MIR503HG 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 MIR503HG 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, MIR503HG.

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^{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 long intergenic non-coding RNA and
7 MIR503 host gene MIR503HG as among the most differentially expressed genes in HGSC
8 tumors of the ovary. MIR503HG may be a gene of interest when prioritizing the study of target
9 genes and pathways for the development of novel therapeutic interventions in high-grade serous
10 ovarian cancers.
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15 **Methods**

16 We used microarray data from datasets and GSE124766² and GSE146556³ for this
17 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
18 and Hochberg method of p-value adjustment was used for ranking of differential expression but
19 raw p-values were used for assessment of statistical significance of global differential
20 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
21 platform annotation was used. GSE124766 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. GSE146556 was generated using
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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 MIR503HG 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.

MIR503HG is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified the gene encoding MIR503HG as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that MIR503HG 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, MIR503HG ranked 104 out of 41093 total transcripts (Table 1).

Differential expression of MIR503HG in HGSC tumors was statistically significant (Table 1; $p=2.31E-06$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of MIR503HG could be observed in tumors from a separate group of patients. We again found that MIR503HG 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, MIR503HG ranked 2755 out of 29088 total transcripts (Table 2). Differential expression of MIR503HG in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=2.47E-03$).

MIR503HG is expressed at lower levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed MIR503HG transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. MIR503HG was expressed at lower levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 0.6715 ± 0.0710 in MIR503HG expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that MIR503HG was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that

MIR503HG expression was 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 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, MIR503HG was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

The miR-503 host gene, MIR503HG is a 5-exon gene encoding a long intergenic non-coding RNA with high conservation in non-human primates⁷. In a global microarray analysis of tissues from patients with systemic sclerosis (SSc), MIR503HG was identified as significantly up-regulated in fibrotic tissues from the skin, liver and lungs of Ssc patients. MIR503HG could be induced within 6 hours of treatment with TGF- β *in vitro*, and induction of MIR503HG by TGF- β was impaired by depletion of SMAD3, demonstrating importance of the TGF- β pathway in MIR503 expression⁸. Depletion of MIR503HG in skin fibroblasts resulted in decreased expression of COLA1A1, fibronectin, and α SMA, suggesting MIR503HG might function as a regulator of the myofibroblast phenotype⁸.

MIR503HG is implicated in a number of human cancers. A study identifying lncRNA expression in anaplastic lymphoma kinase (ALK)-negative anaplastic large-cell lymphoma (ALCL) found that ALK-negative ALCL cell lines expressed relatively high levels of MIR503HG⁹. Over-expression of MIR503HG promoted tumor growth while depletion of MIR503HG impaired proliferation of ALK-negative ALCL cell lines FePD and MAC-9. Depletion of MIR503HG enhanced degradation of the TGF- β receptor (TGFBR), and over-expression of MIR503HG promoted miR-503 expression; miR-503 expression promoted stabilization of the TGFBR by decreasing Smurf2 expression and Smurf2-mediated ubiquitination of the TGFBR⁹. In the mammary gland tumor cell line HCC1954, treatment with 5-Aza-dC increased expression of MIR503HG, suggesting MIR503HG expression could be regulated by DNA methylation¹⁰. MIR503HG was expressed at relatively high levels in the JEG-3 choriocarcinoma cells¹⁰ and its expression was significantly higher in placental tissues from women with pre-eclampsia than in normal placenta. Ectopic expression of MIR503HG in HTR-8/SVneo trophoblast cells resulted in decreased expression of MMP2, MMP9, snail, and E-cadherin. In a study of 12 hepatocellular carcinomas, MIR503HG was among the most differentially expressed long non-coding RNAs¹¹. MIR503HG expression was significantly decreased in HCC¹¹. miR503HG could interact with the heterogenous ribonucleoprotein HNRNPA2B1 to promote its degradation through the ubiquitin-proteasome pathway; this in turn reduced stability of NF-kB molecules p52 and p65 to impair NF-kB signaling¹². Depletion of MIR503HG significantly increased invasion and migration of HCC cell lines SMMC-7221 and

Huh⁷¹². Patients with low expression of MIR503HG had significantly higher percent survival and probability of recurrence as compared to those with high expression of MIR503HG¹². In bladder cancer, low expression of MIR503HG was associated with lymph node metastasis, advanced histological grade and advanced tumor stage, and in SW780 cells, ectopic expression of MIR503HG resulted in impairment of proliferation, invasion, migration and support cell death through apoptosis¹³. A study of the Han Chinese showed that MIR503HG was down-regulated in colorectal cancers and lower expression of MIR503HG was associated with worse survival within 5 years of hospital admission for patients with CRC¹⁴. In a CRC cell line *in vitro*, over-expression of MIR503HG resulted in decreased migration and invasion, as well as down-regulation of TGF- β ¹⁴. A study of triple-negative breast cancers demonstrated that MIR503HG expression was significantly decreased in TNBC tumors and cell lines. Lower expression of MIR503HG was associated with metastasis to lymph nodes and to distant sites, as well as late clinical stage¹³. Low expression of MIR503HG was found to be, independently, a poor prognostic factor for overall survival in TNBC patients. *In vitro* studies suggested that the influence of MIR503HG on migration and invasion might be through miR-103 and OLFM4; authors concluded that MIR503HG functioned as a tumor-suppressive long-non coding RNA in TNBC¹⁵. MIR503HG expression has also been found to be dysregulated in non-small cell lung cancer; MIR503HG expression was significantly up-regulated in NSCLC cell lines, and unlike other cancer types studied, over-expression of MIR503HG enhanced proliferation and impaired apoptosis in NCI-H1975 NSCLC cells¹⁶. Mechanistically, authors argued MIR503HG might

function by negatively regulating expression of miR-489-3p and miR-625-5p¹⁶. However, a separate study of NSCLC found that MIR503HG was down-regulated in NSCLC, with low expression of MIR503HG associated with worse survival in NSCLC patients¹⁷. Expression of Cyclin D1 and MIR503HG were inversely correlated and over-expression of MIR503HG resulted in cell cycle arrest¹⁷. In oral squamous cell carcinoma, MIR503HG expression was up-regulated in cancer tissue and higher expression of MIR503HG was associated with worse disease-free survival¹⁸. MIR503HG could interact with Oct4 and MIR503HG expression correlated with Oct4 expression in OSCC tumor tissue¹⁸.

In a Cancer Genome Atlas dataset, 50-fold down-regulation of MIR503HG was observed in ovarian cancer tumors and lower expression of MIR503HG was predictive of worse survival¹⁹. Authors demonstrated a direct interaction between miR-31-5p and MIR503HG; expression of miR-31-5p was inversely correlated with that of MIR503HG and up-regulated in ovarian cancers¹⁹. In the A2780 ovarian cancer cell line, over-expression of MIR503HG resulted in decreased cell invasion and migration, while over-expression of miR-31-5p resulted in increased cell invasion and migration¹⁹. Thus, down-regulation of MIR503HG can be observed in multiple human cancers with up-regulation negatively affecting tumor cell proliferation and invasive properties; however, up-regulation of MIR503HG has been documented in at least two cancers^{16,18}.

Differential expression of MIR503HG in HGSC should be validated in larger and

1 separate cohorts of women with HGSC. If validated, mouse models of high-grade serous
2 ovarian cancers²⁰⁻²² can be utilized to assess the effect of conditional expression of MIR503HG
3 on HGSC tumor growth post-development and on survival *in vivo*.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
104	A_23_P159986	2.31E-06	-8.5034164	5.27328	0.6715 ± 0.0710	MIR503HG	MIR503 host gene

Table 1: MIR503HG 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 MIR503HG 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
2755	8175256	2.47E-03	-3.2103973	-1.9054323	MIR503HG	MIR503 host gene

Table 2: MIR503HG 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 MIR503HG in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: MIR503HG is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of MIR503HG 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 MIR503HG expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.