TRPM2 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 melastatin-related transient receptor potential channel 2, TRPM2 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, TRPM2.

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 TRPM2 as among the
7 most differentially expressed in HGSC tumors of the ovary. TRPM2 may be a gene of interest
8 when prioritizing the study of target genes and pathways for the development of novel
9 therapeutic interventions in high-grade serous ovarian cancers.
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14 **Methods**

15 We used microarray data from datasets GSE124766² and GSE146556³ for this
16 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
17 and Hochberg method of p-value adjustment was used for ranking of differential expression but
18 raw p-values were used for assessment of statistical significance of global differential expression.
19 Log-transformation of data was auto-detected, and the NCBI generated category of platform
20 annotation was used. GSE124766 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. GSE146556 (platform GPL6244) was generated
23 using Affymetrix Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 for
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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 TRPM2 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.

TRPM2 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified TRPM2 as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that TRPM2 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, TRPM2 ranked 228 out of 41093 total transcripts (Table 1). Differential expression of TRPM2 in HGSC tumors was statistically significant (Table 1; $p=1.64E-05$).

1 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
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3 tumors from women diagnosed with HGSC to determine whether differential expression of
4 TRPM2 could be observed in tumors from a separate group of patients. We again found that
5 TRPM2 was differentially expressed in primary HGSC tumors (Table 2). When sorting all of the
6 transcripts measured by microarray based on change in expression between HGSC and the
7 normal ovary, in this data set, TRPM2 ranked 2781 out of 29088 total transcripts (Table 2).
8 Differential expression of TRPM2 in HGSC tumors in this second microarray dataset was
9 statistically significant (Table 2; $p=2.53E-03$).
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13 TRPM2 is expressed at higher levels in HGSC when compared to the normal ovary.

14 We obtained exact mRNA expression levels for the differentially expressed TRPM2
15 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. TRPM2
16 was expressed at higher levels in high-grade serous ovarian cancers when compared to the
17 normal ovary; this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a
18 mean fold change of 1.3351 ± 0.0779 in TRPM2 expression when comparing HGSC tumors to
19 normal ovarian tissue (Table 1).
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23 Thus, we found using published microarray data that TRPM2 was among the genes most
24 differentially expressed in tumors from women with high-grade serous carcinomas and that
25 TRPM2 expression was higher in HGSC tumors when compared to the normal ovary.
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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, TRPM2 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

Calcium flux across cell membranes is mediated by cation channels termed transient receptor potential proteins (TRP). Melastatin-related transient receptor potential channel 2 (TRPM2) is a mammalian TRP Ca^{2+} membrane-permeable channel with ligands including nicotinamide adenine dinucleotide (NAD^+) and ADP-ribose (ADPR)⁷⁻¹⁰. TRPM2 has been described as a sensor of reactive oxygen species (ROS) and oxidative stress⁸. Intracellular Ca^{2+} is an activator of TRPM2 and the only known activator of spliced TRPM2 isoforms¹¹. The CaM-binding motif is necessary for activation of TRPM2 by both intracellular Ca^{2+} and by ADPR¹¹. TRPM2 has important roles associated with effector function in the immune system. In monocytes, TRPM2 can regulate chemokine secretion induced by exposure to reactive oxygen species (ROS)¹². In macrophages, activation of the NLRP3 inflammasome by liposomes induces calcium flux through TRPM2 triggered by ROS exposure¹³.

1 In pancreatic β cells, TRPM2 localizes to lysosomes where it can be activated by
2 adenosine diphosphoribose; TRPM2 both in the lysosome and at the plasma membrane were
3 important for execution of pancreatic β cell death following exposure to peroxide¹⁴. Depletion of
4 TRPM2 in cultured rat neurons impaired both H₂O₂-mediated Ca²⁺ influx as well as H₂O₂-
5 induced cell death¹⁵. Thus, in more than one cell type, TRPM2 is important for cell death
6 responses following exposure to oxidative stress such as peroxides.
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10 TRPM2 is implicated in a variety of human cancers¹⁶⁻²⁰. Investigators reported enriched
11 expression of an antisense transcript of TRPM2 in melanomas, TRPM2-AS, as well as a
12 proximally located tumor enriched TRPM2 transcript termed TRPM2-TE. Methylation analysis
13 of a CpG island shared by TRPM2-AS and TRPM2-TE reveled that CpG island methylation
14 status correlated with transcript expression levels for both antisense and tumor-enriched TRPM2
15 transcripts in melanoma cell lines including MR299, DettMel and Mel Juso. The functional
16 consequence of TRPM2-TE depletion mirrored that of TRPM2 over-expression in increasing
17 levels of apoptotic and necroptotic cell death¹⁶. TRPM2 is reported as over-expressed in prostate
18 cancers during androgen-independent progression and following androgen withdrawal.
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21 Investigators assessed whether depletion of TRPM2 in PC-3 prostate cancer cells using TRPM2
22 antisense oligonucleotides (ODN) targeting TRPM2 could restore chemrsensitivity. In PC-3
23 xenografts, one ODN conferred 80% reduction of tumor volume when delivered in conjunction
24 with paclitaxel and 60% reduction of tumor volume when delivered in conjunction with
25 mitoxantrone¹⁷.
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ROS exposure can lead to activation of autophagy; while LC3-II levels decrease in PC3 cells following ROS treatment, LC3-II levels in PC3 cells depleted of TRPM2 are induced; decreases in LC3-II following ROS treatment could also be reduced by pre-treatment with clotrimazole, a TRPM2 antagonist¹⁸. Thus in PC3 prostate cancer cells expressing TRPM2, oxidative stress lead to induction of cell death and inhibition of autophagy, connecting calcium flux to cell fate under conditions of oxidative stress¹⁸. In AGS gastric cancer cells, depletion of TRPM2 impaired invasion and migration *in vitro*, and activation of the protein kinase Akt rescued this impairment in the context of TRPM2 loss. *In vivo*, depletion of TRPM2 in AGS cells implanted into NOD/SCID xenografts resulted in significant decreases in tumor weight, tumor volume, and in blunted expression of EMT markers N-cadherin, Twist and ZEB1¹⁹.

Neutrophil secretion of peroxide can induce cancer cell death; this is dependent on Ca²⁺, as chelation of intracellular calcium protects against cytotoxicity in multiple cell lines including 4T07, AT3, and 168FARN breast cancer cell lines, the MC38 colon cancer cell line, and in the LLC Lewis lung cancer cell line²⁰. Cancer cell death induced by neutrophil secretion of H₂O₂ is dependent on TRPM2; in an orthotopic breast cancer mouse model using the MDA-MD-231 cell line, TRPM2 was absolutely required for cell death triggered by Ca²⁺ influx following neutrophil H₂O₂ secretion²⁰. Moreover, depletion of TRPM2 in the 4T1 cell line impaired metastasis *in vivo*. In human tumors of the bladder, breast, colon, lung and prostate, TRPM2 is over-expressed at the mRNA level when compared to primary tissues²⁰. Thus, TRPM2 is over-expressed in primary tumors in multiple human cancers, and its expression appears to be

1 important for metastatic and invasive properties.

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3 A study assessing the presence of macrophages in the rat ovary documented TRPM2
4 expression in the ovary²¹ but we could not identify information describing a role for TRPM2 in
5 high-grade serous ovarian cancer. Differential expression of TRPM2 in HGSC should be
6 validated in larger and separate cohorts of women with HGSC. If validated, mouse models of
7 high-grade serous ovarian cancers²²⁻²⁴ can be utilized to assess the effect of conditional
8 expression of TRPM2 in HGSC tumor growth post-development and on survival *in vivo*.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
228	A_24_P27977	1.64E-05	6.9802832	3.38582	1.3351 ± 0.0779	TRPM2	transient receptor potential cation channel subfamily M member 2

Table 1: TRPM2 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 TRPM2 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
2781	8069085	2.53E-03	3.2014883	-1.928545	TRPM2	transient receptor potential cation channel subfamily M member 2

Table 2: TRPM2 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 TRPM2 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: TRPM2 is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of TRPM2 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 TRPM2 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.