SYTL4 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 synaptotagmin-like 4 (SYTL4) 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, SYTL4.

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 synaptotagmin-like
7 4 (SYTL4) as among the most differentially expressed in HGSC tumors of the ovary. SYTL4
8 may 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.
10

11 12 **Methods**

13 We used microarray data from datasets GSE146556² and GSE124766³ for this
14 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
15 and Hochberg method of p-value adjustment was used for ranking of differential expression but
16 raw p-values were used for assessment of statistical significance of global differential
17 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
18 platform annotation was used. GSE146556² was generated using Affymetrix Human Gene 1.0
19 ST Array with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade
20 serous ovarian cancer. GSE124766³ was generated using Agilent-014850 Whole Human
21 Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors
22 from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels
23 only for the top 250 most differentially expressed genes.
24

25 A statistical test was performed to evaluate whether SYTL4 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
28

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.

SYTL4 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified synaptotagmin-like 4 (SYTL4) 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, SYTL4 ranked 126 out of 29088 total transcripts (Table 1). Differential expression of SYTL4 in HGSC tumors was statistically significant (Table 1; $p=1.84E-08$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of SYTL4 could be observed in tumors from a separate group of patients. We again found that SYTL4 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, SYTL4 ranked 35 out of 41093 total transcripts (Table 2). Differential expression of SYTL4 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=1.52E-07$).

SYTL4 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 SYTL4 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. SYTL4 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 0.1698 ± 0.1176 in SYTL4 expression when comparing HGSC tumors to normal ovarian tissue
3 (Table 1).

4 We then obtained exact mRNA expression levels for the differentially expressed SYTL4
5 transcript from both normal ovarian tissue and in high-grade serous ovarian tumors from the
6 second microarray dataset used to identify differential expression of SYTL4 in HGSC tumors.
7 SYTL4 was again expressed at significantly lower levels in high-grade serous ovarian cancers
8 when compared to the normal ovary (Figure 2; $p < 0.0001$). We calculated a mean fold change
9 of 0.6670 ± 0.0500 in SYTL4 expression when comparing HGSC tumors to normal ovarian
10 tissue (Table 2).

11 Thus, we found using published microarray data that SYTL4 was among the genes most
12 differentially expressed in tumors from women with high-grade serous carcinomas and that
13 SYTL4 expression was significantly lower in HGSC tumors when compared to the normal ovary.
14

15 Discussion

16 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
17 classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated,
18 proliferative, and mesenchymal⁸. We sought to continue to describe the transcriptional
19 landscape of high-grade serous ovarian cancers⁸ and identify genes whose differential
20 expression was associated with HGSC by using published microarray data from primary tumors
21 of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}.
22 In both datasets analyzed, SYTL4 was among the genes whose expression changed most
23 significantly when comparing the normal ovary to primary HGSC tumors.
24

25 SYTL4, also known as Slp or Slp-4 or granuphilin, is an effector of the Rab27A pathway,
26 functions in exocytosis and is important for the regulated secretion of insulin, stored in
27 conventional dense-core granules^{7,8}. SYTL4 can be found on the dense-core vesicles of
28 neuroendocrine cells, regulating their exocytosis through interactions between the linker domain

1 of SYTL4 and the Munc18-1 syntaxin-1a complex⁹. SYTL4 can interact with GTP-bound
2 Rab27a through its amino-terminal synaptotagmin-like protein homology domain (SHD), and
3 both localize to the membrane of insulin granules⁷⁻¹⁰. SYTL4 can also interact with SNARE
4 proteins on the plasma membrane through its linker domain and with phosphatidylinositol-(4,5)-
5 biphosphate through its C-terminal C2 domains (C2A and C2B)¹⁰. Silencing of SYTL4
6 phenocopies the negative effect of silencing of Rab27a on the secretion of exosomes⁸.

7
8 SYTL is important for the secretion of another soluble factor, von Willebrand Factor
9 (VWF), stored in rod-shaped granules known as Weibel-Palade bodies (WBP)¹¹. Investigators
10 demonstrated that SYTL4 associated with STXBP1, syntaxin-2 and syntaxin-3, that SYTL4
11 functioned in the recruitment of STXBP1 and that depletion of STXBP1 could impair exocytosis
12 of WBP¹¹. Interestingly, one study demonstrated that SYTL4 has been identified as a sexually
13 dimorphic gene in the brain, possessing hormone-regulated expression, significantly higher in
14 the bed nucleus of the stria terminalis (BNST) of the hypothalamus¹². Mice deficient in SYTL4
15 possessed defects in male sexual behaviors (i.e., mating), including chemoinvestigation
16 (sniffing), ultrasonic vocalization (USV), mounting, and intromission (penetration)¹².

17
18 Differential expression of SYTL4 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 cancers¹³⁻¹⁵ can be utilized to assess the effect of conditional expression of SYTL4 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	FC	Gene	Gene name
126	8173955	1.84E-08	-6.846589	9.3314417	0.1698 ± 0.1176	SYTL4	synaptotagmin like 4

Table 1: SYTL4 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 SYTL4 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	FC	Gene	Gene name
35	A_24_P122337	1.52E-07	-11.0053299	7.77444	0.6670 ± 0.0500	SYTL4	synaptotagmin like 4

Table 2: SYTL4 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 SYTL4 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: SYTL4 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of SYTL4 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 SYTL4 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.