The regulation network and network motif analysis in ovarian cancer

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Summary

Objective: Several gene alterations have been identified associated with ovarian cancer (OC) development. However, how these genetic elements are coordinated in transcription network during OC initiation and progression is poorly understood. Thus, the objective of this study was to interpret the transcription regulation network of OC. *Materials and Methods:* The GSE14407 microarray data was used for analysis of the transcription regulation network of OC. *Results:* The results showed that the TP53 (tumor protein p53) was the most crucial transcription factor in the transcriptome network. P53 could down-regulate CDC14A (CDC14 cell division cycle 14 homolog A [S. cerevisiae]) and FAS (TNF receptor superfamily, member 6) expression, but up-regulate SFN (stratifin) and THBS1 (thrombospondin 1) expression to involve in pathways in cancer, cell cycle, p53 signaling pathway, and apoptosis pathway. *Conclusion:* This transcriptional regulation may provide a better understanding of molecular mechanism and some potential therapeutic targets in the treatment of OC.

Key words: Regulation network; Network motif analysis; Ovarian cancer.

Introduction

Ovarian cancer (OC) is the leading cause of death from gynecologic cancer for women worldwide. OC comprises of four main histological subtypes. 1) serous cystadenocarcinoma, 2) mucinous, 3) endometrioid, and 4) clear cell [1]. The majority of patients are diagnosed with advanced disease, which is cured with surgery and postoperative chemotherapy [2]. However, some patients exhibit resistance to chemotherapy, resulting in an overall five-year survival of 10%-30% [3].

A number of gene alterations have been identified and frequently encountered during ovarian tumorigenesis, including *KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog), *BRAF* (v-raf murine sarcoma viral oncogene homolog B1), *TP53* (tumor protein p53), *RB* (retinoblastoma), *PTEN* (phosphatase and tensin homolog) [4], *MYC* (v-myc myelocytomatosis viral oncogene homolog [avian]) [5], *E2F-1* (E2F transcription factor 1), *EGFR* (epidermal growth factor receptor), *etc*. Approximately 60%-70% of low-grade serous carcinomas carry KRAS or BRAF genes mutations [6], but deregulation of the tumor-suppressing pathways p53 and BRCA1/2 is more common in high-grade serous tumors [7]. Expression level of E2F-1 is elevated in all the OC cell lines studied when compared with control cells. High expression of E2F-1 is found to be associated with histopathologic grade 3 tumors and residual tumor over two cm in diameter in OC patients [8, 9]. EGFR is reported over-expression in the majority of OC [10] and has been implicated in both the growth and progression of this disease. Targeting the EGF receptor via antisense transfection or tyrosine kinase inhibitor in OC reduces the expression of EGFR and suppresses OC cell to grow and responsiveness to exogenous EGF [11, 12]. EGF activated in vivo binding of E2F1 to the B-Myb promoter and subsequent activation of B-Myb gene expression [13], whose expression is also involved in OC [14, 15]; however, relatively little is known about how these genetic elements are coordinated in transcription network during OC initiation and progression.

Therefore, the objective of this study was to identify potential transcription regulation relationships between transcription factors and differentially expressed target genes in OC by using the microarray data and transcriptional network analysis. Network motif was used to represent the different interaction type. Moreover, the authors characterized their underlying molecular mechanisms by KEGG pathway enrichment analysis.

Materials and Methods

Data source - affymetrix microarray data

The transcription profile of GSE14407 was obtained from NCBI GEO database (http://www.ncbi.nlm.nih.gov/geo/) which is based on the Affymetrix Human Genome U133 Plus 2.0 Array. A total of 24 chips, purchased from Cancer Development and Evolution in Georgia Institute of Technology, were used for this analysis. Twelve healthy ovarian surface epithelia samples (OSE) were compared to twelve laser-captured microdissected serous OC epithelia samples (CEPI) via Affymetrix 3' expression array.

Regulation data

A total of 774 pairs of regulatory relationship between 219 transcription factors (TFs) and 265 target genes were collected from TRANSFAC [16] (http://www.gene-regulation.com/pub/data bases.html). A total of 5,722 pairs of regulatory relationships between 102 TFs and 2,920 target genes were collected from TRED [17] (http://rulai.cshl.edu/TRED/). By integrating the

Revised manuscript accepted for publication August 14, 2012

above two regulation datasets, a total of 6,328 regulatory relationships between 276 TFs and 3,002 target genes were ultimately obtained.

Differentially-expressed genes (DEGs) analysis

For the GSE14407 dataset, the limma method [18] was used to identify DEGs. The original expression datasets from all conditions were extracted into expression estimates and then to construct the linear model. The DEGs only with a fold change value larger than 2 and *p* value less than 0.05 were selected.

Co-expression analysis

For demonstrating the potential regulatory relationship, the Pearson correlation coefficient (PCC) [19] was calculated for all pair-wise comparisons of gene-expression values between TFs and the DEGs. The regulatory relationships whose absolute PCC were larger than 0.75 were considered significant.

Regulation network construction

Using the regulation data that have been collected from TRANSFAC [16] database and TRED [17] database, the authors matched the relationships between differentially-expressed TFs and their target genes.

Based on the above two regulation datasets, the significant relationships (PCC > 0.6 or PCC < – 0.6) between TFs and their target genes, the authors constructed the regulation networks by Cytoscape [20].

Network motif

Fanmod [21] is a tool for finding so-called networks motifs in a network, that is, it locates small vertex-induced subgraphs that occur significantly more often than in random networks.

Enumeration algorithm was applied to search for five sizes of subgraphs, which were found more than five times, |Z-Score| ≥ 5, and *p* value ≤ 0.05. The Z-Score is the original frequency minus the random frequency divided by the standard deviation. The higher the Z-Score, the more significant the motif is. The *p* value of a motif is the number of random networks in which it occurred more often than in the original network, divided by the total number of random networks. Therefore, *p* values range from 0 to 1; the smaller the *p* value, the more significant the motif is.

Pathway analysis

DAVID [22], a high-throughput and integrated data-mining environment, analyzes gene lists derived from high-throughput genomic experiments. DAVID was used to identify KEGG pathway analysis.

Results

Microarray data analysis

Publicly-available microarray data set GSE14407 was obtained from GEO. A total of 10,836 DEGs with the fold change value > 2 and *p* value < 0.05 were selected using the limma method. All of these genes are positive expression genes.

Regulation network analysis

To obtain regulation network, based on the significant relationships (PCC > 0.6 or PCC < - 0.6) between TFs and their target genes, 160 expression relationships including 44 TFs and 138 target genes were selected. By integrating expression relationships above, a regulation network was built between TFs and their target genes (Figure 1). TP53 (tumor protein p53), E2F1 (E2F transcription factor 1), NFKB (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1), MYC (v-myc myelocytomatosis viral oncogene homolog [avian]), and SPI1 (spleen focus forming virus [SFFV] proviral integration oncogene spi1) were hub nodes in the transcriptome network. Among them, TP53 had the most interaction regulation relationships with its target genes, such as p53 could down-regulate CDC14A (CDC14 cell division cycle 14 homolog A [S. cerevisiae]) and FAS (TNF receptor superfamily, member 6) expression, but up-regulate SFN (stratifin) and THBS1 (thrombospondin 1) expression.

Table 1. — *Top 10 KEGG pathways*.

Term	Description	Count	*p* value	FDR
hsa05200	Pathways in cancer	32	8.23E-12	9.57E-09
hsa05221	Acute myeloid leukemia	12	7.12E-08	8.28E-05
hsa05222	Small cell lung cancer	12	3.46E-06	0.004024
hsa04110	Cell cycle	13	3.18E-05	0.036994
hsa05212	Pancreatic cancer	10	4.10E-05	0.047633
hsa04115	p53 signaling pathway	9	1.72E-04	0.200131
hsa04210	Apoptosis	10	1.83E-04	0.213119
hsa05215	Prostate cancer	10	2.19E-04	0.253811
hsa05218	Melanoma	9	2.34E-04	0.271445
hsa05220	Chronic myeloid leukemia	9	3.43E-04	0.397652

Network motif analysis

To search for five sizes of subgraphs, which were found more than five times, |Z-Score| ≥ 5, *p* value ≤ 0.05 between TFs and their target genes, 175 expression relationships including 42 TFs and 114 target genes were selected. Network motif subgraphs were built between TFs and their target genes (Figure 2). Figure 2 lists the top five motif subgraphs. From these results, the authors also suggest the more important role of TP53 in regulation network.

Function analysis of the network

Using the KEGG pathways to describe the function of the regulation network, several KEGG pathways were enriched among these pathways in the regulation network, including pathways in cancer (hsa05200), acute myeloid leukemia (hsa05221), small cell lung cancer (hsa05222), cell cycle (hsa04110), p53 signaling pathway (hsa04115), and apoptosis (hsa04210). Table 1 only lists the top ten enriched KEGG pathways.

Term represents pathway ID. Description is a pathway symbol. Count is the number of enrichment pathways. The *p* value is the probability of obtaining a test statistic. The smaller the *p* value is, the more enrichment the pathway is. False discovery rate (FDR) control is a statistical method used in multiple hypothesis testing to correct for multiple comparisons. The smaller the FDR is, the higher the correctness is.

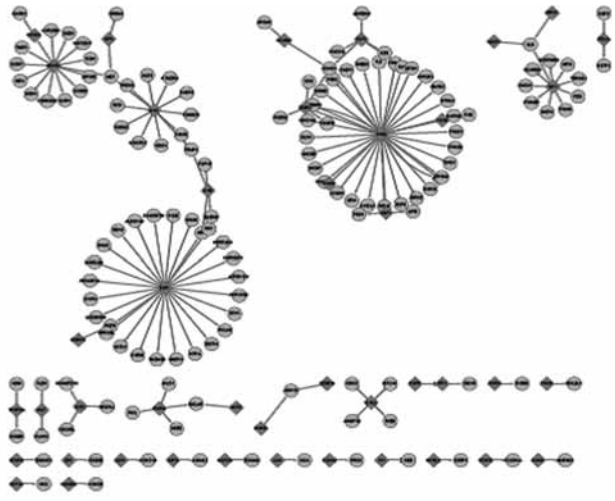

Figure 1. — Figure 1 Regulation network. The diamond nodes indicate TFs and the circle node indicates target genes. The lines indicate up-regulation and the lines indicate down-regulation.

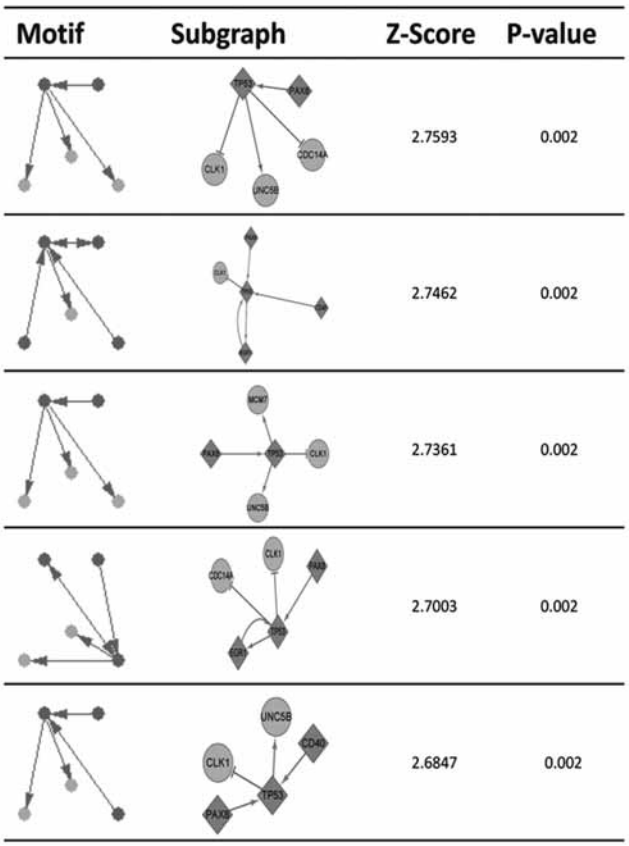

Figure 2. — Network motif subgraph. The circle nodes and diamonds indicate TFs and the circle node indicates target genes. The lines indicate up-regulation and the lines indicate down-regulation. The Z-Score is the original frequency minus the random frequency divided by the standard deviation. The *p* value of a motif is the number of random networks in which it occurred more often than in the original network, divided by the total number of random networks.

Discussion

In this study, the authors systemically investigated the regulation network of OC between TFs and target genes and their underlying molecular pathways. The authors have shown that the gene TP53 was a crucial TF in the transcriptome network. The gene *TP53* that encodes the tumor suppressor protein p53 is amongst the most commonly altered genes in human cancer, including OCs [23]. The loss of tumor-suppressor function of the TP53 protein, subsequent to a mutation in the coding sequence (missense or nonsense mutations) [24, 25], seems to be a common feature in a majority of epithelial OCs. A mutation of *p53* in early stage of OCs is associated with a short-term improvement in overall survival and progression-free survival [26, 27]. However, in advanced-stage cancers, *p53* mutation seems to confer a more aggressive biology related to metastasis and poor clinical outcome [28]. Missense TP53 mutations have been reported to result in p53 accumulation. Therefore, high-level expression of nuclear p53 protein is detected in malignant or benign epithelial OC [29]. A positive correlation was observed between PAX8 and p53 expression in endometrial carcinomas [30]. TP53 expression could also be regulated by PAX8 in the present study, which is highly-expressed in benign and malignant epithelial OC when compared to normal ovarian samples [31, 32].

In response to DNA damage, p53 is imported into the nucleus, binds to target genes, and alters their transcription to involve in cell cycle arrest and apoptosis [33]. In this study, the authors found TP53 could down-regulate CDC14A and FAS expression, but up-regulated SFN and THBS1expression. These genes were proposedly involved in cell cycle, P53 signaling pathway, and apoptosis pathway based on previous reports.

CDC14A protein is a member of the dual specificity protein tyrosine phosphatase family, which plays pleiotropic roles during the cell cycle, including the initiation of DNA replication [34] and the exit from mitosis [35]. Cdc14A is found differentially-expressed in human tissues with high-protein expression in brain, heart, small intestine, and skeletal muscle, moderate expression in spleen, and low or undetectable expression in kidney, liver, lung, testis, and pancreas. Low expression of Cdc14A occurs in cancer cell lines harboring wild-type p53 [36]. This protein has been proved to interact with, and dephosphorylate tumor suppressor protein p53 at ser315 site, and is thought to regulate the function of p53 in cancer [37]. In this study, the authors predicted that Cdc14A was also of low expression in OCs.

Fas (CD95/APO-1), a type I transmembrane cell surface protein with a molecular weight of 48 kD, is generally regarded as the prototypical cell death receptor of tumor necrosis factor family. It initiates apoptosis following engagement by Fas ligand, FasL, which has been

described as trigger molecules of apoptosis, such as caspase-8, resulting in downstream cell death caspase cascades [38]. Serum soluble Fas levels are significantly increased in women with OC compared with healthy controls. Increased pretreatment serum soluble Fas levels were associated with shortened disease-free and overall survival [39]. Decreased sensitivity to Fas-mediated apoptosis could contribute to ovarian tumorigenesis [40]. There is a p53-responsive element within the first intron of the Fas gene, as well as three putative elements within the promoter [41, 42]. Wild-typep53 binds to and trans-activates the Fas gene, whereas mutant p53 fails to induce apoptosis via activation of the Fas gene [42, 43], this is, down-regulated by mutant p53.

SFN protein, also known as 14-3-3σ, plays a crucial role in the G2 checkpoint by sequestering the mitotic initiation complex, cdc2-cyclin B1, in the cytoplasm after DNA damage [44]. This prevents cdc2-cyclin B1 from entering the nucleus in which the protein complex would normally initiate mitosis. In this manner, 14-3-3 induces G2 arrest and allows the repair of damaged DNA. Recently, the expression of 14-3-3σ protein has been reported to be frequently methylated and inactivated in ovarian carcinoma tissues [45]. Treatment of ovarian cell with demethylating agent resulted in the demethylation of the promoter CpG islands and restored the expression of 14-3-3σ gene. Decreased 14-3-3σ expression was significantly associated with positive p53 immunoreactivity, that is, an inverse correlation between14-3-3σ and p53 expression [46].

TSP-1, a 420 kDa extracellular matrix-bound adhesive glycoprotein, is the first protein recognized as an endogenous inhibitor of angiogenesis. Several studies have indicated that TSP-1 has tumor suppressive properties in vivo and overexpression of TSP-1 in human various cancer cells blocks tumor progression. *TSP-1* is found to be up-regulated upon THY-1 induction (a tumor suppressor gene) in human OC [47]. TSP-1 mRNA and protein levels are significantly decreased by hepatocyte growth factor (HGF) induction through MAPK signaling pathways, leading to the induction of MMP-9 and subsequent invasion of OC cell [48]. *P53* expression is found inversely-correlated with TSP-1 staining in OC cases. The reduction of TSP-1 expression associated with overexpression of *p53* may be coupled with the development of a pro-angiogenic environment and malignant phenotype [49].

In conclusion, the present findings shed new light on the regulation network of OC. The authors showed that TP53 TF could play an important role in OC via up-regulating or down-regulating target genes, such as SFN, THBS1, CDC14A, and FAS genes which are all associated with breast cancer progression.

Acknowledgements

This study was supported by the Biomedical Engineering Cross Research Funding of Shanghai Jliao Tong University (YG2011MS42).

References

[1] Soslow R.A.: "Histologic subtypes of ovarian carcinoma: an overview". *Int. J. Gynecol. Pathol.,* 2008, *27,* 161.
[2] Vergote I., Tropé C.G., Amant F., Kristensen G.B., Ehlen T., Johnson N. *et al*.: "Neoadjuvant chemotherapy or primary surgery in Stage IIIC or IV ovarian cancer". *New Engl. J. Med*., 2010, *363,* 943.
[3] Ozols R.F., Bundy B.N., Greer B.E., Fowler J.M., Clarke-Pearson D., Burger R.A. *et al*.: "Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected Stage III ovarian cancer: a Gynecologic Oncology Group study". *J. Clin. Oncol*., 2003, *21,* 3194.
[4] Shan W., Liu J.: "Epithelial ovarian cancer". *Cell. Cycle,* 2009, *8,* 731.
[5] Chen C.H., Shen J., Lee W.J., Chow S.N.: "Overexpression of cyclin D1 and c Myc gene products in human primary epithelial ovarian cancer". *Int. J. Gynecol. Cancer,* 2005, *15*, 878.
[6] Singer G., Oldt III R., Cohen Y., Wang B.G., Sidransky D., Kurman R.J. *et al*.: "Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma". *J. Nat. Cancer Inst*., 2003, *95,* 484.
[7] Landen C.N. Jr., Birrer M.J., Sood A.K. "Early events in the pathogenesis of epithelial ovarian cancer". *J. Clin. Oncol*., 2008*, 26,*995.
[8] Reimer D., Sadr S., Wiedemair A., Goebel G., Concin N., Hofstetter G. *et al*.: "Expression of the E2F family of transcription factors and its clinical relevance in ovarian cancer". *Ann. N.Y. Acad. Scie*., 2006, *1091*, 270.
[9] Suh D., Yoon M., Choi K., Kim J.: "Significance of E2F 1 overexpression in epithelial ovarian cancer". *Int. J. Gynecol. Cancer,* 2008, *18,* 492.
[10] Nielsen J.S., Jakobsen E., Hølund B., Bertelsen K., Jakobsen A.: "Prognostic significance of p53, Her 2, and EGFR overexpression in borderline and epithelial ovarian cancer". *Int. J. Gynecol. Cancer,* 2004, *14,* 1086.
[11] Brader K., Wolf J., Chakrabarty S., Price J.: "Epidermal growth factor receptor (EGFR) antisense transfection reduces the expression of EGFR and suppresses the malignant phenotype of a human ovarian cancer cell line". *Oncol. Rep*., 1998, *5,* 1269.
[12] Sewell J., Macleod K., Ritchie A., Smyth J., Langdon S.: "Targeting the EGF receptor in ovarian cancer with the tyrosine kinase inhibitor ZD 1839 ('Iressa')". *Br. J. Cancer,* 2002, *86*, 456.
[13] Hanada N., Lo H.W., Day C.P., Pan Y., Nakajima Y., Hung M.C.: "Co regulation of B Myb expression by E2F1 and EGF receptor". *Mol. Carcinogen*., 2006, *45,* 10.
[14] Barletta C., Lazzaro D., Prosperi P.R., Testa U., Grignani F., Ragusa R. *et al*.: "C-MYB activation and the pathogenesis of ovarian cancer". *Eur. J. Gynaecol. Oncol*., 1992, *13,* 53.
[15] Ghosh-Choudhury T., Xiao W., Gunaratne P., Anderson M.: "Loss of miR-148a/b expression promotes ovarian cancer by targeting Erbb3 and MYB". *Gynecol. Oncol.,* 2012, *125*, S98.
[16] Matys V., Fricke E., Geffers R., Gossling E., Haubrock M., Hehl R. *et al*.: "TRANSFAC: transcriptional regulation, from patterns to profiles". *Nucleic. Acids Res*., 2003, *31,* 374.
[17] Jiang C., Xuan Z., Zhao F., Zhang M.Q.: "TRED: a transcriptional regulatory element database, new entries and other development". *Nucleic Acids Res*., 2007, *35,* 137.
[18] Smyth G.K.: "Linear models and empirical bayes methods for assessing differential expression in microarray experiments". *Stat. Appl. Genet. Mol. Biol*., 2004, *3*, Epub Feb. 12.
[19] Derrick T.R., Bates B.T., Dufek J.S.: "Evaluation of time-series data sets using the Pearson product-moment correlation coefficient". *Med. Sci. Sports Exerc.,* 1994, *26*, 919.
[20] Shannon P., Markiel A., Ozier O., Baliga N.S., Wang J.T., Ramage D. *et al*.: "Cytoscape: a software environment for integrated models of biomolecular interaction networks". *Genome Res.,* 2003, *13,* 2498.
[21] Wernicke S., Rasche F.: "FANMOD: a tool for fast network motif detection". *Bioinformatics,* 2006, *22,* 1152.
[22] Huang da W., Sherman B.T., Lempicki R.A.: "Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources". *Nat. Protoc*., 2009, *4*, 44.
[23] Schuijer M., Berns E.M.J.J.: "TP53 and ovarian cancer". *Hum. Mut.,* 2003, *21,* 285.

[24] Ahmed A.A., Etemadmoghadam D., Temple J., Lynch A.G., Riad M., Sharma R. *et al*.: "Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary". *J. Pathol*., 2010, *221,* 49.
[25] Salani R., Kurman R., Giuntoli R. II, Gardner G., Bristow R., Wang T.L. *et al*.: "Assessment of TP53 mutation using purified tissue samples of ovarian serous carcinomas reveals a higher mutation rate than previously reported and does not correlate with drug resistance". *Int. J. Gynecol. Cancer,* 2008, *18,* 487.
[26] Havrilesky L., Darcy K.M., Hamdan H., Priore R..L, Leon J., Bell J. *et al*.: "Prognostic significance of p53 mutation and p53 overexpression in advanced epithelial ovarian cancer: a Gynecologic Oncology Group Study". *J. Clin. Oncology,* 2003, *21,* 3814.
[27] Wang Y., Helland Å., Holm R., Skomedal H., Abeler V., Danielsen H. *et al*.: "P53 mutations in early-stage ovarian carcinoma, relation to long-term survival". *Br. J. Cancer,* 2004, *90,* 678.
[28] Shahin M.S., Hughes J.H., Sood A.K., Buller R.E.: "The prognostic significance of p53 tumor suppressor gene alterations in ovarian carcinoma". *Cancer,* 2000, *89,* 2006.
[29] Marks J., Davidoff A., Kerns B., Humphrey P., Pence J., Dodge R. *et al*.: "Overexpression and mutation of p53 in epithelial ovarian cancer". *Cancer Res*., 1991, *51*, 2979.
[30] Brunner A.H., Riss P., Heinze G., Meltzow E., Brustmann H.: "Immunoexpression of PAX 8 in endometrial cancer: relation to high-grade carcinoma and p53". *Int. J. Gynecol. Pathol*., 2011, *30,* 569.
[31] Bowen N.J., Logani S., Dickerson E.B., Kapa L.B., Akhtar M., Benigno B.B. *et al*.: "Emerging roles for PAX8 in ovarian cancer and endosalpingeal development". *Gynecol. Oncol*., 2007, *104,* 331.
[32] Tickle T.L., Vachris J.C., Zhang J., Gurlov S., McKinney K.Q., Tait D.L. *et al*.: "Pax8, a human paired box gene, is over expressed in ovarian cancer". *P. Am. Asso. Cancer Res*., 2006, *1,* 768.
[33] Astanehe A., Arenillas D., Wasserman W.W., Leung P.C.K., Dunn S.E., Davies B.R. *et al*.: "Mechanisms underlying p53 regulation of PIK3CA transcription in ovarian surface epithelium and in ovarian cancer". *J. Cell. Sci*., 2008, *121*, 664.
[34] Fitzpatrick P., Toyn J., Millar J., Johnston L.: "DNA replication is completed in Saccharomyces cerevisiae cells that lack functional Cdc14, a dual-specificity protein phosphatase". *Mol. General. Genetics, MGG*, 1998, *258*, 437.
[35] Bembenek J., Yu H.: "Regulation of the anaphase-promoting complex by the dual specificity phosphatase human Cdc14a". *J. Biol. Chem*., 2001, *276,* 48237. Epub 2001 Oct. 11.
[36] Paulsen M.T., Starks A.M., Derheimer F.A., Hanasoge S., Li L., Dixon J.E. et al.: "The p53-targeting human phosphatase hCdc14A interacts with the Cdk1/cyclin B complex and is differentially expressed in human cancers". *Mol. Cancer,* 2006, *5*, 25.
[37] Li L., Ljungman M., Dixon J.E.: "The human Cdc14 phosphatases interact with and dephosphorylate the tumor suppressor protein p53". *J. Biol. Chem*., 2000, *275*, 2410.
[38] Kischkel F., Hellbardt S., Behrmann I., Germer M., Pawlita M., Krammer P. *et al*.: "Cytotoxicity-dependent APO-1 [Fas/CD95]-associated proteins form a death-inducing signaling complex [DISC] with the receptor". *EMBO J*., 1995, *14*, 5579.
[39] Abrahams V.M., Straszewski S.L., Kamsteeg M., Hanczaruk B., Schwartz P.E., Rutherford T.J. *et al*.: "Epithelial ovarian cancer cells secrete functional Fas ligand". *Cancer Res*., 2003, *63,* 5573.
[40] Baldwin R.L., Tran H., Karlan B.Y.: "Primary ovarian cancer cultures are resistant to Fas-mediated apoptosis". *Gynecol. Oncol*., 1999, *74,* 265.
[41] Munsch D., Watanabe-Fukunaga R., Bourdon J.C., Nagata S., May E., Yonish-Rouach E. *et al*.: "Human and mouse Fas [APO-1/CD95] death receptor genes each contain a p53-responsive element that is activated by p53 mutants unable to induce apoptosis". *J. Biol. Chem*., 2000, *275*, 3867.
[42] Müller M., Wilder S., Bannasch D., Israeli D., Lehlbach K., Li-Weber M. *et al*.: "p53 activates the CD95 [APO-1/Fas] gene in response to DNA damage by anticancer drugs". *J. Exp. Med*., 1998, *188,* 2033.
[43] Bennett M., Macdonald K., Chan S.W., Luzio J.P., Simari R., Weissberg P.: "Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis". *Science,* 1998, *282,* 290.
[44] Chan T.A., Hermeking H., Lengauer C., Kinzler K.W., Vogelstein B.: "14-3-3 is required to prevent mitotic catastrophe after DNA damage". *Nature,* 1999, *401,* 616.
[45] Kaneuchi M., Sasaki M., Tanaka Y., Shiina H., Verma M., Ebina Y. *et al*.: "Expression and methylation status of 14-3-3 sigma gene can characterize the different histological features of ovarian cancer". *Biochem. Biophy Res. Comm*., 2004, *316*, 1156.
[46] Akahira J., Sugihashi Y., Suzuki T., Ito K., Niikura H., Moriya T. *et al*.: "Decreased expression of 14-3-3 is associated with advanced disease in human epithelial ovarian cancer". *Clin. Cancer Res*., 2004, *10,* 2687.
[47] Abeysinghe H.R., Li L.Q., Guckert N.L., Reeder J., Wang N.: "THY-1 induction is associated with up-regulation of fibronectin and thrombospondin-1 in human ovarian cancer". *Cancer Gen. Cytog*., 2005, *161,* 151.
[48] Wei W., Kong B., Yang Q., Qu X.: "Hepatocyte growth factor enhances ovarian cancer cell invasion through downregulation of thrombospondin-1". *Cancer Biol. Ther*., 2010, *9,* 79.
[49] Alvarez A.A., Axelrod J.R., Whitaker R.S., Isner P.D., Bentley R.C., Dodge R.K. *et al*.: "Thrombospondin-1 expression in epithelial ovarian carcinoma: association with p53 status, tumor angiogenesis, and survival in platinum-treated patients". *Gynecol. Oncol*., 2001, *82,* 273.

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