Tumor suppressor genes associated with drug resistance in ovarian cancer (Review)

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Abstract. Ovarian cancer is a fatal gynecological cancer and a major cause of cancer-related mortality worldwide. The main limitation to a successful treatment for ovarian cancer is the development of drug resistance to combined chemotherapy. Tumor suppressor genes (TSGs) are wild-type alleles of genes which play regulatory roles in diverse cellular activities, and whose loss of function contributes to the development of cancer. It has been demonstrated that TSGs contribute to drug resistance in several types of solid tumors. However, an overview of the contribution of TSGs to drug resistance in ovarian cancer has not previously been reported. In this study, 15 TSGs responding to drug resistance in ovarian cancer were reviewed to determine the relationship of TSGs with ovarian cancer drug resistance. Furthermore, gene/protein-interaction and bio-association analysis were performed to demonstrate the associations of these TSGs and to mine the potential drug resistance-related genes in ovarian cancer. We observed that the 15 TSGs had close interactions with each other, suggesting that they may contribute to drug resistance in ovarian cancer as a group. Five pathways/processes consisting of DNA damage, apoptosis, cell cycle, DNA binding and methylation may be the key ways with which TSGs participate in the regulation of drug resistance. In addition, ubiquitin C (*UBC*) and six additional TSGs including the adenomatous polyposis coli gene (*APC*), death associated protein kinase gene (*DAPK*), pleiomorphic adenoma gene-like 1 (*PLAGL1*), retinoblastoma susceptibility gene (*RB1*), a gene encoding an apoptosis-associated speck-like protein (*PYCARD/ASC*) and tumor protein 63 (*TP63*), which had close interactions with the 15 TSGs, are potential drug resistance-related genes in ovarian cancer.

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Contents

1. Introduction

Ovarian cancer is the most aggressive cancer of the female reproductive system, and a leading cause of cancer-related mortality worldwide every year. Early-stage malignancy is frequently asymptomatic and difficult to detect, and thus diagnosis usually occurs after the disease has disseminated beyond the ovaries (1). Therefore, approximately 70% of ovarian cancer cases are diagnosed at advanced stage and only 40% of women with this type of cancer survive 5 years (2). Although cisplatin-centered chemotherapy, which is the current preferred treatment modality in human ovarian cancer, significantly reduces the mortality rates and prolongs the survival time of patients, the main obstacle to a successful treatment for ovarian cancer is the development of drug resistance to combined chemotherapy (3).

Drug resistance, both intrinsic and acquired, results from a variety of factors including individual variations in patients and somatic cell genetic differences in tumors (4). Several molecular mechanisms have been implicated in the increase of resistance in cellular models of ovarian cancer. Johnson *et al* (5) considered that three general categories consisting of decreased cell-associated drugs, altered drug inactivation, and increased DNA damage tolerance/repair would be the platinum-based resistance mechanisms in ovarian cancer. Sorrentino *et al* (3) reported that increased antiapoptotic regulator activity, growth factor receptor deregulation, defective DNA damage response, and increased DNA repair activity would respond to drug resistance in ovarian cancer. It is now widely accepted that the apoptotic capacity of cancer cells is crucial in determining the response to chemotherapeutic agents (6). At the same time, apoptosis is the cellular underpinning of cisplatin-induced cell death, which associates

with the expression of specific 'death' genes and downregulation of 'survival' counterparts (7). However, regardless of mechanisms, abnormal expression of drug resistance-related genes often plays an important role in drug resistance. Among all these drug resistance-related genes, tumor suppressor genes (TSGs) are clearly the key players.

TSGs are wild-type alleles of genes which play regulatory roles in diverse cellular activities, including cell proliferation, differentiation, migration, cell cycle checkpoint responses, protein ubiquitination and degradation, detection and repair of DNA damage, mitogenic signaling, and tumor angiogenesis (8,9), and whose loss of function contributes to the development of cancer (9). It has been proved that TSGs contribute to drug resistance in several types of solid tumors. For example, TSGs including *E1A, p53, Fhit, IL-24, Fus1* and *BiKDD* are associated with drug resistance in ovarian, lung and pancreatic cancer, and these genes are potential genes for gene therapy (10). In this study, on the basis of published reports, 15 TSGs which contributed to drug resistance in ovarian cancer were reviewed to provide an overview of the relationship of TSGs with ovarian cancer drug resistance; meanwhile, protein interactions and bio-association analysis were performed to demonstrate the associations of these TSGs and to mine the potential drug resistance-related genes which are closely related to the 15 TSGs.

2. Overall information on the 15 TSGs that contribute to drug resistance in ovarian cancer

To comprehensively collect the drug resistance-related TSGs in ovarian cancer, we first summarized a total of 39 ovarian cancer-related TSGs from the PubMed online database (http://www.ncbi.nlm.nih.gov/pubmed/), the online Dragon database for exploration of Ovarian Cancer Genes (DDOC, October, 2012) (http://apps.sanbi.ac.za/ddoc/) (11), six candidate gene lists produced by large-scale genomic platforms on ovarian cancer from the Cancer Genome Atlas (TCGA) (12), one comprehensive expert review on ovarian cancer-related genes from Nature Reviews Cancer (13) and one bioinformatics study on ovarian cancer-related genes from PLOS One (14), followed by an advanced search with 'ovarian cancer' or 'ovarian carcinoma', 'drug resistance' or 'multi-drug resistance' or 'chemoresistance' or 'resistance' and 'name of the TSGs' performed in the PubMed database to acquire the drug resistance-related TSGs in ovarian cancer.

A total of 15 TSGs including *BRCA1, BRCA2, CHEK2/Chk2, FBXO32, MLH1, SULF1, IL24/MDA-7, p16/CDKN2A, p21/CDKN1A, p53/TP53, TP73, PDCD4, PTEN, RASSF1* and *WWOX* which contributed to drug resistance in ovarian cancer were summarized. As shown in Table I, the modifications of the TSGs, the responding drugs, and the ways for TSGs to regulate the drug resistance in ovarian cancer were integrated. We observed that both genetic and epigenetic changes of the TSGs were contributed to drug resistance in ovarian cancer, but the latter apparently played dominant roles. As it is challenging to treat ovarian cancer through a genetic strategy, due, in part, to its heterogeneity, the reversibility of epigenetic mechanisms involved in ovarian cancer opens potential new avenues for treatment (15). The epigenomics of ovarian cancer has become a rapidly expanding field leading to intense investigation, and the reversion of epigenetic changes of TSGs has already proved to be effective in reversing the drug resistance clinically in ovarian cancer. It has been reported that low-dose decitabine alters DNA methylation restoring sensitivity to carboplatin in patients with heavily pre-treated ovarian cancer, resulting in a high response rate and prolonged progression free survival, and demethylation of the *MLH1* and *RASSF1a* in tumors from day 1 to 8 is positively correlated with progression free survival ($P<0.05$) (16). These results were promising and encouraged further study on epigenetic changes of TSGs associated with drug resistance in ovarian cancer. In addition, with the exception of *FBXO32* and *WWOX*, the other 13 TSGs participated in the regulation of drug resistance in ovarian cancer through certain pathways, in particular, through apoptosis and DNA damage-related pathways. However, regardless of pathways, the 15 TSGs responded to drug resistance in ovarian cancer through 'growth' (including cell proliferation, cell growth and cell survival) and 'death' (including cell death and cell apoptosis) (Table I).

3. Gene/protein interaction network of the 15 TSGs

Gene/protein function predictions based on bioinformatics analysis is a potential, feasible and valuable way for gene/protein function mining, and numerous large-scale networks of molecular interactions within the cell have made it possible to go beyond one dimensional approaches to study gene/protein function in the context of a network (46). GeneMANIA is a web-based database and tool for prediction of gene/protein function on the basis of multiple networks derived from different genomic or proteomic data/sources, and it is fast enough to predict gene/protein function with significant accuracy (47). Protein-protein interactions of the 15 TSGs were analyzed using the GeneMANIA online tool. Except for *IL24* and *SULF1*, all 15 TSGs had direct interactions (co-expression, co-localization, genetic interactions, shared pathway, physical interactions and shared protein domains) or indirect interactions (through direct interactions with an intermediate gene) with each other (Fig. 1). For example, *TP53* had direct interactions with *MLH1, BRCA1, BRCA2, CHEK2, CDKN1A, CDKN2A, PTEN, TP73* and *WWOX*, and it had indirect interactions with *FBXO32* and *RASSF1*; *BRCA1* had direct interactions with *BRCA2, MLH1, CHEK2, PTEN, WWOX* and *p53*; *RASSF1* had indirect interactions with *BRCA1, CDKN1A, CDKN2A, CHEK2, PTEN, TP53, TP73* and *WWOX*; *PDCD4* had indirect interactions with *BRCA1, BRCA2, CDKN1A, CDKN2A, FBXO32, IL24, MLH1, PTEN, TP53, TP73* and *WWOX*. These results suggested that the 15 TSGs may contribute to drug resistance as a whole.

There were an additional 6 TSGs, adenomatous polyposis coli gene (*APC*), death associated protein kinase gene (*DAPK*)1, pleiomorphic adenoma gene-like 1 (*PLAGL1*), retinoblastoma susceptibility gene (*RB1*), a gene encoding an apoptosis-associated speck-like protein (*PYCARD)*, and tumor protein 63 (*TP63*), which had close interactions with the 15 TSGs in the network (Table II), suggesting that these 6 TSGs may be involved in drug resistance and would be potential drug resistance-related TSGs in ovarian cancer. With the exception of *PLAGL1*, the other 5 TSGs have been confirmed to associate with drug resistance in several

Table I. General overview of the 15 TSGs that contribute to drug resistance in ovarian cancer.

TSG abbreviation	Full name of the TSGs	Modifications of the TSGs	Drugs	Regulation manner of drug resistance	Pathways associated with drug resistance
BRCA1	Breast cancer susceptibility gene 1	Mutation, DNA methylation (17)	Taxol	DNA repairing (18)	DNA damage response (19)
BRCA2	Breast cancer susceptibility gene 2	Mutation, DNA methylation	Platinum	Cell survival (17)	DNA damage response (19)
CHEK2/Chk2	Checkpoint kinase 2 gene	Mutation (20)	Cisplatin	Inducible degradation of CHEK2 protein (21)	Ubiquitin-proteasome pathway (21)
FBXO32	F-box only protein 32 gene	DNA methylation	Platinum	Cell proliferation, cell apoptosis (22)	-
MLH1	DNA mismatch repair gene 1	DNA methylation	Cisplatin	Mismatch repairing (23,24)	DNA mismatch repair (24)
SULF1	Sulfatase 1 gene	DNA methylation, Histone acethylation (25)	Platinum	Cell proliferation, cell apoptosis	HSPG-related signal transduction pathways (26)
IL24/MDA-7	Interleukin 24 gene	Ubiquitinated (27)	Taxol	Cell growth, cell apoptosis (28)	Ubiquitin-proteasome system (27)
p16/CDKN2A	Cyclin-dependent kinase inhibitor 2A gene	Gene mutation	Platinum, Taxol	Cell growth, cell death	Cell cycle (29)
p21/CDKN1A	Cyclin-dependent kinase inhibitor 1A gene	Histone modification (30)	Cisplatin	Cell apoptosis, DNA mismatch repair (31,32)	DNA mismatch repair and apoptosis pathways (32)
PDCD4	Programmed cell death 4 gene	miRNA (33)	Platinum	Tumor growth, apoptosis (34)	Death receptor pathway (34)
PTEN	Phosphatase and tensin homolog gene	miRNA (35)	Cisplatin	Cell proliferation, cell apoptosis (36-38)	PI3K/AKT signaling pathway, p53 signaling pathway (36-38)
RASSF1A	Ras association domain family 1A gene	DNA methylation	Taxol	Cell apoptosis, cell survival, stabilization of microtubules (39)	Apoptosis (39)
TP53	Tumor protein 53 gene	Mutation, DNA methylation (40)	Platinum	Cell cycle, cell apoptosis (41)	Cell cycle, apoptosis, p53 signaling pathway (41)
TP73	Tumor protein 73 gene	DNA methylation (42)	Cisplatin	Apoptosis (43)	Apoptosis (43)
WWOX	WW domain-containing oxidoreductase gene	DNA methylation (44)	Cisplatin	Cell proliferation, cell apoptosis (45)	-

TSG, tumor suppressor gene.

Table II. The interactions of the additional 6 TSGs with the 15 TSGs in the gene/protein interaction network.

The additional 6 TSGs in the network	Member of the 15 TSGs (Direct interactions)	Member of the 15 TSGs (Indirect interactions through an intermediate gene)
APC	*CDKN1A*, *PTEN*, *TP53*, *TP73* and *WWOX*	*CDKN2A*, *CHEK2*, *PDCD4* and *RASSF1*
DAPK1	*FBXO32*, *PDCD4*, *PTEN*, *TP53* and *TP73*	*BRCA1*, *BRCA2*, *CDKN1A*, *IL24*, *MLH1*, *PDCD4*, *PTEN*, *SULF1*, *TP53* and *TP73*
PLAGL1	*BRCA1* and *TP53*	*CDKN1A*, *CDKN2A*, *CHEK2*, *FBXO32*, *PDCD4*, *PTEN* and *RASSF1*
RB1	*BRCA1*, *CDKN1A*, *CDKN2A*, *CHEK2*, *TP53*, *TP73* and *WWOX*	*RASSF1* and *PDCD4*
PYCARD	*TP53*	*IL24* and *WWOX*
TP63	*CHEK2*, *TP53* and *TP73*	*BRCA1*, *BRCA2*, *CDKN1A*, *MLH1*, *PDCD4*, *PTEN*, *TP53* and *TP73*

TSG, tumor suppressor gene.

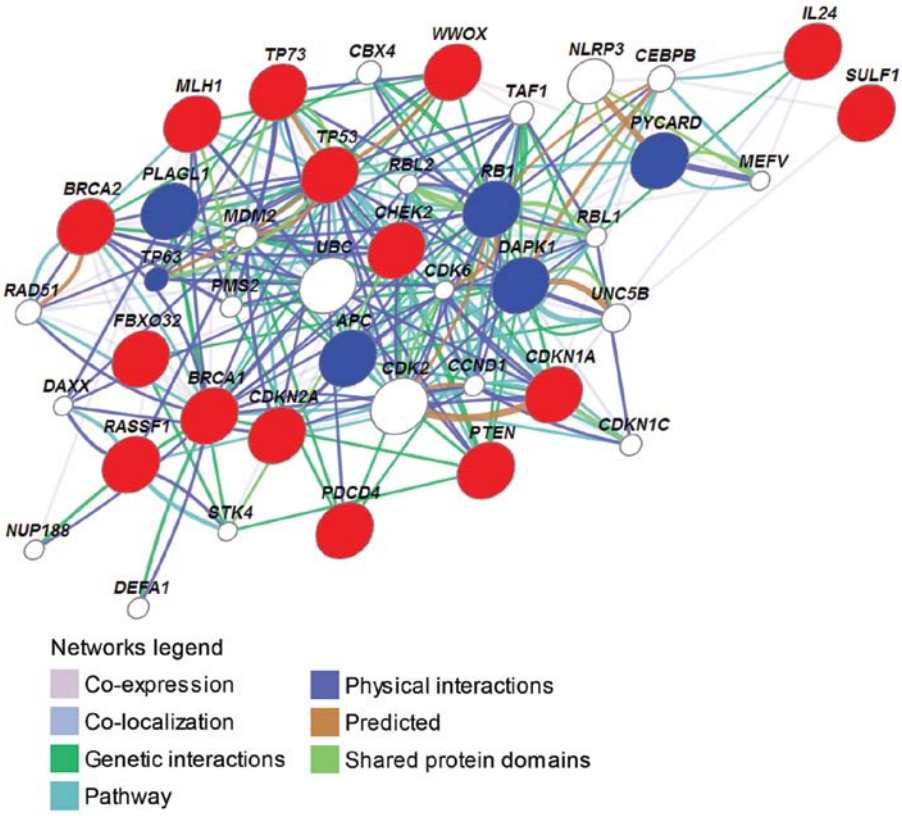

Figure 1. Gene/protein interaction network of the 15 tumor suppressor genes (TSGs) based on GeneMANIA online tool. Genes/proteins are depicted as colored circles and experimentally detected relationships between genes/proteins as connecting lines. Red circles are the 15 TSGs, blue circles are the additional 6 TSGs, and white circles are the other genes/proteins that had interactions with the 15 TSGs.

types of solid cancer. The expression status of the *APC* determines the relative sensitivity of colon cancer cells to histone deacetylase inhibitor-induced apoptosis which may relate to drug resistance (48); *DAPK* is hypermethylated in drug-resistant non-small cell lung cancer cell lines and head and neck squamous cell carcinoma cell lines. Restoration of *DAPK* into the resistant non-small cell lung cancer cells by stable transfection can re-sensitize the cells to both erlotinib and cetuximab. Conversely, siRNA-mediated knockdown of *DAPK* induces resistance in the parental sensitive cells. These results demonstrate that *DAPK* plays important roles in both cetuximab and erlotinib resistance (49); point mutations of the *RB1* encode nuclear proteins with impaired ability to induce apoptosis compared to wild-type pRb *in vitro*. Notably, three out of four tumors harboring *RB1* mutations display primary resistance to treatment with either 5-FU/mitomycin or doxoru-

Table III. Annotated functions of the 15 TSGs according to the protein interaction network.

Annotated function	False discovery rate	No. of the 15 TSGs	Other TSGs	Other genes
Cell cycle related	1.19e-13~9.82e-8	8 (*BRCA1, BRCA2, CDKN1A, CDKN2A, PTEN, RASSF1, TP53* and *TP73*)	*APC, RB1* and *TP63*	*MDM2, UBC, CDK2, CDK6, TAF1, CCND1* and *CDKNIC*
DNA binding related	8.91e-6~1.13e-2	8 (*BRCA1, BRCA2, CDKN2A, MLH1, PDCD4, PTEN, TP53* and *TP73*)	*APC, TP63, RB1* and *PYCARD*	*RAD51, PMS2, UBC, TAF1* and *NLRP3*
DNA damage related	6.05e-11~1.84e-3	6 (*BRCA1, CDKN1A, CDKN2A, TP53, TP73* and *CHEK2*)	*TP63*	*MDM2, UBC, CDK2* and *CCND1*
Apoptosis	2.45e-6~2.45e-2	6 (*BRCA1, CDKN1A, CDKN2A, CHEK2, TP53* and *TP73*)	*TP63*	
Signal transduction by TP53	8.71e-10~3.67e-3	5 (*BRCA1, CDKN1A, CDKN2A, TP53* and *TP73*)	*TP63*	*MDM2, CDK2* and *UBC*
Aging	2.5e-7~2.99e-2	5 (*CDKN1A, CDKN2A, CHEK2, PDCD4* and *TP53*)		*CDK6*
DNA repair	1.43e-6~4.35e-3	5 (*BRCA1, BRCA2, CHEK2, MLH1* and *TP73*)		*RAD51, PMS2* and *UBC*
Cell growth	6.34e-3~9.1e-2	3 (*CDKN1A, CDKN2A* and *TP53*)	*RB1*	

TSG, tumor suppressor gene.

bicin (50); upregulated expression of *PYCARD/ASC* leads to enhanced sensitivity to cisplatin, gemcitabine and docetaxel in bladder cancer cells and pancreatic cancer cells (51,52); *TP63*, with high homology with *TP53*, is critical for the development of stratified epithelial tissues such as epidermis, breast, and prostate (53). Expression analysis in long-term survivors reveals a significant upregulation of *TP63, PTEN, GADD45a* and *MAPK1* in metastatic gastric cancer, suggesting that these genes may be involved in drug metabolism and resistance (54).

In addition, ubiquitin C (*UBC*) had direct physical interactions with 7 of the 15 TSGs, *BRCA1, BRCA2, CDKN1A, MLH1, PTEN, TP53 and TP73*, and it had direct physical interactions with other genes including *MDM2, DAPK1, TP63, PMS2* and *DAXX*; meanwhile, *MDM2* (murine double minute 2 gene) had direct interactions with 7 of the 15 TSGs, i.e., *CDKN2A, CHEK2, PDCD4, RASSF1, TP53, TP73* and *WWOX*, and it had direct physical interactions with other genes including *APC, CDK2, DAXX, RB1, TAF1, TP63* and *UBC*. Among these other genes which had interactions with *UBC* and *MDM2, APC, RB1* and *TP63* were TSGs associated with drug resistance in cancer, and death domain-associated protein (DAXX) can lead to apoptosis in cancer (55). These results indicated that *UBC* and *MDM2* may play roles in drug resistance in ovarian cancer. It has previously been reported that *MDM2* negatively regulates tumor suppressor p53 via binding to the transactivation and the DNA-binding domains of p53 (56), and contributes to drug resistance in ovarian cancer through regulation of p53 (57). *UBC* is low in normal kidneys but is increased in malignant tumors *in vivo* and in several established renal carcinoma cell lines, indicating that high expressions of the *UBC* are important in the cancerous state of these cells (58). Similarly, *UBC* is highly expressed in human nasopharyngeal carcinoma drug-resistant cell lines (59). However, the studies on *UBC* associated with drug resistance are limited.

Among the annotated functions in accordance with the GeneMANIA network, eight were closely related to drug resistance (Table III). The cell cycle-related function which covered 8 of the 15 TSGs and 3 of the additional 6 TSGs was annotated with the highest false discovery rate (FDR), followed by the DNA binding-related function which also covered 8 of the 15 TSGs and 4 of the additional 6 TSGs, suggesting that these 3 pathways (functions) may be the major ways with which TSGs participate in the regulation of drug resistance in ovarian cancer. Furthermore, DNA damage and apoptosis, which both covered 6 of the 15 TSGs and *TP63*, may also be crucial ways for the contribution of the TSGs to the regulation of drug resistance in ovarian cancer.

Table IV. Analysis of process associations of the 15 TSGs using PubGene bio-associations.

Annotated process	No. of the 15 TSGs	Article (P-value)
Methylation	12 (*BRCA1*, *BRCA2*, *CDKN1A*, *CDKN2A*, *CHEK2*, *MLH1*, *PDCD4*, *PTEN*, *RASSF1*, *TP53*, *TP73* and *WWOX*)	0
Mismatch repair	11 (*BRCA1*, *BRCA2*, *CDKN1A*, *CDKN2A*, *CHEK2*, *MLH1*, *PTEN*, *RASSF1*, *TP53*, *TP73* and *WWOX*)	0
Cell cycle checkpoint	11 (*BRCA1*, *BRCA2*, *CDKN1A*, *CDKN2A*, *CHEK2*, *MLH1*, *PTEN*, *RASSF1*, *TP53*, *TP73* and *WWOX*)	0
DNA replication checkpoint	3 (*CDKN1A*, *CHEK2* and *TP53*)	0
Response to DNA damage stimulus	10 (*BRCA1*, *BRCA2*, *CDKN1A*, *CDKN2A*, *CHEK2*, *MLH1*, *PTEN*, *RASSF1*, *TP53* and *TP73*)	0
DNA damage checkpoint	9 (*BRCA1*, *BRCA2*, *CDKN1A*, *CDKN2A*, *CHEK2*, *MLH1*, *TP53*, *TP73* and *WWOX*)	0
Cell cycle arrest	14 (*BRCA1*, *BRCA2*, *CDKN1A*, *CDKN2A*, *CHEK2*, *FBXO32*, *IL24*, *MLH1*, *PDCD4*, *PTEN*, *RASSF1*, *TP53*, *TP73* and *WWOX*)	0
DNA methylation	13 (*BRCA1*, *BRCA2*, *CDKN1A*, *CDKN2A*, *CHEK2*, *MLH1*, *PDCD4*, *PTEN*, *RASSF1*, *SULF1*, *TP53*, *TP73* and *WWOX*)	4.64e-191
G2/M transition checkpoint	7 (*BRCA1*, *BRCA2*, *CDKN1A*, *CDKN2A*, *CHEK2*, *MLH1* and *TP53*)	3.22e-164
Apoptosis	12 (*BRCA1*, *BRCA2*, *CDKN1A*, *CDKN2A*, *CHEK2*, *IL24*, *MLH1*, *PDCD4*, *PTEN*, *RASSF1*, *TP53* and *TP73*)	2.1e-119

TSG, tumor suppressor gene.

4. Process associations of the 15 TSGs

The process associations of the 15 TSGs were analyzed by PubGene Bio-associations (www.pubgene.org). Pubgene is a gene/protein database and web-based tool for literature mining. It carries out automated extraction of experimental and theoretical biomedical knowledge from publicly available gene and text databases to create a gene-to-gene co-citation network for 13,712 named human genes by automated analysis of titles and abstracts in over 10 million MEDLINE records (60). Therefore, gene and protein names are cross-referenced to each other and to terms that are relevant to understanding their biological function and importance in disease. Using PubGene process associations, we annotated the 15 TSGs to biological processes by probabilistic scoring, and the 10 most annotated processes of the 15 TSGs are shown in Table IV. It appears that most of the 15 TSGs played roles in processes of methylation/DNA methylation, DNA damage and repair, cell cycle and apoptosis ($P<0.001$), suggesting that these biological processes may be the most common manner with which the 15 TSGs contribute to drug resistance in ovarian cancer.

5. Conclusion

TSGs play crucial roles in several aspects of cancer development including cell cycle control, signal transduction, angiogenesis, development and drug resistance, indicating that they contribute to a broad array of normal and tumor-related functions. It is proposed that TSGs provide a vast untapped resource for anticancer therapy (8). It has been reported that a total of 33 TSGs contribute to ovarian cancer development through DNA damage, DNA repair, regulating macromolecule metabolism, cell cycle, and apoptosis (14). However, an overview of the TSGs associated with drug resistance in ovarian cancer had yet to be reported. In this study, the 15 TSGs which are involved in drug resistance were comprehensively

reviewed. Furthermore, gene/protein interaction and bio-association analysis were performed. The 15 TSGs may contribute to drug resistance as a whole in ovarian cancer, since they have close interactions with each other in accordance with the gene/protein interaction network. An additional 6 TSGs including *APC, DAPK1, PLAGL1, RB1, PYCARD* and *TP63*, which had close interactions with the 15 TSGs (Table II) and which were associated with drug resistance in other types of solid cancer, may be potential drug resistance-related genes in ovarian cancer. *UBC*, which had direct physical interactions with a number of the 15 TSGs, may be associated with drug resistance in ovarian cancer, although its drug resistance-related functions have yet to be reported.

DNA damage and apoptosis play important roles in drug resistance in several solid tumors. DNA damage results in genetic alterations that underlie drug resistance, disabled repair and resistance to apoptosis (61). Apoptosis plays an important role in the maintenance of physiological homeostasis in response to stimuli, and failure of apoptosis would result in unopposed tissue growth and, eventually, fatal disease such as cancer (62). It has been proved that apoptosis is involved in drug resistance in several solid tumors including ovarian cancer. In this study, on the basis of gene/protein-interaction and process-association analysis, DNA damage and apoptosis were the main annotated functions/processes for the 15 TSGs, suggesting that these two biological processes may be the main manner for the participation of TSGs in drug resistance in ovarian cancer. These results are partly supported by previous studies that reported that *BRCA1, BRCA2, MLH1* and *p21* contributed to ovarian cancer drug resistance via DNA damage and repair, and *p21, RASSF1, TP53* and *TP73* contributed to ovarian cancer drug resistance via apoptosis (Table I). Furthermore, cell cycle was also a leading annotated function/process for the 15 TSGs on the basis of gene/protein-interaction and process-association analysis. It has been proved that cell cycle can create drug resistance and therefore reduce combination chemotherapeutic efficacy (63). In addition, DNA binding-related functions, which covered a total of 12 TSGs (Table III), may be another crucial process for TSGs responding to drug resistance in ovarian cancer. Methylation/DNA methylation was also important for the 15 TSGs based on process association (Table IV); this finding was supported by previous studies revealing that 9 of the 15 TSGs were regulated by DNA methylation when they participated in the regulation of drug resistance in ovarian cancer (Table I). Collectively, five pathways/processes comprising DNA damage, apoptosis, cell cycle, DNA binding, and methylation, may be the major means by which TSGs respond to drug resistance in ovarian cancer.

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