Downregulation of NEK11 is associated with drug resistance in ovarian cancer

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Abstract. NEKs [NIMA (never in mitosis gene A)-related expressed kinase] are involved in ovarian cancer development and progression, while their association with drug resistance is limited, especially NEK11, and its relationship with drug resistance has never been reported. In this study, on the basis of comprehensive bioinformatic analyses, including mRNA expression according to microarray data, protein/gene interaction, protein-small molecule interaction, annotation of biological process and microRNA-mRNA interaction analysis, we revealed that the NEK11 mRNA was significantly downregulated in 586 cases of ovarian serous cystadenocarcinomas and cisplatin-resistant A2780 ovarian cancer cells, and interacted with 22 proteins and 4 small molecules which all were contributed to drug resistance in ovarian cancer. Furthermore, seven cell cycle-related biological processes were annotated with NEK11, drug resistance and ovarian cancer, suggesting that NEK11 potentially was involved in the drug resistance in ovarian cancer via its regulatory roles in the cell cycle. In addition, among the eight microRNAs predicted to be most strongly targeting NEK11, the majority were involved in drug resistance in ovarian and other cancers. All those results provide a very strong possibility that the notable downregulation of NEK11 in cisplatin-resistant ovarian cancer cells was involved in drug resistance, via its interactions with drug resistance-related genes, proteins, small molecules, microRNAs and biological processes, particularly the cell cycle-related processes. To our knowledge, this is the first report of the association of NEK11 with drug resistance in cancer, and it would pave the way for further investigation of the drug resistance-related functions of this gene.

Introduction

Ovarian cancer is the most lethal cancer of the female reproductive system, with high rate of mortality worldwide. Likewise, ovarian cancer is one of the five leading types of cancer death in women (1,2). In the last 80 years (1930-2010), the overall death rate of ovarian cancer patients declined very slightly (2). The main obstacle to a successful treatment for ovarian cancer is the development of drug resistance to combined chemotherapy (3). Drug resistance results from a variety of factors including decreased cell-associated drugs, altered drug inactivation, increased DNA damage tolerance/repair, increased anti-apoptotic regulator activity and growth factor receptor deregulation (4,5). Besides, abnormal expressions of genes mediated by microRNA regulation also play critical roles in development of drug resistance in ovarian cancer (6,7). However, regardless of mechanisms, abnormal expression of drug resistance-related genes often play important roles in drug resistance (8). Thus, mining and exploring of potentially drug resistance-related genes would be a feasible and reasonable way to meet the challenge of the drug resistance in ovarian cancer (9).

NEK [NIMA (never in mitosis gene A)-related expressed kinase] family is the never-in-mitosis A (NIMA) protein of *Aspergillus nidulans*, which was first identified by Oakley and Morris in a genetic screen for cell division cycle mutants (10). NEKs have now been identified in a wide range of organisms, and eleven genetically distinct proteins named NEK1 to NEK11 are expressed in humans. The functions of NEKs mainly are involved in the regulation of cell cycle progression, in particular, NEK2, NEK6, NEK7 and NEK9 contribute to the establishment of the microtubule-based mitotic spindle, whereas NEK1, NEK10 and NEK11 have been implicated in the DNA damage response (11). NEKs have been proven to be associated with cancer development and progression, in a cell cycle-dependent manner, and thus the NEKs are considered as potential therapeutic targets in cancer (11-13).

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The association of NEKs with drug resistance in cancer is limited, with only a few studies. For example, NEK2 down-regulation may improve the sensitivity of breast cancer cells during chemotherapy treatments (14), and its upregulation may induce drug resistance mainly through activation of efflux drug pumps in myeloma and other cancers (15). NEK4 is downregu-lated at 8 and/or 24 h in colon cancer cell lines in response to 5-fluorouracil (16), and its suppression sensitize cancer cells to taxol and vincristine, via regulation on mitosis and microtubule homeostasis (17). In ovarian cancer, we revealed that the upregulation of NEK2 is associated with drug resis-tance in ovarian cancer, via its direct or indirect interaction with a number of genes, proteins, microRNAs and biological processes (18), and further analysis indicated that the NEK2 expression is regulated by NR2F2 (19). Additionally, NEK1 expression can be induced by paclitaxel (20), NEK4 is identi-fied as a candidate gene as potentiators of cisplatin (21), NEK6 can be affected by paclitaxel through preventing phosphoryla-tion of Thr389 (22), and NEK8 is significantly downregulated in DDP-resistant ovarian cell line IGROV-1 (23). These results indicated that the NEK1, 4, 6 and NEK8 were chemo-treatment related proteins/genes.

However, among all the NEKs, the association of NEK11 with cancer is rare, with one study considering it as a potential tumor suppressor gene (24), and it is significantly downregul-ated in ovarian cancer, mediated by methylation or mutation (25,26), but its association with drug resistance in cancer has not been reported. In this study, on the basis of comprehensive bioinformatics analyses, we aimed to illustrate the association of NEK11 with drug resistance in ovarian cancer.

Materials and methods

The microarray data of NEK11 in ovarian cancer tissues was retrieved from the TCGA Ovarian array data deposited in the Oncomine online database (https://www.oncomine.org/resource/main.html) (27). The microarray data of NEK11 in ovarian cancer cells was retrieved from the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geoprofiles/) (28,29). There were three probe sets targeting NEK11, while only two probe sets with significant variability in statistics were kept. Unpaired, two-tailed t-test assuming homogeneity of the variances was performed with Excel software.

The protein/gene interaction analysis was performed using GeneMANIA online tool (http://www.genemania.org/) (30-32). Protein-small molecule/chemical interaction analysis was performed using STITCH 4.0 beta (http://stitch-beta.embl.de/) (33-35) and DrugBank online database (36,37). Annotation of biological process was performed by Coremine Medical online database (http://www.coremine.com/medical/) (38). The microRNAs targeted to the gene were predicted by miRWalk online tool which including 10 prediction tools (DIANAmT, miRanda, miRDB, miRWalk, RNAhybrid, PICTAR4, PICTAR5, PITA, RNA22, Targetscan) (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/) (39).

Results

NEK11 is significantly downregulated in ovarian cancer tissues and cisplatin-resistant cells. The mRNA expres-sion of NEK11 in ovarian cancer tissues and cells was retrieved from TCGA ovarian array and DataSet Record GDS3754 which have been deposited in Oncomine and GEO online database, respectively. As shown in Fig. 1A and C, compared with the expression in 8 cases of normal ovaries, the NEK11 mRNA was significantly downregu-lated in 586 cases of ovarian serous cystadenocarcinomas, with a 3.05-fold change (P=3.45E-5). Similarly, the expres-sion of the NEK11 in cisplatin-resistant ovarian cancer cells was measured (Fig. 1B and D). As shown in the figure, the mRNA expression of NEK11 was decreased in cisplatin-resistant ovarian cancer cells compared with expression in its sensitive counterpart, with at least a 1.8-fold change (P<0.001). These results suggested that the downregulation of NEK11 might potentially play crucial roles in develop-ment of ovarian cancer and drug resistance.

Prediction and analysis of function based on protein/gene interactions. Protein/gene-protein/gene interactions performed with GeneMANIA was used to reveal the drug resistance-related functions of NEK11. As shown in Fig. 2, NEK11 had direct interactions with NEK2, MET, JUN, ERBB2, EGFR, AKT1, AKT2, CHEK2, WWOX, IKBKE, ELF3 and MAST2, among those, 10 proteins/genes have been proven to be involved in the regulation of drug resis-tance in ovarian cancer. NEK2, MET, JUN, ERBB2, EGFR, AKT1, AKT2 and IKBKE are oncogenes associated with drug resistance in ovarian cancer (18,41-50). For example, IKBKE is found to be upregulated in ovarian, breast and prostate cancer, and its upregulation biologically and clini-cally relevant to the cancer development and progression, as well as the chemoresistance (50). Downregulation of the AKT2 sensitizes the ovarian cancer cells to paclitaxel-induced apoptosis, and inhibits the survivin expression which can induce drug-resistance to paclitaxel (49). NEK2 shared protein domains and had very strong physical inter-action with NEK11, and has been proven to be responsible for the development of drug resistance not only in ovarian cancer (18), but also in breast (14) and colorectal cancer (51). CHEK2 and WWOX are the tumor suppressor genes which directly interacted with NEK11. CHEK2 is one of the critical kinases governing cell apoptosis, cell cycle checkpoint and DNA damage repair. In ovarian cancer cells, CHEK2 is degraded at the protein level in response to cisplatin through the ubiquitin-proteasome pathway, suggesting that degrada-tion or decreased expression of CHEK2 is partially responsible for chemoresistance (52). WWOX suppression by RNA interference reverses platinum resistance in DDP-resistant SKOV3 ovarian cancer cells (53). In addition to the direct interactions, NEK11 indirectly interacted with 15 proteins/genes in the network. Of which, 10 including BRCA1, BRCA2, PTEN, CDKN2A, MLH1, KRAS, MYC, HGF, PMF2 and MLH3 are associated with drug resistance in ovarian cancer (54-63).

Taken together, based on the protein/gene interaction network, total of 28 proteins/genes were identified to be directly/indirectly interacted with NEK11, and of which, 22 were contributed to drug resistance in ovarian cancer. These results strongly supported the idea that NEK11 might asso-ciate with drug resistance in ovarian cancer.
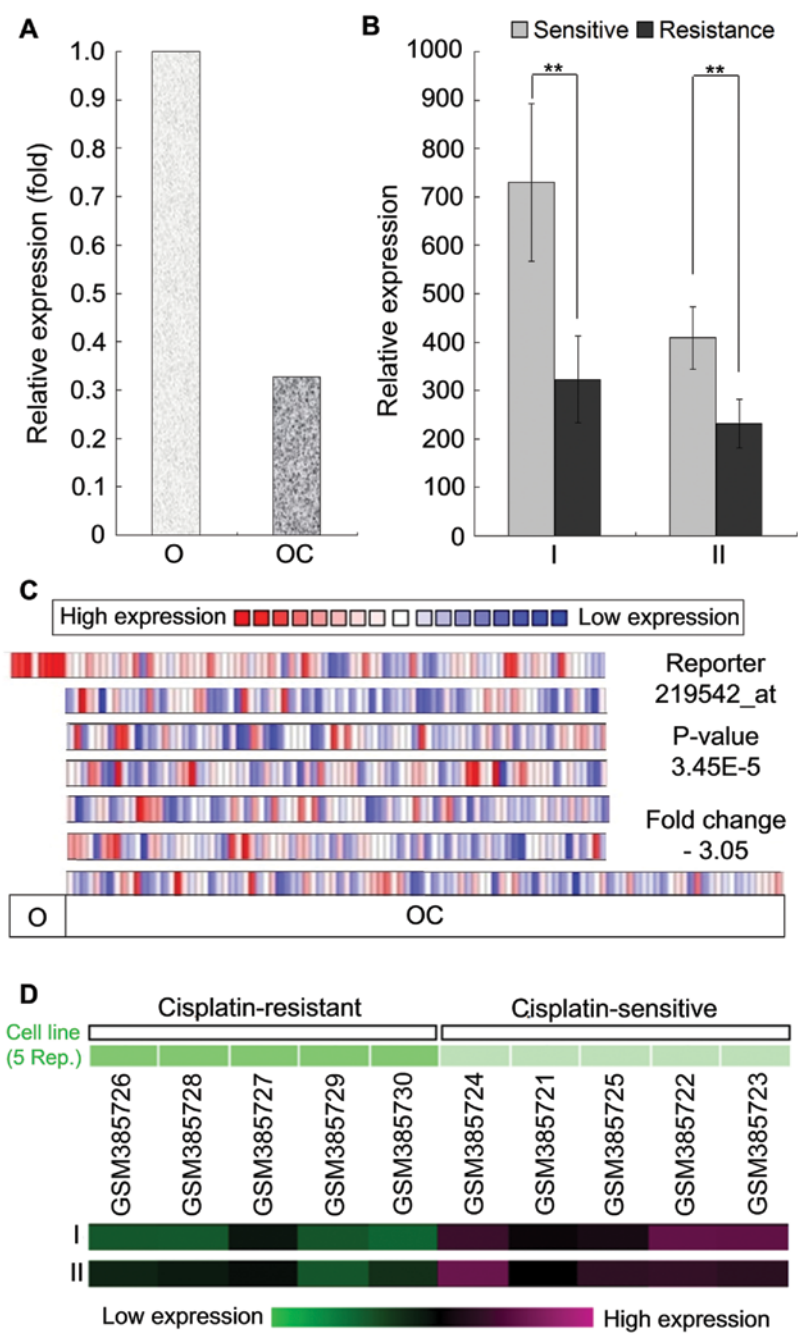
Figure 1. The relative expression of NEK11 in ovarian cancer tissues and cells. (A and C) mRNA expression of NEK11 in 586 cases of ovarian serous cystad-enocarcinomas compared with the expression in 8 cases of ovaries used as normal controls (P=3.45E-5), on the basis of TCGA Ovarian array data retrieved from Oncomine online database. The NEK11 expression data in the Oncomine are presented as fold changes, in the present study, the expression of NEK11 in normal ovaries was normalized to 1.0. O, 8 ovaries; OC, 586 ovarian serous cystadenocarcinomas. (B and D) mRNA expression of NEK11 in cisplatin-sensitive and -resistant A2780 ovarian cancer cells (with 5 biological replications each), based on the array data retrieved from the GEO Profiles database (GDS3754). The data have been processed and normalized (40). I, GDS3754/219542_at/NEK11; II, GDS3754/1555082_a_at/NEK11. **Significantly different in statistics.

Prediction and analysis of function based on protein-small mole-cule/chemical interaction. The protein-small molecule/chemical interaction was performed to further explain the drug resis-tance-related functions of NEK11 in ovarian cancer. As shown in Table I, five chemicals including CC 243, staurosporine, dasatinib, flavin mononucleotide and dabrafenib were found to interact with NEK11. Except for the CC 243, all the chemi-cals are associated with drug resistance in ovarian and other cancers. For example, treatment of ovarian cancer cells with staurosporine induces apoptosis in a time-dependent manner

(64), and reduces P-glycoprotein expression and modulates multidrug resistance (65). Dasatinib is an antitumor agent for many solid tumors (66), and can significantly enhance the sensitivity to carboplatin in ovarian cancer cells (67). As for the flavin mononucleotide, a previous study showed that the overexpression of riboflavin kinase increases the levels of flavin mononucleotide and render cell resistance not only to cisplatin but also to hydrogen peroxide and diamide (68), suggesting a role of flavin mononucleotide in regulation of cisplatin resistance. Dabrafenib is an inhibitor of BRAF
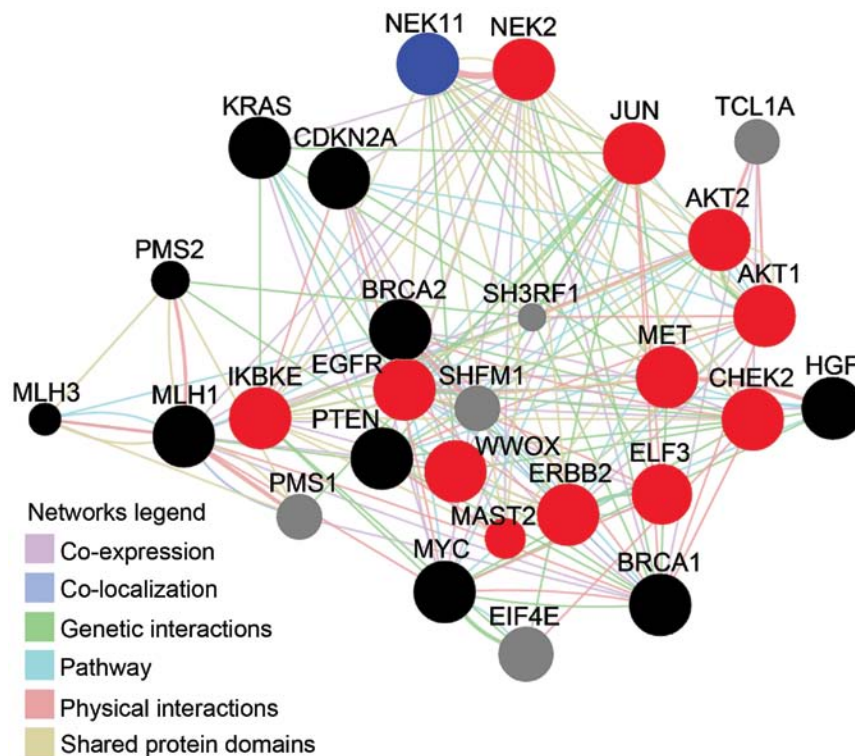
Figure 2. Protein/gene interaction analysis of NEK11 using GeneMANIA online tool. The query in blue is the target NEK11; the queries in red are the proteins/genes which directly interacted with NEK11; the queries in black are the indirectly interacted proteins/genes which associated with drug resistance in ovarian cancer. The type of interactions between genes/proteins is illustrated as the network legend indicates.
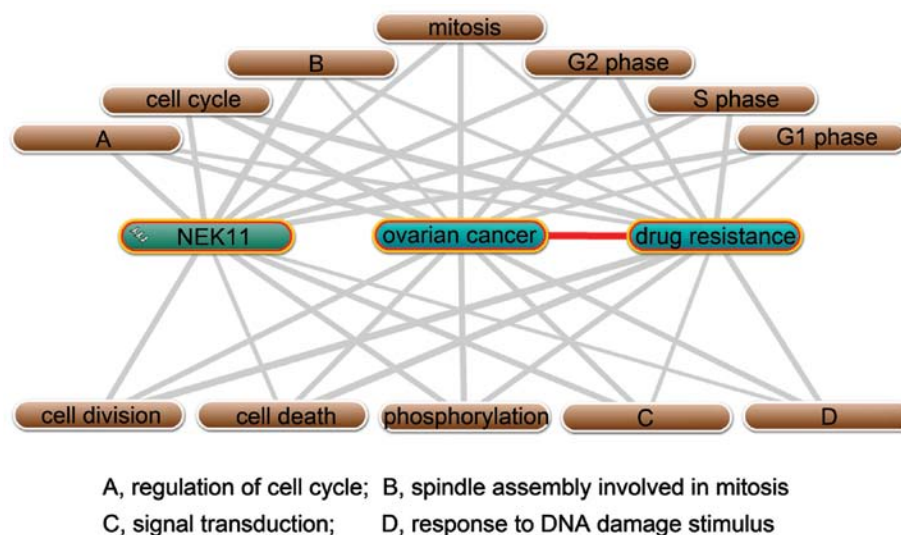

A, regulation of cell cycle; B, spindle assembly involved in mitosis
C, signal transduction; D, response to DNA damage stimulus

Figure 3. Biological process annotation of NEK11, ovarian cancer and drug resistance (P<0.01). The input terms were NEK11, ovarian cancer and drug resistance. The line in red indicates the links between input terms; the line in gray indicates the links between the input terms and their annotated biological processes.

leading to constitutive activation of the MAPK signaling pathway (69,70), while the BRAF and MAPK signaling have been proven to play important roles in drug resistance in ovarian cancer (43,71-73).

Prediction and analysis of function based on the annotation of biological processes. The Gene Ontology (GO) (74) provides a valuable source of structured knowledge of protein function

in terms of molecular function, biological process, and cellular component. Among other things, each gene may be involved in one or more biological processes (75), and the involvement of a gene in a given biological process can be used to predict the biological role and function of the gene (76). Coremine Medical online database is designed to be used by anyone seeking information on health, medicine, and biology (38), and it analyzed the associations between the genes, biological

Table I. The small molecules or chemicals interacting with NEK11 and their drug resistance-related functions in cancer.

Small molecule/ chemical	Drug resistance-related associations of the chemicals in cancer (refs.)
NEK11	
CC 243	-
Staurosporine	Associated with multidrug resistance (65)
Dasatinib	Involved in drug resistance (66,67)
Flavin mononucleotide	Involved in drug resistance (68)
Dabrafenib	Involved in drug resistance (43,69-73)

The protein-small molecule/chemical interaction was analyzed using STITCH 4 beta online tool, except that the interaction of Dabrafenib with NEK11 was analyzed by DrugBank online database.

processes and drug resistance as well as ovarian cancer. As shown in Fig. 3, the annotation of biological process indicated there is no direct linkage between NEK11 and ovarian cancer and drug resistance, indicating the limited study among them. Total of 12 biological processes significantly associated with NEK11, ovarian cancer and drug resistance were annotated (P<0.01) (Fig. 3). Given the intimate relationships of NEK11 with the 12 biological processes, and the intimate relationships of those processes with ovarian cancer and drug resistance, we concluded that NEK11 probably is involved in the regulation of drug resistance in ovarian cancer via its regulatory roles in those biological processes. Among the 12 biological processes, 7 of them including cell cycle, regulation of cell cycle, spindle assembly involved in mitosis, G2 phase, S phase and G1 phase are cell cycle-related processes, indicating that the NEK11 might perform drug resistance-related functions in ovarian cancer mainly via its involvement in the cell cycle.

Functional prediction based on the functionality of the microRNAs that target the gene. MicroRNA-mediated post-transcriptional gene regulation is considered as a significant regulator of many cellular processes, both physiological and pathological (77,78). The microRNAs perform their functions through the regulation on their target genes, and it has been well established that microRNAs represent a class of genes with a great potential for use in diagnostics, prognosis and therapy (79). Therefore, the function of a gene can be predicted based on functionality of the microRNAs that target it (9). A microRNA-mRNA interaction analysis was performed with miRWalk to predict the microRNAs that target NEK11. Using this tool, we identified 443 microRNAs predicted to transcriptionally target NEK11. These results suggesting that the microRNAs might be an important regulation mechanism on NEK11. As shown in Table II, among the top 8 microRNAs most strongly targeted NEK11, 3 microRNAs including miR-21, -590-5p and -149 were shown to be involved in drug resistance in ovarian and other cancers (80-84). For example, the expression of miR-21 is notably upregulated in the paclitaxel-resistant ovarian cancer A2780/taxol cells compared with the parental A2780 cells. Inhibition of miR-21 in A2780/taxol cells decreased the expression of P-gp and HIF-1α proteins, and increased the sensitivity of the A2780/taxol cells to paclitaxel. These results suggested that miR-21 may be involved in the development of drug resistance and the regulation of MDR1/P-gp expression, at least in part, by targeting HIF-1α in ovarian cancer cells (80). The other microRNAs did not indicate direct involvement in drug resistance, however, except for the miR-876-5p, they all play important roles in cancer development and progression (as shown in Table II), and might also be involved in the drug resistance. For example, miR-376b controls mTOR inhibition-related autophagy (85), while the autophagy plays an important role in wt p53 and mutant p53-immediated multidrug resistance in ovarian cancer (86).

Table II. The top 8 microRNAs targeting NEK11, as predicted by microRNA-mRNA interactions and their drug resistance-related functions in cancer.

Gene	MicroRNA (hsa-)	The microRNA-mRNA prediction tool								Drug resistance-related functions of the microRNAs in cancers (refs.)
		A	B	C	D	E	F	G	H	
NEK11	miR-376b	1	1	1	1	0	1	0	1	Controls autophagy (85)
	miR-21	1	1	1	1	0	1	0	1	Drug resistance (80-82)
	miR-590-5p	1	1	1	1	0	1	0	1	Drug resistance (83)
	miR-876-5p	1	1	0	1	0	1	0	1	-
	miR-1255b	0	1	1	1	0	1	0	1	Tumor suppressor (87)
	miR-1290	0	1	1	1	0	1	0	1	Impairs cytokinesis and affects the reprogramming of cancer cells (88)
	miR-149	1	1	0	1	0	1	0	1	Drug resistance and progression-free survival (84)
	miR-370	1	1	0	1	0	1	0	1	Tumor suppressor associated with tumor progression and carcinogenesis (89-91)

A, DIANAmT; B, miRanda; C, miRDB; D, miRWalk; E, RNAhybrid; F, PICTAR5; G, PITA; H, Targetscan. 1, predicted; 0, not predicted.
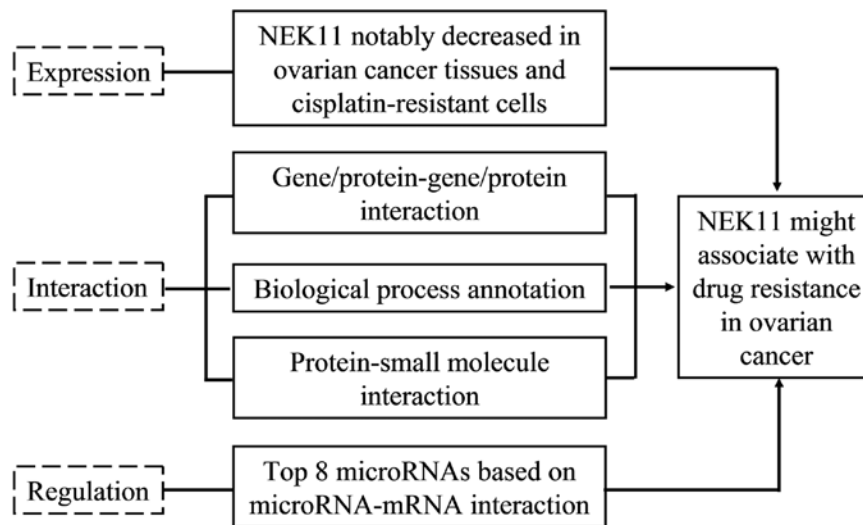
Figure 4. The overall procedure of bioinformatics analyses of NEK11 associated with drug resistance in ovarian cancer.

Discussion

Functional annotation of proteins/genes is a fundamental problem in the post-genomic era, and determining the functions of proteins encoded in genome sequences represents a major challenge in current biology (92). Experimental determination of protein function is expensive and time-consuming. Thus, computational approaches based on the diverse genomic and proteomic datasets can facilitate more rapid annotation of protein function and guide laboratory experiments (92,93). The computational approaches to gene function prediction have relied on a variety of genomic and proteomic data, at least including usage of microarray expression data (94), protein-protein interaction networks (95), protein-small molecule/chemical interactions (33-35) and the annotation of gene with biological processes (93). Computational methods for inferring protein function, which exploit the context of a protein in cellular networks, can provide both a first hand hint into the functional role of a protein and offer complementary insights to understanding the function of proteins (96). Thus, on the basis of many large-scale databases and networks, gene function prediction based on bioinformatics analysis is a potential, feasible and valuable way for gene function prediction (92). Using the comprehensive bioinformatics analyses, we identified that two genes CCL21 and SPARCL1 are associated with drug resistance in ovarian cancer (9). Similarly, we identified that the upregulation of NEK2 and E2F3 are associated with drug resistance in ovarian cancer and poor prognosis in hepatocellular carcinoma, respectively (18,97).

The association of NEK11 with drug resistance in ovarian cancer was analyzed, on the basis of comprehensive bioinformatics analysis (Fig. 4), including microarray data retrieving, protein/gene interaction, protein-small molecule/chemical interaction, biological process annotation and microRNA-mRNA interaction. The database/tool/software used in the analysis included GeneMANIA online tool (30-32), Coremine Medical (38), Oncomine online database (27), GEO profiles (28,29), STITCH 4.0 beta (33-35), DrugBank online database

(36,37) and miRWalk (39), which all are regularly used and reliable databases/tools. For example, GeneMANIA is a web-based database and a tool for prediction of gene functions on the basis of multiple networks derived from different genomic or proteomic data/sources (30). With a query gene, GeneMANIA can find a small set of genes that are most likely to share function with that gene based on their interactions with it (32). The GEO at the National Center for Biotechnology Information (NCBI) has emerged as the leading fully public repository for gene expression data, predominantly gene expression data generated by DNA microarray technology (28), and about a billion individual gene expression measurements are stored, submitted by over 1500 laboratories, addressing a wide range of biological phenomena (98). Thus, the protein/gene functions predicted by these online tools/databases/software were accurate and reliable.

NEK11 mRNA was significantly downregulated in both ovarian cancer tissues and cisplatin-resistant cells (Fig. 1), indicating the potential roles of NEK11 in regulation of drug resistance in ovarian cancer. Protein/gene interaction indicated that among the total 28 proteins/genes which interacted with NEK11, 22 were contributed to drug resistance in ovarian cancer (Fig. 2). Protein-small molecule interaction suggested that among the 5 small molecules/chemicals which interacted with NEK11, 4 were directly/indirectly associated with drug resistance in ovarian and other cancers (Table I). Annotation of biological process indicated that NEK11 probably were involved in the regulation of drug resistance in ovarian cancer via its regulatory roles in 12 biological processes (Fig. 3). MicroRNA-mRNA interaction analysis indicated that among the eight microRNAs most strongly targeting NEK11, the majority were involved in drug resistance in ovarian and other cancers (Table II). All these results provide a very strong possibility that NEK11 is involved in drug resistance in ovarian cancer, via its interactions with a number of genes, proteins, small molecules, microRNAs and related biological processes.

Cell cycle-mediated drug resistance is best described as a relative insensitivity to a chemotherapeutic agent because

of the position of the cells in the cell cycle. Cell cycle is closely involved in chemosensitivity for combination chemotherapy, and the chemotherapeutic agents correlated with cell cycle events include taxanes, platinum, camptothecin and fluorouracil (99). Cell cycle is closely related to drug resistance in ovarian cancer. For instance, integration of DNA methylation and gene expression reveals specific platinum resistance related signaling pathways in ovarian cancer, which include cell growth-promoting pathways PI3K/Akt and cell cycle progression (40). Besides, curcumin and cisplatin or oxaliplatin can induce cell cycle inhibition (100), and cell cycle synchronization can reverse taxol resistance of human ovarian cancer cell lines (101). In addition, comprehensive bioinformatics analysis indicates that 15 TSGs perform their drug resistance-related functions through 5 pathways including cell cycle (8). Annotation of NEK11 with ovarian cancer and drug resistance generated 12 biological processes, of which, 7 were cell cycle-related processes. Thus, given the important roles of NEK11 in regulation of cell cycle (11,12), the important roles of the cell cycle in drug resistance (99), and the close linkages between NEK11 and drug resistance (Fig. 3), we concluded that the NEK11 might be associated with drug resistance in ovarian cancer via regulation of the cell cycle.

Taken together, for the first time, we illustrated that downregulation of NEK11 in drug resistant cells might contribute to drug resistance, via their interactions with a number of drug resistance-related genes, proteins, small molecules and microRNAs, and probably through regulation of the cell cycle. This study set the stage for further investigation into the drug resistant-related functions of NEK11, in ovarian and other cancers.

Acknowledgements

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