AMERICAN SOCIETY of
GENE & CELL
THERAPY

# Drug Resistance-Related Competing Interactions of lncRNA and mRNA across 19 Cancer Types

Haizhou Liu,[1,4] Shuyuan Wang,[2,4] Shunheng Zhou,[1] Qianqian Meng,[2] Xueyan Ma,[2] Xiaofeng Song,[1] Lihong Wang,[3] and Wei Jiang[1]

[1]College of Automation Engineering, Nanjing University of Aeronautics and Astronautics, Nanjing 211106, China; [2]College of Bioinformatics Science and Technology, Harbin Medical University, Harbin 150081, China; [3]Department of Pathophysiology, School of Medicine, Southeast University, Nanjing 210009, China

**Drug resistance is a common cause of treatment failure in cancer therapy, and molecular mechanisms need further exploration. Competing endogenous RNAs (ceRNAs) can influence drug response by participating in various biological processes, including regulation of cell cycle, signal transduction, and so on. In this study, we systematically explored resistance from the perspective of ceRNA modules. First, we constructed a general ceRNA network, involving 83 long non-coding RNAs (lncRNAs) and 379 mRNAs. Next, we identified the drug resistance-related modules for 138 drugs and 19 cancer types, totaling 758 drug-cancer conditions. Function analysis showed that resistance-related biological processes were enriched in these modules, such as regulation of cell proliferation, DNA damage repair, and so on. Pan-drug and pan-cancer analyses revealed some common and specific modules across multiple drugs or cancers. In addition, we also found that drug pairs with common modules have similar structure, indicating high risk for multidrug resistance (MDR). Finally, we speculated that ceRNA pair GAS5-RPL8 could regulate drug resistance because low expression of GAS5 would enhance microRNA (miRNA)-mediated inhibition of RPL8. In total, we investigated the drug resistance by using ceRNA modules and proposed that ceRNA modules may be new markers for drug resistance that indicated a possible novel mechanism.**

## INTRODUCTION

Resistance to chemotherapy usually exists in the process of treating cancers.[1] The understanding of the mechanism is inadequate, and the solution is limited. Hitherto, many genes have been proved to contribute to drug resistance through affecting hallmarks of drug resistance, such as increased ability to repair DNA damage, altered proliferation, and so on.[2] For example, upregulation of NEK2 was associated with cisplatin resistance via establishment of the microtubule-based mitotic spindle in the S and G2 phases of the cell cycle in ovarian cancer.[3] FOXM1 directly activated ABCG2 to increase drug efflux activation and drug resistance of doxorubicin in bladder cancer cells.[4] In addition, long non-coding RNAs (lncRNAs) are currently a hotspot in biomedical research. They play important roles in regulation of proliferation, differentiation, apoptosis, cell cycle, and so on,[5,6] and have been proved to be associated with drug

resistance in cancer.[7,8] For example, gemcitabine treatment causes resistance of pancreatic cancer stem-like cells via induction of lncRNA HOTAIR.[9] Overexpression of lncRNA UCA1 correlates with resistance to cisplatin.[10]

lncRNAs utilize their functions through multiple mechanisms, such as affecting chromatin remodeling, controlling transcription and translation, as well as competing for microRNA (miRNA) binding with mRNAs.[11,12] Thereinto, lncRNAs could act as competing endogenous RNAs (ceRNAs) to regulate gene expression through sharing miRNA recognition elements (MREs) with mRNAs. Many studies have demonstrated that ceRNAs were involved in the process of development and treatment of diseases.[13–15]

Recently, an increasing number of researchers attempted to identify the drug resistance-related ceRNA interactions in a specific cancer. For example, lncRNA CASC2 upregulated PTEN as a ceRNA of miR-21 and played an important role in cervical cancer sensitivity to cisplatin.[16] lncRNA TUG1 mediated methotrexate resistance as ceRNA in colorectal cancer via the miR-186/CPEB2 axis.[17] However, there is no global analysis of ceRNAs in drug resistance across many cancer types and many drugs. Here, we first constructed a ceRNA network, from which the significant ceRNA modules related to drug resistance were identified in 758 conditions (one condition meant that specific cancer type was treated with specific drug). Then, we analyzed the conserved and specific significant modules in different conditions (the workflow diagram was shown in Figure 1). These analyses and validations demonstrated how ceRNAs could be used as biomarkers and potential therapeutics.
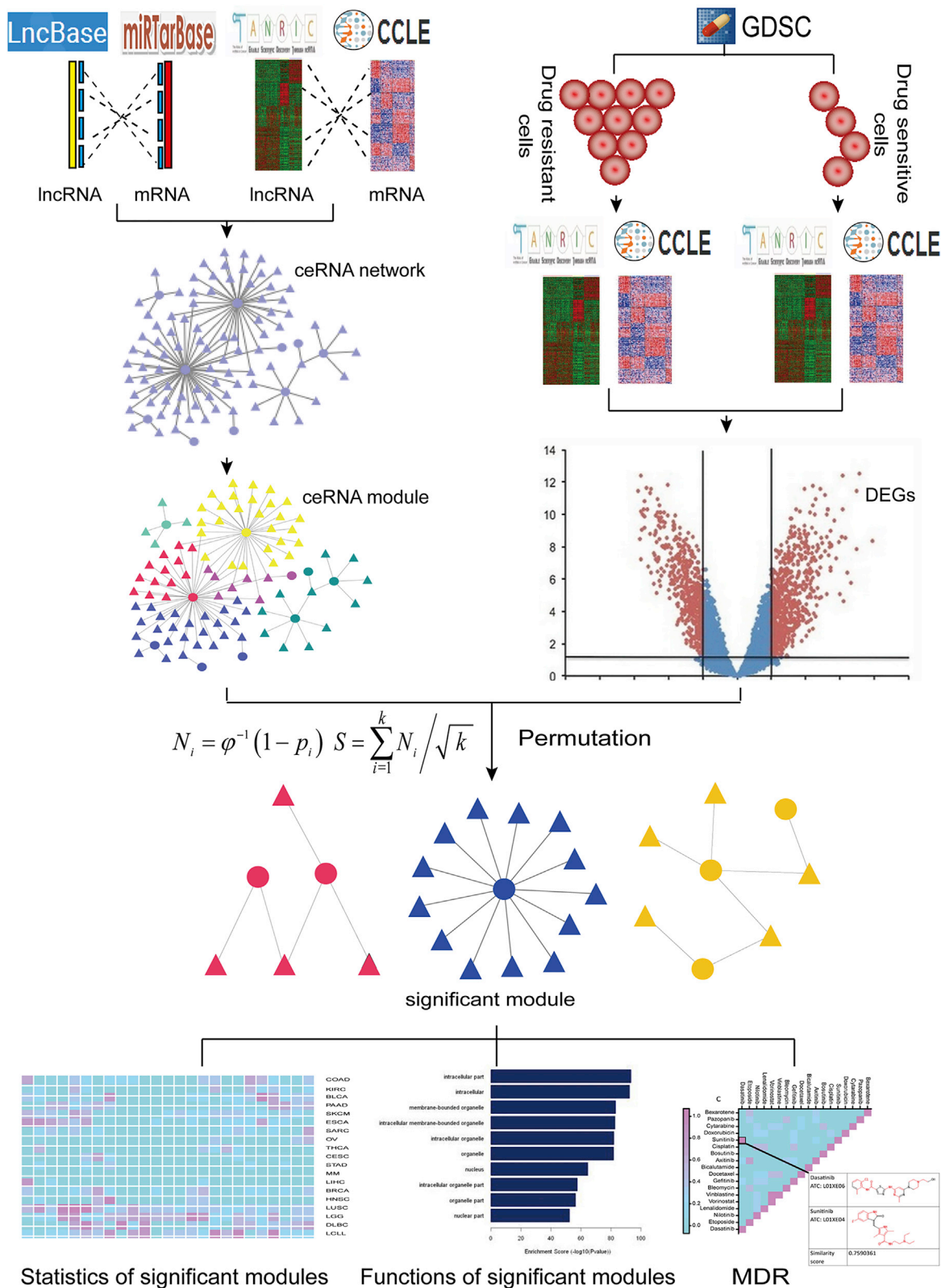
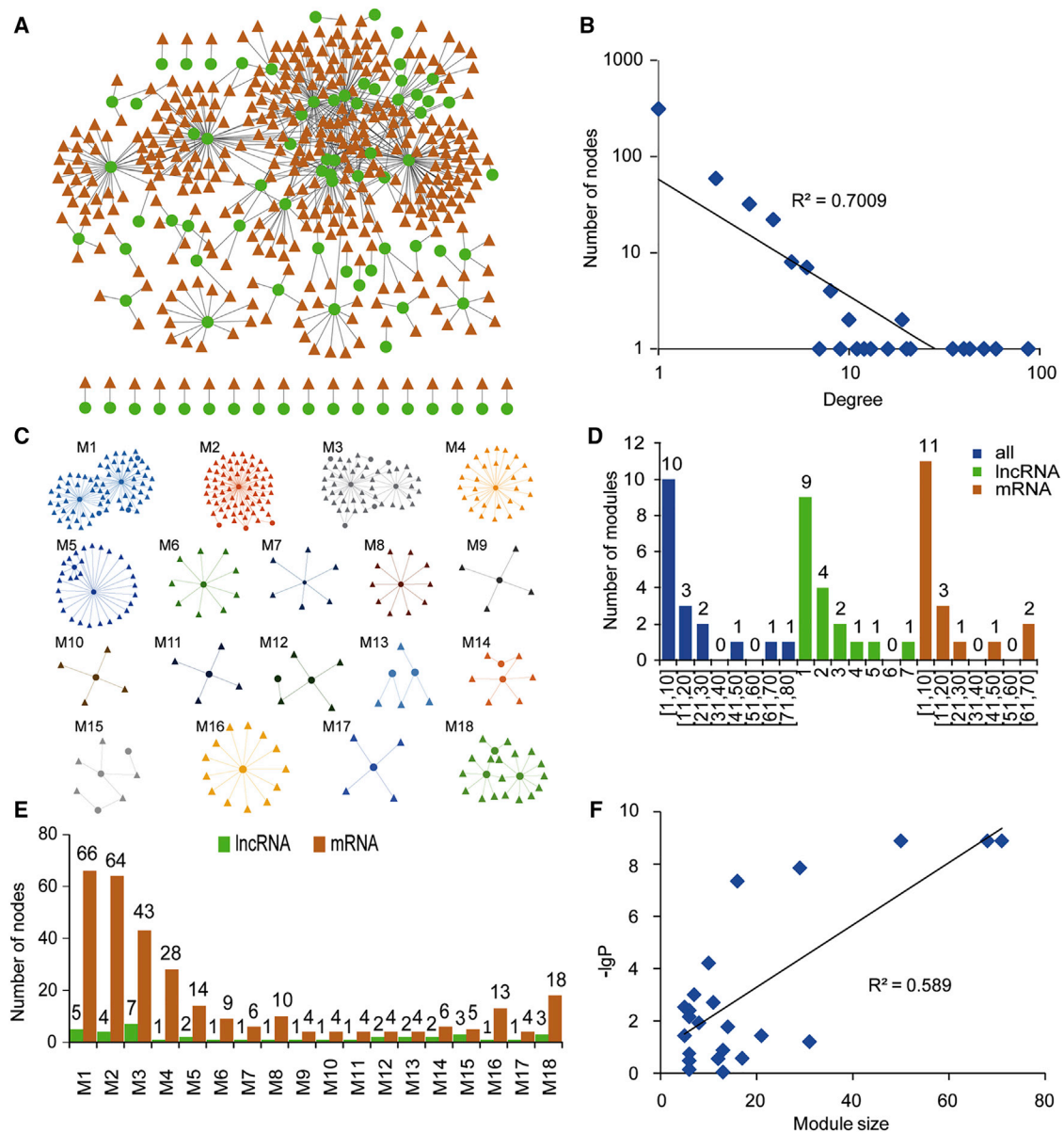**Figure 1. The Workflow Diagram for This Study**

**Figure 2. Overview and Characteristics of the ceRNA Network and Modules**

(A) ceRNA network. Green circular nodes represent lncRNAs, and yellow triangular nodes represent mRNAs. Lines represent lncRNA-mRNA ceRNA interactions. (B) The degree of distribution of the ceRNA network. (C) The 18 modules mined from the ceRNA network. (D) The distribution of the number of all nodes, lncRNAs, and mRNAs in the module, respectively. The x axis represents the number of nodes in the module. y axis represents the number of modules. Blue, green, and yellow colors represent all nodes, lncRNAs, and mRNAs, respectively. (E) The number of lncRNAs and mRNAs in each module. (F) The correlation of the module size and the module p values.

## RESULTS

### ceRNA Network and Modules

According to the shared miRNAs and the expression correlation of lncRNA and mRNA, we identified 605 ceRNA interactions and constructed the ceRNA network, including 83 lncRNAs and 379 mRNAs (Figure 2A). Next, we investigated the topological properties of the ceRNA network. The degree distribution approximately displayed a power law distribution (Figure 2B), which meant that the ceRNA network satisfied scale-free topology, as similar as most biological networks. About 71.8% of mRNAs in the ceRNA network had competitive relationships with only one lncRNA, and 73.5% of lncRNAs connected with no more than four neighbors (Figures S1 and S2). We also observed that 82.6% of ceRNA pairs were regulated by no more than 10 miRNAs (Figure S3).
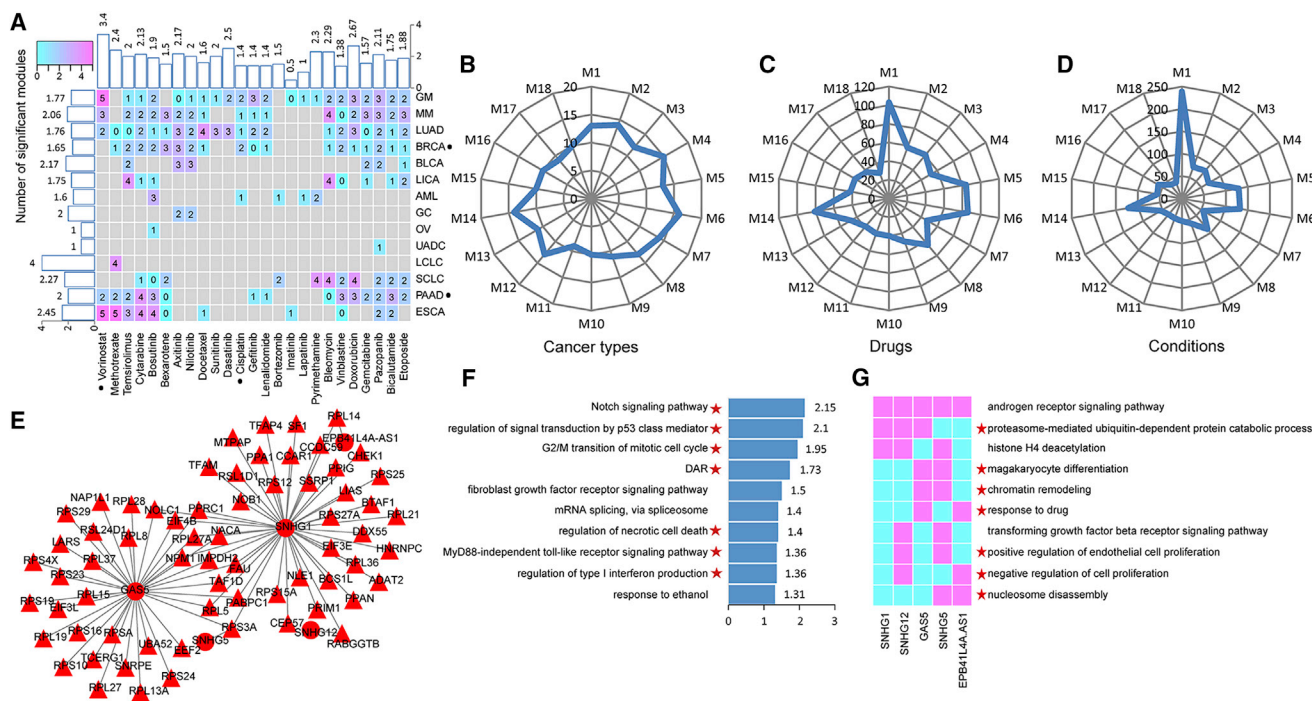
**Figure 3. Significant Modules Related to Drug Resistance**

(A) The number of significant modules for FDA-approved drugs in different cancers. The gray color represents that the treatment is not performed. The color bar encodes the number of significant modules. The histogram represents the number of significant modules on average of each row and column, respectively. The examples mentioned in the manuscript were marked with dots. (B–D) The distribution of the number of (B) cancers, (C) drugs, and (D) conditions related to the significant modules, respectively. (E) Module 1. The circular nodes represent lncRNAs, and the triangular nodes represent mRNAs. (F) The function annotation of the genes in module 1. (G) The function annotation of the lncRNAs in module 1. The functions related to hallmark of drug resistance are marked as red stars. DAR, DNA damage repair.

Moreover, we used ClusterONE to identify network modules from the ceRNA network. The ClusterONE method calculated a p value for each module, and a lower p value reflected a more cohesive module. In order to ensure sufficient lncRNAs and mRNAs for further analysis in each module, we also set the minimum size of module as 5. Thus, we obtained 18 modules with $p < 0.05$ and more than five nodes (Figure 2C). The sizes of the 18 modules ranged from 5 to 71, and the median value was 9 (Figures 2D and 2E). We also observed that the bigger the module size was, the more significant the module was (Figure 2F).

## Significant Modules Related to Drug Resistance

The above identified network modules were general instead of condition specific. So, we performed the differential expression analysis for all lncRNAs and mRNAs between drug-resistant and drug-sensitive cell lines for each condition. Then, we calculated the module score for each module by integrating p values of all nodes in this module and obtained the significance level of this module by permutation test (details in Materials and Methods). As a result, we identified the significant modules in 758 conditions relating to 19 cancers and 138 drugs (including 25 US Food and Drug Administration [FDA]-approved drugs) (Data S1). Here, we found that the lncRNAs in these significant modules were significantly enriched (p value < 0.001) in

the experimentally validated drug-resistant-related lncRNAs (Figure S4), which were obtained from ncDR, a comprehensive resource of non-coding RNAs involved in drug resistance.[18]

Next, we showed the number of significant modules in 19 cancers of 25 FDA-approved drugs by heatmap (Figure 3A). Intuitively, more than one module was significantly identified in most (63%) conditions, which indicated that drug resistance was so complex that there might be many functions or pathways participating in this process. In addition, we also found that the number of significant modules of cancer-specific drugs, which means the drugs used for treatment of one specific or a few cancers, were larger than that of the broad-spectrum anti-cancer drugs. For example, vorinostat, which is one class of histone deacetylase inhibitors for the treatment of patients with cutaneous T cell lymphoma, had the most significant modules (~3.4) on average. The broad-spectrum anti-cancer drug cisplatin had been widely used for treatment of breast cancer (BRCA), ovarian cancer, cervical cancer, and so on, which had only ~1.4 significant modules on average. On the other hand, the cancer types with bad prognosis were more likely to have more significant modules for FDA-approved drugs, which might be because of their high degree of malignancies. For example, pancreas adenocarcinoma (PAAD) is one of the deadliest cancers whose median survival period is ~3–6 months and
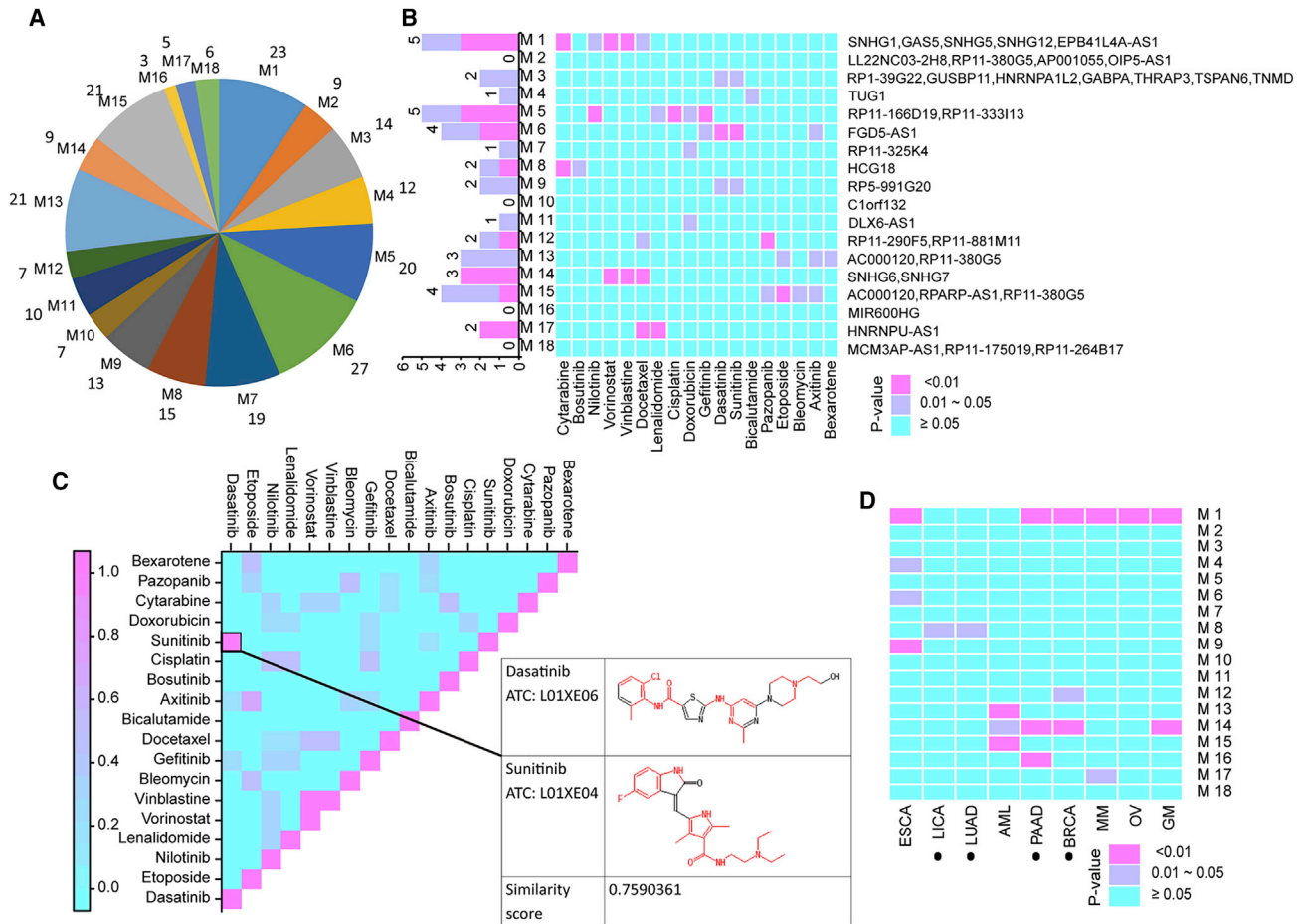
**Figure 4. Drug Resistance for Pan-Drugs and Pan-Cancers**

(A) The numbers and proportions of drugs that were significantly related to each module in LUAD. (B) Significance of modules in LUAD for FDA-approved drugs. lncRNAs in each module are presented on the right, and the histogram on the left is the number of FDA-approved drugs that were significantly related to this module. (C) MDR analysis in LUAD. The color bar encodes the Jaccard index of the significant modules related to drug resistance. The box indicates drug structure, and red parts mean similar structure. (D) Significance of modules for bosutinib resistance. The examples mentioned in the manuscript were marked with dots.

5-year survival rate is less than 5%. We found that 73.3% of the drugs had two or more significant modules in PAAD. However, for BRCA, with a 5-year survival rate of more than 90%, 50% of the drugs had no more than one significant module.

Furthermore, we analyzed the distribution of the significant modules in cancer types, drugs, and conditions separately (Figures 3B–3D). We found that several modules were conservatively identified in cancers, drugs, and conditions, such as modules 1 and 14, and some other modules, such as modules 11 and 17, appeared in only a few cancers, drugs, or conditions. Here, module 1 (Figure 3E) was identified in the most (31.5%) conditions, including 103 drugs in 13 cancer types. The hub lncRNA *GAS5* in this module had been proved to be related to drug resistance of many drugs in many cancers.[19–21] For example, *GAS5* silencing in LNCaP and 22Rv1 cells (human prostate cancer cells) decreased the sensitivity to mTOR inhibitors, whereas transfection of *GAS5* sensitized human prostate cancer cells to agents.[22] Stim-

ulation of expression of *GAS5* sensitized much of the cytotoxic and cytostatic effects of rapalogs in mantle cell lymphoma.[23] Finally, we also performed functional annotation for lncRNAs and mRNAs in module 1, respectively (Figures 3F and 3G). Amounts of functions were related to hallmarks of anti-cancer drug resistance, such as regulation of cell cycle and DNA damage response, which illustrated that module 1 might play important roles in drug resistance through many ways.

**Pan-Drug Analysis of Significant Modules in LUAD**

Taking lung adenocarcinoma (LUAD) as an example, we analyzed the significant modules across different drugs in a certain cancer (Figure 4A). We found that module 1 was significantly related to the resistance of the second most (23) drugs, which was similar to the above observation in all cancer types (Figures 3B–3D). Next, the chi-square test revealed that some modules were related to resistance of more drugs in LUAD than in other cancer types (p < 0.01), which indicated

these modules might play important roles specifically in LUAD. For example, functional annotation showed that the genes in module 11 enriched in the function of regulation of Rho protein signal transduction (Data S2), which had been demonstrated to induce the invasion of human non-small-cell lung cancer (NSCLC) cells.[24] Also, the genes in module 7 enriched in IRE1-mediated unfolded protein response (Data S2), which enhanced tumor cell survival in LUAD.[25] In addition, we investigated the significant modules related to resistance of FDA-approved drugs (Figure 4B). Intuitively, we found that some drugs shared the same modules, indicating that they might have similar mechanisms of resistance. For example, dasatinib and sunitinib shared three common modules (modules 3, 6, and 9). They have the same mechanism acting as receptor tyrosine kinase inhibitors (TKIs).

As we know, tumor cells resistant to one drug are usually resistant to several other drugs.[26] The multidrug resistance (MDR) decreases the drug efficacy and limits cancer therapy. In a previous study, we predicted MDR by sharing regulated communities related to drug resistance.[18] Here, we used the Jaccard index to systematically measure the similarity of modules of all FDA-approved drugs (Figure 4C). The drug pairs with a high Jaccard index meant that they shared more significant modules and had high possibilities to form MDR. For example, the Jaccard index of dasatinib and sunitinib was 1. Recently, they were recognized to form MDR.[27] In addition, the bioactivity of a drug directly depends on its molecular structure.[28] We further calculated the structural similarity between dasatinib and sunitinib by Tanimoto coefficient.[29] Interestingly, they have a similar structure whose similarity score is up to ∼0.76. Moreover, we calculated the correlation between the number of common modules and the structure similarity of all drug pairs. The result showed that there was a significantly positive correlation (Pearson correlation coefficient was 0.512, p value was $1.33 \times 10^{-11}$). Finally, functional annotation of the common modules (modules 3, 6, and 9) revealed that the cellular nitrogen compound metabolic process and regulation of cell proliferation might be the causes of drug resistance and MDR, which had been validated in previous studies.[30,31]

### Pan-Cancer Analysis of Significant Modules for Bosutinib

We also analyzed the significant modules for a certain drug bosutinib across 19 cancer types (Figure 4D). We found that eight modules were cancer type specific, whereas modules 1, 8, and 14 were identified in multiple cancers. From another axis, we found that some cancers had similar significant modules, indicating that they might have a similar pathologic process. For example, PAAD and BRCA had two significant modules, modules 1 and 14. In clinical, there were many cases reported in which BRCA and PAAD co-occurred.[32,33] LUAD and large intestine cancer (LICA) shared the significant module 8. For LICA patients, one of the most common positions of metastasis is the lung.[34] The analysis indicated that the cancers sharing similar significant modules might have relevant clinical features because of their similar mechanism of drug response, which could help us to guide cancer therapy better.

### Case Study of Potential Mechanism of ceRNA in Drug Resistance

lncRNA can compete for miRNAs with targeted genes, which creates an indirect interaction among miRNA targets, eventually influencing expression level.[35] Drug resistance is a complex process, so we cannot blame it on one model or one factor. We suggested that dysregulation of ceRNA interaction might be one of the mechanisms leading to drug resistance.

In a previous study, low expression of *GAS5* was demonstrated to be related to drug resistance in multiple cancers,[36] and low-expression *RPL8* were reported to be involved in poor response to the chemotherapy.[37] In this study, *GAS5* and *RPL8* were identified to form a ceRNA interaction in module 1. The mechanism of drug resistance might be that low-expression *GAS5* was targeted by less miRNAs, and more free miRNAs repressed *RPL8* expression to form resistance (Figure 5). Furthermore, some other nodes in module 1 were also reported to be associated to drug resistance. For example, *SNRPE* was found to enhance *hsp70* promoter activation, which was associated with cell survival and drug resistance,[38] and *SNHG1* was also predicted to be related to doxorubicin resistance in colon adenocarcinoma (COAD).[18] So we suggested that the ceRNA interactions should participate in the regulation of drug resistance.

### DISCUSSION

Drug resistance is one of causes of cancer treatment failure. Previous studies suggested that ceRNA took part in the regulation of drug resistance. However, these studies just focused on individual ceRNA interaction in a specific cancer or drug, lacking a global view of properties across drugs in pan-cancer. In this study, we dissected drug resistance from a ceRNA module perspective and performed a systematic analysis across 19 major cancer types and 138 drugs. First, we constructed a general ceRNA network. The network approximately displayed a power law distribution. In addition, most of mRNAs in the ceRNA network connected with only one lncRNA. About three-quarters of lncRNAs had no more than four neighbors. Also, we observed that more than 80% of ceRNA pairs were regulated by less than 10 miRNAs. We considered that this phenotype was consistent with the energy-conservation principle of living organisms. The amount of sharing miRNAs should not be too large because the process of driving miRNAs to combine with ceRNA consumes energy.

Then, we mined 18 modules and identified significant ceRNA modules related to 758 conditions. We got a systematic view of ceRNA modules across 19 cancers and 138 drugs. Herein, we found that the number of significant modules might be associated with drug spectrum and tumor malignant degree. In general, fewer modules were identified for broad-spectrum drugs and in low-degree malignant tumors, respectively. The reasons might be that the broad-spectrum drugs had good responses for many cancers, and it was relatively easy to treat the low-degree malignant tumors. Also, we analyzed the distribution of the significant modules and found several conserved and specific modules for cancer types, drugs, and conditions.
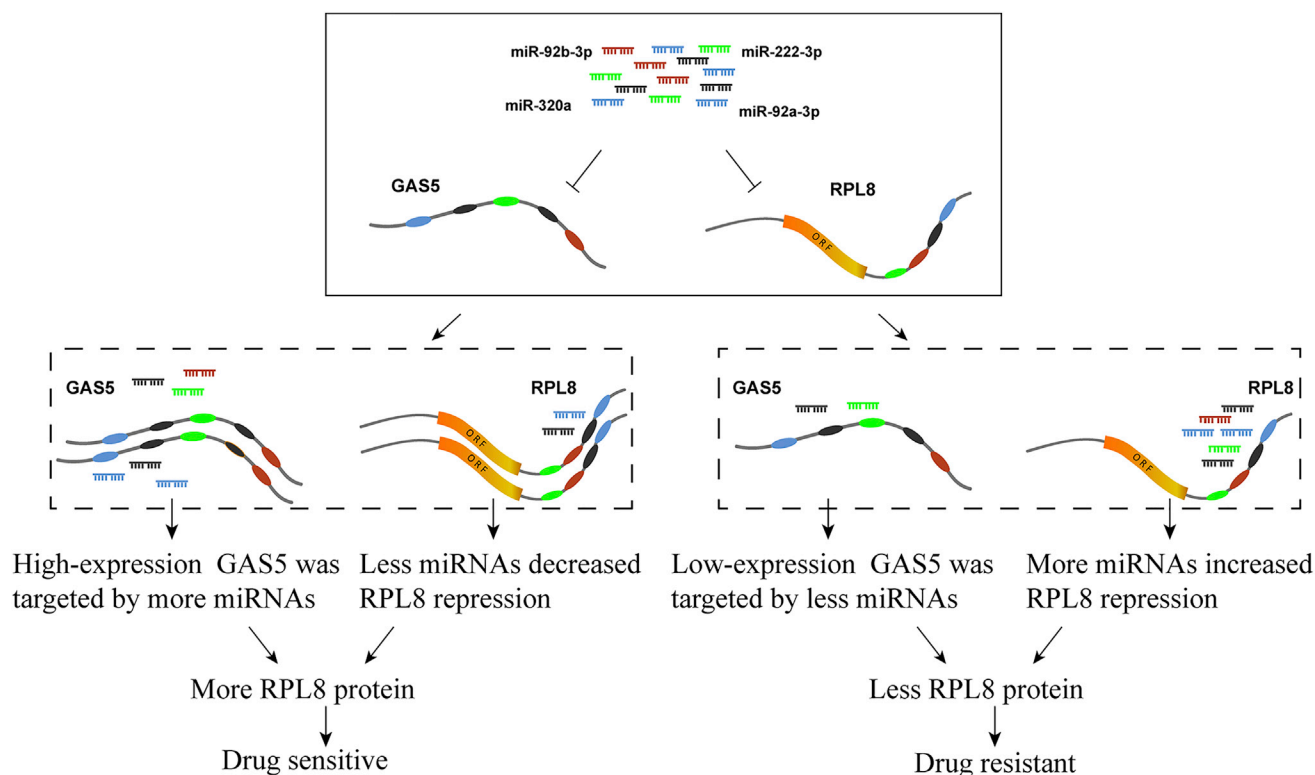
**Figure 5. Case Study of Potential Mechanism of ceRNA in Drug Resistance**

Next, we specifically analyzed the significant modules for pan-drugs, especially FDA-approved drugs. Taking LUAD as an example, we found that some modules were related to resistance of more drugs in LUAD than in other cancers such as module 11 (showing function related to lung cancer invasion) and module 7 (showing function of enhancing tumor cell survival in LUAD). In addition, we investigated the significant modules related to resistance of FDA-approved drugs and found that some drugs shared the same modules, such as dasatinib and sunitinib, which indicate that they might have similar mechanisms of resistance. As we know, MDR, which meant that tumor cells were resistant to many drugs at the same time, could decrease the drug efficacy and limit cancer therapy. To go a step further, we used the Jaccard index of all FDA-approved drugs to measure the possibility of MDR. We found that some drug pairs with a high Jaccard index had similar structures. It meant that drugs with similar structures might have similar drug responses and were more likely to form MDR. This result could help to give a better choice of treatment for drug-resistant tumors.

Meanwhile, we specifically analyzed the significant modules for pan-cancers. Taking bosutinib as an example, we found cancer-specific and conserved modules. Herein, eight modules were cancer type specific, and modules 1, 8, and 14 were identified in multiple cancers. Moreover, we found that some cancers had similar significant modules, indicating that they might have a similar pathologic process.

For example, PAAD-BRCA and LUAD-LICA shared common significant modules, respectively, and they had been reported to co-occur in clinical.

Last, we explored the mechanism of drug resistance. *GAS5* and *RPL8* had both been reported to be involved in anticancer drug resistance. In this study, *GAS5* and *RPL8* were predicted to form ceRNA in module 1. So we suggested that the ceRNA interaction might participate in the regulation of drug resistance. Here, we proposed a general hierarchical model to systematically understand the ceRNA interactions in anticancer drug resistance (Figure S5). When cancer patients were treated by anticancer drugs, resistance usually occurred. In this process, ceRNA interactions might be dysregulated. Consequently, the ceRNA subnetworks or modules were perturbed and further influencing some hallmark functions of anticancer drug resistance. Therefore, not only direct regulation but also indirect stimuli, such as ceRNA, could change individual response to chemotherapy in cancers.

This study provided candidate biomarkers of drug resistance in different conditions, but it still needed further experimental validation. Also, the unbalance of miRNA regulation to mRNA and lncRNA led to a small number of lncRNAs in the modules. Meanwhile, because of data limitation, we did not integrate miRNA expression profiles to construct the ceRNA network. In the following studies,

**Table 1. Summary of miRNA-Target Information**

| Database | No. of miRNAs | No. of lncRNAs | No. of mRNAs | No. of Regulations |
|---|---|---|---|---|
| LncBase | 960 | 6,580 | – | 59,947 |
| miRTarBase | 2,599 | – | 14,843 | 410,365 |

we will combine the related multi-omics data to strengthen our approach and prediction.

Above all, resistance limits clinical drug use for cancer therapy. Here, we performed a systemic analysis of drug response from the new perspective of ceRNA modules. We proposed that the significant ceRNA modules may be regarded as new markers for drug resistance, which indicated a possible novel mechanism. Taken together, the construction of the ceRNA networks and identification of significant modules will provide more chances for development of cancer therapeutics and clinical drug use, and enrich the drug resistance mechanisms.

## MATERIALS AND METHODS

### Data Collection and Preprocessing

We collected lncRNA expression data of cell lines from The Atlas of non-coding RNA in cancer (TANRIC)[39] and mRNA expression data of cell lines from Cancer Cell Line Encyclopedia (CCLE).[40] The regulations of miRNA to lncRNA and miRNA to gene were collected from DIANA-LncBase[41] and miRTarBase,[42] respectively (details in Table 1). We converted lncRNA and mRNA symbols to Ensembl ID. Finally, we got 1,946 lncRNAs (common in LncBase and TANRIC), 13,563 mRNAs (common in CCLE and miRTarBase), and 2,588 miRNAs for further analysis.

We collected drug response data from Genomics of Drug Sensitivity in Cancer (GDSC),[43] including 138 drugs and 707 cell lines that were classified into 45 cancer types according to tissue descriptor. Half maximal inhibitory concentration ($IC_{50}$) values were used to distinguish drug-resistant and -sensitive cell lines. Finally, 348 common cell lines in GDSC (707 cell lines) and CCLE (504 cell lines) were used for further analysis.

### Construction of the ceRNA Network

According to the hypothesis presented in a previous study,[44] we first identified lncRNA-mRNA pairs sharing more than three miRNAs and then computed the significance of shared miRNAs using a hypergeometric test. Here, the lncRNA-mRNA pairs with $p < 0.05$ were considered as candidate ceRNA pairs. Next, for each candidate ceRNA pair, the Pearson correlation coefficient (R) of expression of lncRNA and mRNA in all cell lines was calculated. Only candidate ceRNA pairs with $R > 0.5$ and $p < 0.01$ were retained and were used to construct a ceRNA network, where a node represented a lncRNA or mRNA, and an edge represented a competing interaction. The obtained ceRNA network was visualized by Cytoscape 3.4.0.[45]

**Table 2. Number of Cell Lines in 19 Cancers**

| Cancer Short Name | Cancer Full Name | No. of Cell Lines |
|---|---|---|
| LUAD | lung adenocarcinoma | 36 |
| BRCA | breast cancer | 29 |
| MM | malignant melanoma | 23 |
| GM | glioma | 23 |
| SCLC | small cell lung carcinoma | 16 |
| ESCA | esophageal carcinoma | 15 |
| LICA | large intestine cancer | 15 |
| PAAD | pancreas adenocarcinoma | 14 |
| BLCA | bladder carcinoma | 12 |
| AML | acute myeloid leukemia | 12 |
| OV | ovary cancer | 11 |
| GC | gastric cancer | 9 |
| UADC | upper aerodigestive tract cancer | 8 |
| NB | neuroblastoma | 8 |
| OSTC | other soft tissue cancer | 7 |
| OLN | other lymphoid neoplasm | 7 |
| LTCL | lymphoblastic T cell leukemia | 7 |
| LCLC | large cell lung carcinoma | 7 |
| ENCA | endometrium carcinoma | 7 |

### Identification of Modules in the ceRNA Network

Network modules are subgraphs that are more important parts of larger complete network. They should contain many interactions inside the module and be well separated from the rest of the network. We used ClusterONE[46] to identify ceRNA modules. ClusterONE is a user-friendly plugin in Cytoscape for locating and visualizing modules, and performed a greedy growth process to bring together the cohesive nodes in the level of topological structure.

### Identification of Drug-Resistant and -Sensitive Cell Lines

We first defined drug treatment condition as the cell lines of one cancer type treated with one drug. For each condition, a cell line was considered as a resistant cell line of the specific drug if its $IC_{50}$ value was greater than the mean value plus 0.8 times of the SD of all cell lines of the specific cancer type. Otherwise, a cell line with $IC_{50}$ less than the mean value minus 0.8 times of the SD was defined as a drug-sensitive cell line. Moreover, the numbers of both resistant and sensitive cell lines should be not less than three for each condition. Thus, 758 conditions satisfied the above criteria, which included 19 cancer types and 138 drugs (details in Data S3). The total numbers of resistant and sensitive cell lines of the 19 cancer types were shown in Table 2.

### Identification of Significant Modules Related to Drug Resistance

We defined the significant modules related to drug resistance as the modules having more differentially expressed genes (lncRNA and mRNA) between drug-resistant and -sensitive cell lines. For each

condition, differential expression analysis was performed for lncRNA and mRNA by t test. Based on the t test p value, the node score was calculated by Equation 1,[47]

$$Ni = \varphi^{-1}(1 - p_i), \qquad \text{(Equation 1)}$$

where $p_i$ represents the significance of differential expression determined by t test, and $\varphi^{-1}$ is the inverse normal cumulative distribution function. Then, the module score was defined as the weighted sum of node scores.[47] The detailed formula was displayed as follows:

$$S = \sum_{i=1}^{k} Ni \Big/ \sqrt{k}, \qquad \text{(Equation 2)}$$

where k is the number of nodes in the module.

Next, we performed the permutation analysis to estimate the significance of the module score. For each module, we first randomly selected the same number of lncRNAs and mRNAs in the module to construct a random module. This process was repeated 1,000 times. Then, we calculated the module score for each random module according to the above equations and generated the null distribution of module scores. The empirical p value was defined as the proportion of random module scores (Srandom) larger than the real module score (S): p value = $(N_{S_{random} > S})/N_P$, where $N_{S_{random} > S}$ was the number of random modules that had larger scores than the real module, and Np was the number of permutations (here, Np was 1,000). The modules with $p < 0.05$ were considered as significant modules related to drug resistance for one condition (Data S1).

### Functional Analysis of Significant Modules

For each module, functional enrichment analysis of genes was performed based on gene ontology (GO) by DAVID (Database for Annotation, Visualization, and Integrated Discovery) online tools.[48] The lncRNA functions were obtained from the knowledgebase of inferred functions of non-coding RNA transcripts (FARNA).[49]

### Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available in the Results and Supplemental Information.

## SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2019.03.011.

## AUTHOR CONTRIBUTIONS

W.J., L.W., and X.S. designed the study; H.L. and S.W. carried out data acquisition and analysis, and drafted the manuscript; S.Z. performed the statistical analysis; Q.M. and X.M. provided scientific advice and contributed to results interpretations; all authors read and approved the final manuscript.

## CONFLICTS OF INTEREST

The authors declare no competing interests.

## REFERENCES

1. Goodman, L.S., Wintrobe, M.M., Dameshek, W., Goodman, M.J., Gilman, A., and McLennan, M.T. (1984). Landmark article Sept. 21, 1946: Nitrogen mustard therapy. Use of methyl-bis(beta-chloroethyl)amine hydrochloride and tris(beta-chloroethyl) amine hydrochloride for Hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. By Louis S. Goodman, Maxwell M. Wintrobe, William Dameshek, Morton J. Goodman, Alfred Gilman and Margaret T. McLennan. JAMA *251*, 2255–2261.

2. Cree, I.A., and Charlton, P. (2017). Molecular chess? Hallmarks of anti-cancer drug resistance. BMC Cancer *17*, 10.

3. Liu, X., Gao, Y., Lu, Y., Zhang, J., Li, L., and Yin, F. (2014). Downregulation of NEK11 is associated with drug resistance in ovarian cancer. Int. J. Oncol. *45*, 1266–1274.

4. Roh, Y.G., Mun, M.H., Jeong, M.S., Kim, W.T., Lee, S.R., Chung, J.W., Kim, S.I., Kim, T.N., Nam, J.K., and Leem, S.H. (2018). Drug resistance of bladder cancer cells through activation of ABCG2 by FOXM1. BMB Rep. *51*, 98–103.

5. Hwang, H.W., and Mendell, J.T. (2006). MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br. J. Cancer *94*, 776–780.

6. Chen, Y., and Stallings, R.L. (2007). Differential patterns of microRNA expression in neuroblastoma are correlated with prognosis, differentiation, and apoptosis. Cancer Res. *67*, 976–983.

7. Xia, H., and Hui, K.M. (2014). Mechanism of cancer drug resistance and the involvement of noncoding RNAs. Curr. Med. Chem. *21*, 3029–3041.

8. Heery, R., Finn, S.P., Cuffe, S., and Gray, S.G. (2017). Long Non-Coding RNAs: Key Regulators of Epithelial-Mesenchymal Transition, Tumour Drug Resistance and Cancer Stem Cells. Cancers (Basel) *9*, e38.

9. Wang, L., Dong, P., Wang, W., Huang, M., and Tian, B. (2017). Gemcitabine treatment causes resistance and malignancy of pancreatic cancer stem-like cells via induction of lncRNA HOTAIR. Exp. Ther. Med. *14*, 4773–4780.

10. Wang, H., Guan, Z., He, K., Qian, J., Cao, J., and Teng, L. (2017). LncRNA UCA1 in anti-cancer drug resistance. Oncotarget *8*, 64638–64650.

11. Sarkar, D., Leung, E.Y., Baguley, B.C., Finlay, G.J., and Askarian-Amiri, M.E. (2015). Epigenetic regulation in human melanoma: past and future. Epigenetics *10*, 103–121.

12. Li, J.H., Liu, S., Zhou, H., Qu, L.H., and Yang, J.H. (2014). starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res. *42*, D92–D97.

13. Song, C., Zhang, J., Qi, H., Feng, C., Chen, Y., Cao, Y., Ba, L., Ai, B., Wang, Q., Huang, W., et al. (2017). The global view of mRNA-related ceRNA cross-talks across cardiovascular diseases. Sci. Rep. *7*, 10185.

14. Zhang, Y., Xu, Y., Feng, L., Li, F., Sun, Z., Wu, T., Shi, X., Li, J., and Li, X. (2016). Comprehensive characterization of lncRNA-mRNA related ceRNA network across 12 major cancers. Oncotarget *7*, 64148–64167.

15. Zhou, S., Wang, L., Yang, Q., Liu, H., Meng, Q., Jiang, L., Wang, S., and Jiang, W. (2018). Systematical analysis of lncRNA-mRNA competing endogenous RNA network in breast cancer subtypes. Breast Cancer Res. Treat. *169*, 267–275.

16. Feng, Y., Zou, W., Hu, C., Li, G., Zhou, S., He, Y., Ma, F., Deng, C., and Sun, L. (2017). Modulation of CASC2/miR-21/PTEN pathway sensitizes cervical cancer to cisplatin. Arch. Biochem. Biophys. *623–624*, 20–30.

17. Li, C., Gao, Y., Li, Y., and Ding, D. (2017). TUG1 mediates methotrexate resistance in colorectal cancer via miR-186/CPEB2 axis. Biochem. Biophys. Res. Commun. *491*, 552–557.

18. Dai, E., Yang, F., Wang, J., Zhou, X., Song, Q., An, W., Wang, L., and Jiang, W. (2017). ncDR: a comprehensive resource of non-coding RNAs involved in drug resistance. Bioinformatics 33, 4010–4011.

19. Zhang, H., Guo, Y., Song, Y., and Shang, C. (2017). Long noncoding RNA GAS5 inhibits malignant proliferation and chemotherapy resistance to doxorubicin in bladder transitional cell carcinoma. Cancer Chemother. Pharmacol. 79, 49–55.

20. Dong, S., Qu, X., Li, W., Zhong, X., Li, P., Yang, S., Chen, X., Shao, M., and Zhang, L. (2015). The long non-coding RNA, GAS5, enhances gefitinib-induced cell death in innate EGFR tyrosine kinase inhibitor-resistant lung adenocarcinoma cells with wide-type EGFR via downregulation of the IGF-1R expression. J. Hematol. Oncol. 8, 43.

21. Lucafò, M., Bravin, V., Tommasini, A., Martelossi, S., Rabach, I., Ventura, A., Decorti, G., and De Iudicibus, S. (2016). Differential expression of GAS5 in rapamycin-induced reversion of glucocorticoid resistance. Clin. Exp. Pharmacol. Physiol. 43, 602–605.

22. Yacqub-Usman, K., Pickard, M.R., and Williams, G.T. (2015). Reciprocal regulation of GAS5 lncRNA levels and mTOR inhibitor action in prostate cancer cells. Prostate 75, 693–705.

23. Mourtada-Maarabouni, M., and Williams, G.T. (2014). Role of GAS5 noncoding RNA in mediating the effects of rapamycin and its analogues on mantle cell lymphoma cells. Clin. Lymphoma Myeloma Leuk. 14, 468–473.

24. Kang, C.G., Im, E., Lee, H.J., and Lee, E.O. (2017). Plumbagin reduces osteopontin-induced invasion through inhibiting the Rho-associated kinase signaling pathway in A549 cells and suppresses osteopontin-induced lung metastasis in BalB/c mice. Bioorg. Med. Chem. Lett. 27, 1914–1918.

25. Jorgensen, E., Stinson, A., Shan, L., Yang, J., Gietl, D., and Albino, A.P. (2008). Cigarette smoke induces endoplasmic reticulum stress and the unfolded protein response in normal and malignant human lung cells. BMC Cancer 8, 229.

26. Muller, C., Bailly, J.D., Goubin, F., Laredo, J., Jaffrézou, J.P., Bordier, C., and Laurent, G. (1994). Verapamil decreases P-glycoprotein expression in multidrug-resistant human leukemic cell lines. Int. J. Cancer 56, 749–754.

27. Brózik, A., Hegedüs, C., Erdei, Z., Hegedus, T., Özvegy-Laczka, C., Szakács, G., and Sarkadi, B. (2011). Tyrosine kinase inhibitors as modulators of ATP binding cassette multidrug transporters: substrates, chemosensitizers or inducers of acquired multidrug resistance? Expert Opin. Drug Metab. Toxicol. 7, 623–642.

28. Andrade, C.H., Pasqualoto, K.F., Ferreira, E.I., and Hopfinger, A.J. (2010). 4D-QSAR: perspectives in drug design. Molecules 15, 3281–3294.

29. Chen, X., and Reynolds, C.H. (2002). Performance of similarity measures in 2D fragment-based similarity searching: comparison of structural descriptors and similarity coefficients. J. Chem. Inf. Comput. Sci. 42, 1407–1414.

30. Gromicho, M., Magalhães, M., Torres, F., Dinis, J., Fernandes, A.R., Rendeiro, P., Tavares, P., Laires, A., Rueff, J., and Sebastião Rodrigues, A. (2013). Instability of mRNA expression signatures of drug transporters in chronic myeloid leukemia patients resistant to imatinib. Oncol. Rep. 29, 741–750.

31. Gromicho, M., Dinis, J., Magalhães, M., Fernandes, A.R., Tavares, P., Laires, A., Rueff, J., and Rodrigues, A.S. (2011). Development of imatinib and dasatinib resistance: dynamics of expression of drug transporters ABCB1, ABCC1, ABCG2, MVP, and SLC22A1. Leuk. Lymphoma 52, 1980–1990.

32. Kliiger, J., and Gorbaty, M. (2017). Metastasis to the pancreas and stomach from a breast cancer primary: a case report. J. Community Hosp. Intern. Med. Perspect. 7, 234–237.

33. Bonapasta, S.A., Gregori, M., Lanza, R., Sangiorgi, E., Menghi, A., Scarpini, M., and Modesti, M. (2010). Metastasis to the Pancreas from Breast Cancer: Difficulties in Diagnosis and Controversies in Treatment. Breast Care (Basel) 5, 170–173.

34. Fang, L.T., Lee, S., Choi, H., Kim, H.K., Jew, G., Kang, H.C., Chen, L., Jablons, D., and Kim, I.J. (2014). Comprehensive genomic analyses of a metastatic colon cancer to the lung by whole exome sequencing and gene expression analysis. Int. J. Oncol. 44, 211–221.

35. Riba, A., Bosia, C., El Baroudi, M., Ollino, L., and Caselle, M. (2014). A combination of transcriptional and microRNA regulation improves the stability of the relative concentrations of target genes. PLoS Comput. Biol. 10, e1003490.

36. Pickard, M.R., Mourtada-Maarabouni, M., and Williams, G.T. (2013). Long non-coding RNA GAS5 regulates apoptosis in prostate cancer cell lines. Biochim. Biophys. Acta 1832, 1613–1623.

37. Salas, S., Jézéquel, P., Campion, L., Deville, J.L., Chibon, F., Bartoli, C., Gentet, J.C., Charbonnel, C., Gouraud, W., Voutsinos-Porche, B., et al. (2009). Molecular characterization of the response to chemotherapy in conventional osteosarcomas: predictive value of HSD17B10 and IFITM2. Int. J. Cancer 125, 851–860.

38. Saito, Y., Nakagawa, T., Kakihana, A., Nakamura, Y., Nabika, T., Kasai, M., Takamori, M., Yamagishi, N., Kuga, T., Hatayama, T., and Nakayama, Y. (2016). Yeast Two-Hybrid and One-Hybrid Screenings Identify Regulators of hsp70 Gene Expression. J. Cell. Biochem. 117, 2109–2117.

39. Li, J., Han, L., Roebuck, P., Diao, L., Liu, L., Yuan, Y., Weinstein, J.N., and Liang, H. (2015). TANRIC: An Interactive Open Platform to Explore the Function of lncRNAs in Cancer. Cancer Res. 75, 3728–3737.

40. Marum, L. (2012). Cancer Cell Line Encyclopedia launched by Novartis and Broad Institute. Future Med. Chem. 4, 947.

41. Paraskevopoulou, M.D., Georgakilas, G., Kostoulas, N., Reczko, M., Maragkakis, M., Dalamagas, T.M., and Hatzigeorgiou, A.G. (2013). DIANA-LncBase: experimentally verified and computationally predicted microRNA targets on long non-coding RNAs. Nucleic Acids Res. 41, D239–D245.

42. Hsu, S.D., Lin, F.M., Wu, W.Y., Liang, C., Huang, W.C., Chan, W.L., Tsai, W.T., Chen, G.Z., Lee, C.J., Chiu, C.M., et al. (2011). miRTarBase: a database curates experimentally validated microRNA-target interactions. Nucleic Acids Res. 39, D163–D169.

43. Yang, W., Soares, J., Greninger, P., Edelman, E.J., Lightfoot, H., Forbes, S., Bindal, N., Beare, D., Smith, J.A., Thompson, I.R., et al. (2013). Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. Nucleic Acids Res. 41, D955–D961.

44. Xu, J., Li, Y., Lu, J., Pan, T., Ding, N., Wang, Z., Shao, T., Zhang, J., Wang, L., and Li, X. (2015). The mRNA related ceRNA-ceRNA landscape and significance across 20 major cancer types. Nucleic Acids Res. 43, 8169–8182.

45. Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504.

46. Nepusz, T., Yu, H., and Paccanaro, A. (2012). Detecting overlapping protein complexes in protein-protein interaction networks. Nat. Methods 9, 471–472.

47. Ideker, T., Ozier, O., Schwikowski, B., and Siegel, A.F. (2002). Discovering regulatory and signalling circuits in molecular interaction networks. Bioinformatics 18 (Suppl 1), S233–S240.

48. Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44–57.

49. Alam, T., Uludag, M., Essack, M., Salhi, A., Ashoor, H., Hanks, J.B., Kapfer, C., Mineta, K., Gojobori, T., and Bajic, V.B. (2017). FARNA: knowledgebase of inferred functions of non-coding RNA transcripts. Nucleic Acids Res. 45, 2838–2848.