RESEARCH ARTICLE

WNT7A Regulation by miR-15b in Ovarian Cancer

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Abstract

WNT signaling is well known to play an important role in the regulation of development, cell proliferation and cell differentiation in a wide variety of normal and cancerous tissues. Despite the wealth of knowledge concerning when and where various WNT genes are expressed and downstream events under their control, there is surprisingly little published evidence of how they are regulated. We have recently reported that aberrant WNT7A is observed in serous ovarian carcinomas, and WNT7A is the sole ligand accelerating ovarian tumor progression through CTNNB1 (β-catenin)/TCF signaling in the absence of CTNNB1 mutations. In the present study, we report that WNT7A is a direct target of miR-15b in ovarian cancer. We showed that a luciferase reporter containing the putative binding site of miR-15b in the WNT7A 3'-UTR was significantly repressed by miR-15b. Mutation of the putative binding site of miR-15b in the WNT7A 3'-UTR restored luciferase activity. Furthermore, miR-15b was able to repress increased levels of TOPFLASH activity by WNT7A, but not those induced by S33Y. Additionally, miR-15b dose-dependently decreased WNT7A expression. When we evaluated the prognostic impact of WNT7A and miR-15b expression using TCGA datasets, a significant inverse correlation in which high-expression of WNT7A and low-expression of miR-15b was associated with reduced survival rates of ovarian cancer patients. Treatment with decitabine dose-dependently increased miR-15b expression, and silencing of DNMT1 significantly increased miR-15b expression. These results suggest that WNT7A is post-transcriptionally regulated by miR-15b, which could be down-regulated by promoter hypermethylation, potentially via DNMT1, in ovarian cancer.

Introduction

MicroRNA (miRNAs) are small noncoding RNAs that regulate gene expression by post-transcriptional mRNA silencing. The processes regulated by miRNAs involve a variety of biological pathways, and their dysregulation is a common feature of human cancer [1–3]. The miR-15 family includes six highly conserved members, miR-15a, miR-15b, miR-16-1, miR-16-2, miR-195 and miR-497, which are clustered on three different chromosomes [4, 5]. The miR-15a/16-1
cluster, was originally reported as the target of 13q14 deletions or downregulation in chronic Lymphocytic leukemia (CLL) [6]. Specifically, miR-15a and miR-16-1 directly regulate BCL2, which is an anti-apoptotic oncogene [7], and hence act as tumor suppressors by inducing apoptosis [8]. Further studies have shown that miR-15a and miR-16-1 act as putative tumor suppressors by targeting BCL2, BMI1 CCND1, MCL1 and WNT3A in CLL, melanoma, as well as colon, bladder, ovarian and prostate cancers [4, 9, 10]. The miR-15b/miR-16-2 cluster, which is located in 3q25, has also been reported to act in tumor suppression by targeting BCL2, BM1, CCND1 and SUZ12 [11–13]. Reduction of miR-15b was observed in CLL, melanoma, gastric and chemoresistant tongue cancer, as well as cancer stem cells [11–15]. Deletion of miR-15b and miR-16-2 promotes B-cell pathogenesis [11]. Thus, the direct targets of the miR-15 family members are likely to be critical oncogenes.

We have recently reported that the upregulation of WNT7A (uniquely among 19 WNT ligands) results in accelerated development and progression of ovarian cancer (OvCa), and plays a critical role in tumor progression mediated by the WNT7A/CTNNB1 signaling pathway [16, 17]. Our studies further indicate that FGF1 is a direct downstream target of WNT7A/CTNNB1 signaling, and that this pathway has potential as a therapeutic OvCa target [16]. One of our findings clearly showed that high expression of WNT7A and FGF1 were correlated in OvCa, especially in serous carcinomas, and poor overall patient survival [16]. Thus, WNT7A encodes a potent oncogenic factor of relevance to OvCa. However, we still do not know the answer as to why WNT7A becomes specifically overexpressed in serous OvCa.

In the present study, we report that abundant WNT7A, present in OvCa, is post-transcriptionally down-regulated by miR-15b. In support of its role in cancer inhibition, OvCa patients had poor overall survival rate in the group with high expression of WNT7A and low expression of miR-15b. Furthermore, our results indicate that DNMT1 modulates miR-15b transcription through promoter methylation.

**Materials and Methods**

**Reagents and plasmids**

The 3'-UTR segments of the endogenous WNT7A gene and its mutant form were amplified by PCR, and subcloned into the pMIR-REPORT vector (Thermo Fisher) using the SpeI and HindIII restriction sites to generate pMIR-WNT7A and pMIR-WNT7A mutation 3'-UTR-containing plasmids. Decitabine (5'aza-2'deoxycytidine, aka: 5-aza-2'-dC), mirVana miRNA mimic (negative control and hsa-miR-15b-5p), DNMT1 siRNAs, pre-miR-15b expression constructs and Dual Luciferase Reporter Assay System were purchased from Cayman Chemical, Thermo Fisher, Qiagen, System Biosciences and Promega, respectively.

**Cell lines**

OVCAR3, OVCAR5, SKOV3, and ES2 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). OVCAR4 cells were gifted from Dr. Joanna Burdette (University of Illinois at Chicago). KURAMOCHI, OVKATE and OVSAHO were purchased from the JCRB cell bank (Osaka, Japan). SKOV3.ip1 cells were purchased from the cell bank at The University of Texas MD Anderson Cancer Center. All cells were authenticated by short tandem repeat (STR) analysis and passaged within 6 months of receipt. All cells were tested routinely for cell proliferation and BrdU incorporation as well as mycoplasma contamination. All cell lines exhibited similar morphology, characteristic growth rates, and remained negative for mycoplasma contamination throughout all experiments. OVCAR4, KURAMOCHI, OVKA TE and OVSAHO cells were grown in RPMI 1640 with 10% FBS and penicillin/streptomycin, and
other cells were cultured in DMEM with 10% FBS, 2mM glutamine and penicillin/streptomycin. All cell lines were grown in a humidified incubator at 37°C and constant 5% CO2.

**Quantitative real-time PCR (qPCR) assay**

Total RNA was extracted from cells, and cDNA was synthesized from total RNA using the High-Capacity cDNA Reverse Transcription Kit from Thermo Fisher. Relative gene expression was determined by SYBR green (Bio Rad) incorporation using a Bio-Rad myCycler as described previously [18]. Micro RNA was extracted from cells using Pure Link miRNA isolation kit (Thermo Fisher), and cDNA was synthesized by using miScript II RT Kit (Qiagen). Relative miRNA expression was determined by miScript SYBR Green PCR kit (Qiagen), and miR-15b and SNORD68 specific primers (Qiagen). A table of oligonucleotides used for each gene is presented in S1 Table.

**Cell proliferation and adhesion assays**

Cell proliferation, adhesion and migration assays were performed following our previously described methods [16, 17]. To assess cell proliferation, cells were seeded in 24-well plates, and counted 24, 48 and 72 hours by Countess II FL Automated Cell Counter (Thermo Fisher) with trypan blue exclusion. To assess cell adhesion, cells were seeded in 24-well plates and harvested after 4 h incubation.

**Statistical analyses**

All experimental data were subjected to one-way ANOVA and differences between individual means were tested by a Tukey multiple-range test using Prism 5.0 (Graphpad). QPCR data were corrected for differences in sample loading using the RPL19 data as a covariate. Tests of significance were performed using the appropriate error terms according to the expectation of the mean squares for error. A p-value of 0.05 or less was considered significant. Data are presented as means with standard error of the means (SEM). The Kaplan-Meier method was used to calculate the survival rates and was evaluated by the log-rank test using a TCGA dataset, TCGA-OV that contained 554 primary ovarian tumors with completed data sets, was selected for survival analysis.

**Results**

**WNT7A expression in OvCa cells depending on genetic background or features**

When we reported the clinical significance of WNT7A during malignant transformation of OvCa, our results showed that WNT7A was highly expressed in serous carcinomas, the most common/aggressive subtype of OvCa [16, 17]. Thus, aberrant WNT7A could be induced by abnormal genetic background or correlated with aggressive characters in OvCa. When we examined WNT7A expression levels in OvCa cells, abundant WNT7A (>1000 fold) was observed in invasive or high-grade serous OvCa cells (SKOV3.ip1, KURAMOCHI, OVCAR4 and OVSAHO, S1 Fig). Note: KURAMOCHI, OVCAR4 and OVSAHO cells have been recently confirmed as high-grade serous OvCa cell lines by genomic profiling [19]. SKOV3.ip1 cells possess highly invasive and metastatic features as these cells are isolated from ascites fluids [20]. ES2, OVCAR3 and OVCAR5 cells possess genomic profiles that are partially similar to serous OvCa tumors.
WNT7A is a direct target of miR-15b

We examined transcriptional activation of the WNT7A promoter by using luciferase reporter assays, however, our deletion analyses did not reveal any critical sites or potential regulators within a distance of 10 kb up- or down-stream of the WNT7A promoter (data not shown). Therefore, we subjected the WNT7A 3′-UTR to an in silico analysis using 3 different algorithms (TargetScan, PicTar and miRanda) to identify putative miRNA seed-matching sequences. All three search engines detected miR-15a, miR-15b and miR-195 consensus binding sequences (Fig 1A) in the 3′-UTR of WNT7A (Fig 1B). We next evaluated the prognostic impact of WNT7A and/or miR-15b expression using TCGA datasets (total patient number is 554). While high-expression of miR-15b (n = 278) showed a good prognosis (P = 0.0394) compared with low-expression of miR-15b (n = 276), WNT7A did not show any correlation (high-expression of WNT7A, n = 275, P = 0.2364). A significant inverse correlation in which high-expression of WNT7A and low-expression of miR-15b (n = 147 vs n = 146 of low-expression of WNT7A and high-expression of miR-15b) was associated with reduced survival rate of ovarian cancer patients by log-rank test (P = 0.0297, Fig 1C). NOTE: high-expression of WNT7A/miR-15b n = 132 and low-expression of WNT7A/miR-15b n = 129 were not included in inverse correlation analysis. However, no inverse correlation was seen between WNT7A and miR-15a or miR-197 (data not shown). Therefore, we focused on miR-15b for further analyses. Additionally, we examined the inverse correlation between BCL2 and miR-15b, as BCL2 is one of the well-known targets of miR-15 family and acts as an oncogene. However, no significant association between BCL2 and miR-15b concerning the survival rate of ovarian cancer patients using TCGA-OV datasets (P = 0.2760) was observed, suggesting that WNT7A is a more relevant critical target of miR-15b with respect to OvCa.

We used TargetScan 7.0 [21], to identify miR-15b target sites within the WNT7A 3′-UTR and found one putative miR-15b binding site (Fig 1B). To examine whether miR-15b binds to this sequence, OvCa cells were transfected with pMIR-REPORT plasmid containing the putative binding site of miR-15b in the WNT7A 3′-UTR, and mirVana miRNA mimic (negative control or miR-15b), and then luciferase reporter activity was measured (Fig 2A). Luciferase activity was significantly repressed by miR-15b compared with negative control. In the previous study, we have shown that WNT7A activates the canonical CTNNB1 signaling pathway [16, 17]. In order to determine whether miR-15b inhibits WNT7A’s action, we examined CTNNB1 mediated transcriptional activity with TOPFLASH reporter construct (Fig 2B). We found that miR-15b significantly repressed TOPFLASH reporter activity. When TOPFLASH activity was increased by WNT7A or S33Y-mutated CTNNB1 (an established positive control for activation of the TOPFLASH reporter) in ES2 cells, that lowly or undetectably possess endogenous WNT7A, miR-15b was able to repress increased levels of TOPFLASH activity by WNT7A, but not those induced by S33Y (Fig 2B). Furthermore, mutation of the putative binding site of miR-15b in the WNT7A 3′-UTR (5′TGCTGCT3′ to 5′TaaTGCT3′) restored the luciferase activity previously repressed by miR-15b (Fig 2C). In support of these findings, miR-15b dose-dependently decreased WNT7A mRNA levels in OvCa cells (Fig 3). These results suggest that WNT7A expression is directly regulated by miR-15b in OvCa. Because BMI1 and BCL2 have been reported as target genes of miR-15b in cancer [12, 13], their mRNA levels in OvCa were also examined. While miR-15b was able to decrease BCL2, BMI1 was not regulated by miR-15b in OvCa cells.

MiR-15b inhibits cell proliferation and adhesion

The role of miR-15b as a tumor suppressor has been characterized, as loss of miR-15b in mice leads to the development of B-cell malignancy [11], and overexpression of miR-15b suppresses metastasis dissemination using tongue cancer xenografts [12]. In the present study, miR-15b...
Fig 1. (A) Bioinformatics prediction of miRNA interaction with seeded sequences from the 3'-UTR of \textit{WNT7A} using three different algorithms. (B) Schematic of the putative \textit{miR-15b} binding sequence in the \textit{WNT7A} 3'-UTR. (C) \textit{TCGA2012} survival calculated with TCGA-OV datasets (total n = 554, high \textit{WNT7A}/\textit{miR-15b}, n = 132; high \textit{WNT7A}/low \textit{miR-15b}, n = 147; low \textit{WNT7A}/high \textit{miR-15b}, n = 146; low \textit{WNT7A}/\textit{miR-15b}, n = 129) by Kaplan-Meier method using Prism 5.0. The P-value was determined by the log rank test.

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overexpressing OvCa cells were time-dependently less proliferative (Fig 4A), and reduced cell adhesion (Fig 4B), indicating that miR-15b also acts as tumor suppressor in OvCa.

**MiR-15b is regulated by promoter hypermethylation**

Our present results suggest that abundant expression of WNT7A is likely induced due to down-regulation of miR-15b. The prognostic impact of OvCa patients with inverse correlation of
WNT7A and miR-15b further support this hypothesis. Therefore, we tested the possibility that miR-15b might be down-regulated in OvCa through promoter hypermethylation. Treatment with an inhibitor of DNMTs, 5-aza-2’dC, dose-dependently increased miR-15b expression and decreased WNT7A expression in OvCa cells (Fig 5A). When we examined the expression levels of three active DNMT isoforms (DNMT1, DNMT3A and DNMT3B) in 5-aza-2’dC (5 μM) treated OvCa cells, only DNMT1 was reduced in SKOV3.ip1 and OVCAR4 cells (Fig 5B), indicating that DNMT1 could be functional to methylate miR-15b. Indeed, silencing of DNMT1 significantly increased miR-15b expression in OvCa cells (Fig 5C), suggesting that miR-15b is potentially down-regulated, especially in high WNT7A-expressing cells, by promoter hypermethylation via DNMT1 in OvCa.

Discussion

WNT signaling is well known to play an important role in cancer biology [22]. While CTNNB1 is the key mediator of WNT signaling, we have demonstrated that WNT7A is the sole ligand activating intact CTNNB1/TCF signaling (i.e. within cells lacking activation by
mutation of CTNNB1), especially the serous OvCa subtype [16, 17]. Despite the wealth of knowledge concerning the expression of various WNT ligands and downstream events under their control, there is surprisingly little published evidence of how WNT genes are regulated, and why some members are upregulated in specific tumor types. Recently, WNT3A, which promotes tumorigenesis via accelerated cellular proliferation and invasion [23], is found to be directly regulated by the miR-15a/16-1 cluster, and upregulation of WNT3A is inversely correlated with decreased miR-15a and miR-16-1 in advanced prostate tumors [10]. In the present

Fig 4. (A) Cell proliferation or (B) adhesion with either miR-15b expressing or control cells.

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Fig 5. (A) 5-aza-2’-dC treatment induces miR-15b expression (left) and decreases WNT7A expression (right) in OvCa cells. The cells were treated with 5-aza-2’-dC for 3 days, and miR-15b or WNT7A was assessed by qPCR compared to 0 µM control (set to background level of 1 in each cell). Different letters denote transcripts that have statistically significant (P<0.05) differences in
study, bioinformatics seed-matching programs identified three highly conserved miR-15 family members, miR-15a, miR-15b and miR-195, which could be critical regulators of WNT7A. However, neither miR-15a nor miR-195 exhibited significant inverse correlations with WNT7A in the OvCa patient survival data sets. Thus, WNT7A expression is most likely subject to regulation by miR-15b in OvCa.

Previous work in CLL has demonstrated the tumor suppressor activity of the miR-15a/16-1 cluster is dependent on repression of BCL2 [8]. BCL2 has also been reported as a direct target of miR-15b using a model of drug resistant gastric cancer cells [13]. It has been characterized that miR-15b/16-2 knockout mice develop B-cell malignancy, whereas BCL2 faintly up-regulated in B-cells from knockout mice [11]. Our results showed that miR-15b repressed BCL2 expression in OvCa cells. However, no inverse correlation between BCL2 and miR-15b was observed in the analysis of patient survival in OvCa. These results suggest that regulation of BCL2 by miR-15b has less impact on ovarian tumorigenesis.

The actions of miR-15b as a tumor suppressor have been clearly demonstrated in the pathogenesis of B-cells in CLL [11]. In addition, overexpression of miR-15b inhibits metastasis dissemination via epithelial-mesenchymal transition in the model of chemoresistant tongue cancer cell xenografts [12]. Downregulation of miR-15b occurs in breast cancer stem cells, and overexpression of miR-15b inhibits their growth and differentiation [15]. Inhibition of cell proliferation targeting of cyclin D1, and induction of apoptosis by miR-15b have also been reported [11–14, 24]. Our results further support miR-15b’s tumor suppressor function, and add inhibition of OvCa cell proliferation and cell adhesion to its list of relevant tumors.

In the present study, we showed that 5-aza-2’-dC, an inhibitor of DNMT activity, increased miR-15b expression. Similarly, decreased DNMT1 was observed by the treatment of 5-aza-2’-dC, and silencing of DNMT1 increased miR-15b in OvCa cells. Increased DNMT1 levels have been reported in OvCa, with lower expression in primary stage I/II tumors and peak expression occurring at stage III/IV [25]. DNMT1-mediated promoter hypermethylation induces reduction of E-cadherin and progresses invasive feature of OvCa [26]. There is one report examining the correlation between alteration in copy number at the chromosomal location of miR-15b and the changes in miR-15b promoter methylation status for breast, ovarian, head and neck, lung and kidney cancer using TCGA data sets [27]. This group found significant correlation between miR-15b expression and copy number variation at those loci, as well as between miR-15b expression and methylation levels in the relevant cancer types and the pooled data from all cancer types. Note: No methylation data are available for ovarian cancer in TCGA (only expression and copy number alteration). Furthermore, the promoter region of miR-16-2, which is present in a cluster with miR-15b located on chromosome 3q25, is methylated in polycythemia vera CD34+ cells [28]. Although the epigenetic regulation of miR-15b in OvCa remains to be investigated, DNMT1 may be one of the regulators to suppress miR-15b.

In summary, we found that WNT7A is directly regulated by miR-15b in OvCa. As WNT7A activates tumor growth and progression in OvCa via the WNT7A/CTNNB1 signaling pathway [16, 17], downregulation of miR-15b allows aberrant WNT7A expression is further support the impact of WNT7A and its mechanisms in OvCa.
Supporting Information

S1 Fig. Relative WNT7A expression was assessed by qPCR in OvCa cells. Data are expressed as fold above ES2 expression levels, which were the near background and arbitrarily set to 1. (EPS)

S1 Table. Primers for qPCR (PDF)

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Author Contributions

Conceived and designed the experiments: JAM MLK KH. Performed the experiments: JAM MLK HO KH. Analyzed the data: JAM HO KH. Wrote the paper: JAM KH.

References

1. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116(2):281–97. Epub 2004/01/28. PMID: 14744438.
2. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annual review of pathology. 2014; 9:287–314. Epub 2013/10/02. doi: 10.1146/annurev-pathol-012513-104715 PMID: 24079833; PubMed Central PMCID: PMC4009396.
3. Suzuki Hi, Katsura A, Matsuyama H, Miyazono K. MicroRNA regulons in tumor microenvironment. Oncogene. 2015; 34(24):3085–94. Epub 2014/08/19. doi: 10.1038/onc.2014.254 PMID: 25132266; PubMed Central PMCID: PMC4761641.
4. Pekarsky Y, Croce CM. Role of miR-15/16 in CLL. Cell death and differentiation. 2015; 22(1):6–11. Epub 2014/06/28. doi: 10.1038/cdd.2014.87 PMID: 24971479; PubMed Central PMCID: PMC4262785.
5. Yue J, Tiyi G. Conservation of miR-15a/16-1 and miR-15b/16-2 clusters. Mammalian genome: official journal of the International Mammalian Genome Society. 2010; 21(1–2):88–94. Epub 2009/12/17. doi: 10.1007/s00335-009-9240-3 PMID: 20013340; PubMed Central PMCID: PMC2820079.
6. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99(24):15524–9. Epub 2002/11/16. doi: 10.1073/pnas.050665499 PMID: 12434020; PubMed Central PMCID: PMC137750.
7. Sanchez-Beato M, Sanchez-Aguilera A, Piris MA. Cell cycle deregulation in B-cell lymphomas. Blood. 2003; 101(4):1220–35. Epub 2002/10/24. doi: 10.1182/blood-2002-07-2009 PMID: 12393483.
8. Cimmino A, Calin GA, Fabbrini M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(39):13944–9. Epub 2005/09/17. doi: 10.1073/pnas.0506654102 PMID: 16166262; PubMed Central PMCID: PMC1236577.
9. Bhattacharya R, Nicolo M, Arvizo R, Wang E, Cortez A, Rossi S, et al. MiR-15a and MiR-16 control Bmi-1 expression in ovarian cancer. Cancer research. 2009; 69(23):9090–5. Epub 2009/11/12. doi: 10.1158/0008-5472.CAN-09-2552 PMID: 19903841; PubMed Central PMCID: PMC2859686.
10. Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memm L, et al. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nature medicine. 2008; 14(11):1271–7. Epub 2008/10/22. doi: 10.1038/nn.1880 PMID: 18931683.
11. Lovat F, Fassan M, Gasparini P, Rizzotto L, Cascione L, Pizzi M, et al. miR-15b/16-2 deletion promotes B-cell malignancies. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112(37):11636–41. Epub 2015/09/02. doi: 10.1073/pnas.1514954112 PMID: 26324892; PubMed Central PMCID: PMC4577143.
12. Sun L, Yao Y, Liu B, Lin Z, Lin L, Yang M, et al. MiR-200b and miR-15b regulate chemotherapy-induced epithelial-mesenchymal transition in human tongue cancer cells by targeting BMI1. Oncogene. 2012; 31(4):432–45. Epub 2011/07/05. doi: 10.1038/onc.2011.263 PMID: 21725369.

13. Xia L, Zhang D, Du R, Pan Y, Zhao L, Sun S, et al. miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. International journal of cancer. 2008; 123(2):372–9. Epub 2008/05/02. doi: 10.1002/ijc.23501 PMID: 18449891.

14. Satzger I, Matern A, Kueattler U, Weinspach D, Voelker B, Kapp A, et al. MicroRNA-15b represents an independent prognostic parameter and is correlated with tumor cell proliferation and apoptosis in malignant melanoma. International journal of cancer. 2010; 126(11):2553–62. Epub 2009/10/16. doi: 10.1002/ijc.24960 PMID: 19830692.

15. Polytarchou C, Iliopoulos D, Struhl K. An integrated transcriptional regulatory circuit that reinforces the breast cancer stem cell state. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109(36):14470–5. Epub 2012/08/22. doi: 10.1073/pnas.1212811109 PMID: 22908280; PubMed Central PMCID: PMC3437881.

16. King ML, Lindberg ME, Stodden GR, Okuda H, Ebers SD, Johnson A, et al. WNT7A/beta-catenin signaling induces FGF1 and influences sensitivity to niclosamide in ovarian cancer. Oncogene. 2015; 34(26):3452–62. Epub 2014/09/02. doi: 10.1038/onc.2014.277 PMID: 25174399; PubMed Central PMCID: PMC4345161.

17. Yoshioha S, King ML, Ran S, Okuda H, MacLean JA 2nd, McAsey ME, et al. WNT7A regulates tumor growth and progression in ovarian cancer through the WNT/beta-catenin pathway. Mol Cancer Res. 2010; 10(3):469–82. Epub 2010/11/11. doi: 10.1158/1541-7786.MCR-10-0177 PMID: 22232518; PubMed Central PMCID: PMC3307825.

18. Hayashi K, Erikson DW, Tilford SA, Bany BM, Maclean JA 2nd, Rucker EB 3rd, et al. Wnt genes in the mouse uterus: potential regulation of implantation. Biology of reproduction. 2009; 80(5):899–1000. Epub 2009/01/24. doi: 10.1095/biolreprod.108.075416 PMID: 19164167; PubMed Central PMCID: PMC2804842.

19. Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. Nature communications. 2013; 4:2126. Epub 2013/07/11. doi: 10.1038/ncomms3126 PMID: 23839242; PubMed Central PMCID: PMC3331705.

20. Yu D, Wolf JK, Scanlon M, Price JE, Hung MC. Enhanced c-erbB-2/neu expression in human ovarian cancer cells correlates with more severe malignancy that can be suppressed by E1A. Cancer research. 1993; 53(4):891–8. Epub 1993/02/15. PMID:8094034.

21. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. eLife. 2015; 4. Epub 2015/08/13. doi: 10.7554/eLife.05005 PMID: 26267216; PubMed Central PMCID: PMC4532895.

22. Polakis P. Wnt signaling in cancer. Cold Spring Harbor perspectives in biology. 2012; 4(5). Epub 2012/03/23. doi: 10.1101/cshperspect.a008052 PMID: 22438566; PubMed Central PMCID: PMC3331705.

23. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2006; 127(3):469–80. Epub 2006/11/04. doi: 10.1016/j.cell.2006.05.018 PMID: 17081971.

24. Polakis P. Drugging Wnt signalling in cancer. The EMBO journal. 2012; 31(12):3469–82. Epub 2012/06/25. doi: 10.1038/emboj.2012.126 PMID: 22617421; PubMed Central PMCID: PMC3380214.

25. Gu Y, Yang P, Shao Q, Liu X, Xia S, Zhang M, et al. Investigation of the expression patterns and correlation of DNA methyltransferases and class I histone deacetylases in ovarian cancer tissues. Oncology letters. 2013; 5(2):452–8. Epub 2013/02/20. doi: 10.3892/ol.2012.1057 PMID: 23400501; PubMed Central PMCID: PMC3573157.

26. Cheng JC, Auersperg N, Leung PC. Inhibition of p53 represses E-cadherin expression by increasing DNA methyltransferase-1 and promoter methylation in serous borderline ovarian tumor cells. Oncogene. 2011; 30(37):3930–42. Epub 2011/04/12. doi: 10.1038/onc.2011.117 PMID: 21478913.

27. Sehgal V, Sefirou EG, Moss TJ, Mills GB, Azencott R, Ram PT. Robust Selection Algorithm (RSA) for Multi-Omic Biomarker Discovery; Integration with Functional Network Analysis to Identify mRNA Regulated Pathways in Multiple Cancers. PloS one. 2015; 10(10):e0140072. Epub 2015/10/28. doi: 10.1371/journal.pone.0140072 PMID: 26505200; PubMed Central PMCID: PMC4623517.

28. Guglielmelli P, Tozzi L, Bogani C, Iacobucci I, Ponziani V, Martinelli G, et al. Overexpression of microRNA-16-2 contributes to the abnormal erythropoiesis in polycythemia vera. Blood. 2011; 117(25):6923–7. Epub 2011/04/30. doi: 10.1182/blood-2010-09-306506 PMID: 21527532.