Thyroid hormone receptor (TR α) / NR1A1 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 the gene encoding the thyroid hormone receptor, THRA, also known as nuclear receptor subfamily 1, group A, member 1 (NR1A1) 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, THRA.

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 the thyroid hormone
7 receptor alpha (THRA) as among the most differentially expressed in HGSC tumors of the
8 ovary. THRA 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.
11

12 **Methods**

13
14 We used microarray data from datasets GSE146556² and GSE124766³ for this
15 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
16 and Hochberg method of p-value adjustment was used for ranking of differential expression but
17 raw p-values were used for assessment of statistical significance of global differential
18 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
19 platform annotation was used. GSE146556² was generated using Affymetrix Human Gene 1.0
20 ST Array with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade
21 serous ovarian cancer. GSE124766³ was generated using Agilent-014850 Whole Human
22 Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors
23 from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels
24 only for the top 250 most differentially expressed genes.
25

26
27 A statistical test was performed to evaluate whether THRA expression was significantly
28 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.

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

We identified THRA 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, THRA ranked 215 out of 29088 total transcripts (Table 1). Differential expression of THRA in HGSC tumors was statistically significant (Table 1; $p=2.45E-07$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of THRA could be observed in tumors from a separate group of patients. We again found that THRA 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, THRA ranked 72 out of 41093 total transcripts (Table 2). Differential expression of THRA in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=8.61E-07$).

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

We obtained exact mRNA expression levels for the differentially expressed THRA transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. THRA 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

0.4100 $\pm$ 0.1055 in THRA expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

We then obtained exact mRNA expression levels for the differentially expressed THRA transcript from both normal ovarian tissue and in high-grade serous ovarian tumors from the second microarray dataset used to identify differential expression of THRA in HGSC tumors. THRA was again expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p=0.0045$). We calculated a mean fold change of 0.8527 $\pm$ 0.0166 in THRA expression when comparing HGSC tumors to normal ovarian tissue (Table 2).

Thus, we found using published microarray data that THRA was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that THRA 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 mesenchymal⁸. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers⁸ 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, THRA/NR1A1 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors

TR α can activate gene expression through interactions with the general transcription factor TFIIB through the amino-terminal A/B domain of TR α . On the other hand, TR α can exert transcriptional repression in the absence of ligand by interactions with TATA-binding protein

(TBP)⁸. In chicken, expression of THRA is ubiquitous and could be observed as early as embryonic day 4⁹. Mice engineered to model TR β mutations found in humans at the endogenous TR α locus have growth defects and develop as dwarfs^{10,11}. TR α -/- mice manifest a red blood cell phenotype, with reduced numbers of erythroid progenitors in the fetal liver; adult TR α -/- mice have decreased hematocrit levels, perturbed stress erythropoiesis, and abnormal progenitor cellularity in the spleen¹². Oligodendrocyte numbers in optic nerve from postnatal day 7 and 14 TR α -/- mice are lower than in wild-type mice, and oligodendrocyte precursor cells (OPC) from postnatal TR α -/- mice fail to differentiate in response to thyroid hormone (TH)¹³. TR α -/- mice with *bona fide* TR α deficiency (as compared to a dominant negative TR α mutation) have a heart-rate 20% lower than wild-type mice, with prolonged QRS- and QT_{end}- durations on electrocardiogram, body temperature 0.5 degrees lower than normal, and display mild hypothyroidism¹⁴. In humans, mutation of TR α was found in a child with hypothyroidism (growth and developmental retardation, severe constipation and skeletal dysplasia), but possessing only marginally abnormal TH levels¹⁵. This mutation results in the production of a receptor with dominant negative activity¹⁵.

TR α expression in human surface ovarian epithelial cells has been documented¹⁶. In cancers¹⁷, mutation of TR α is found in hepatocellular carcinoma^{18,19}, renal clear cell carcinoma²⁰, thyroid papillary cancer²¹, and non-functioning pituitary tumors²². TR α expression is associated with metastasis in gastric cancer²³, and elevated expression of TR α has been reported in nasopharyngeal carcinoma²⁴. Mice deficient in all thyroid hormone receptors (TR α -/- - TR β -/-) develop metastatic follicular thyroid carcinoma in adulthood, demonstrating tumor suppressive properties of thyroid hormone receptors²⁵.

Differential expression of THRA/NR1A1 in HGSC should be validated in larger and separate cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian cancers²⁶⁻²⁸ can be utilized to assess the effect of conditional expression of THRA in HGSC 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
215	8007008	2.45E-07	-6.0850277	6.8424035	0.4100 ± 0.1055	THRA	thyroid hormone receptor, alpha

Table 1: THRA 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 THRA 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
72	A_23_P207742	8.61E-07	-9.3517304	6.197	0.8527 ± 0.0166	THRA	thyroid hormone receptor, alpha

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

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

The mRNA expression of THRA in normal ovarian tissues from control subjects (left side of each graph) and in the primary tumors of patients with HGSC (right side of each graph) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in THRA expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.