Transcriptional and Epigenetic Regulation of \textit{KIF14} Overexpression in Ovarian Cancer

Brigitte L. Thériault\textsuperscript{1}, Halesha D. Basavarajappa\textsuperscript{2}, Harvey Lim\textsuperscript{1,3}, Sanja Pajovic\textsuperscript{1}, Brenda L. Gallie\textsuperscript{1,3,4,5*}, Timothy W. Corson\textsuperscript{2,6*}

\textsuperscript{1}Campbell Family Cancer Research Institute, Princess Margaret Cancer Centre, Toronto, Ontario, Canada, \textsuperscript{2}Eugene and Marilyn Glick Eye Institute, Department of Ophthalmology, and Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, United States of America, \textsuperscript{3}Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada, \textsuperscript{4}Division of Visual Science, Toronto Western Hospital Research Institute, Toronto, Ontario, Canada, \textsuperscript{5}Departments of Molecular Genetics and Ophthalmology, University of Toronto, Toronto, Ontario, Canada, \textsuperscript{6}Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, Indiana, United States of America

Abstract

\textit{KIF14} (kinesin family member 14) is a mitotic kinesin and an important oncogene in several cancers. Tumor \textit{KIF14} expression levels are independently predictive of poor outcome, and in cancer cells \textit{KIF14} can modulate metastatic behavior by maintaining appropriate levels of cell adhesion and migration proteins at the cell membrane. Thus \textit{KIF14} is an exciting potential therapeutic target. Understanding \textit{KIF14}’s regulation in cancer cells is crucial to the development of effective and selective therapies to block its tumorigenic function(s). We previously determined that close to 30% of serous ovarian cancers (OvCa tumors) exhibit low-level genomic gain, indicating one mechanism of \textit{KIF14} overexpression in tumors. We now report on transcriptional and epigenetic regulation of \textit{KIF14}. Through promoter deletion analyses, we identified one cis-regulatory region containing binding sites for Sp1, HSF1 and YY1. siRNA-mediated knockdown of these transcription factors demonstrated endogenous regulation of \textit{KIF14} overexpression by Sp1 and YY1, but not HSF1. ChIP experiments confirmed an enrichment of both Sp1 and YY1 binding to the endogenous \textit{KIF14} promoter in OvCa cell lines with high \textit{KIF14} expression. A strong correlation was seen in primary serous OvCa tumors between Sp1, YY1 and \textit{KIF14} expression, further evidence that these transcription factors are important players in \textit{KIF14} overexpression. Hypomethylation patterns were observed in primary serous OvCa tumors, suggesting a minor role for promoter methylation in the control of \textit{KIF14} gene expression. miRNA expression analysis determined that miR-93, miR-144 and miR-382 had significantly lower levels of expression in primary serous OvCa tumors than normal tissues; treatment of an OvCa cell line with miRNA mimics and inhibitors specifically modulated \textit{KIF14} mRNA levels, pointing to potential novel mechanisms of \textit{KIF14} overexpression in primary tumors. Our findings reveal multiple mechanisms of \textit{KIF14} upregulation in cancer cells, offering new targets for therapeutic interventions to reduce \textit{KIF14} in tumors, aiming at improved prognosis.

Introduction

\textit{KIF14} was first identified as an oncogene and a contributor to malignant transformation in the childhood cancer retinoblastoma [1]. Located on chromosome 1q32.1, genomic gain of \textit{KIF14} occurs in up to 50% of retinoblastomas [1–3]. Our group and others have previously shown that \textit{KIF14} protein and mRNA are overexpressed in multiple cancers including ovarian cancers (OvCa tumors) [4–10]. We also reported that overall outcome of serous OvCa patients can be predicted based on \textit{KIF14} mRNA expression levels in their primary tumors. Further analysis of these samples showed that expression of \textit{KIF14} mRNA and protein exceed the levels expected based on the copy number gain alone, suggesting an up-regulation in the transcriptional control in cancer cells versus their respective normal counterparts.

There are currently 45 human kinesins classified into 14 distinct families. In humans, \textit{KIF14} is a mitotic kinesin belonging to the N-3 family. Although its cellular function has not yet been fully elucidated, \textit{KIF14} plays a vital role in the completion of cytokinesis, and may also function in the primary cilium [11]. It interacts with protein-regulator of cytokinesis 1 (PRC1) and citron kinase through specific domains to support proper cell division [12,13]. Silencing \textit{KIF14} using siRNA induces cytokinesis failure resulting in multinucleated cells, aneuploidy, and apoptosis [12]. A temporary accumulation of \textit{KIF14} protein is observed in mitotic cells, consistent with its function [12]. We recently demonstrated in breast cancer cells lines, direct interaction of \textit{KIF14} with Radil, a crucial mediator of Rap1a-mediated integrin inside-out signaling, thereby controlling Radil-Rap1a activity at the cell membrane and promoting cell adhesion and migration [14].
Whereas the structure and function of other kinesins are fairly well understood, much less is known about their regulation at the DNA level. One study described roles of the transcription factors Sp1 and E2F1 in respectively activating and repressing the transcription of the human mitotic centromere-associated kinesin (MCAK) promoter [13]. No studies have yet been reported on KIF14. Understanding the mechanism of its gene regulation is crucial as KIF14 emerges as an important target in cancer therapy.

We now report identification of a putative cis-regulatory region of the KIF14 promoter harbouring binding sites for the transcription factors Sp1, YY1 and HSF1. Through siRNA knockdown and ChIP assays, we show that Sp1 and YY1, but not HSF1 directly bind to the KIF14 promoter and control endogenous KIF14 levels in OvCa cell lines. Furthermore, both Sp1 and YY1 expression levels correlate with KIF14 mRNA expression in primary serous OvCa tumors, demonstrating their potential role in maintaining high KIF14 levels in OvCa tumor cells. Methylation analysis revealed that the human KIF14 promoter is largely hypomethylated in OvCa primary tumors, normal ovary tissues, and cell lines, indicating that methylation is unlikely to regulate KIF14 overexpression within OvCa tumors. However, a differential methylation pattern may exist in OvCa tumors expressing very high KIF14.

miRNA expression analysis of primary OvCa tumors and cell lines revealed three putative regulators (miR-93, miR-144 and miR-382) of KIF14 expression that have documented roles in tumorigenesis in cancer cells. We determined that these candidate miRNAs could directly modulate KIF14 mRNA expression, indicating their importance in maintaining elevated KIF14 tumor levels. Our results unveil a complexity of regulatory mechanisms driving KIF14 overexpression in OvCa tumors, and underscore the importance of understanding KIF14 regulation to ultimately target this gene for therapeutic benefit.

Materials and Methods

Construction of Luciferase Reporter Plasmids

Sixteen different lengths of the KIF14 promoter (548 bp, 966 bp, 1514 bp, 1666 bp, 1787 bp, 1898 bp, 2032 bp, 2150 bp, 2245 bp, 2327 bp, 2366 bp, 2401 bp, 2466 bp, 2536 bp, 2898 bp, 4555 bp) all sharing common sequences at their proximal end were amplified by polymerase chain reaction (PCR) from genomic DNA of healthy retina using KOD Hot Start DNA Polymerase (Novagen) (Table S1). Promoter fragments were subcloned into StrataClone™ Blunt PCR Cloning Vector pSC-B (Stratagene) and verified for correct insertion and orientation by restriction digest. PCR product was excised out using Kpn1/Sma1 (Stratagene) and verified for correct insertion and orientation by restriction digest. PCR product was excised out using Kpn1/Sma1 restriction sites and inserted into pGL3-Basic vector (Promega) at complementary sites upstream of the luciferase gene generating the reporter constructs pGL3-548, pGL3-966, pGL3-1514, pGL3-1666, pGL3-1787, pGL3-1898, pGL3-2032, pGL3-2150, pGL3-2245, pGL3-2237, pGL3-2366, pGL3-2401, pGL3-2466, pGL3-2536, pGL3-2898, and pGL3-4555. Sequences of all 16 constructs aligned with the reference sequences from the NCBI website. The pRsv-Luciferase reporter plasmid was kindly provided by Dr. A. Schimmer, Ontario Cancer Institute.

Cell Culture

SKOV3 (a kind gift from Dr. Mark Nachtigal, University of Manitoba) [16] and HeLa cells (ATCC) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% fetal bovine serum (FBS) and penicillin/streptomycin/L-glutamine (P/S/G) (Invitrogen). OvCa 429 cells (Dr. Mark Nachtigal) [16] were cultured in alpha-MEM containing 10% FBS and P/S/G. WERI-Rb1 retinoblastoma cells (positive control for Sp1 binding) were cultured in Iscove’s modified Dulbecco’s medium (IMDM) containing 10% FBS (PAA laboratories), P/S/G, 10 μg/mL insulin, and 55 μM β-mercaptoethanol. Cell cultures were maintained at 5% CO2 with humidity in a 37°C incubator. Cell line identity was confirmed by short tandem repeat profiling.

Transfection of Reporter Constructs and siRNA

Trypsinized cells were seeded at a density of 5×10^4 cells/mL 24 hours after seeding, equal amounts (0.5 μg) of both reporter and cytomegalovirus-β-galactosidase (pCMV-β-gal, Promega) constructs were cotransfected into triplicate 12-well plates using Gene Juice (Novagen). To evaluate the endogenous effect of transcription factor knockdown on KIF14 levels, a set of 3 siRNAs (Sigma Aldrich) for HSF1, Sp1, and YY1 were transfected at an amount of 0.5 nmol into 60 mm dishes. Target sequences for each siRNA are listed in Table S2. Transfections were carried out in triplicate according to the manufacturers’ instructions and repeated at least three times.

Luciferase and β-galactosidase Assay

Cells that were cotransfected with reporter and pCMV-β-gal constructs were harvested 24 hours after transfection. Cell lysates were prepared and assayed for luciferase activity using the Luciferase Assay System (Promega) in accordance to its protocol. Luciferase activity was normalized to β-galactosidase activity (measured by hydrolysis of the colorimetric substrate ONPG [O-nitrophenyl β-d-galactopyranoside], Sigma) and expressed as a relative percentage of pRsv-Luc control.

Clinical Samples

Twenty-six fresh frozen OvCa tumor samples, ten fresh frozen normal non-neoplastic ovaries from patients undergoing oophorectomy for non-oncological conditions and four normal tubal epithelium tissues were obtained from the University Health Network (UHN) Biobank. The UHN Research Ethics Board approved the study of tissue samples and associated clinical data (#08-0884-TE), and all tissues were banked with written informed consent. Clinical data associated with the UHN Biobank samples were reviewed by a gynecological oncologist and gynecologic pathologist to ensure data integrity and quality, and all UHN Biobank tissues (adjacent HE stained slides) were reviewed by a gynecologic pathologist to ensure samples contained >80% tumor cells.

Real-time PCR

Seventy-two hours following transfection with siRNA, cell lines were harvested and total RNA isolated by TRizol reagent (Invitrogen) according to manufacturer’s instructions. OvCa tumor and normal ovary and tubal epithelial RNAs were also isolated by TRizol reagent as previously described [8]. First-strand cDNA was prepared from 1 μg of total cellular RNA with 100 μM random hexamer primers, 20 U Ribolock RNase Inhibitor (Fermentas), and 200 U Superscript II reverse transcriptase (Invitrogen) in a final volume of 20 μL. 1 μL of synthesized cDNA was added to 1X TaqMan PCR master mix (ABI), and 1X TaqMan Gene Expression Assay primer-probe mix for KIF14 (Hs00978216_m1), Sp1 (Hs00916521_m1), YY1 (Hs00231533_m1), and HSF1 (Hs01027608_g1). Mean expression of three housekeeping genes was used as an endogenous control: TBP (Tata-box binding protein, Hs_99999910_m1), HPRT (Hypoxanthine phosphoribosyl transferase, Hs99999909_m1) and GAPDH (Glyceraldehyde-3-phosphate dehydrogenase, Hs99999905_m1). Triplicate reactions were
were calculated using Photoshop CS3.

**Signal intensity measurements**

were detected using a chemiluminescence reagent (Denville) and incubated with horseradish peroxidase-labeled secondary antibodies (Chemicon). Horseradish peroxidase-labeled secondary antibodies (Chemicon) were detected using a chemiluminescence reagent (Denville) and incubated with photographic film (Denville). Signal intensity measurements were calculated using Photoshop CS3.

**Western Blot Analysis**

Seventy-two hours after transfecting siRNA, cells were harvested and lysed with buffer containing 20 mM Tris HCl pH 7.6, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100, and the protease inhibitors aprotinin, leupeptin, and phenylmethylsulphonyl fluoride (PMSF). Total cellular protein was quantified by Bradford assay (BioRad). 30 μg was loaded onto a 4–12% gradient SDS-PAGE precast gel (Lona) and transferred onto a polyvinylidene fluoride (PVDF) membrane and blocked with 5% BLOTTO (BioRad) in Tris-Buffered Saline-0.05% Tween-20 (TBST). Primary antibodies against KIF14 (Bethyl), Sp1, YY1 and HSF1 (Abcam) and β-tubulin (Sigma) were employed to detect endogenous protein expression. Horseradish peroxidase-labeled secondary antibodies (Chemicon) were detected using a chemiluminescence reagent (Denville) and incubated with photographic film (Denville). Signal intensity measurements were calculated using Photoshop CS3.

**Chromatin Immunoprecipitation (ChIP) Assay**

ChIP assays were conducted with the Imprint® Chromatin Immunoprecipitation Kit (Sigma) according to manufacturer instructions. Briefly, 1x10⁵ cells were cultured, and the protein-DNA complexes cross-linked with 1% formaldehyde (final concentration), followed by nuclear fractionation and DNA shearing via sonication. Immunoprecipitations were carried out for 90 minutes at RT with antibodies against Sp1, YY1, HSF1 (Abcam), RNA polymerase II (positive control) and IgG (negative control). The cross-links were reversed, DNA was extracted and was used for both endpoint and real time PCR. Endpoint PCR was performed in 25 μL reactions with KOD Hot Start DNA Polymerase (Novagen) and the appropriately designed primer pair (target promoter sequence: Forward: tga cta ggt act gat gta; Reverse: ctc ttc ccc, ccc cc g; RNA Pol II target sequence: gtgGAPDH-Forward: cta ccc atc taa ggt gtc; hGAPDH-Reverse: tag tag ccc ggt cct act tt, 246 bp). The PCR conditions were as follows: 95°C for 2 min followed by 40 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 30 sec, and then a final extension period of 72°C for 10 min. Products were visualized using agarose gel electrophoresis. Signal intensity measurements were calculated using Photoshop CS3. Real time PCR was performed in triplicate using 1 μL of ChIP DNA per reaction, along with 1X TaqMan Master Mix (ABI), 500 nM each primer and 250 nM probe. Primers were Forward: tga cca cca cct cca cga gg and Reverse: tct cgt aat ggt gga ctc gc, and the probe was cac ggc cac gca gca ggg cag, labeled with FAM and ZEN quencher (Integrated DNA Technologies, Coralville, IA, USA). Reactions were run using standard parameters on a ViiATM 7 real-time PCR system. The relative expression values (RQ) of KIF14 or miRNAs normalized to endogenous control genes and relative to control treatment were calculated using ViiATM 7 version 1.2 software.

**miRNA mimics and inhibitors**

SKOV3 cells in 12-well plates were transfected with 50 nM of miR93, miR144 and miR382 mimics or inhibitors or corresponding non-coding control mimic or inhibitor (Applied Biosystems) using 3 μL of Lipofectamine 2000 (Invitrogen) reagent as per the manufacturer’s instructions. After 72 hours of transfection, total RNA was isolated and cDNA synthesized as described above. Expression levels of KIF14 and miRNAs of interest were analyzed as above, with qPCR performed using an Applied Biosystems ViiATM 7 real time PCR system. The relative expression values (RQ) of KIF14 or miRNAs normalized to endogenous control genes and relative to control treatment were calculated using ViiATM 7 version 1.2 software.

**Methylation analysis**

Genomic DNA was isolated from primary OvCa tumor tissues and normal ovary controls as previously described [8]. Genomic DNA (200 ng) was modified and purified using a commercially available bisulphite modification kit [Imprint® DNA Modification Kit, Sigma] according to manufacturer’s instructions. A CpG island was identified (http://cgisilonse.usc.edu/) within the 4500 bp KIF14 promoter region (−2371 to −1129) (Figure S1A), and methylation-specific primers (−120 bp) against the largest CpG island within the KIF14 promoter using the online software Methprimer were designed (http://www.uoregon.edu/~methprimer/) (Figure S1B). Primer sequences were as follows: Methylation-specific: Forward: att taa agg ggt tta att ttt acg; Reverse: gat aat taa act ccc ata ace gtc; Unmethylated-specific: Forward: att taa agg ggt att ttt atg; Reverse: cca taa tta acc ctt acat cc. One quarter of the purified bisulphite-treated DNA (5 μL of a 20 μL eluate) was used in the methylation PCR reaction. End-point PCR reactions were conducted in 25 μL final volume with Maxima Hot Start Taq Polymerase (Fermentas). The PCR conditions were as follows: 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec, and then a final extension period of 72°C for 10 min. Products (−120 bp) were visualized using agarose gel electrophoresis. Densitometry was performed on grayscale images using Image J. Verification of bisulphite conversion of the DNA was conducted with unmethylated calponin-specific primers (Figure S2) as described [17].

**Statistical Analysis**

Expression values ( luciferase activity, miRNA, miRNA or protein expression) were assessed between normal tissue controls and OvCa tumor tissues, or controls and treated groups using paired or unpaired t-tests. KIF14 expression after miRNA inhibitor/mimic treatment was analyzed by one-way ANOVA. Unless otherwise specified, all statistical analyses were conducted using Graph Pad Prism 4.0. Correlations between KIF14 gain vs.
**KIF14** no gain categories and **Sp1**, **YY1** and **HSF1** mRNA expression were analyzed using Pearson’s Correlation Coefficient (r).

**Results**

**Identification of a KIF14 cis regulatory region in ovarian cancer cell lines**

A 4500 bp region of genomic DNA immediately upstream of the human **KIF14** transcriptional start site was amplified from normal human DNA, and deletional reporter analyses were conducted by cloning sequentially smaller regions of the **KIF14** promoter into the pRSV-Luc promoter region. Luciferase activity was measured in OvCa429, SKOV3 and HeLa cells, and promoter activity was calculated in relation to pRSV-Luc expression (set at 100%). The entire promoter region construct (pGL3-4555) showed luciferase activity close to that of the positive control (pRSV-Luc). One highly active region was identified between -2366 and -2245 bp, demonstrating similar activity to the full promoter construct, but significantly more luciferase activity compared to all other deletion constructs for all three cell lines (P<0.05, Figure 1). Bioinformatic analysis of this active region through online analysis software (Genomatix) identified putative transcription factor binding sites for **Sp1**, **YY1** and **HSF1** (Figure 1), suggesting the presence of a potential cis regulatory region within the **KIF14** promoter.

**Sp1 and YY1 endogenously regulate KIF14 expression in OvCa cell lines**

We tested whether these transcription factors affected **KIF14** mRNA expression by siRNA-mediated knockdown of endogenous **Sp1**, **HSF1** and **YY1** in SKOV3 and OvCa429 cells. Transient knockdown resulted in a significant reduction of transcription factor (**Sp1**, **HSF1**, **YY1**) mRNA (Figure 2A, B). While knockdown of **HSF1** had no significant effect on **KIF14** gene expression, **Sp1** and **YY1** knockdown resulted in significantly decreased **KIF14** mRNA, suggesting that both **Sp1** and **YY1** act as enhancers of **KIF14** transcription. In response to **Sp1** and **YY1** knockdown, a decrease in transcription factor protein expression and associated **KIF14** protein was confirmed via immunoblots (Figure 3) in SKOV3 cells. While **HSF1** protein levels decreased in response to knockdown, no significant change in **KIF14** protein expression was seen. Similar results were seen for OvCa429 cells (data not shown), indicating that **Sp1** and **YY1** may control the expression of **KIF14** in OvCa cell lines. The reduction in **KIF14** mRNA and protein expression in response to **Sp1** knockdown was less than with **YY1** knockdown, and could likely be related to the efficiency of transcription factor knockdown (Figure 2A, B and Figure 3A, B). The reduction in **KIF14** mRNA and protein was more

---

**Figure 1. KIF14 promoter activity.** Promoter deletion constructs fused to Luciferase were tested for activity in HeLa, SKOV3 and OvCa429 cells. pRSV-Luc, positive control, 0, empty vector control. Numbers are base pairs relative to the transcriptional start site (0). Bioinformatic analysis (Genomatix) of the most active region within the KIF14 promoter (−2366 to −2245) identified putative binding sites for transcription factors YY1, HSF1 and Sp1. Specific transcription factor recognition sites are underlined, and space within sequence denotes location of deletion constructs. N = 3, * Significance at P<0.05, unpaired t-test. P = 0.01 (HeLa); P = 0.01 (SKOV3); P = 0.03 (OvCa429).

doi:10.1371/journal.pone.0091540.g001
pronounced in response to YY1 knockdown, suggesting that YY1 may be a major player in the regulation of KIF14 overexpression in these cells.

To verify whether Sp1 and YY1 could associate directly with the KIF14 promoter, chromatin immunoprecipitation (ChIP) assays were conducted in SKOV3, OvCa429 and HeLa cells. We found that both Sp1 and YY1, but not HSF1 exhibited a much higher binding affinity to the KIF14 promoter region than the IgG control (higher relative expression values; Figure 4). OvCa429 cells showed the greatest enrichment of binding for both Sp1 and YY1 (over 10-fold; Figure 4A, B), while SKOV3 and HeLa cells showed more modest enrichment (on average 4-fold; Figure 4A, B). Binding of HSF1 to the KIF14 promoter was much less pronounced (on average 2-fold for all cell lines; Figure 4C). Enrichment in Sp1 and YY1 binding was also shown via endpoint PCR (Figure S3). These results confirm our mRNA and protein expression results by showing that both Sp1 and YY1 can bind directly to the KIF14 promoter. Combined, these data indicate that Sp1 and YY1 can endogenously regulate KIF14 expression in OvCa cell lines.

Figure 2. Knockdown of endogenous SP1 and YY1 mRNA inhibits KIF14 transcription. siRNA knockdown of endogenous Sp1 (orange), HSF1 (green), and YY1 (red) transcription factors, and measurement of their mRNA expression along with corresponding KIF14 mRNA levels (blue) via real-time PCR in A SKOV3 and B OvCa429 cells. Y-axes: normalized mRNA expression relative to MOCK. GL2, control siRNA; N = 3, * Significance at \( P < 0.05 \), unpaired t-test. Three different siRNA molecules (A, B, C) were used to knock down each gene. P values for panel A (SKOV3 cells): \( P = 0.02 \) for Sp1 expression (orange) with Sp1 siRNAs A, B, and C; \( P = 0.03 \) (siRNA–A), \( P = 0.047 \) (siRNA–B), \( P = 0.04 \) (siRNA–C) for KIF14 expression (blue), \( P = 0.001 \) for HSF1 expression (green) with HSF1 siRNAs A, B, and C; \( P = 0.23 \) (siRNA–A), \( P = 0.12 \) (siRNA–B), \( P = 0.4 \) (siRNA–C) for KIF14 expression (blue), \( P = 0.01 \) for YY1 expression (red) with YY1 siRNAs A, B and C; \( P = 0.006 \) for KIF14 expression (blue) with YY1 siRNAs A, B, and C. P values for panel B (OvCa429 cells): \( P = 0.03 \) for Sp1 expression (orange) with Sp1 siRNAs A, B, and C; \( P = 0.05 \) (siRNA–A), \( P = 0.04 \) (siRNA–B), \( P = 0.045 \) (siRNA–C) for KIF14 expression (blue), \( P = 0.003 \) for HSF1 expression (green) with HSF1 siRNAs A, B, and C; \( P = 0.31 \) (siRNA–A), \( P = 0.45 \) (siRNA–B), \( P = 0.39 \) (siRNA–C) for KIF14 expression (blue), \( P = 0.02 \) (siRNA–A), \( P = 0.01 \) (siRNA–B), \( P = 0.01 \) for YY1 expression (red); \( P = 0.02 \) (siRNA–A), \( P = 0.001 \) (siRNA–B), \( P = 0.006 \) (siRNA–C) for KIF14 expression (blue).

doi:10.1371/journal.pone.0091540.g002
Sp1 and YY1 overexpression correlates with KIF14 overexpression

We have previously documented genomic gain of KIF14 in up to 30% of primary OvCa tumors that correlates with very high overexpression of *KIF14* mRNA (KIF14\textsuperscript{HIGH}) [8]. These data suggest that genomic gain is one mechanism through which KIF14 is overexpressed in these tumors. To determine whether KIF14 overexpression in OvCa tumors could also be linked to transcriptional regulation, we measured mRNA expression of Sp1, YY1, and HSF1 in a subset of OvCa tumor tissues with (15 samples) and without (50 samples) KIF14 genomic gain. In OvCa tumors without genomic gain (50), we dichotomized the samples into two groups based on median expression, into either KIF14\textsuperscript{HIGH} or KIF14\textsuperscript{LOW} groups, as we have previously reported [8]. Many of the 19 KIF14\textsuperscript{HIGH} OvCa tumors expressed significantly higher levels of Sp1 and YY1 mRNA (Figure S5) than those with KIF14\textsuperscript{LOW} overexpression (31), indicating potential roles for Sp1 and YY1 to maintain high KIF14 levels in tumors. HSF1 mRNA levels were similar in all OvCa tumors, thus less likely to be important in regulating KIF14 mRNA. The mean YY1 expression level in primary OvCa tumors is much higher than the mean Sp1 expression level; together with the YY1 knockdown data in cell lines (Figures 2 and 3), these results suggest that YY1 is an important regulator of KIF14 overexpression in OvCa tumors. In support of this, a strong correlation was seen between KIF14 and Sp1 or YY1 expression, whereas none existed for HSF1 (Table 1 and Figure S4D–F). Tumors with KIF14 genomic gain demonstrated no transcription factor correlation (Table 1 and Figure S4A–C), confirming that genomic gain is the mechanism controlling KIF14 overexpression in these tumors. Interestingly, a proportion (approximately 40%) of KIF14\textsuperscript{HIGH} tumors showed Sp1 and YY1 expression close to normal tissue levels (Figure 5), further implicating other biological mechanisms to potentially control KIF14 overexpression in OvCa tumors.

The KIF14 promoter is differentially hypomethylated in primary serous OvCa tumors and cell lines

Epigenetic mechanisms of gene regulation, such as aberrant DNA methylation have been identified to upregulate oncogene expression and silence tumor suppressors in many cancers [18]. In OvCa tumors, DNA methylation patterns have been investigated as potential biomarkers for tumor progression and response to therapy [19–25]. To study KIF14 promoter methylation, we documented the KIF14 promoter region using the online software CpG Island Searcher (http://www.cpgislands.com). We identified a CpG island within the 4500 bp KIF14 promoter region (−2371 to −1129) (Figure S1A), and designed methylation-specific primers (−120 bp) against the largest CpG island using the online software Methprimer (Figure S1B). Interestingly the location of this major CpG island coincides with the above-analyzed Sp1 and YY1 binding sites (Figure S1A). In support of our findings, it is well known that Sp1 recognition sites are commonly located within CpG islands [26]. Bisulphite conversion of tumor DNA from 20 serous OvCa tumors (10 KIF14\textsuperscript{HIGH} and 10 KIF14\textsuperscript{LOW}), 7 normal ovary tissues, and 4 OvCa cell lines (including 2 IOSE samples) was performed, followed by PCR using primers directed against
methylated/unmethylated residues. Complete bisulphite conversion of the DNA was verified with the use of unmodified calponin-specific primers (Figure S2). Most tissue and cell line samples tested, including normal ovary controls, demonstrated higher abundance of unmethylated product than methylated product (Figure 6), as evidenced by the unmethylated/methylated (U/M) ratios above 1 (Table S4). Interestingly, analysis of the U/M ratios between KIF14\textsuperscript{HIGH} and KIF14\textsuperscript{LOW} samples revealed a statistically significant difference, wherein KIF14\textsuperscript{HIGH} tumors demonstrated on average less unmethylated product (lower U/M means more methylated product) than KIF14\textsuperscript{LOW} (higher U/M ratio means less methylated product; $P = 0.009$; Table S4). The methylation patterns in normal ovary were much more variable, and not statistically different from KIF14\textsuperscript{LOW} tumors ($P = 0.27$) but approaching significance for KIF14\textsuperscript{HIGH} tumors ($P = 0.054$). A marked difference was also seen in U/M ratios between OvCa cell lines and IOSE cells, following the same trend as for the KIF14\textsuperscript{HIGH} vs KIF14\textsuperscript{LOW} tumors; however sample size ($n = 2$) precluded statistical analysis. These results indicate that differential methylation patterns exist between OvCa tumors. Although in need of further investigation, the fact that unmethylated product is present in all normal and tumor tissues suggests a minor role for promoter methylation (of the particular region studied) in the control of KIF14 overexpression in OvCa tumor cells.

miR93, miR144 and miR382 expression are associated with KIF14 expression in primary OvCa tumors

MicroRNAs (miRNAs) are a large, extensively studied group of small non-coding RNAs that downregulate protein expression by targeting mRNA translation and/or stability [27]. miRNAs have been implicated in the control of expression of many crucial cellular gene expression pathways in normal and cancer cells, and have demonstrated roles in ovarian cancer pathogenesis and progression [28]. To determine whether KIF14 expression may be under miRNA control, we analyzed the KIF14 gene region using 7 online miRNA binding site prediction tools (Table S5) that revealed multiple potential candidate miRNAs binding to the KIF14 gene. The expression of 16 candidate miRNAs presenting

Figure 4. SP1 and YY1 bind endogenously to the human KIF14 promoter in cancer cell lines. ChIP assays of endogenous YY1, Sp1 and HSF1 followed by real time PCR with the KIF14 promoter region (−2300 to −2133) in cell lines SKOV3, OvCa429, and HeLa compared to IgG (negative control). Values represent average quantity of promoter region product relative to IgG control. Error bars represent standard deviation of triplicate assays. doi:10.1371/journal.pone.0091540.g004
one or more hits was studied on 6 OvCa cell lines (including 2 immortalized ovarian surface epithelium (OSE) controls) and 26 primary serous OvCa tumors; half (13) with low KIF14 overexpression (KIF14<sup>HIGH</sup>, red) and KIF14<sup>LOW</sup> (green) mRNA expression (but no KIF14 genomic gain) for Sp1 (circle), YY1 (triangle), and HSF1 (diamond) normalized to normal ovary expression (set as 1, black dashed line). Mean, black line. Individual tumors represented by symbols. * Significance at \( P < 0.05 \), paired t-test. \( P = 0.03 \) (Sp1), \( P = 0.01 \) (YY1), \( P = 0.32 \) (HSF1).

Figure 5. KIF14<sup>HIGH</sup> OvCa tumors overexpress Sp1 and YY1. Quantitative mRNA expression analysis of primary OvCa tumors with KIF14<sup>HIGH</sup> (red) and KIF14<sup>LOW</sup> (green) mRNA expression (but no KIF14 genomic gain) for Sp1 (circle), YY1 (triangle), and HSF1 (diamond) normalized to normal ovary expression (set as 1, black dashed line). Mean, black line. Individual tumors represented by symbols. * Significance at \( P < 0.05 \), paired t-test. \( P = 0.03 \) (Sp1), \( P = 0.01 \) (YY1), \( P = 0.32 \) (HSF1).

miR93, miR144 and miR382 modulate KIF14 mRNA expression in an OvCa cell line

To determine whether the identified candidate miRNAs could modulate KIF14 mRNA expression, we treated SKOV3 cells with either the candidate miRNA mimic or inhibitor, and measured KIF14 mRNA expression via real time PCR. miR93 and miR144 mimics caused a modest but reproducible decrease in KIF14 mRNA expression that did not achieve statistical significance, while treatment with miR382 mimic resulted in a significant inhibition of KIF14 mRNA expression (Figure 8). Correspondingly, treatment with miR93 inhibitor resulted in a modest increase in KIF14 mRNA expression (Figure 8), while treatment with a miR382 inhibitor produced a substantial increase in KIF14 mRNA expression (Figure 8). These results point to the potential that these candidate miRNAs (especially miR382) are modulators of KIF14 mRNA levels in OvCa that could contribute to the overexpression of KIF14 mRNA in OvCa tumors.

Discussion

We and others identified KIF14 as an important onco- gene, prognostic indicator, biomarker and therapeutic target in the progression of multiple cancers, including retinoblastoma, glioma, breast, lung, renal, hepatocellular and ovarian cancers [1,4–9,14,29–32]. KIF14 mRNA is highly overexpressed in the majority of OvCa tumors [8], and has recently been identified as a marker for taxane resistance in a small cohort of OvCa tumors [33]. Thus insight into the regulation of its overexpression is crucial for developing therapeutic approaches to reduce KIF14 expression in OvCa cells. We have previously shown that a proportion of OvCa tumors demonstrate genomic gain of KIF14 (up to 30%, depending on histological subtype) that correlates with high mRNA overexpression, indicating genomic gain as one mechanism of KIF14 overexpression. Since over 90% of OvCas overexpress KIF14 mRNA, other mechanisms of overexpression are likely to exist within these tumors. The goal of this study was to characterize additional mechanisms of KIF14 overexpression in

| Table 1. Pearson correlation between KIF14 gain/no gain tumors and Sp1, HSF1 and YY1 mRNA. |
|---------------------------------|------------------|------------------|------------------|
| **gain** | **Sp1** | **HSF1** | **YY1** |
| \( r \) | \(-0.07\) | \(-0.13\) | \(-0.08\) |
| \( \rho \) | 0.8 | 0.62 | 0.77 |
| N | 15 | 15 | 15 |
| **no gain** | **Sp1** | **HSF1** | **YY1** |
| \( r \) | 0.4 | 0.2 | 0.35 |
| \( \rho \) | 0.004 | 0.06 | 0.01 |
| N | 50 | 50 | 50 |

\( r \) = correlation coefficient; \( \rho \) significance at \( < 0.05 \); N = number of samples.

doi:10.1371/journal.pone.0091540.t001
OvCa cells. This type of comprehensive gene regulation analysis is important prior to development of new therapeutic strategies.

We first characterized the regulation of the KIF14 promoter region in OvCa cell lines. We identified via luciferase reporter activity a cis-regulatory region within the promoter region to harbor putative binding sites for the transcription factors Sp1, HSF1, and YY1. Through transient siRNA knockdown of these transcription factors in OvCa cell lines, we determined that inhibition of Sp1 and YY1, but not HSF1, significantly decreased KIF14 mRNA expression. Furthermore, we show that both endogenous Sp1 and YY1 can bind to the KIF14 promoter in OvCa cell lines, indicating their potential importance in OvCa progression. The consensus binding site for the Sp1 family of transcription factors is GGGGCGGGG. In our analysis of the KIF14 promoter region, only two partial Sp1 binding sites were found within the putative cis-regulatory region; the siRNA, ChIP and expression data confirm a regulatory role for Sp1 in enhancing KIF14 expression, similar to previous data showing Sp1 regulation of the MCAK promoter [15]. Sp1 is a ubiquitously expressed transcription factor that is involved in modulating normal and tumorigenic gene expression in multiple cancers. It is overexpressed in breast, thyroid, hepatocellular, prostate, pancreatic, gastric, lung and ovarian cancers [34–37], and small molecule inhibitors of Sp1 have been tested in pre-clinical models with successful reduction in tumor burden [38,39]. Our data demonstrate not only that Sp1 can enhance KIF14 expression in OvCa cell lines, but that Sp1 is overexpressed and correlates with high KIF14 overexpression in primary OvCa tumors. Targeting Sp1 expression may reduce KIF14 levels in tumor cells, and could contribute to a therapeutic strategy against OvCa progression.

YY1 is a transcription factor that also has documented roles in either enhancing or repressing gene expression in multiple cancers [40–42], however its tumorigenic overexpression and prognostic value have been the subject of debate due to conflicting reports for multiple cancers, including in breast, colon, prostate, osteosarcoma, cervical and ovarian cancers [42]. Interestingly, YY1 has been shown to interact with general transcription factors such as Sp1 [43–47] to enhance gene expression. YY1 can bind to DNA and directly modulate transcriptional activity, and also act as a cofactor to recruit into an active transcriptional complex other factors, including Sp1 [42]. Based on our data, one can easily envision YY1 and Sp1 cooperating to enhance KIF14 overexpression in OvCa tumors. Detailed mechanisms of their interaction remain to be investigated.

HSF1 is a heat-shock response transcription factor that plays a tumorigenic role in multiple cancers [48]. Although we found HSF1 binding sites within the identified cis-regulatory region of the KIF14 promoter, our data demonstrate no effect of HSF1 knockdown on KIF14 gene or protein expression, and very weak...
endogenous binding of HSF1 to the KIF14 promoter in OvCa cell lines.

Methylation analysis of the largest CpG island within the human KIF14 promoter revealed that the KIF14 promoter is largely hypomethylated in OvCa primary tumors, normal ovary tissues, and cell lines, indicating that methylation may not be a mechanism regulating KIF14 overexpression within OvCa tumors. This result is not surprising, as the KIF14 gene is crucial in normal and tumor cells to control the last stages of cytokinesis [12,13], and thus there is most likely a need for some expression (albeit low in normal tissues) to maintain normal proliferation and viability. However, the presence of more methylated product in KIF14HIGH tumors than KIF14LOW tumors indicates that a differential methylation pattern may exist between low and high KIF14

Figure 7. Candidate miRNA expression in primary OvCa tumors and OvCa cell lines. Mean miRNA expression in KIF14HIGH (dark blue) and KIF14LOW (light blue) primary serous OvCas, and established OvCa cell lines (red). Expression normalized to the endogenous control gene RNU44, relative to normal ovary tissues (primary OvCas) or IOSE (OvCa cell lines). * Significance, P<0.05, unpaired t-test. P = 0.002 (miR-93), P = 0.01 (miR-144), P = 0.006). doi:10.1371/journal.pone.0091540.g007

Figure 8. Candidate miRNAs modulate KIF14 mRNA expression in an OvCa cell line. SKOV3 cells were treated with either candidate miRNA mimics that increased miRNA expression 200–6000-fold, or miRNA inhibitors that decreased expression by 70–100%. KIF14 mRNA expression was measured using real time PCR. Graph represents KIF14 expression relative to negative control transfections. Average ± SD of three separate experiments shown. Significance: *, P<0.05; **, P<0.01 compared with control, one-way ANOVA with Dunnett’s post hoc tests. doi:10.1371/journal.pone.0091540.g008
tumors. Although warranting further investigation given that promoter methylation is generally seen as a mechanism of transcriptional repression [19], these results raise the intriguing possibility of a feedback mechanism, attempting, but inadequate to reduce KIF14 levels in tumors with high KIF14 expression. Although the other minor CpG islands were not tested, our results indicate that other mechanisms of gene regulation, such as genomic gain, transcriptional activation, or miRNA regulation are more likely to control KIF14 overexpression in serous OvCa tumors.

The knockdown of YY1 produced a much larger decrease in KIF14 protein than mRNA, which prompted us to investigate the possibility that KIF14 could be regulated by miRNAs. Through miRNA binding site prediction software, we identified 16 putative miRNAs to bind to the KIF14 promoter region. Expression analysis of these miRNAs in OvCa cell lines and primary OvCa tumors in comparison to normal ovary tissues and IOSE cells revealed that miR93, miR144 and miR382 were expressed significantly lower in KIF14-high primary OvCa tumors than in KIF14-low tumors. These miRNAs were also expressed lower in established OvCa cell lines compared to IOSE cells, suggesting that these miRNAs are interesting potential regulators of KIF14 levels in the promotion of OvCa tumor progression worthy of further characterization. We determined that treatment of SKOV3 cells with candidate miRNA inhibitors specifically increased KIF14 mRNA expression. Although the role of these candidate miRNAs in the modulation of KIF14 protein levels has yet to be assessed, our results clearly implicate these miRNAs in the maintenance of KIF14 mRNA levels, and potential KIF14 overexpression in OvCa tumors. Interestingly, the largest increase in KIF14 mRNA expression resulted from miR382 inhibitor treatment; this candidate miRNA also showed the strongest inverse correlation with KIF14 mRNA expression in primary OvCa tumors, the lowest level of KIF14 mRNA expression in OvCa cell lines, and the greatest decrease in KIF14 mRNA expression when overexpressed. Our results thus highlight the potential importance of miR382 in the progression of OvCa tumors.

miR93 has been recently shown to have a role modulating cisplatin resistance in OvCa cell lines by targeting the PTEN tumor suppressor [49]. This study was conducted in established OvCa cell lines treated with chemotherapy, as opposed to the tumor suppressor [49]. This study was conducted in established tumors.

miR93 has been recently shown to have a role modulating cisplatin resistance in OvCa cell lines by targeting the PTEN tumor suppressor [49]. This study was conducted in established OvCa cell lines treated with chemotherapy, as opposed to the tumor suppressor [49]. This study was conducted in established tumors.

The dismal prognosis associated with many solid tumors arises from poorly effective therapies for treatment of recurrent disease. The identification of potential regulatory transcription factors and miRNAs will open the door for development of more precise, less invasive methods to predict outcome, as miRNAs especially are known to be stably detected in serum [54]; these candidate tumor miRNAs could be developed into non-invasive detection and/or prognostic markers. With further validation, these putative transcriptional regulators may be targets for selective anti-KIF14 therapy.

Supporting Information

Figure S1 Methylation specific PCR. A CpG island analysis of the KIF14 promoter identified one CpG island between −2371 to −1129 (1243 bp). Blue line delineates the CpG island, while red lines represent potential methylated CpG residues. B Design of methylation-specific primers for the KIF14 promoter. (TIF)

Figure S2 Calponin PCR. Calponin PCR of primary OvCa tumors and cell lines. L, molecular weight ladder; n, normal ovary tissue; H, KIF14-high (tumor); L, KIF14-low (tumor); red, tumors with KIF14 gain. (TIF)

Figure S3 SP1 and YY1 bind endogenously to the human KIF14 promoter in OvCa cell lines. ChIP assays of endogenous YY1, Sp1 and HSF1 with the KIF14 promoter region (−2150 to −2366 = 216 bp ampiclon, black arrow) in cell lines SKOV3, OvCa429, and WERI-Rb1 (positive control for Sp1 binding), compared to IgG (negative control and RNA pol II (positive control for ChIP, 250 bp ampiclon). Numbers represent relative expression values (normalized to RNA pol II, relative to IgG) Black asterisk, 200 bp marker. (TIF)

Figure S4 KIF14 mRNA expression correlates with SP1 and YY1 expression in OvCa tumors without genomic gain. Pearson correlation analysis between KIF14 gain (A–C) or no gain (D–F) OvCa tumors and Sp1 (A, D), HSF1 (B, E) and
YY1 (C, F) mRNA expression. r = correlation coefficient; P significance at <0.05; N = number of samples. (TIF)

Table S1 Primer sequences for PCR of KIF14 promoter fragment. (TIF)

Table S2 Target sequences for siRNAs. (TIF)

Table S3 Sequences for miRNA specific assays. (TIF)

Table S4 Unmethylated/Methylated DNA ratios. (TIF)

Table S5 miRNA database predictions for miRNA candidates binding to the KIF14 promoter. (TIF)

Table S6 miRNA expression analyses of primary OvCas and OvCa cell lines. Highlighted miRNAs represent significant difference in expression between KIF14HIGH (H) and KIF14LOW (L) OvCas. AVG, average; STDEV, standard deviation; t-test, comparison of miRNA expression between KIF14HIGH and KIF14LOW groups. Pearson correlation, comparison of miRNA expression to KIF14 expression in the entire tumor cohort. P<0.05, significant difference. (TIF)

Acknowledgments

We thank Dr. Patricia Shaw and Dr. Marcus Bernardini for pathology review of the tumor issues.

Author Contributions

Conceived and designed the experiments: BT HB HL SP BG TC. Performed the experiments: BT HB HL. Analyzed the data: BT HB HL SP BG TC. Contributed reagents/materials/analysis tools: BT HB HL SP BG TC. Wrote the paper: BT TC. Editing of manuscript: BT HB HL SP BG TC.

References

1. Corson TW, Huang A, Tsao MS, Gallie BL (2005) KIF14 is a candidate oncogene in the 1q41 minimal region of genomic gain in multiple cancers. Oncogene 24: 4741–4753.
2. Bowles E, Corson TW, Bayani J, Squire JA, Wong N, et al. (2007) Profiling genomic copy number changes in retinoblastoma beyond loss of RB1. Genes Chromosomes Cancer 46: 118–129.
3. Theriault BL, Corson T.W. (2012) Kinesin Family Member 14 (KIF14). Atlas of Genetics and Cytogenetics in Oncology and Haematology.
4. Corson TW, Gallie BL (2006) KIF14 mRNA expression is a predictor of grade and outcome in breast cancer. Int J Cancer 119: 1088–1094.
5. Corson TW, Zhu CQ, Lau SK, Shepherd FA, Tsao MS, et al. (2007) KIF14 messenger RNA expression is independently prognostic for outcome in lung cancer. Clin Cancer Res 13: 3229–3234.
6. Markska J, Oczko-Wojciechowska M, Gierek T, Jazraw M, Paluch J, et al. (2009) Gene expression profile analysis in laryngeal cancer by high-density oligonucleotide microarrays. J Physiol Pharmacol 60 Suppl 1: 57–63.
7. Markska J, Tyszkiewicz T, Jazraw M, Oczko-Wojciechowska M, Gierek T, et al. (2009) Metalloproteinase ADAM12, kinesin 14 and checkpoint suppressor 1 as new molecular markers of laryngeal carcinoma. Eur Arch Otorhinolaryngol 266: 1501–1507.
8. Theriault BL, Pajovic S, Bernardzini MO, Shaw PA, Gallie BL (2012) Kinesin family member 14: an independent prognostic marker and potential therapeutic target for ovarian cancer. Int J Cancer 130: 1844–1854.
9. Wang Q, Wang L, Li D, Deng J, Zhao Z, et al. (2013) Kinesin family member 14 is a candidate prognostic marker for outcome of glioma patients. Cancer Epidemiol 37: 79–84.
10. Yang T, Zhang XB, Zheng ZM (2013) Suppression of KIF14 expression inhibits hepatocellular carcinoma progression and predicts favorable outcome. Cancer Sci 104: 552–557.
11. Filges I, Nosowa E, Bruder E, Tecranli S, Townsend K, et al. (2013) Exome sequencing identifies mutations in KIF14 as a novel cause of an autosomal recessive lethal cardio-deaf phenotype. Clin Genet.
12. Carleton M, Mao M, Biery M, Warrener P, Kim S, et al. (2006) RNA interference-mediated silencing of mitotic kinesin KIF14 disrupts cell cycle progression and induces cytokinesis failure. Mol Cell Biol 26: 3853–3863.
13. Gruneberg U, Neef R, Li X, Chan EH, Chalamalasetty RB, et al. (2006) KIF14 negatively regulates Rap1a-Radil signaling during breast cancer progression. J Cell Biol 172: 563–572.
14. Ahmed SM, Theriault BL, Uppalapati M, Chiu CW, Gallie BL, et al. (2012) KIF14 negatively regulates Rap1a-Radil signaling during breast cancer progression. J Cell Biol 199: 951–967.
15. Jun DY, Park HS, Lee JY, Kim YH (2008) Regulation of the human mitotic centromere-associated kinesin (MCAK) promoter by the transcription factor Sp1 and E2F1. Biochim Biophys Acta 1779: 356–361.
16. Shepherd TG, Theriault BL, Campbell EJ, Nachigal MW (2006) Primary culture of ovarian surface epithelial cells and ascites-derived ovarian cancer cells from patients. Nat Protoc 1: 2634–2645.
17. Srinsaks R, Chaopatchayakul P, Jearanaikoon P, Leelayuwat C, Limpaboch T (2010) Verification of complete bisulfite modification using Calponin-specific primer sets. Clin Biochem 43: 528–530.
18. Sava ML, Raggi N, Bernstein BE (2013) Epigenetic reprogramming in cancer. Science 339: 1567–1570.
19. Bijourn JG, Bel GM, Verheijen RH, van Diest PJ (2012) Epigenetic biomarkers in the diagnosis of ovarian cancer. Expert Opin Med Diag 6: 421–430.
20. Chiang YC, Chang MC, Chen PJ, Wu MM, Hsieh CY, et al. (2013) Epigenetic silencing of BLU through interfering apoptosis results in chemoresistance and poor prognosis of ovarian versus carcinoma patients. Endocr Relat Cancer 20: 213–227.
21. Heichman KA, Warren JD (2012) DNA methylation biomarkers and their utility for solid cancer diagnostics. Clin Chem Lab Med 50: 1707–1721.
22. Ho CM, Huang CJ, Huang CY, Wu YY, Chang SF, et al. (2012) Promoter methylation status of HIN-1 associated with outcomes of ovarian clear cell adenocarcinoma. Mol Cancer 11: 53.
23. Joung JG, Kim D, Kim KH, Kim JH (2013) Extracting coordinated patterns of DNA methylation and gene expression in ovarian cancer. J Am Med Inform Assoc.
24. Stefansson OA, Villanueva A, Vidal A, Marri L, Esteller M (2012) BRCA1 epigenetic inactivation predicts sensitivity to platinum-based chemotherapy in breast and ovarian cancer. Epigenetics 7: 1225–1229.
25. Wang YQ, Yan Q, Zhang JR, Li SD, Yang YX, et al. (2013) Epigenetic inactivation of BRCA1 through promoter hypermethylation in ovarian cancer progression. J Obstet Gynaecol Res 39: 549–554.
26. Deaton AM, Bird A (2011) CpG islands and the regulation of transcription. Genes Dev 25: 1010–1022.
27. Thorsen SB, Olaf S, Jensen NF, Steenberg J, Kauppinen S (2012) The therapeutic potential of microRNAs in cancer. Cancer J 18: 273–284.
28. Zaman MS, Maher DM, Khan S, Jagg J, Chauhan SG (2012) Current status and implications of microRNAs in ovarian cancer diagnosis and therapy. J Ovarian Res 5: 44.
29. Dimaras H, Khetan V, Halliday W, Orlic M, Prigoda NL, et al. (2008) Loss of RB1 induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. Hum Mol Genet 17: 1363–1372.
30. Kim TM, Yim SH, Shin SH, Xu HD, Jung YC, et al. (2008) Clinical implication of recurrent copy number alterations in hepatocellular carcinoma and putative oncogenes in recurrent gains on 1q. Int J Cancer 123: 2008–2015.
31. Madhavan J, Mitra M, Mallikarjuna K, Pranav O, Sinivasan R, et al. (2009) KIF14 and EZF3 mRNA expression in human retinoblastoma and its phenotypic association. Mol Vis 15: 235–240.
32. Szpolar A, Zabokov D, Pavlik J, Jauch A, Kovacs G (2009) Three genetic developmental stages of papillary renal cell tumors: duplication of chromosome 1q marks fatal progression. J Intern J Cancer 124: 2071–2076.
33. Ehrlichova M, Mehlhofer-Kachouche B, Hrdl J, Brenychova V, Mrhalova M, et al. (2018) The association of taxane resistance genes with the clinical course of ovarian carcinoma. Genomics 102: 96–101.
34. Chung JY, Wu CH, Lai MD, Chang WC, Hung JJ (2009) Overexpression of Sp1 leads to β3-dependent apoptosis in cancer cells. Int J Cancer 125: 2066–2076.
35. Davie JR, He S, Li L, Sekhavat A, Espino P, et al. (2008) Nuclear organization and chromatin dynamics—Sp1, Sp3 and histone deacetylases. Adv Enzyme Regul 48: 189–200.
36. Kong LM, Liao CG, Fei F, Guo X, Xing JL, et al. (2010) Transcription factor Sp1 regulates expression of cancer-associated molecule CD44 in human lung cancer. Cancer Sci 101: 1463–1470.
37. Ho CM, Huang CJ, Huang CY, Wu YY, Chang SF, et al. (2012) Promoter methylation status of HIN-1 associated with outcomes of ovarian clear cell adenocarcinoma. Mol Cancer 11: 53.
38. Malek A, Nunez LE, Magistri M, Brambilla L, Jovic S, et al. (2012) Modulation of the activity of Sp transcription factors by mithramycin analogues as a new strategy for treatment of metastatic prostate cancer. PLoS One 7: e35130.
39. Previti S, Malek A, Albertini V, Risa C, Capella C, et al. (2010) Inhibition of Sp1-dependent transcription and antitumor activity of the new aureolic acid analogues mithramycin SDK and SK in human ovarian cancer xenografts. Gynecol Oncol 118: 182–188.

40. Simon JA, Lange CA (2008) Roles of the EZH2 histone methyltransferase in cancer epigenetics. Mutat Res 647: 21–29.

41. Yu H, Mashtalir N, Daou S, Hammond-Martel I, Ross J, et al. (2010) The ubiquitin carboxyl hydrolase BAP1 forms a ternary complex with YY1 and HCF-1 and is a critical regulator of gene expression. Mol Cell Biol 30: 5071–5085.

42. Zhang Q, Stovall DR, Inoue K, Sui G (2011) The oncogenic role of Yin Yang 1. Crit Rev Oncog 16: 163–197.

43. Zhou Q, Gedrich RW, Engel DA (1995) Transcriptional repression of the c-fos gene by YY1 is mediated by a direct interaction with ATF/CREB. J Virol 69: 4323–4330.

44. Usheva A, Shenk T (1996) YY1 transcriptional initiator: protein interactions and association with a DNA site containing unpaired strands. Proc Natl Acad Sci U S A 93: 13571–13576.

45. Seto E, Lewis B, Shenk T (1993) Interaction between transcription factors Sp1 and YY1. Nature 365: 462–464.

46. Austen M, Luscher B, Luscher-Firzlaff JM (1997) Characterization of the transcriptional regulator YY1. The bipartite transactivation domain is independent of interaction with the TATA box-binding protein, transcription factor IIIB, TAFII55, or cAMP-responsive element-binding protein (CRE)-binding protein. J Biol Chem 272: 1769–1777.

47. Renna M, Caporaso MG, Bonatti S, Kaufman RJ, Remondelli P (2007) Regulation of ERGIC-53 gene transcription in response to endoplasmic reticulum stress. J Biol Chem 282: 22499–22512.

48. Cocca DR, Arrigo AP, Calderwood SK (2013) Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. Arch Toxicol 87: 19–48.

49. Fu X, Tian J, Zhang L, Chen Y, Hao Q (2012) Involvement of microRNA-93, a new regulator of PTEN/Akt signaling pathway, in regulation of chemotherapeutic drug cisplatin chemosensitivity in ovarian cancer cells. FEBS Lett 586: 1279–1286.

50. Iwaya T, Yokobori T, Nishida N, Kogo R, Sudoh T, et al. (2012) Downregulation of miR-144 is associated with colorectal cancer progression via activation of mTOR signaling pathway. Carcinogenesis 33: 2391–2397.

51. Sarver AL, Thayanithy V, Scott MC, Cleton-Jansen AM, Hogendoorn PC, et al. (2013) MicroRNAs at the human 14q32 locus have prognostic significance in osteosarcoma. Orphanet J Rare Dis 8: 7.

52. Kim SW, Kim JW, Kim YT, Kim JH, Kim S, et al. (2007) Analysis of chromosomal changes in serous ovarian carcinoma using high-resolution array comparative genomic hybridization: Potential predictive markers of chemoresistant disease. Genes Chromosomes Cancer 46: 1–9.

53. Campbell MJ, Turner BM (2013) Altered histone modifications in cancer. Adv Exp Med Biol 754: 81–107.

54. Healy NA, Heneghan HM, Miller N, Osborne CK, Schiff R, et al. (2012) Systemic mirnas as potential biomarkers for malignancy. Int J Cancer 131: 2213–2222.