Frizzled class 7 receptor is differentially expressed in high-grade serous ovarian cancer and based on patient survival.

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High-grade serous ovarian cancer (HGSC) is the most common type of the most lethal gynecologic malignancy¹. To identify genes whose expression was associated with survival outcomes in HGSC, we used published data from patients enrolled in the ICON7 trial to compare the global gene expression profiles of primary HGSC tumors from women with the best and worst progression-free survival (PFS)². We found that the Frizzled class 7 (Fzd7) receptor was among the genes most differentially expressed in HGSC tumors when comparing tumor transcriptomes based on superior or inferior PFS. In two independent datasets, Fzd7 was among the genes most differentially expressed in HGSC tumors when comparing primary tumor to the normal ovary^{3,4}. Wnt pathway signaling through Fzd7 may be relevant to the biology of high-grade serous ovarian cancers.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, Fzd7.

High-grade serous ovarian cancer (HGSC) is the most prevalent type of the most lethal gynecologic malignancy: ovarian cancer^{1,5,6}. The five-year survival rate for women diagnosed with high-grade serous ovarian cancer is between 30-40% and has not changed significantly in decades^{5,6}. Understanding how the gene expression of tumors differs from the organ from which it is derived, and how tumor transcriptomes differ when compared based on patient survival outcomes can provide insights into the mechanisms by which cancers are initiated and maintained. We used published microarray data²⁻⁴ to identify differentially expressed genes in HGSC. The Frizzled class 7 receptor emerged as among the genes most differentially expressed in HGSC tumors, both when comparing tumor to control ovarian tissue and when comparing patient tumor global gene expression profiles based on survival outcomes.

Methods

We used microarray data from datasets GSE140082², GSE124766³ and GSE146556⁴ for this differential gene expression analysis of high-grade serous carcinomas. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. GSE140082² was generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with $n=31$ for tumors from patients with progression-free survival less than 200 days and $n=36$ for tumors from patients with progression-free survival greater than 1000 days. GSE124766³ was generated using Agilent-014850 Whole

Human Genome Microarray 4x44K G4112F with $n=3$ of for normal ovarian tissue and $n=8$ for tumors from patients with high-grade serous ovarian cancer. GSE146556⁴ was generated using Affymetrix 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 Fzd7 expression was significantly different when comparing tumor tissue to normal ovary, or when comparing tumor tissue based on progression-free survival greater than 1100 days or less than 200 days using a two-tailed, unpaired t-test with Welch's correction. GEO2R provides exact mRNA expression levels only for the top 250 most differentially expressed genes. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray data 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, to identify differentially expressed genes both based on survival outcomes² and when comparing tumor to the normal ovary^{3,4}.

Fzd7 expression in HGSC associates with survival outcomes.

To identify genes associated with greater patient survival in ovarian cancer, we used tumor microarray data from patients enrolled in the ICON7 trial to compare global gene expression profiles of tumors from patients surviving less than 200 days to that of patients

1 surviving greater than 1100 days². We found that the Frizzled class 7 receptor encoded by Fzd7
2 was among the genes most differentially expressed in HGSC based on survival outcomes (Table
3 1 and Figure 1). When sorting all transcripts measured by microarray based on significance in
4 change in expression between tumors from women with PFS greater than 1100 days or PFS less
5 than 200 days, Fzd7 ranked 163 out of 29377 total transcripts. Differential expression of Fzd7
6 in HGSC tumors based on patient survival less than 200 days or greater than 1100 days was
7 statistically significant (Table 1; $p=0.0025901$).

11 Fzd7 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

12 We used separate microarray data³ to identify genes associated with the HGSC tumor
13 state by comparing primary HGSC tumors to the normal ovary. We identified Fzd7 as among
14 the genes whose expression was most different when comparing primary HGSC tumors to
15 normal ovarian tissue (Table 2). When sorting all of the transcripts measured by microarray
16 based on change in expression between HGSC and the normal ovary, Fzd7 ranked 411 out of
17 41093 total transcripts (Table 2). Differential expression of Fzd7 in HGSC tumors was
18 statistically significant (Table 2; $6.31E-05$).

22 We analyzed a second microarray dataset⁴ generated using normal ovarian tissues and
23 tumors from women diagnosed with HGSC to determine whether differential expression of Fzd7
24 could be observed in tumors from a different group of patients. We again found that Fzd7 was
25 differentially expressed in primary HGSC tumors (Table 3). When sorting all of the transcripts
26 measured by microarray based on change in expression between HGSC and the normal ovary, in
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1 this data set, Fzd7 ranked 3060 out of 29088 total transcripts (Table 3). Differential expression
2 of Fzd7 in HGSC tumors was statistically significant (Table 3; $p=3.4E-03$).
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4 Fzd7 is expressed at significantly lower levels in HGSC tumors from women with
5 progression-free survival greater than 1100 days.
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8 We obtained exact mRNA expression levels² for the differentially expressed Fzd7
9 transcript in tumors from with progression-free survival greater than 1100 days when
10 compared to tumors from women with PFS less than 200 days. Fzd7 was expressed at
11 significantly lower levels in high-grade serous tumors from women with PFS greater than
12 1100 days when compared to tumors from women with PFS less than 200 days (Figure 1;
13 $p=0.0188$). We calculated a mean fold change of $.9146 \pm 0.1040$ in Fzd7 expression when
14 comparing HGSC tumors from women with PFS greater than 11100 days when compared to
15 tumors from women with PFS less than 200 days (Table 1).
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19 Thus, we found using published microarray data² that Fzd7 was among the most
20 differentially expressed genes when comparing primary HGSC tumors based on patient survival,
21 expressed at significantly lower levels in the tumors of patients surviving greater than 200 days
22 when compared to that of patients surviving less than 1100 days. We also observed significant
23 differential expression of Fzd7 in HGSC tumors when compared to the normal ovary^{3,4}.
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Discussion

The Frizzled class 7 receptor is a membrane receptor for the Wnt signaling pathway and the most abundantly expressed FZD gene in human embryonic stem cells^{7,8}. Fzd7 functions in maintenance of pluripotency in human embryonic stem cells by transmitting a Wnt3a signal through β -catenin to maintain hES cells in an undifferentiated state^{7,8}.

In addition to Wnt3a, another described ligand of Fzd7 is Wnt7a. Wnt7a signaling can activate the planar-cell-polarity pathway during stem cell expansion in skeletal muscle repair. Addition of Wnt7a to differentiated C2C12 myotubes resulted in increased myotube diameter, indicating that Wnt7a could drive hypertrophy in differentiated myofibers⁹. Wnt7a signaling leads to activation of the mTOR/protein kinase Akt pathway. In C2C12 myotubes, Fzd7 can be found in complex with phosphoinositide-3-kinase (PI3K) and the G protein $G\alpha s$ ⁹.

Fzd7 is implicated in a number of human cancers. In the Stem-A sub-variety of ovarian cancer, Fzd7 expression was found to be enriched in patient tumors when compared to Stem-B, epithelial-A (Epi-A), Epi-B, or mesenchymal types, and among cell lines Fzd7 expression was relatively highest in the Stem-A cell lines PA1, CH1 and OV-17R¹⁰. Depletion of Fzd7 in these cell lines resulted in decreases in cell proliferation and an increase in percentage of cells in G_0 but with no change in frequency of apoptotic cells. Importantly, Fzd7-depleted cells became more epithelial in morphology and polarized; tensile contractility increased after Fzd7 depletion, evidenced by relocalization of phosphorylated myosin light chain from sites of cell-cell

1 contact to colony periphery, as well as loss of stress fibers¹⁰. A number of planar-cell-polarity
2 genes were expressed at higher levels in Stem-A tumors compared to Stem-B tumors, including
3 CELSR3, PRICKLE4, PFN2, PCDHB17, PTK7 and SPRY1. Fzd7 depletion by small
4 interfering RNA (siRNA) resulted in inhibition of RhoA but activation of Rac1, and there was a
5 significant decrease in localization of CK1 ϵ at the γ -tubulin centrosome¹⁰. Together, the data
6 suggested that Fzd7 influenced cell cycle and proliferation in Stem-A ovarian cancers, as well as
7 cell migration phenotypes through the planar cell polarity and non-canonical Wnt pathways¹⁰. A
8 separate study demonstrated that depletion of Fzd7 in the OV-17R and CH1 ovarian cell lines
9 using small hairpin RNA (shRNA) depletion resulted in acquisition of epithelial morphology
10 and the down-regulation of epithelial mesenchymal transition genes including TWIST¹¹. The
11 ability to avoid cell death in environments where adherence signals are deficient, anoikis
12 resistance, was significantly decreased in cells depleted of Fzd7 and spheroid formation by
13 OV-17R and CH1 cells was also reduced¹¹.

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20 Fzd7 promotes cell proliferation in triple negative breast cancer¹². Depletion of Fzd7 in
21 TNBC cell lines MDA-MD-231 and BT-20 resulted in decreased cell proliferation and colony
22 formation¹². One study demonstrated that Wilms' tumors (WT) could be distinguished based
23 on response to Fzd7 blockade by Fzd7 antibody¹³. Fzd7+ cells sorted from Fzd7-resistant WT
24 were highly proliferative and clonogenic with significantly increased expression of stemness
25 genes SIX2, SALL1 and OCT-4¹³.

1 Fzd7 expression is also increased in gliomas, with expression associated with more
2 advanced tumor stage¹⁴. Fzd7 could activate the transcriptional coactivator TAZ and
3 proliferation of gliomas supported by Fzd7 required TAZ expression¹⁴. Patients whose glioma
4 tumors expressed highest levels of Fzd7 had significantly shorter survival¹⁴. In the U-87MG
5 cell line ectopically expressing Fzd7 in xenografts *in vivo*, tumor weight and tumor volume was
6 significantly greater. Together, the data suggest a role for Fzd7 in promotion of tumor growth
7 and stemness.
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11 We found significant changes in expression of the Frizzled class 7 receptor in the tumors
12 of patients with high-grade serous ovarian cancer. Fzd7 expression was also differentially
13 expressed when comparing tumors based on progression-free survival, with significantly lower
14 expression of Fzd7 in tumors from patients with progression-free survival greater than 1100
15 days. Differential expression of Fzd7 in HGSC should be validated in larger and separate
16 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
17 cancers¹⁵⁻¹⁷ can be utilized to assess the effect of conditional expression of Fzd7 on HGSC
18 tumor growth post-development and on survival *in vivo*.
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Rank	ID	p-value	t	B	FC	Gene
167	ILMN_2108823	0.0025901	-3.20	-1.6674	.9146 ± 0.1040	FZD7

Table 1: Fzd7 is differentially expressed in high-grade serous ovarian carcinomas when comparing tumors based on progression-free survival greater than 1100 days or less than 200 days.

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 Fzd7 in patients with PFS >1100 days when compared to patients with PFS <200 days, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
411	A_23_P209449	6.31E-05	-6.0464001	2.06592	FZD7	frizzled class receptor 7

Table 2: Fzd7 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 gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
3060	8047487	3.4E-03	-3.0955394	-2.2003367	FZD7	frizzled class receptor 7

Table 3: Fzd7 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 gene and gene name are listed in this chart.

Figure 1: Fzd7 is expressed at significantly lower levels in HGSC tumors of patients surviving greater than 1100 days when compared to that of tumors from patients surviving less than 200 days.

The mRNA expression of Fzd7 in tumors from HGSC patients surviving less than 200 days (left) and in the primary tumors of HGSC patients surviving greater than 1100 days (right) is represented with mean mRNA expression level marked and the result of a statistical test, a p-value, listed above.