Downregulation of HNF1 homeobox B is associated with drug resistance in ovarian cancer

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Abstract. The expression of HNF1 homeobox B (HNF1B) is associated with cancer risk in several tumors, including ovarian cancer, and its decreased expression play roles in cancer development. However, the study of HNF1B and cancer is limited, and its association with drug resistance in cancer has never been reported. On the basis of array data retrieved from Oncomine and Gene Expression Omnibus (GEO) online database, we found that the mRNA expression of HNF1B in 586 ovarian serous cystadenocarcinomas and in platinum-resistant A2780 epithelial ovarian cancer cells was significantly decreased, indicating a potential role of HNF1B in drug resistance in ovarian cancer. Based on this finding, comprehensive bioinformatics analyses, including protein/gene interaction, protein-small molecule/chemical interaction, biological process annotation, gene co-occurrence and pathway enrichment analysis and microRNA-mRNA interaction, were performed to illustrate the association of HNF1B with drug resistance in ovarian cancer. We found that among the proteins/genes, small molecules/chemicals and microRNAs which directly interacted with HNF1B, the majority was associated with drug resistance in cancer, particularly in ovarian cancer. Biological process annotation revealed that HNF1B closely related to 24 biological processes which were all notably associated with ovarian cancer and drug resistance. These results indicated that the downregulation of HNF1B may contribute to drug resistance in ovarian cancer, via its direct interactions with these drug resistance-related proteins/genes, small molecules/chemicals and microRNAs, and via its regulations on the drug resistance-related biological processes. Pathway enrichment analysis of 36 genes which co-occurred with HNF1B, ovarian cancer and drug resistance indicated that the HNF1B may perform its drug resistance-related functions through 4 pathways including ErbB signaling, focal adhesion, apoptosis and p53 signaling. Collectively, in this study, we illustrated for the first time that HNF1B may contribute to drug resistance in ovarian cancer, potentially through the 4 pathways. The present study may pave the way for further investigation of the drug resistance-related functions of HNF1B in ovarian cancer.

Introduction

Ovarian cancer is the most lethal cancer of the female reproductive system, with a high rate of mortality worldwide. Approximately 70% of ovarian cancers are diagnosed at advanced stage and only 40% of women with such cancers can expect to survive 5 years (1). The current therapy for ovarian cancer is debulking surgery followed by cisplatin-centered chemotherapy (2). Although cisplatin-centered chemotherapy, which is the currently preferred treatment modality in human ovarian cancer, can achieve a complete response rate of 40-60% in advanced ovarian cancer patients, the main obstacle to a successful treatment for ovarian cancer is the development of drug resistance to combined chemotherapy, and that finally leads to mortality (3-5).

Drug resistance, including intrinsic and acquired resistance, generally develops after the treatments to advanced stage cancer patients with chemotherapies, and results from a variety of factors including individual variations in patients and somatic cell genetic differences in tumors (5,6). Several molecular mechanisms implicated in the rise of resistance in cellular models of ovarian cancer include decreased cell-associated drugs, altered drug inactivation, increased DNA damage tolerance/repair, increased anti-apoptotic regulator activity and growth factor receptor deregulation (4,7). In addition, apoptosis, which is associated with the expression of specific ‘death’ genes and downregulation of ‘survival’ counterparts, is crucial in determining the response to chemotherapeutic agents (8,9). However, regardless of the
mechanisms, abnormal expression of drug resistance-related genes often plays important roles in drug resistance (10).

HNF1 homeobox B (HNF1B), a transcription factor (11-13), is identified as a transforming oncogene required for the survival of cancer cells (14). However, another study indicated that HNF1B may function as a tumor suppressor gene in chromophobe renal cell carcinogenesis through control of PKHD1 expression (15). It has been proven that HNF1B is downregulated in ovarian, gastric, pancreatic and colorectal cancers (16,17), and its suppression influences cellular phenotypes associated with tumor-related properties in prostate cancer cells (18). The associations of HNF1B with cancer focus mainly on the single nucleotide polymorphisms (SNPs). Gudmundsson et al (19) first reported the association of HNF1B variant with prostate cancer risk. Later, the two SNPs (rs4430796 and rs1649743) in HNF1B associated with prostate cancer risk were identified (20,21). Further study indicated that the rs4430796 is also associated with endometrial cancer risk in women of European background (22). Similarly, rs7501939 in HNF1B is associated with the risk of prostate cancer (22) and endometrial cancer (22). In ovarian cancer, HNF1B is identified as a subtype-specific susceptibility gene (24-26). The different SNPs associate with invasive serous (rs7405776) and clear cell (rs1651755) epithelial ovarian cancer, and the risk alleles for the serous subtype associate with HNF1B high methylation and downregulation, and unmethylated and expressed HNF1B is presented in clear cell tumors (24).

Collectively, the expression of HNF1B is associated with cancer risk in several tumors, and its decreased expression play roles in cancer development. However, studies of HNF1B with ovarian cancer are limited, and its association with drug resistance in cancer has yet to be reported. In the present study, we demonstrated that the expression of HNF1B was significantly decreased in serous cystadenocarcinomas and platinum-resistant A2780 ovarian cancer cells, according to the microarray data retrieved from the Oncomine and Gene Expression Omnibus (GEO) online database, respectively, and it indicated that HNF1B may be involved in the drug resistance in ovarian cancer. Following this premise, the present study illustrated that the downregulation of HNF1B may contribute to drug resistance in ovarian cancer, based on our comprehensive bioinformatics analyses.

Methods and database

The microarray data of HNF1B in ovarian cancer tissues was retrieved from the Oncomine online database (https://www.oncomine.org/resource/main.html) (27,28). The microarray data of HNF1B in ovarian cancer cells was retrieved from the GEO (http://www.ncbi.nlm.nih.gov/geo/profiles/) (28,29). The protein/gene-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 BiologicalNetworks2 (http://biological-networks.org/) (36,37). Annotation of biological process and gene co-occurrence analysis were performed using Coremine Medical online database (http://www.coremine.com/medical/) (38). The pathway enrichment analysis was performed using the DAVID online tool (http://david.abcc.ncifcrf.gov/) (39,40). The microRNAs targeted to the gene were predicted by miRWalk online tool which included 10 prediction tools (DIANAmT, miRanda, miRDB, miRWalk, RNAhybrid, PICTAR4, PICTAR5, PITA, RNA22 and Targetscan) (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/) (41).

Results

mRNA expression of HNF1B is notably decreased in ovarian cancer tissues and in platinum-resistant cells. The mRNA expression data of HNF1B in ovaries used as normal controls (p=7.06E-6; fold-change=-5.776). The expression of HNF1B in the Oncomine database is presented as fold-changes (ovarian cancer vs. normal). In the present study, the expression of HNF1B in sensitive cancer cells was normalized to 1.0, and all data are presented as relative expression. (B) Based on the array data retrieval from GEO profiles (GDS3754), the mRNA expression of HNF1B in platinum-resistant A2780 epithelial ovarian cancer cells was notably decreased compared with the expression in their sensitive counterpart (with 5 replicates each; fold-change=-2.16). The expression data in the GEO Profiles database is presented as expression values. In the present study, the expression of HNF1B in sensitive cancer cells was normalized to 1.0, and all data are presented as relative expression.

Figure 1. mRNA expression of HNF1B in ovarian cancer tissues and drug-resistant cells. (A) Based on the TCGA ovarian array data retrieved from the Oncomine online database, the mRNA expression of HNF1B in 586 ovarian serous cystadenocarcinomas was significantly decreased compared with the expression in 8 ovaries used as normal controls (p=7.06E-6; fold-change=-5.776). The expression of HNF1B in the Oncomine database is presented as fold-changes (ovarian cancer vs. normal). In the present study, the expression of HNF1B in the normal control was normalized to 1.0, and all data are presented as relative expression. (B) Based on the array data retrieved from GEO profiles (GDS3754), the mRNA expression of HNF1B in platinum-resistant A2780 epithelial ovarian cancer cells was notably decreased compared with the expression in their sensitive counterpart (with 5 replicates each; fold-change=-2.16). The expression data in the GEO Profiles database is presented as expression values. In the present study, the expression of HNF1B in sensitive cancer cells was normalized to 1.0, and all data are presented as relative expression.

Protein/gene interaction analysis indicating the association of HNF1B with drug resistance in ovarian cancer. The protein/gene interaction of HNF1B with other proteins/genes was analyzed using the GeneMANIA online database. As shown in Fig. 2, HNF1B has direct interactions with 10 proteins/genes; among these, HNF1B shared protein domain,
shared pathways and co-expressed with ONECUT1, had genetic interactions with NFKB1, PTEN, EGFR, PDCD4 and MLH1, had genetic interactions and co-expressed with RAD51B, had physical interactions and shared the pathway with HNF1A, had physical interactions with ATF1 and co-expressed with BRCA1. With the exception of the RAD51B and ONECUT1, the other 8 proteins/genes have all been proven to be closely associated with drug resistance in ovarian cancer. For example, BRCA1 is a well-known TSG and its downregulation contributes to the enhancement of drug resistance in ovarian cancer (42,43). PTEN also is a TSG, and its downregulation results in the development of drug resistance in OVCAR-3 cells and the alterations conferred resistance to cisplatin through the activation of PI3K/Akt and the inhibition of Bax translocation (44). Further research indicated that overexpression of PTEN reverses chemoresistance to cisplatin in human ovarian cancer cells through inactivation of the PI3K/AKT cell survival pathway and may serve as a potential molecular target for the treatment of chemoresistant ovarian cancer (45). NFKB1 functions as a biphasic regulator, either suppressing or enhancing the development of ovarian cancer. As a tumor suppressor in ovarian cancer cell lines, NFKB1 regulates MAPK, while in the aggressive chemoresistant isogenic variants of these lines it plays a role in apoptosis (46). In addition, PDCD4 enhances chemosensitivity of ovarian cancer cells by activating death receptor pathway in vitro and in vivo (47), and the loss of MLH1 mediated by methylation can lead to the cisplatin-resistance in ovarian cancer (48,49). In addition, EGFR (50,51), ATF1 (52,53) and HNF1A (55) are all associated with drug resistance in ovarian and other types of cancer.

In addition to the direct interactions, there were another 10 proteins/genes in network which indirectly interacted with HNF1B; among those, 6 proteins/genes including REL (55,56), CRCC2 (57), PMS2 (58), ZC3H11A (10), FOXA1 (59) and RAD51D (60) have been proven to be associated with drug resistance in ovarian and other cancers. For example, REL contributes directly to elevated uPA gene expression in human ovarian cancer cells (55), thereby promoting the multiple functions of uPA during tumor growth and metastasis, including drug resistance (56). Similarly, a naturally occurring genetic variant of human XRCC2 confers increased resistance to cisplatin-induced DNA damage in ovarian cancer (57).

Collectively, among the total 20 proteins/genes that interacted with HNF1B, 14 were associated with drug resistance in cancers, of which 9 were associated with drug resistance in ovarian cancer. Thus, given the strong interactions of HNF1B with those proteins/genes, we concluded that HNF1B may be involved in the drug resistance in cancer, particularly in ovarian cancer.
Protein-small molecule/chemical interaction analysis indicating the association of HNF1B with drug resistance in ovarian cancer. Protein-small molecule/chemical interaction analysis was performed using STITCH 4.0 beta and BiologicalNetworks2 to further elucidate the associations of HNF1B with drug resistance in ovarian cancer. A total of 6 small molecules/chemicals were identified to interact with HNF1B. Among these, 4 chemicals, including menadione, SB203580, retinoic acid and cyclic AMP, have been proven to be closely related to drug resistance in ovarian cancer. Menadione is identified as a substrate of P-gp, which, presumably, acts as the mechanism for the chemosensitizing effect (61). The treatment of ovarian cancer cells with ascorbate:menadione resulted in the degradation of nuclear and DNA, which finally led to the cell death (62,63). Thus, menadione is considered to be a promising chemotherapeutic enhancer by its ability to circumvent drug resistance, in addition to its own anticancer activity (61). Menadione activated HNF1B according to protein-chemical interaction (Fig. 3A), suggesting that the expression of HNF1B sensitizes the cancer cells to the anticancer drug; this, in turn, indicated that the decreased expression of HNF1B would contribute to drug resistance. Furthermore, HNF1B had physical interaction and co-citation with retinoic acid. Retinoic acid is identified as a suppressor of ovarian carcinoma cell growth (64); it sensitizes cancer cells to paclitaxel in part through survivin downregulation and the promotion of aberrant mitotic progression results in apoptosis (65). Retinoic acid also potentiates the chemotherapeutic effect of cisplatin by inducing differentiation of tumor initiating cells (66). In addition, HNF1B interacts with SB203580 and cyclic AMP. SB203580 is an inhibitor of p38MAPK, which is related to paclitaxel resistance of ovarian carcinoma, and blockade of the p38MAPK pathway can promote the apoptosis of the drug-resistant cells and reverse the drug resistance (67). The cyclic AMP can reduce the induction of AP-1 binding, which is required for the activation of IL8 by paclitaxel (68). The presence of IL8 in paclitaxel-treated ovarian cancer cells contributed to the development of paclitaxel resistance (69). These results indicated that the cyclic AMP is also involved in the development of drug resistance in ovarian cancer. Collectively, of the 6 small molecules/chemicals that interacted with HNF1B, 4 were associated with drug resistance in ovarian cancer, suggesting that HNF1B may contribute to the development of drug resistance in ovarian cancer.

Biological process annotation indicating the association of HNF1B with drug resistance in ovarian cancer. The biological process annotation was performed using Coremine Medical online database/tool. As shown in Fig. 4, a total of 24 biological processes were annotated with HNF1B, ovarian cancer and drug resistance (p<0.01). The top 24 biological processes which closely related to the three terms were annotated. *** Regulation of cell cycle; **** epithelial to mesenchymal transition.
ovarian cancer and drug resistance were varied, while it could still be sub-grouped. As shown in Fig. 4, cell growth related biological processes (covered 5 processes including cell proliferation, cell growth, growth, cell division and regeneration), cell cycle-related (covered 4 processes including cell cycle, regulation of cell cycle, cell cycle arrest and S phase) and gene expression regulation-related (covered 4 processes including gene expression, gene silencing, RNA interference and reverse transcription) may be the main processes by which HNF1B performs its drug resistance-related functions in ovarian cancer.

Pathway enrichment analysis of the genes co-occurring with HNF1B, drug resistance and ovarian cancer, in accordance with Coremine Medical.

<table>
<thead>
<tr>
<th>KEGG pathway</th>
<th>P-value (&lt;0.01)</th>
<th>Benjamini (&lt;0.01)</th>
<th>The genes co-occurring with HNF1B, drug resistance and ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ErbB signaling pathway</td>
<td>3.2E-8</td>
<td>2.8E-7</td>
<td>CDKN1A, EGFR, JUN, PIK3CA, AKT1, ERBB2, ERBB3, MYC</td>
</tr>
<tr>
<td>Focal adhesion</td>
<td>6.8E-7</td>
<td>3.9E-6</td>
<td>BCL2, COL11A2, CCND1, EGFR, JUN, PTEN, PIK3CA, AKT1, ERBB2</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>2.1E-5</td>
<td>1.1E-4</td>
<td>BCL2, BCL2L1, CASP3, PIK3CA, TP53, AKT1</td>
</tr>
<tr>
<td>p53 signaling pathway</td>
<td>1.5E-4</td>
<td>6.8E-4</td>
<td>CASP3, CCND1, CDKN1A, PTEN, TP53</td>
</tr>
</tbody>
</table>

Table II. The 7 microRNAs most strongly targeting HNF1B, and their functions in cancer.

<table>
<thead>
<tr>
<th>MicroRNAs (hsa-)</th>
<th>10 microRNAs-mRNA interaction prediction tools</th>
<th>Drug resistance and related functions in cancer (refs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-24</td>
<td>A: DIANAmT; B: miRanda; C: miRDB; D: miRWalk; E: RNAhybrid; F: PICTAR4; G: PICTAR5; H: PITA; I: RNA22; J: Targetscan</td>
<td>Drug resistance-related (76-78)</td>
</tr>
<tr>
<td>miR-32</td>
<td>1, predicted by the software; 0, not predicted</td>
<td>Inhibit invasion (73); promote growth and migration (74)</td>
</tr>
<tr>
<td>miR-217</td>
<td>1, 0</td>
<td>Suppress cell proliferation and migration (75)</td>
</tr>
<tr>
<td>miR-194</td>
<td>1, 0</td>
<td>Drug resistance-related (79)</td>
</tr>
<tr>
<td>miR-367</td>
<td>1, 0</td>
<td>Drug resistance-related (70)</td>
</tr>
<tr>
<td>miR-25</td>
<td>1, 0</td>
<td>Drug resistance-related (80)</td>
</tr>
<tr>
<td>miR-375</td>
<td>1, 0</td>
<td>Drug resistance-related (71,72)</td>
</tr>
</tbody>
</table>

Among the transcriptional targets of HNF1B were 426 microRNAs as predicted through miRWalk, which is made up of ten miRNA-mRNA prediction tools. Seven microRNAs, i.e., those yielding the highest score for HNF1B, were selected for subsequent analysis (Table II). As shown in Table II, among the top 7 microRNAs, 5 of them, including miR-24, -194, -367, -25 and -375 that targeted HNF1B influenced drug resistance in ovarian and other types of cancers. For example, miR-367 is specifically involved in the drug resistance in paclitaxel-sensitive ovarian cancer cells (70). Similarly, miR-375 is associated with drug resistance in ovarian (71) and cervical cancer (72). Although no study has reported the role of miR-32 and -217 in drug resistance, it is associated with drug resistance-related processes such as cell proliferation, invasion and migration (73-75). Collectively, among the 7 microRNAs most strongly targeting HNF1B, the majority were involved in drug resistance in ovarian and other types of cancers, suggesting that the gene also mediates drug resistance.
Discussion

The increasing number of sequenced genomes makes it important to develop methods that can assign functions to newly discovered genes in a timely and cost-effective manner. Experimental determination of protein functions is not only expensive but also time-consuming. Thus, computational approaches that utilize diverse biological datasets to generate automated predictions are useful, as they can guide laboratory experiments and facilitate more rapid annotation of genomes (81,82). 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 (83), protein-protein interaction networks (84), protein-small molecule/chemical interactions (33-35), and the annotation of gene with biological processes (81). 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 (82). Using the comprehensive bioinformatics analyses, Yin et al (10) performed an integrated analysis of tumor suppressor genes with drug resistance in ovarian cancer, and two genes CCL21 and SPARCL1 associated with drug resistance were identified (85). Using similar bioinformatics analysis, upregulation of NEK2 was identified to be associated with drug resistance in ovarian cancer (86), and upregulation of E2F3 was identified to be associated with poor prognosis in HCC (87).

The association of HNF1B with drug resistance in ovarian and other cancers has yet to be reported. In the present study, a comprehensive bioinformatics analysis was performed to illustrate the associations of HNF1B with drug resistance in ovarian cancer, including array data retrieving, protein/gene interaction, protein-small molecule/chemical interaction, biological process annotation, gene co-occurrence and pathway enrichment analysis, and microRNA-mRNA interaction. The database/tool/software used in this analysis including Oncomine online database (27,28), GEO profiles (28,29), GeneMANIA online tool (30-32), STITCH 4.0 beta (33-35), BiologicalNetworks2 (36,37), Coremine Medical (38), DAVID online tool (39,40) and miRWalk (41), which are all 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). Seven organisms including Homo sapiens are currently supported, and hundreds of data sets have been collected from GEO, BioGRID, IRefIndex and I2D, as well as organism-specific functional genomics data sets (32). With a query gene, GeneMANIA could find a small set of genes that are most likely to share function with that gene based on their interactions with it, and with a query gene list, GeneMANIA could extend the list with functionally similar genes that it identifies using available genomics and proteomics data (32).

On the basis of comprehensive bioinformatics analyses (Fig. 5), we found that the mRNA expression of HNF1B in 586 ovarian cancer tissues and in drug-resistant cells is significantly decreased compared with their control counterparts, with 5.776- and 2.16-fold-changes, respectively. Protein/gene interaction analysis indicated that among the total 20 proteins/genes that interacted with HNF1B, 14 of them were associated with drug resistance. Protein-small molecule/chemical interactions analysis indicated that 4 of 6 chemicals that directly interacted with HNF1B were associated with drug resistance in ovarian cancer. MicroRNA-mRNA interaction analysis suggested that among the 7 microRNAs most strongly targeting HNF1B, the majority were involved in drug resistance in ovarian and other cancers. The biological process annotation indicated that a total of 24 biological processes were annotated with the HNF1B, ovarian cancer and drug resistance, and gene co-occurrence revealed that a total of 36 genes notably co-occurred with HNF1B, ovarian cancer and drug resistance. Collectively, given the strong interactions of HNF1B with proteins, genes, small molecules, microRNAs and biological processes, which were all associated with drug resistance in ovarian and other cancers, we concluded that the downregulation of HNF1B in ovarian serous cystadenocarcinomas and drug resistant ovarian cancer cells may potentially be involved in the development of drug resistance.

Several studies indicated that HNF1B is a downstream transcription activator of Wnt signaling pathway, and performs its functions via the interaction with the signaling (88-90). However, the pathway with which HNF1B is involved
in cancer development is less understood. In the present study, pathway enrichment analysis of 36 genes which co-occurred with HNF1B, ovarian cancer and drug resistance, was performed. In addition to the pathways in cancer and pathways related to specified cancers (such as prostate and colorectal cancer), 4 pathways including ErbB signaling, focal adhesion, apoptosis and p53 signaling were enriched, suggesting that HNF1B may contribute to drug resistance in ovarian cancer via those pathways. ErbB signaling (91), focal adhesion (92,93), apoptosis (94,95) and p53 signaling (96,97) have been reported to associate with drug resistance in ovarian cancer. For example, miR-21 regulates drug resistance via apoptosis and cellular survival pathways (94), and loss of DOK2 induces carboplatin resistance in ovarian cancer via suppression of apoptosis (98).

In summary, on the basis of comprehensive bioinformatics analysis, for the first time, we illustrated that the downregulation of HNF1B may associate with drug resistance in ovarian cancer. The present study may set the stage for further investigation of the drug resistance-related functions of HNF1B in ovarian cancer.

Acknowledgements

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References


