Cytidine-5'-triphosphate synthase 1 (CTPS1) is differentially expressed in high-grade serous ovarian cancers.

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Ovarian cancer is the most lethal gynecologic cancer¹. We sought to identify genes associated with high-grade serous ovarian cancer (HGSC) by comparing global gene expression profiles of normal ovary with that of primary tumors from women diagnosed with HGSC using published microarray data^{2,3}. We found significant differential expression of CTPS1 in high-grade serous ovarian tumors.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, CTPS1.

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 the gene encoding cytidine-5'-
7 triphosphate synthase 1, CTPS1, as among the most differentially expressed genes in HGSC
8 tumors of the ovary. CTPS1 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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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 CTPS1 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.

CTPS1 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified the gene encoding cytidine-5'-triphosphate synthase 1 (CTPS1) as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that CTPS1 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, CTPS1 ranked 236 out of 41093

total transcripts (Table 1). Differential expression of CTPS1 in HGSC tumors was statistically significant (Table 1; $p=1.74E-05$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of CTPS1 could be observed in tumors from a separate group of patients. We again found that CTPS1 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, CTPS1 ranked 772 out of 29088 total transcripts (Table 2). Differential expression of CTPS1 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=3.63E-05$).

CTPS1 is expressed at lower levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed CTPS1 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. CTPS1 was expressed at lower levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 0.8229 ± 0.0419 in CTPS1 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that CTPS1 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that CTPS1 expression was lower 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, CTPS1 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

CTPS1, or cytidine-5'-triphosphate synthase, is the enzyme that catalyzes the *de novo* synthesis of pyrimidine nucleotide CTP from UTP, ATP and glutamine⁷. While an important precursor molecule for RNA and DNA metabolism, CTP can also influence phospholipid synthesis⁸. CTPS1 contains an amino-terminal synthetase domain as well as a carboxy-terminal glutaminase domain⁹. CTPS1 can be found in large, discrete filamentous cellular structures known as cytoophidia both in the cytoplasm and nucleus of eukaryotic cells¹⁰. One group used human autoantibodies to identify cellular structures known as rods and rings (RR) in HEp-2 cells that were not enriched in actin or tubulin but were enriched in both CTPS1 and inosine monophosphate dehydrogenase 2 (IMPDH2), another enzyme involved in *de novo* nucleotide synthesis¹¹. CTPS1 inhibitor 6-diazo-5-oxo-L-norleucine could result in induction of RR in a vast majority of cell lines assayed, suggested that RR formation could be induced by changes in

cellular CTP concentration. In mouse embryonic stem cells, RR could be observed upon retinoic acid differentiation but disassembled upon treatment with Acivicin¹¹. CTPS1 can be regulated through phosphorylation by glycogen synthase kinase-3 beta (GSK3 β)¹². Interaction of CTPS1 with peptidyl prolyl isomerase (PIN1) and with tubulin has been demonstrated; binding of PIN1 by CTPS1 is dependent on phosphorylation of CTPS1 at serine 575¹³. Patients with deficiencies in CTPS1 exhibit severe immunodeficiencies characterized by failure of activated T lymphocytes to proliferate following TCR-CD3 receptor signaling; B-lymphocyte proliferation is also significantly impaired by CTPS1 deficiency¹³. Highest expression of CTPS1 was found in activated T-lymphocytes when compared to the relative low-level expression observed across tissues¹⁴. Thus, CTPS1 has important functions in RNA and DNA metabolism and its deficiency in humans results in severe immunodeficiencies of the adaptive immune system.

In 1978, a report documented increased activity of CTPS1 in all hepatomas tested; CTPS1 activity was highest in tumors with higher proliferation rates¹⁵. Increased CTP synthetase activity was also observed in renal cell carcinomas¹⁵. In pancreatic cancer, protein expression of CTPS1 in conjunction with that of ENT1, UMP-CMP kinase and dCK were correlated with progression-free survival¹⁶. A study investigating YBX1 in breast cancer and potential gene expression targets of YBX1 found that CTPS1 expression was significantly associated with worse prognosis; patients with low expression of CTPS1 survived significantly longer than those whose tumors were deemed to have high expression of CTPS1¹⁷. In a study of hepatocellular carcinomas, cytoophidia were observed in 28% (56/203) of primary human tumor

1 samples; no cytoophidia were observed in adjacent control hepatocytes¹⁸. We could identify one
2 report of perturbed expression of CTPS1 in ovarian cancers. CTPS1 expression was linked with
3 that of CLIC1; CTPS1 expression was increased in ovarian cancers but significantly decreased
4 upon CLIC1 depletion in the A2780 cell line¹⁹. Thus, CTPS1 controls the synthesis and
5 concentration of an important precursor metabolite for RNA and DNA, and investigators have
6 documented perturbed expression of CTPS1 in multiple human cancers.
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10 Differential expression of CTPS1 in HGSC should be validated in larger and separate
11 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
12 cancers²⁰⁻²² can be utilized to assess the effect of conditional expression of CTPS1 in HGSC
13 tumor growth post-development and on survival *in vivo*.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
236	A_23_P21706	1.74E-05	-6.9365732	3.32693	0.8229 ± 0.0419	CTPS1	CTP synthase 1

Table 1: CTPS1 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 CTPS1 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
772	7900510	3.63E-05	4.5900689	2.057867	CTPS1	CTP synthase 1

Table 2: CTPS1 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 CTPS1 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: CTPS1 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of CTPS1 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 CTPS1 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.