Odd-skipped related 2 (OSR2) 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 previously identified forkhead box L2⁴, (FOXL2) as among the genes whose expression was most different in HGSC ovarian tumors when compared to the ovary. Here, we find that potential FOXL2 transcriptional target^{5,6} odd-skipped related gene OSR2⁷ is differentially expressed in high-grade serous ovarian cancer, and could be observed in independent tumor microarray data^{2,3}. These data reveal perturbed expression of a target gene of a key transcription factor and specifier of ovarian cell fate in high-grade serous ovarian cancers.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of 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 decades^{8,9}. 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 odd-skipped related
7 2, *OSR2*, as among the most differentially expressed in HGSC tumors of the ovary. *OSR2* 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 *OSR2* 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.

OSR2 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified OSR2 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, OSR2 ranked 81 out of 29088 total transcripts (Table 1). Differential expression of OSR2 in HGSC tumors was statistically significant (Table 1; $p=1.69E-09$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of OSR2 could be observed in tumors from a different group of patients. We again found that OSR2 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, OSR2 ranked 102 out of 41093 total transcripts (Table 2). Differential expression of OSR2 in HGSC tumors was statistically significant (Table 2; $p=2.12E-06$).

OSR2 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 OSR2 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. OSR2 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p<0.0001$). We calculated a mean fold change of

0.4191 $\pm$ 0.1401 in OSR2 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Analysis of OSR2 mRNA levels in a second microarray dataset revealed that OSR2 was 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 0.6621 $\pm$ 0.0680 in OSR2 expression when comparing HGSC tumors to normal ovarian tissue (Table 2).

Thus, we found using published microarray data that OSR2 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that OSR2 expression was significantly 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 mesenchymal¹⁰. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers¹⁰ 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^{2,3} OSR2 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

Osr2 possesses developmentally patterned expression in the limbs, the kidneys, and the craniofacial structure⁷. Osr2 expression is first detected at embryonic day 9.25 (E9.25) in the mesonephric vesicles and by E10.0, expression of Osr2 can be found in the rostro-lateral mandibular mesenchyme adjacent to the maxillary process⁷. In Osr2-deficient mice, tissue-specific ablation of Osr1 in the developing limb mesenchyme of leads to fusion of multiple joints, demonstrating that Osr1 and Osr2 function redundantly to regulate synovial joint formation¹¹.

1 Genes important for joint formation and induced by Osr1 and Osr2 included Wnt4, Wnt9b, and
2 Gdf5¹¹. Experiments using Osr2^{-/-} mice with an Osr1 cDNA knock-in at the endogenous Osr2
3 locus demonstrated that Osr2 functioned through Fgf10 signaling to regulate eyelid
4 development; Osr1 was able to rescue Osr2 function in induction of Fgf10 expression during
5 eyelid development¹².

6 Osr2 has important roles in the development of the palate: specifically, in secondary
7 palate growth¹³. Osr2 was expressed in the palatal mesenchyme at the start of palatal
8 outgrowth¹³. Null mutation of Osr2 in mice results in delay of palatal shelving and subsequent
9 cleft palate¹³. In Osr2 mutant embryos at embryonic day 13.5, there is a decrease in
10 proliferation of the palatal mesenchyme in the medial halves of downward growing palatal
11 shelves¹³. Pax9-deficient embryos significantly down-regulate Osr2 expression, in the
12 developing palatal mesenchyme, and Osr2 expression can rescue posterior palatal
13 morphogenesis in the absence of Pax9, demonstrating that Pax9 can regulate Osr2 expression
14 to control palatal shelf morphogenesis¹⁴.

15 A study of FOXL2 transcriptional targets identified OSR2 as a FOXL2-induced gene⁶.
16 FOXL2 is a key transcriptional specifier of the ovarian lineage and important for female sex
17 determination in mammals¹⁵⁻¹⁷. Nearly all patients with blepharophimosis/ptosis/epicanthus
18 inversus syndrome (BPES), a developmental condition associated with droopy eyelids (ptosis),
19 increased distance between the corners of the eyelids (telecanthus), and skin folds of the lower
20 eyelid adjacent to the nasal bridge (epicanthus inversus), are thought to possess mutations in
21 FOXL2¹⁸. While female patients with type II BPES only exhibit eyelid abnormalities, female
22 patients with type I BPES also suffer from premature ovarian insufficiency (POI)^{18,19}. However,
23 literature documents a case of POI in the absence of BPES in a patient carrying the FOXL2
24 variant G187D¹⁹. *In vitro*, FOXL2 G187D maintained strong transcriptional control over a
25 construct containing the promoter of Osr2, and the authors argued this was consistent with an
26 absence of palpebral defects in the patient, and further that this suggested that disrupted
27 interactions between Osr2 and FOXL2 might contribute to cranofacial/palpebral defects
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1 observed in BPES patients¹⁹. Like FOXL2-deficient mice, Osr2-deficient mice are born with
2 open eyelids¹². Osr2-deficient mice are also born with cleft palate¹². Interestingly, studies using
3 Wnt4-deficient mice demonstrated that Osr2 expression is typical of indifferent gonads that have
4 chosen the male sexual differentiation pathway and testicular fate²⁰.

5 Differential expression of OSR2 in HGSC should be validated in larger and separate
6 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
7 cancers²¹⁻²³ can be utilized to assess the effect of conditional expression of OSR2 in HGSC
8 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
81	8147573	1.69E-09	-7.5539586	11.6274356	0.4191 ± 0.1401	OSR2	odd-skipped related transcription factor 2

Table 1: OSR2 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 OSR2 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
102	A_23_P71570	2.12E-06	-8.5740195	5.35343	0.6621 ± 0.0680	OSR2	odd-skipped related transcription factor 2

Table 2: OSR2 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 OSR2 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: OSR2 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of OSR2 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 OSR2 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.

Figure 2: OSR2 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of OSR2 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 OSR2 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.