E-06	7.5361217	4.11104	1.2870 ± 0.0586	CXXC5	CXXC finger protein 5

Table 2: CXXC5 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.

Figure 1: CXXC5 expression is significantly elevated in HGSC tumors.

The mRNA expression of CXXC5 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 CXXC5 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p -value, listed above.

Figure 2: CXXC5 expression is significantly elevated in HGSC tumors.

The mRNA expression of CXXC5 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 CXXC5 expression between the ovary of control subjects and primary tumors from patients with HGSC, a *p*-value, listed above.