NR2C Subfamily as a Potential Prognostic Biomarker for Hormone Receptor-Positive Breast Cancer

Xiaofen Li
Fujian Medical University Union Hospital
Kaiyan Huang
Fujian Medical University Union Hospital
Wenfen Fu
Fujian Medical University Union Hospital
Yushan Liu
Fujian Medical University Union Hospital
Yuxiang Lin
Fujian Medical University Union Hospital
Jie Zhang
Fujian Medical University Union Hospital
Chuangui Song (Songcg1971@hotmail.com)
Fujian Medical University Union Hospital https://orcid.org/0000-0001-5703-9123

Research Article

Keywords: Hormone receptor-positive breast cancer, NR2C1, NR2C2, Prognosis, Biomarker

Posted Date: September 3rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-862497/v1

NR2C subfamily as a potential prognostic biomarker for hormone receptor-positive breast cancer

Xiao-Fen Li[1,3], Kai-Yan Huang[1,3], Wen-Fen Fu[1], Yu-Shan Liu[1], Yu-Xiang Lin[1,2], Jie Zhang[1,2*] and Chuan-Gui Song[1,2*]

[1] Department of Breast Surgery, Fujian Medical University Union Hospital, Fuzhou, Fujian Province, 350001, China；
[2] Department of General Surgery, Fujian Medical University Union Hospital, Fuzhou, Fujian Province, 350001, China

[3] These authors contributed equally

[*] Corresponding authors: Jie Zhang: zjie1979@gmail.com; Chuan-Gui Song: songchuangui@yahoo.com

Summary

The overall outcome of hormone receptor-positive (HR+) breast cancer is relatively good, but it still faces the dilemmas of drug resistance and long-term recurrence. The NR2C subfamily are nuclear receptors like ER, PR and AR. However, the prognostic value and molecular mechanism of NR2Cs remain unclear. In our study, we identified the association between expression of NR2C1 or NR2C2 and survival of BRCA patients using Kaplan-Meier plotter. Next, miRNAs-NR2Cs regulatory networks were predicted by ENCORI. Furthermore, functional enrichment analysis was performed via PPI networks and KEGG pathways. Finally, CellMiner was used to obtain the correlation analysis of NR2Cs expression and drug sensitivity. We found that both NR2C1 and NR2C2 were significantly down-regulated in BRCA compared to normal tissues. Survival difference between high and low NR2C1 group only observed in luminal B, while high expression levels of NR2C2 were significantly associated with good prognosis in luminal A, luminal B, LAR and ER+ breast cancer. Moreover, only NR2C1-has-miR-196a network was related to the prognosis of BRCA. For NR2C2, there were thirteen miRNAs of low expression associating with good prognosis, such as hsa-miR-21, hsa-miR-656, hsa-miR-103a. The main biological functions of NR2Cs were metabolic pathways, drug metabolism, steroid hormone biosynthesis, and so on. Altogether, NR2C1 and NR2C2, especially the latter, might be novel important prognostic factors like ER/PR/AR in HR+ breast cancer.

Keywords Hormone receptor-positive breast cancer • NR2C1 • NR2C2 • Prognosis • Biomarker

Declarations Not applicable.

Funding This research was funded by Fujian Young Teacher Education Research Project, Fujian Province (JAT170228).

Conflicts of Interest The authors declare no conflict of interest.

Authors' contributions Xiaofen Li, Jie Zhang and Chuangui Song designed the study. Xiaofen Li, Kaiyan Huang and Wenfen Fu collected and analyzed the data. Xiaofen Li wrote the main manuscript text. All authors contributed to the revision of the manuscript and approved the final version.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

Consent for publication All authors consent to the publication of this study.

Introduction

Hormone receptor-positive (HR+) breast cancer is a special type of breast cancer which can express a group of hormone receptors that are closely related to prognosis, such as: estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR) and so on. In general, luminal (ER-positive or PR-positive) breast cancer has a significant better prognosis than non-luminal [1]. However, long-term follow-up has shown that one out of four patients with luminal breast cancer are prone to relapse during or after five-year endocrine therapy [2]. In addition, Loibl S, Muller BM et al. showed that AR-positive breast cancer was associated with a better prognosis compared to AR-negative breast cancer [3]. HR+ breast cancer is heterogeneous, and further distinction of patient's prognosis is needed to determine whether appropriate intervention or continue follow-up is required. However, there are few prognostic biomarkers for HR+ breast cancer.

ERα (NR3A1) / ERβ (NR3A2) / PR (NR3C3) / AR (NR3C4) all belong to the nuclear receptors (NRs) which are a family of ligand-dependent transcription factors that mediate various biological processes, such as proliferation, apoptosis, metabolism and immunity. The NRs are typically divided into three categories :(1) Steroid hormone receptors, such as AR, ER, PR and glucocorticoid receptor (GR); (2) Nonsteroidal hormone receptors, including thyroid hormone receptor (TR), retinoic acid receptor (RAR), vitamin D receptor (VDR); (3) Orphan nucleus receptors (ONRs), including nuclear receptor subfamily 2, group C, member 1 and member 2 (NR2C1 and NR2C2), and so on [4].

As a unique subfamily of NRs, ONRs, which are initially identified without known cognate ligand, act through homodimerization or heterodimerization and then bind to specific DNA sequences called hormone response elements (HREs) to regulate transcription. Although not widely reported, certain ONRs have been shown to have prognostic significance for breast cancer [5,6]. For example, NR2F2 (COUP-TFII) is associated with better survival of luminal A breast cancer and tamoxifen-treated ER+ breast cancer patients. Moreover, NR2F2 plays a key role in ERα-mediated transcription [5]. Like NR2F2, NR2C1 or NR2C2 can also block ER-ER binding by forming an ER-NR2C1/NR2C2 heterodimer to inhibit ER-mediated transcription instead of binding to the HREs [6,7]. It happens that there is a similar case that the heterodimerization of AR and NR2C2 is able to repress AR target gene expression [8].

NR2C1 and NR2C2, also known as testicular receptor 2 and 4 (TR2 and TR4), were first cloned in 1989 and 1994, respectively. The sequence comparison between NR2C1 and NR2C2 shows that they have a homology of 65% in the whole structure, 51% in the N-terminal, 82% in the DNA-binding domain (DBD) and 65% in the ligand binding domain (LBD). What's more, they also share a certain degree of structural identity with other members of NRs, such as ER, PR, AR and GR. NR2C1 and NR2C2 are expressed in a verity of tissues, particularly in testis, prostate, kidney and skeletal muscle. As important physiological regulators, NR2Cs have been reported to involve in fertility, sugar and lipid metabolism, insulin resistance, osteogenesis and tumorigenesis [9,10]. A study reported that NR2C2 expression was significantly associated with adverse clinicopathological features of non-small cell lung cancer (NSCLC) [11]. Other studies have shown that both NR2C1 and NR2C2 are capable of controlling the progression of prostate cancer [12].

The role of steroid nuclear receptor superfamily including ER, PR and AR in regulating the growth of breast tumors has been well established previously, while the clinical significance of NR2C1 and NR2C2 in breast cancer especially HR+ breast cancer is poorly understood. Therefore, the focus of this study was to explore the expression pattern and prognostic value of NR2C subfamily in HR+ breast cancer, and then identify their potential molecular mechanism and therapeutic targets based on a series of bioinformatics analyses.

Materials and methods

TCGA database

NR2C1 and NR2C2 expression data were extracted from BRCA datasets of The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/).

TIMER database

The mRNA expression of NR2C1 or NR2C2 in different types of cancer, including BRCA, were analyzed via TIMER database (https://cistrome.shinyapps.io/timer/) [13].

UALCAN database

The analysis of NR2C1 and NR2C2 expression across normal and tumor samples in various subgroups based on cancer stages, subclasses or other clinicopathological features was performed using UALCAN (http://ualcan.path.uab.edu) [14].

Kaplan–Meier plotter database

Kaplan–Meier plotter (http://kmplot.com/analysis/) was utilized for performing the analysis of the potential prognostic significance of NR2Cs in HR+ breast cancer, as well as relevant miRNAs in BRCA. Relapse-free survival (RFS) and overall survival (OS) were mainly used to plot the survival curves of patients. [15].

PPI networks

BioGRID (https://thebiogrid.org) was used to identify the known protein interaction networks of NR2Cs [16]. Then, STRING network was further applied for a PPI network construction of NR2Cs, including direct (physical) as well as indirect (functional) interactions [17]. Confidence score$\geqslant$0.4 was considered as significant.

DAVID

KEGG pathway analysis was downloaded from the Database for DAVID v6.8 (https://david.ncifcrf.gov/home.jsp).

ENCORI database

We took advantage of ENCORI (http://starbase.sysu.edu.cn/) [18] to predict the miRNAs regulating NR2C1 or NR2C2. The Cytoscape software (version 3.7.2) was used for visualizing the NR2Cs-targeted miRNAs regulatory networks [19].

CellMiner database

Gene expression and drug sensitivity data for NR2Cs of NCI-60 cancer cell lines were obtained from CellMiner database (http://discover.nci.nih.gov/cellminer) [20].

Statistical analysis

All analysis was performed using R Studio software (version 3.6.3), ggplot2 package (version 3.3.3) for visualization. For paired observation, Wilcoxon signed rank test was performed. Spearman's test was used to describe the correlation analysis. Significance was statistically considered at ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$ and ns, $p>=0.05$.

Results

Expression levels of NR2Cs in breast cancer

To investigate the possible role of NR2Cs in BRCA, based on TIMER website and TCGA database, we found that both NR2C1 and NR2C2 were significantly down-regulated ($p<0.001$) in BRCA compared with normal tissues (Fig.

1a-b). Moreover, the expression levels of NR2Cs in 112 paired BRCA samples from TCGA cohort were consistent with the above-mentioned results. Then, to determine the diagnostic value of NR2Cs in BRCA, ROC curves were constructed. As displayed in Fig. 1c, the area under ROC curve of NR2C1 was larger than that of NR2C2 not only in BRCA but also in luminal breast cancer. We further assessed the performance of the NR2Cs model. And from Fig. 1d, the AUC of the combined diagnosis in BRCA was 0.848, while in luminal breast cancer was 0.824.

Fig.1 NR2C1 and NR2C2 expression levels in BRCA. **a-b** NR2C1 and NR2C2 expression were down-regulated in BRCA from the TIMER and TCGA database. **c** the ROC curves of NR2Cs in BRCA and luminal breast cancer. **d** the ROC curves of NR2Cs model in BRCA and luminal breast cancer.

The association of NR2Cs expression and clinical pathological features of patients with breast cancer

In the UALCAN database, we investigated various clinicopathological characteristics of TCGA-BRCA samples. Compared with normal controls, the expression levels of NR2C1 and NR2C2 were significantly lower in BRCA patients at any age, stages (1-4), nodal metastasis(N0-N3), tumor histology, subclasses, menopause status and TP53 mutation status (Fig. 2). Metaplastic breast carcinoma (MBC) is a unique histological subtype of breast cancer, which is clinically more aggressive than TNBC [21]. The expression of NR2Cs in MBC was relatively lowest of all although not statistically significant. There were no differences in three main subclasses of BRCA, however, statistically significant difference of NR2C1 expression levels was observed between luminal subtype and LAR subtype. Among all BRCA subtypes, the expression levels of NR2C1 and NR2C2 were the lowest in LAR and relatively high in luminal, but the differences were not statistically significant.

Fig.2 NR2C1 and NR2C2 expression in BRCA based on clinicopathological characteristics. **a-b** the expression levels of NR2C1 and NR2C2 were significantly lower in BRCA patients at age, stages, nodal metastasis, tumor histology, subclasses, menopause status and TP53 mutation status than normal controls.

The potential prognostic values of NR2Cs in HR+ breast cancer

By the Kaplan–Meier plotter database, the association of NR2C1 or NR2C2 expression with relapse-free survival (RFS) and overall survival (OS) in BRCA patients was identified. As shown in Fig 3a-b, NR2C1 overexpression was significantly associated with good prognosis of OS ($p = 0.041$) but not with RFS. Both RFS and OS of BRCA patients with high NR2C2 were significantly higher than that of patients with low expression. For hormone receptor positive-breast cancer, such as luminal A subtype, luminal B subtype and luminal androgen receptor (LAR) subtype, there were significant differences between good survival and high NR2C2 expression (Fig. 3c-d), while high levels of NR2C1 were significantly correlated with favorable outcome only in luminal B subtype. ER and PR as important nuclear receptors in HR+ breast cancer, the data indicated that high expression of NR2C2 was associated with good prognosis in ER+ breast cancer, but not in ER- breast cancer. However, no significant correlation was observed between NR2C2 expression and RFS rate in PR+ breast cancer (Fig. 3d). Taken together, these findings indicated that NR2Cs especially NR2C2 were potential prognostic factors for predicting the survival of HR+ breast cancer.

Fig.3 Kaplan–Meier survival analysis of NR2Cs in BRCA patients. **a-b** the correlation of NR2C1 expression and RFS/OS in BRCA, luminal A, luminal B, LAR subtype. **c-d** the correlation of NR2C2 expression and RFS/OS in BRCA, luminal A, luminal B, LAR subtype. **e** the association of NR2C2 expression and RFS in ER+, ER-, PR+, PR- breast cancer.

MiRNAs-NR2Cs regulatory network and survival analysis

As we all known, the differentially expressed genes with NR2Cs^{high} and NR2Cs^{low} expression groups are differ from those in cancer and para-cancerous groups in BRCA samples. We identified the DEmRNAs and DEmiRNAs with NR2Cs^{high} and NR2Cs^{low} expression groups by BRCA-TCGA database, with $p<0.05$ and |log fold change [FC]| >0.5 as the threshold. Volcano plots visually displayed the distribution of DEmiRNAs (Fig. 4a-b). Additionally, the heatmaps depicted the correlation between the first 15 important miRNAs and NR2Cs expression levels in the BRCA samples. MiR-186, mir-590 and mir-5581 were the top three miRNAs most related to NR2C1 and NR2C2 (Fig. 4c-d). MiRNAs are endogenous, small, non-coding RNAs that negatively regulate mRNA by binding to specific target sites at the posttranscriptional level. Based on a wide range of studies, miRNAs can function as potential diagnostic, prognostic, and predictive biomarkers in cancers including BRCA [22,23]. Though the ENCORI database, a total of eight miRNAs targeting NR2C1 and twenty-eight miRNAs targeting NR2C2 which significantly upregulated in BRCA were found ($p<0.05$) (Supplementary Table 1, 2). Next, we determined the correlation of OS and expression levels of these relevant miRNAs in BRCA using TCGA data by the Kaplan-Meier plotter (Supplementary Table 3, 4). Fig 4e showed that hsa-miR-196a was the only miRNA targeting NR2C1, whose expression was significantly correlated with OS. As for NR2C2, there were thirteen miRNAs with low expression associated with better prognosis, such as hsa-miR-137, hsa-miR-301b, hsa-miR-656, etc. The miRNAs-NR2Cs regulatory networks were established using Cytoscape software, which were expected to become prognostic biomarkers and therapeutic targets in the future.

Fig.4 The miRNAs-NR2Cs regulatory networks in BRCA. **a-b** volcano plots of the distribution of DEmiRNAs. **c-d** heatmaps of the correlation between the first 15 important miRNAs and NR2Cs expression. **e-f** the miRNAs-NR2Cs regulatory networks. The blue oval icons indicated that these miRNAs of low expression were associated with good prognosis in BRCA. **g-j** the Kaplan-Meier survival curves of relevant miRNAs which targeting NR2C1 or NR2C2.

Function enrichment analysis of NR2Cs

We respectively retrieved the protein interaction data of NR2C1 or NR2C2 from BioGRID database. As shown in Fig 5a-b, we found that besides for NR2C1, ESR1 and AR were also the relevant proteins to NR2C2, which could also be observed in NR2C1. Then a protein-protein interaction (PPI) network of NR2C1 and NR2C2 was constructed in the STRING database, and the most enrichment clusters was type II transforming growth factor beta (TGFB) receptor binding (Fig. 5c). To further explore the potential mechanism of NR2C subfamily, KEGG pathway enrichment analysis was performed. The results showed that the DEmRNAs of NR2C1 were particularly enriched in the "neuroactive ligand-receptor interaction," and "metabolic pathways", while the enrichment term related to NR2C2 were the "metabolic pathways" and "ribosome". (Fig. 5d-e). Although the number of enriched genes was not exactly the same, there was a lot of overlap of enrichment pathways.

Fig.5 Function enrichment analysis of NR2Cs. **a-b** the protein interaction of NR2C1 or NR2C2 from BioGRID database. **c** PPI network of NR2C1 and NR2C2 constructed in the STRING database. **d-e** KEGG pathway enrichment analysis of NR2C1and NR2C2 from DAVID.

The correlation of NR2Cs expression levels and drug sensitivity.

To further confirm whether NR2Cs can be a new therapy target in cancer, we explored the correlation of NR2Cs expression levels and drug sensitivity (IC50 values) in NCI-60 cancer cell line including breast cancer cell lines.

From Fig. 6a-b, we found a moderate negative correlation between NR2C1 expression and IC50 value of Okadaic acid, Vinorelbine and ICG-001. Additionally, high expression of NR2C1 was associated with low IC50 value of Okadaic acid, while high NR2C1 expression levels were connected with high IC50 value of ST-3595, 3-Bromopyruvate (acid), 8-Chloro-adenosine and RH1. Interestingly, both NR2C1 and NR2C2 expression levels in NCI-60 cell lines may correlated to sensitivity to ZM-336372 treatment (Fig. 6c).

Fig.6 The correlation of NR2Cs expression levels and drug sensitivity in NCI-60 cancer cell line. **a-b** the correlation of NR2C1 expression and IC50 values of eight drugs. **c** only ZM-336372 treatment may be associated with NR2C2.

Discussion

Hormone receptor-positive breast cancer accounts for more than 60% of all. A review lists published DNA methylation prognostic biomarkers for HR+ breast cancer [24]. Qin Huo et al. reported that high SIRT7 expression was associated with poor prognosis in luminal breast cancer [25]. In addition to these studies, there is limited information on prognostic biomarkers in HR+ breast cancer. Endocrine therapy which mainly by inhibiting the estrogen pathway plays an important role in the treatment of luminal breast cancer, significantly improving the outcome of patients. However, now the challenges of endocrine resistance and late recurrence are emerging continuously, caused the public gaze. More and more researchers are focusing their attention on other pathways and targeted drugs including mTOR pathway and CDK4/6 inhibitors [26]. New prognostic biomarkers and more effective therapeutic approaches are urgently required in HR+ breast cancer.

As members of nuclear receptor family, NR2C1 and NR2C2 share a common structure with ER, PR, and AR. Dysregulation of NR2C1 and NR2C2 has been reported in many cancers, for example, the expression of NR2C2 is higher in hepatocellular carcinoma than in normal tissue, while both NR2C1 and NR2C2 are down-regulated in BRCA. From the ROC analysis (Fig. 1), we found that the diagnostic value of NR2C1 is higher than NR2C2 in both BRCA and luminal breast cancer. Moreover, the combined diagnostic value of NR2Cs was better in BRCA than in luminal. Interestingly, based on TP53-mutation and TP53-non mutation, it's suggested that TP53 mutation might be associated with the abnormal NR2C1 expression in BRCA (Fig. 2). From the Fig 2c, the expression of NR2C2 in BRCA patients with N3 was lower than that of N2, probably indicating that low expression levels of NR2C2 resulted in high invasiveness in BRCA. Similarly, mRNA levels of NR2Cs in MBC which was more aggressive than TNBC were lower than others (Fig. 2). As one of the most common BRCA subtypes, the luminal subtype has a relatively good prognosis. In a sense, the high expression of NR2Cs suggested its possible tumor suppressor effect in luminal breast cancer.

Here our findings indicated that NR2Cs could be a potential indicator for prognosis in HR+ breast cancer. These results enriched current understanding and might contribute to increasing the accuracy of survival prediction and improving the treatment regimens. Our study suggested that high expression of NR2C2 was associated with good prognosis in ER+ breast cancer, but not in ER- breast cancer (Fig. 3e). Previous studies have demonstrated that the inhibition of ER transcription by NR2C1 and NR2C2 results in the suppression of estrogen-induced cell growth in ER+ breast cancer, which could explain why upregulated NR2C2 mRNA levels were associated with better survival in estrogen-dependent breast cancer. Similarly, the heterodimerization of AR and NR2C2 can lead to the mutual repression of transactivation of AR or NR2C2, and from our results, we found that preferable RFS and OS were well related to overexpression of NR2C2 in LAR breast cancer. However, it's reported that NR2C2 fails to suppress the PR-mediated transcription [6,7]. In this study, there was no correlation between NR2C2 expression and prognosis in PR+ or PR- breast cancer ($p>0.05$). Altogether, these findings may support us a way to regulate ER or AR function though interrupting the protein-protein binding with NR2C1 or NR2C2, and especially the latter. Any small peptides or synthetic compounds that simulate the interaction of them could have the possibility to be developed into a new therapeutic approach to better control HR+ breast cancer.

Previous studies have shown that miRNAs regulate gene expression via interacting with the 3' untranslated region (3'UTR) or the 5' promoter region of target mRNAs, then inhibiting their translation or leading to degradation [27,28]. The expression levels of miRNAs vary in cancers, mainly depending on the genes or pathways that regulate them. In our study, we observed that the potential regulatory miRNAs targeting NR2C1 or NR2C2 all meet the following two criteria, increased expression and poor prognosis in BRCA patients. A growing body of evidence indicates that the miRNAs play a vital role in a variety of biological processes in cancer including proliferation, invasion and migration. A study reported that NR2C2 promoted the clear cell renal cell carcinoma (ccRCC) invasion/migration via altering the miR-32-5p/TR4/HGF/Met/MMP2-MMP9 signaling [29]. We found here that miR-32-5p might also be able to alter the NR2C2 expression (Fig. 4e), although the association of its expression and prognosis in BRCA was not significant. The further study needed to be carried out to evaluate the potential role of NR2C2 in BRCA especially HR+ breast cancer. MiRNA-196-5p was the only predictive miRNA targeting NR2C1, whose low expression was correlated with better prognosis in BRCA patients. There is no idea about the mechanism of miRNA-196-5p influencing breast cancer cell via regulating the miR-196-5p/NR2C1 pathway, which remains to be more identified.

As shown in Fig.5, the KEGG pathway enrichment analysis of NR2Cs uncovered their main biological functions in metabolic pathways, drug metabolism, steroid hormone biosynthesis, and so on. From Fig.6, NR2C1 expression was associated with some anti-cancer drug, such as 3-Bromopyruvate (acid), Vinorelbine and 8-Chloro-adenosine. Obviously, high expression of NR2C1 increased drug sensitivity to Okadaic acid which was inhibitor of protein serine/threonine phosphatases. Although these results could not clearly elucidate the role of NR2Cs in drug therapy, they provided some ideas for exploring the molecular mechanism in the future.

Conclusion

Based on the results of the baseline expression and survival analysis, there was no survival difference between high and low NR2C1 in various subtypes HR+ breast cancer, except luminal B, while high expression levels of NR2C2 were significantly correlated with good prognosis in luminal A, luminal B, LAR and ER+ breast cancer. In conclusion, NR2C1 and NR2C2, especially NR2C2, might act as prognostic biomarkers like ER/PR/AR in hormone receptor-positive breast cancer.

Acknowledgments This study was supported by the fund of Fujian Young Teacher Education Research Project, Fujian Province (JAT170228). The authors acknowledge the comments of the reviewers that helped to improve the study.

References

1. Hennigs A et al. (2016) Prognosis of breast cancer molecular subtypes in routine clinical care: A large prospective cohort study BMC Cancer 16:734 doi:10.1186/s12885-016-2766-3
2. (EBCTCG) EBCTCG (2005) Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials The Lancet 365:1687-1717 doi:10.1016/s0140-6736(05)66544-0
3. Loibl S et al. (2011) Androgen receptor expression in primary breast cancer and its predictive and prognostic value in patients treated with neoadjuvant chemotherapy Breast Cancer Res Treat 130:477-487 doi:10.1007/s10549-011-1715-8
4. Bain DL, Heneghan AF, Connaghan-Jones KD, Miura MT (2007) Nuclear receptor structure: implications for function Annu Rev Physiol 69:201-220 doi:10.1146/annurev.physiol.69.031905.160308
5. Erdos E, Balint BL (2020) NR2F2 Orphan Nuclear Receptor is Involved in Estrogen Receptor Alpha-Mediated Transcriptional Regulation in Luminal A Breast Cancer Cells Int J Mol Sci 21 doi:10.3390/ijms21061910
6. Hu YC, Shyr CR, Che W, Mu XM, Kim E, Chang C (2002) Suppression of estrogen receptor-mediated transcription and cell growth by interaction with TR2 orphan receptor J Biol Chem 277:33571-33579 doi:10.1074/jbc.M203531200

7. Shyr CR, Hu YC, Kim E, Chang C (2002) Modulation of estrogen receptor-mediated transactivation by orphan receptor TR4 in MCF-7 cells J Biol Chem 277:14622-14628 doi:10.1074/jbc.M110051200
8. Yi-Fen Lee C-RS, Tin Htwe Thin, Wen-Jye Lin, and Chawnshang Chang (1999) Convergence of two repressors through heterodimer formation of androgen receptor and testicular orphan receptor-4: A unique signaling pathway in the steroid receptor superfamily Proceedings of the National Academy of Sciences of the United States of America 96:14724–14729 doi:10.1073/pnas.96.26.14724.
9. Lee YF, Lee HJ, Chang C (2002) Recent advances in the TR2 and TR4 orphan receptors of the nuclear receptor superfamily J Steroid Biochem Mol Biol 81:291-308 doi:10.1016/s0960-0760(02)00118-8
10. Lin SJ, Yang DR, Yang G, Lin CY, Chang HC, Li G, Chang C (2017) TR2 and TR4 Orphan Nuclear Receptors: An Overview Curr Top Dev Biol 125:357-373 doi:10.1016/bs.ctdb.2017.02.002
11. Zhang L et al. (2015) Testicular orphan receptor 4 (TR4) is a marker for metastasis and poor prognosis in non-small cell lung cancer that drives the EMT phenotype Lung Cancer 89:320-328 doi:10.1016/j.lungcan.2015.06.007
12. Wu D, Cheung A, Wang Y, Yu S, Chan FL (2016) The emerging roles of orphan nuclear receptors in prostate cancer Biochim Biophys Acta 1866:23-36 doi:10.1016/j.bbcan.2016.06.001
13. Li T et al. (2017) TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells Cancer Res 77:e108-e110 doi:10.1158/0008-5472.CAN-17-0307
14. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, Varambally S (2017) UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses Neoplasia 19:649-658 doi:10.1016/j.neo.2017.05.002
15. Lanczky A, Nagy A, Bottai G, Munkacsy G, Szabo A, Santarpia L, Gyorffy B (2016) miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients Breast Cancer Res Treat 160:439-446 doi:10.1007/s10549-016-4013-7
16. Oughtred R et al. (2021) The BioGRID database: A comprehensive biomedical resource of curated protein, genetic, and chemical interactions Protein Sci 30:187-200 doi:10.1002/pro.3978
17. Szklarczyk D et al. (2019) STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets Nucleic Acids Res 47:D607-D613 doi:10.1093/nar/gky1131
18. Li JH, Liu S, Zhou H, Qu LH, Yang JH (2014) starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data Nucleic Acids Res 42:D92-97 doi:10.1093/nar/gkt1248
19. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ (2019) Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data J Proteome Res 18:623-632 doi:10.1021/acs.jproteome.8b00702
20. Reinhold WC et al. (2012) CellMiner: a web-based suite of genomic and pharmacologic tools to explore transcript and drug patterns in the NCI-60 cell line set Cancer Res 72:3499-3511 doi:10.1158/0008-5472.CAN-12-1370
21. El Zein D, Hughes M, Kumar S, Peng X, Oyasiji T, Jabbour H, Khoury T (2017) Metaplastic Carcinoma of the Breast Is More Aggressive Than Triple-negative Breast Cancer: A Study From a Single Institution and Review of Literature Clin Breast Cancer 17:382-391 doi:10.1016/j.clbc.2017.04.009
22. Iorio MV, Croce CM (2012) MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review EMBO Mol Med 4:143-159 doi:10.1002/emmm.201100209
23. Zhang W, Liu J, Wang G (2014) The role of microRNAs in human breast cancer progression Tumour Biol 35:6235-6244 doi:10.1007/s13277-014-2202-8
24. de Ruijter TC, van der Heide F, Smits KM, Aarts MJ, van Engeland M, Heijnen VCG (2020) Prognostic DNA methylation markers for hormone receptor breast cancer: a systematic review Breast Cancer Res 22:13 doi:10.1186/s13058-020-1250-9
25. Huo Q, Li Z, Cheng L, Yang F, Xie N (2020) SIRT7 Is a Prognostic Biomarker Associated With Immune Infiltration in Luminal Breast Cancer Front Oncol 10:621 doi:10.3389/fonc.2020.00621
26. Hanker AB, Sudhan DR, Arteaga CL (2020) Overcoming Endocrine Resistance in Breast Cancer Cancer Cell 37:496-513 doi:10.1016/j.ccell.2020.03.009
27. Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, Pasquinelli AE (2005) Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation Cell 122:553-563 doi:10.1016/j.cell.2005.07.031
28. Correction for Place et al. (2018) MicroRNA-373 induces expression of genes with complementary promoter sequences Proc Natl Acad Sci U S A 115:E3325 doi:10.1073/pnas.1803343115
29. Wang M et al. (2018) Preclinical studies using miR-32-5p to suppress clear cell renal cell carcinoma metastasis via altering the miR-32-5p/TR4/HGF/Met signaling Int J Cancer 143:100-112 doi:10.1002/ijc.31289

Figures

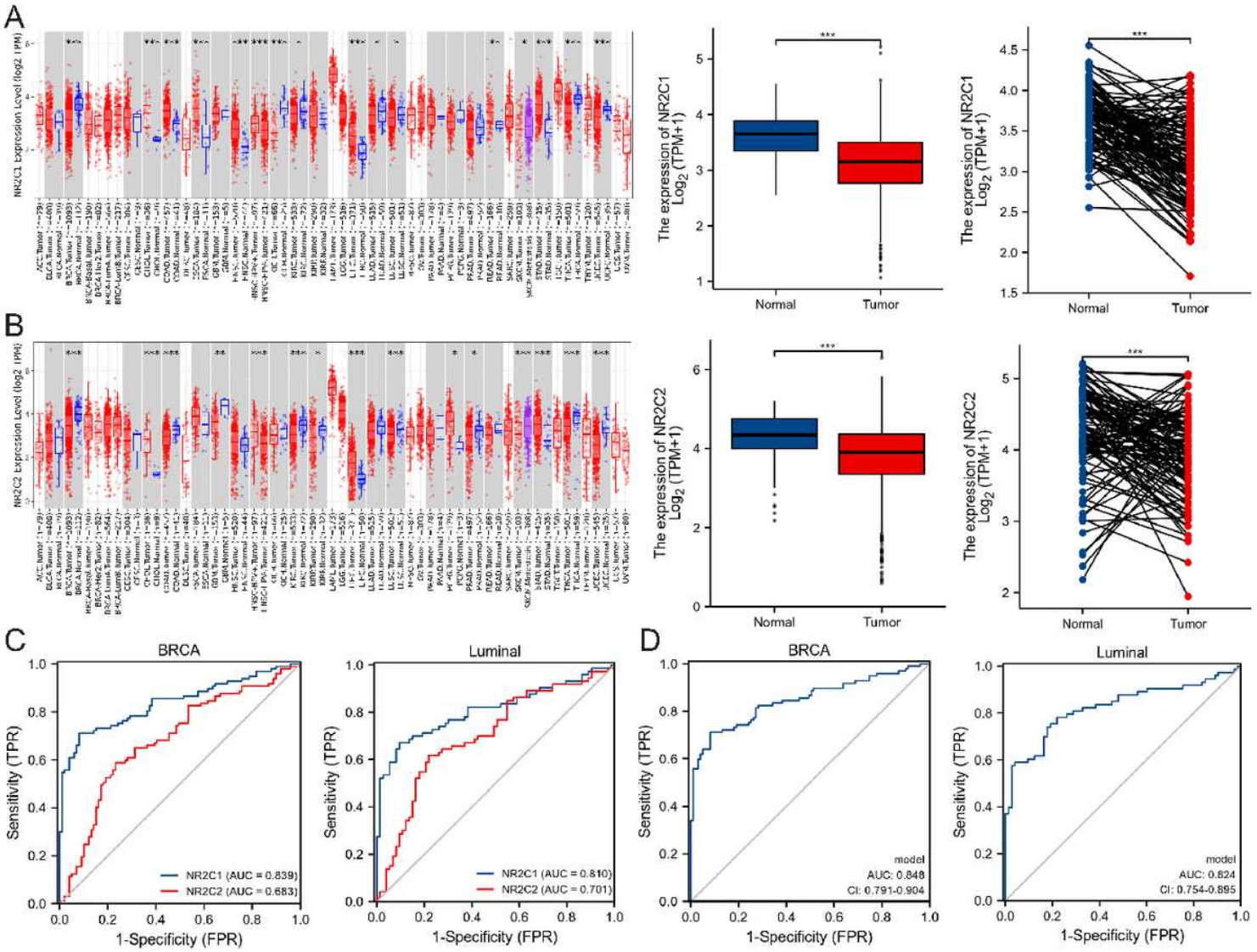

Figure 1

NR2C1 and NR2C2 expression levels in BRCA. a-b NR2C1 and NR2C2 expression were down-regulated in BRCA from the TIMER and TCGA database. c the ROC curves of NR2Cs in BRCA and luminal breast cancer. d the ROC curves of NR2Cs model in BRCA and luminal breast cancer.

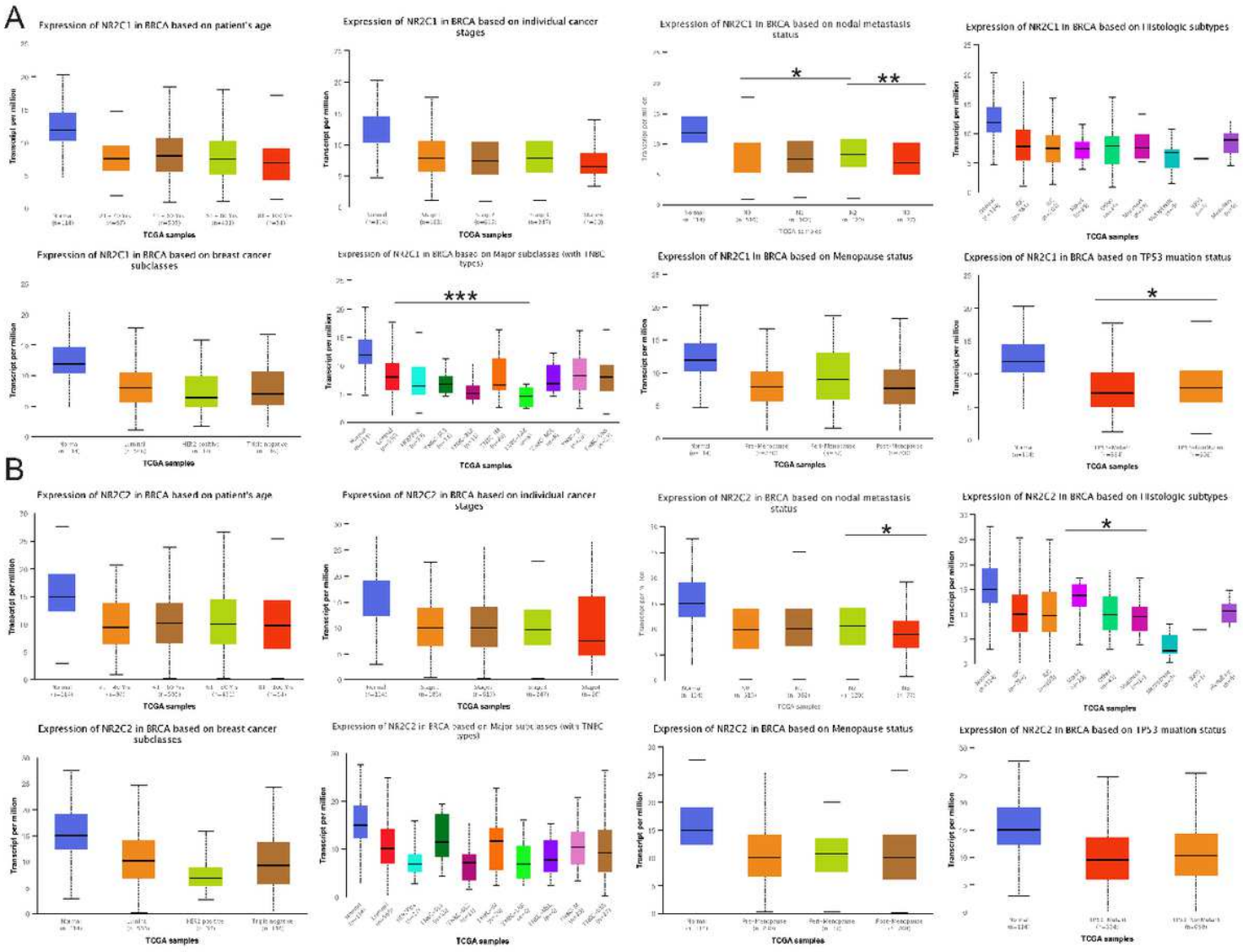

Figure 2

NR2C1 and NR2C2 expression in BRCA based on clinicopathological characteristics. a-b the expression levels of NR2C1 and NR2C2 were significantly lower in BRCA patients at age, stages, nodal metastasis, tumor histology, subclasses, menopause status and TP53 mutation status than normal controls.

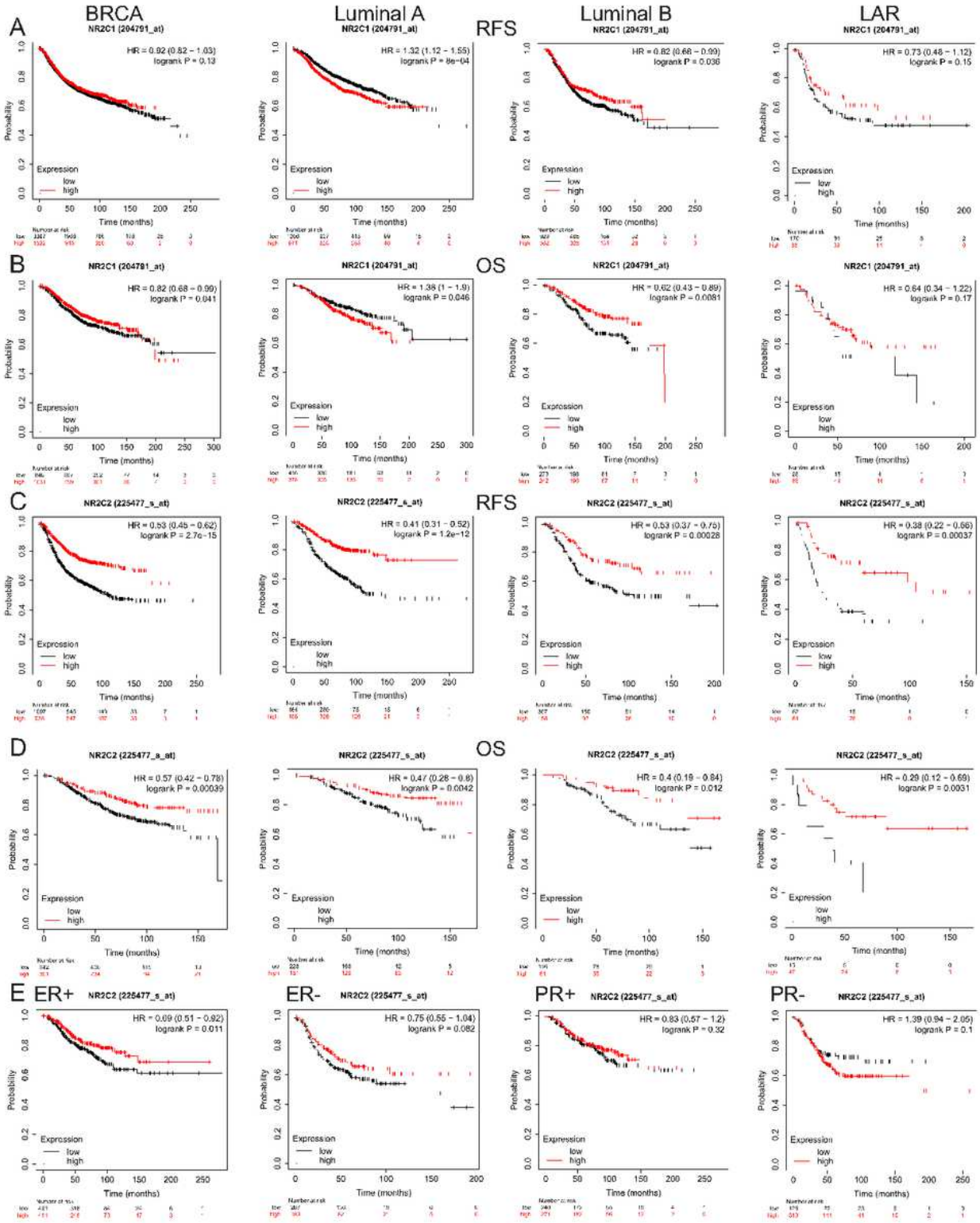

Figure 3

Kaplan–Meier survival analysis of NR2Cs in BRCA patients. a-b the correlation of NR2C1 expression and RFS/OS in BRCA, luminal A, luminal B, LAR subtype. c-d the correlation of NR2C2 expression and RFS/OS in BRCA, luminal A, luminal B, LAR subtype. e the association of NR2C2 expression and RFS in ER+, ER-, PR+, PR- breast cancer.

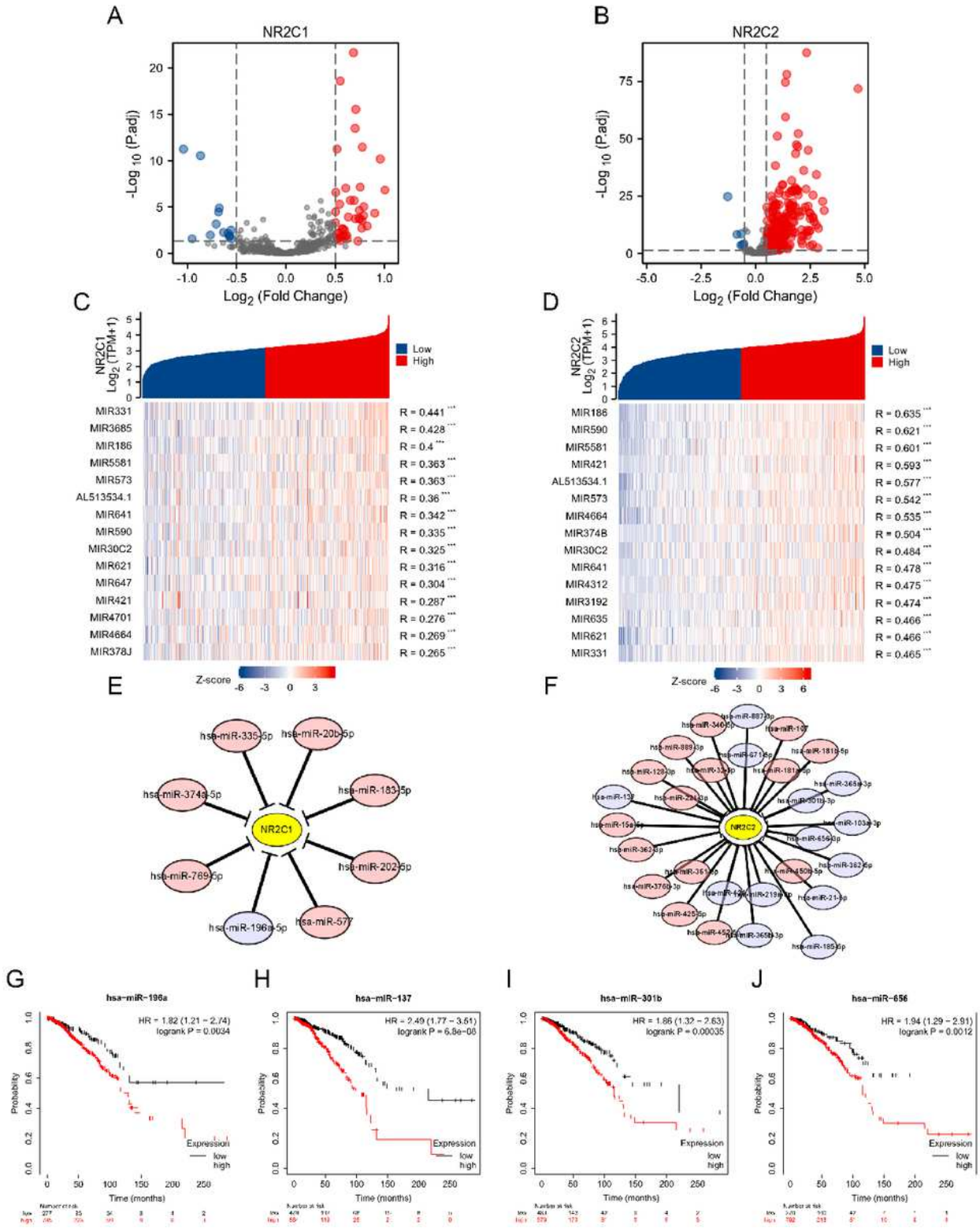

Figure 4

The miRNAs-NR2Cs regulatory networks in BRCA. a-b volcano plots of the distribution of DEmiRNAs. c-d heatmaps of the correlation between the first 15 important miRNAs and NR2Cs expression. e-f the miRNAs-NR2Cs regulatory networks. The blue oval icons indicated that these miRNAs of low expression were associated with good prognosis in BRCA. g-j the Kaplan-Meier survival curves of relevant miRNAs which targeting NR2C1 or NR2C2.

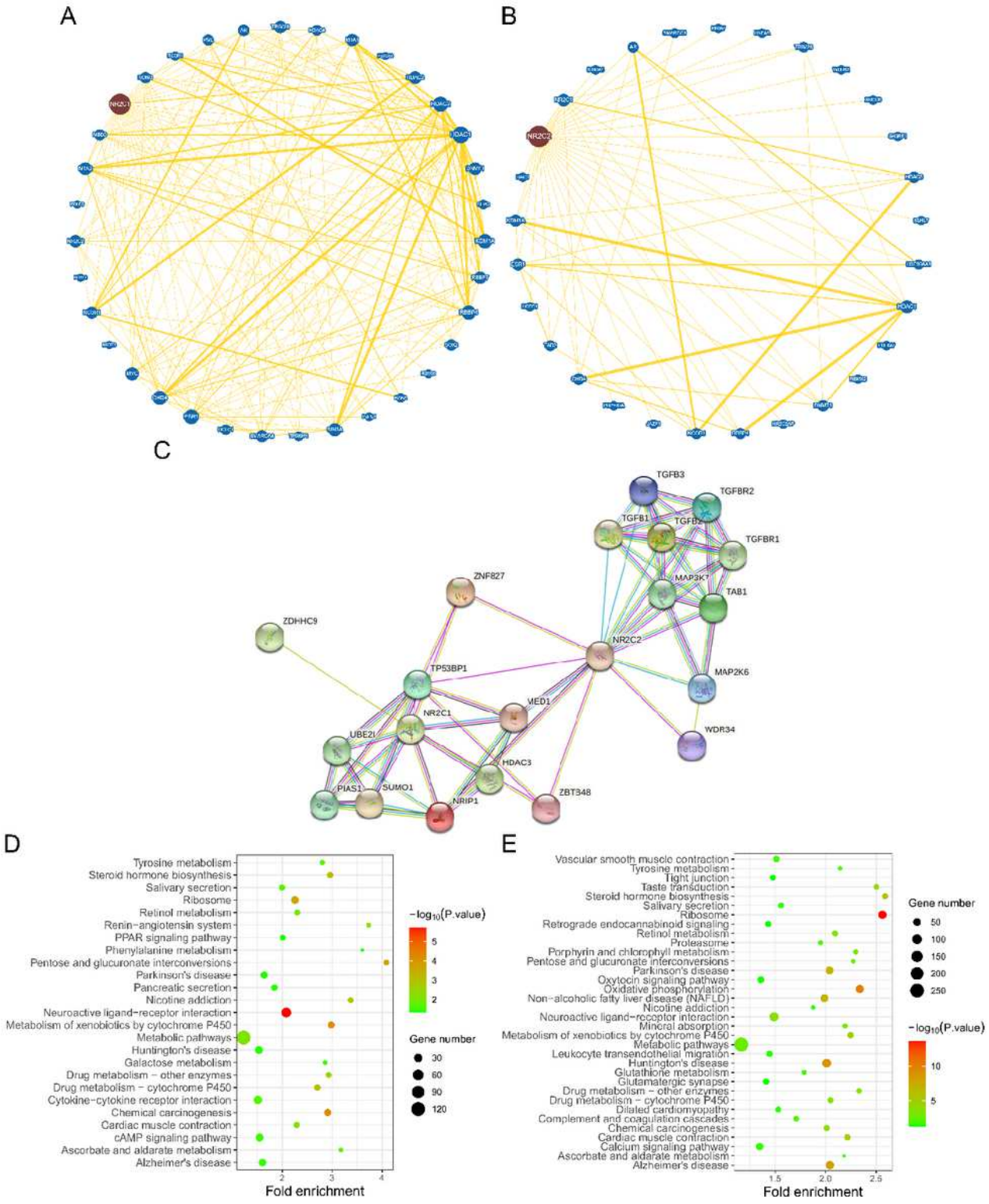

Figure 5

Function enrichment analysis of NR2Cs. a-b the protein interaction of NR2C1 or NR2C2 from BioGRID database. c PPI network of NR2C1 and NR2C2 constructed in the STRING database. d-e KEGG pathway enrichment analysis of NR2C1and NR2C2 from DAVID.

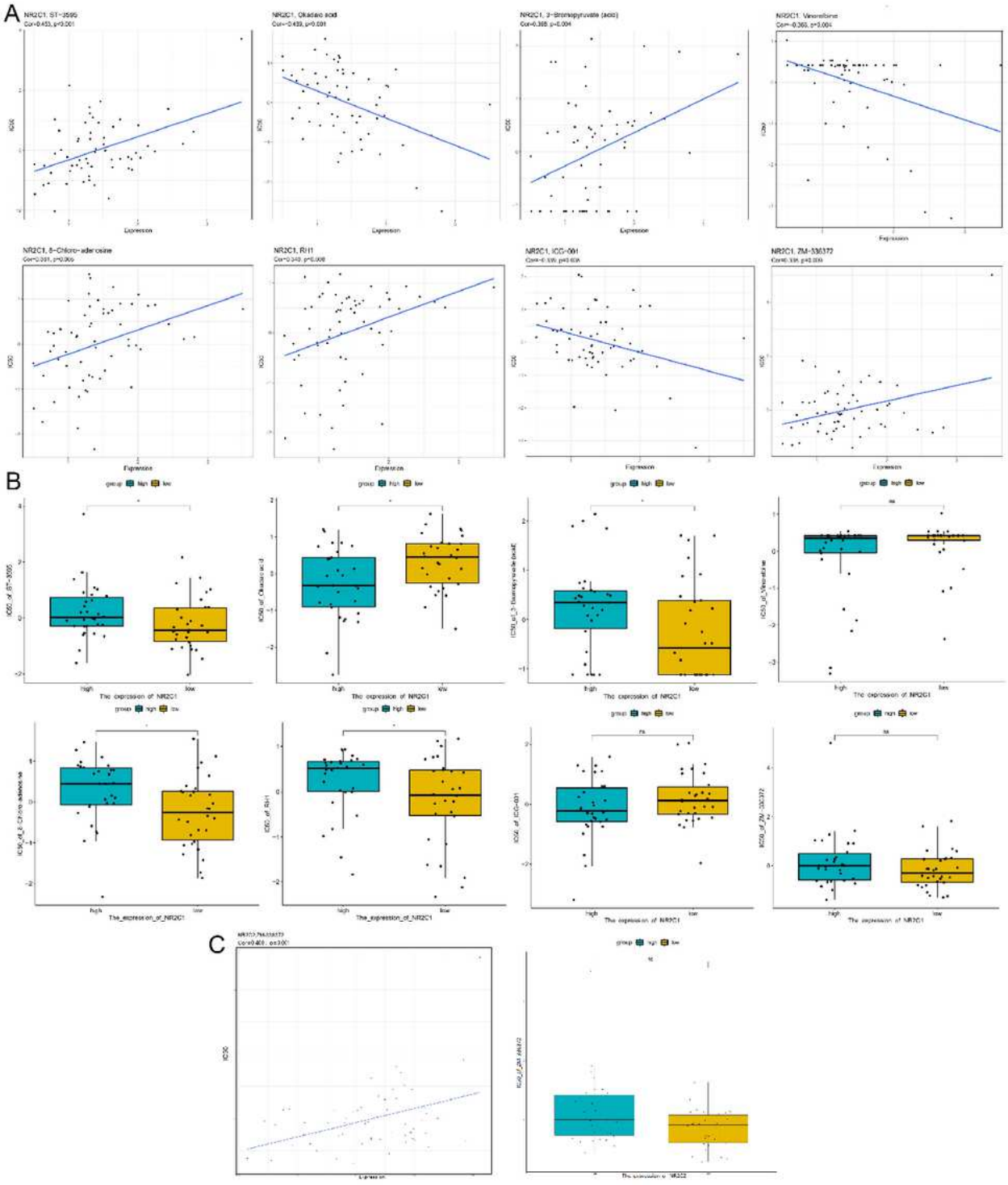

Figure 6

The correlation of NR2Cs expression levels and drug sensitivity in NCI-60 cancer cell line. a-b the correlation of NR2C1 expression and IC50 values of eight drugs. c only ZM-336372 treatment may be associated with NR2C2.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.pdf