RYR2 mutation in non-small cell lung cancer prolongs survival via down-regulation of DKK1 and up-regulation of GS1-115G20.1: A weighted gene Co-expression network analysis and risk prognostic models

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Abstract

RYR2 mutation is clinically frequent in non-small cell lung cancer (NSCLC) with its function being elusive. We downloaded lung squamous cell carcinoma and lung adenocarcinoma samples from the TCGA database, split the samples into RYR2 mutant group (n = 337) and RYR2 wild group (n = 634), and established Kaplan-Meier curves. The results showed that RYR2 mutant group lived longer than the wild group ($p = 0.027$). Weighted gene co-expression network analysis (WGCNA) of differentially expressed genes (DEGs) yielded prognosis-related genes. Five mRNAs and 10 IncRNAs were selected to build survival prognostic models with other clinical features. The AUCs of 2 models are 0.622 and 0.565 for predicting survival at 3 years. Among these genes, the AUCs of DKK1 and GS1-115G20.1 expression levels were 0.607 and 0.560, respectively, which predicted the 3-year survival rate of NSCLC sufferers. GSEA identified an association of high DKK1 expression with TP53, MTOR, and VEGF expression. Several target miRNAs interacting with GS1-115G20.1 were observed to show the relationship with the phenotype, treatment, and survival of NSCLC. NSCLC patients with RYR2 mutation may obtain better prognosis by down-regulating DKK1 and up-regulating GS1-115G20.1.

Keywords

Dickkopf Wnt signalling pathway inhibitor-1, differentially expressed gene, long non-coding RNA, non-small cell lung cancer, prognostic signature, ryanodine receptor 2

Wenjun Ren and Yongwu Li contributed equally.

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1 | INTRODUCTION

Globally, the number of lung cancer cases and deaths is increasing, with GLOBOCAN statistics in 2018 showing approximately more than 2 million new cases of lung cancer, reported to hold 11.6% of all cancer types, and 1.76 million deaths, accounting for 18% of all cancers [1]. Despite the availability of surgery, chemotherapy, and targeted drug therapy [2], the overall survival (OS) of lung cancer is still disappointing, with a survival rate at 5 years of only 19.4% [3]. Non-small cell lung cancer (NSCLC) is the highest prevalent subtype reported to take up 85% of total lung cancers [4]. Depending on the different pathological features, NSCLC can be further classified into three types: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. In NSCLC cells, there are a large number of genetic and epigenetic alterations [5], which have an important impact on the pathogenesis and progression of NSCLC. Understanding the somatic genetic mutations and transcriptome changes of NSCLC is of great significance for improving the prognosis of NSCLC.

Many cancer genetic studies have identified frequent mutations in the genes that encode extremely large protein molecules in cancer cells, and these mutated genes include TTN, RYR2, RYR3, MUC16, MUC4, and DNNH5 [6]. In order to study human cancers at the gene and transcriptional product level, numerous public databases from large patient cohorts have been created to identify various biomarkers related to cancer pathogenesis, progression and therapeutic responses [7, 8]. By analysing public data, we found that RYR2 mutation is a clinically frequent variant in NSCLC. Ryanodine receptor two protein, encoded by the RYR2 gene, is predominantly distributed in heart and involved in excitation–contraction coupling [9], whose mutations are mainly associated with a range of myopathies and arrhythmias [10]. Although RYR2 polymorphisms have been confirmed for underlying functions in three different regions or “hot-spots” of the coding sequence, the study of its assumed function in cancers is still in its initial stages and further studies are expected [11]. Schmitt, K et al. found differential promoter methylation status and expression level of RYR2 in head and neck tumor, suggesting that reduced expression of RYR2 in adjacent tissues and precancerous lesions may potentially indicate poor survival and impending malignancy [6]. In oesophageal cancer, the RYR2 mutation upregulated signalling pathways involved in the immune response and enhanced anti-tumor immunity [12]. The RYR2 rs12594 mutation (occurring in the 3′-UTR) also significantly reduced the risk of developing breast cancer [13]. Nevertheless, its role in NSCLC is currently unknown.

Long non-coding RNAs (lncRNAs), a type of RNA molecule composed of over 200 nucleotides, are structurally similar to messenger RNAs but not translated into proteins [14, 15]. The main ways in which lncRNAs perform biological functions in disease include: RNA decoy, miRNA sponge, constituting RNP, recruiting chromatin modifier as well as influencing transcription, splicing and degradation of mRNA [16]. More and more evidences have shown that lncRNAs are disordered in human cancer [17]. Yang et al. demonstrated that NSCLC cells with up-regulated lncRNA GACAT3 expression have increased the resistance of tumor cells to radiotherapy [18], while Shi’s group uncovered that down-regulated GAS5 expression in NSCLC suggested poor prognosis [19]. Although there is no lncRNA specifically associated with NSCLC, the crucial functions which lncRNAs play towards the diagnosis, treatment and prognostic evaluation of NSCLC are emerging [16].

In this study, we used a series of bioinformatical tools to construct risk prognostic models of mRNAs and lncRNAs in NSCLC with RYR2 mutations. For the first time, significant associations of high DKK1 or low GS1-115G20.1 expressions with the poor outcomes of NSCLC in the presence of RYR2 mutations were uncovered, and a significant negative correlation was identified between the expression of DKK1 and GS1-115G20.1 in RYR2 mutated NSCLC.

2 | MATERIALS AND METHODS

2.1 | Sample preparation and preprocessing

Genomic and transcriptomic data was retrieved from the lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) files which are documented in the Cancer Genome Atlas (TCGA) on the UCSC Xena platform (https://xenabrowser.net/). Samples with no survival status or survival time were removed from our study, while the samples with both genomic and transcriptomic data were retained. According to the mutation data detected by the Varscan2 software [20], those containing RYR2 sense mutations were classified as the mutant group, while those without RYR2 sense mutations were classified as the wild group. We used Kaplan-Meier curves to analyse survival differences between the RYR2 mutant and wild groups and included age, gender, TNM classification of the tumour, disease diagnosis (LUAD or LUSC) and tumour stage as covariates to reduce the effect of confounding factors.

2.2 | Identification of differentially expressed genes (DEGs) and functional enrichment analysis

Differentially expressed mRNAs (DEmRNAs) and lncRNAs (DElncRNAs) were analysed by R package “edgeR” between the RYR2 mutant group (n = 337) and RYR2 wild group (n = 634), the DEmRNAs and DElncRNAs with adjusted p values less than 0.05 were retained [21, 22]. Gene Ontology (GO) analysis [23, 24] and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis were run for differential mRNAs with R package “clusterProfiler” [24].
2.3 | Weighted gene co-expression network analysis (WGCNA)

The modules of co-expressed differential mRNAs and lncRNAs were identified by R package “WGCNA” [25, 26]. First, a suitable soft threshold $\beta = 6$ was calculated. Then, average linkage hierarchical clustering was realised on a dendrogram as a result of DynamicTreeCut analysis based on a Topological Overlap Measure (TOM)-based dissimilarity matrix. A minimum threshold of 40 was set to group the genes of similar expression pattern into the same modules. Module eigengenes (MEs) were calculated, and then the modules were analysed by clustering, and the closer modules were merged into new modules by setting height = 0.5. To determine the association with clinical traits, gene significance (GS) for each module was computed, and higher GS indicated genes with more biologically significant association with clinical features. Module-gene significance (MS) describes the relationship between module gene expression profiles and MEs. Then, MEs were correlated with different clinical features to identify the modules associated with prognosis [27].

2.4 | Risk prognostic model based on multivariate COX proportional hazard model

For DEGs, univariate regression analysis with $p < 0.05$ was used as a filter to identify prognosis-related mRNAs and lncRNAs by combining clinical information. The mRNA genes and lncRNA genes associated with prognosis were screened by the least absolute shrinkage and selection operator (Lasso) Cox penalised regression model. Corresponding coefficients were obtained, and risk prognostic models of the identified mRNAs and lncRNAs were constructed according to the expression levels and coefficients of these genes. According to the models, each patient was scored and then defined as high and low risk by taking the median score as the threshold. Differences in survival rates between the two cohorts were analysed. Time-dependent receiver operating characteristic (ROC) curves were drawn to determine the predictive performance of risk score one and risk score two on the survival of NSCLC patients at 3 years. To further investigate the reliability of the mRNA prediction model, we downloaded the data of the other article for external validation of the 3-year survival rate [28].

2.5 | Analysis of crucial DEmRNA DKK1 and DELncRNA GS1-115G20.1

Multivariate Cox regression models were constructed to validate whether the crucial DEmRNA $\text{DKK1}$ and DELncRNA $\text{GS1-115G20.1}$ are prognostic factors independent of other clinical factors. Statistical significance was defined when $p < 0.05$. According to $\text{TP53}$ mutation, the samples were split into the $\text{TP53}$ mutant group and wild group. Thereafter, expressions of $\text{DKK1}$ and $\text{GS1-115G20.1}$ in both groups were compared. Median expression-based grouping was then performed to classify patients into high and low expression groups, and Kaplan-Meier curves were plotted to compare their survival. ROC analysis was run to evaluate the predictive accuracy of $\text{DKK1}$ and $\text{GS1-115G20.1}$ on 3-year survival in NSCLC.

The transcriptomic data were processed by GSEA software based on the high and low $\text{DKK1}$ expression in the samples [29]. The DEGs between the high and low expression groups of $\text{DKK1}$ were analysed by the R package “edgeR”, and then the DEGs list was annotated by DAVID [30]. To identify the transcription factors (TFs), the DEGs in high and low $\text{DKK1}$ expression groups were analysed by R package “edgeR”, and a total of 1485 DEGs were obtained with the condition that $p < 0.05$ and $|\log2(FC)| \geq 0.8$. The list of DEGs was annotated by DAVID (module UCSC_TFBS of function Protein_Interaction), and the filtering condition was $p < 0.05$. In this way, we can use the UCSC database collection of TFBS (transcription factor binding sites) to understand which transcription factors the genes are enriched to.

To further investigate DELncRNA $\text{GS1-115G20.1}$, we used the StarBase v2.0 database [31] for the competing endogenous RNAs (ceRNAs) of $\text{C1ORF21}$, which took the top 20. The correlation of $\text{C1ORF21}$, $\text{GS1-115G20.1}$ and ceRNAs expression was performed separately based on the data obtained from this analysis. The Custom Prediction function of the miRDB database (http://www.mirdb.org/) was consulted to show the possible miRNA targets of $\text{GS1-115G20.1}$ [32].

2.6 | Ethical statement

Not applicable.

2.7 | Statistical analysis

Statistical tests and plots were completed on R and GraphPad Prism 7.04. A difference of statistical significance was defined at $p < 0.05$. In the graph, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

3 | RESULTS

3.1 | Survival analysis of the RYR2 mutant group and wild group

Figure 1 shows the workflow of the whole study. Totally, 971 analysable NSCLC samples were obtained by the UCSC Xena database retrieval (Table S1), which were split into the $\text{RYR2}$ mutant group ($n = 337$) and the wild group ($n = 634$) (Table 1). See Table S2 for a comparison of the clinical characteristics of the two groups of patients. Between-group comparison for survival was implemented, and it was
demonstrated that the OS of the RYR2 mutant group was longer than that of the RYR2 wild group (HR = 0.778 95% CI = 0.625–0.969) (Figure 2a), with the statistical significance as indicated in the logarithmic rank test (p = 0.025).

### 3.3 Construction of co-expression network to identify prognosis-related modules

DEmRNAs and DElncRNAs were projected onto a weighted co-expression network with the R package “WGCNA”, and then the modules obtained were clustered into 24 modules in total (Figure 4a), with the module sizes ranging from 52 to 2208 genes (Table 3). Independent genes were clustered into grey modules which were excluded from this analysis. The topological overlap of co-expressed genes in each module was shown in the DEGs heat map (Figure 4b), and the relationship of the 24 co-expression modules was shown in the eigengene adjacency heat map (Figure 4c). Pearson correlation coefficients were calculated for the MEs. The numbers displayed in each small cell are the coefficients that reflect the association between the gene modules and the corresponding clinical factors, and the numbers in parentheses indicate the p-value. In Figure 4d, we can conclude significant associations of the blue and light green modules with one-year survival and the red module with OS; hence, genes from these three modules were selected for further analysis.

**TABLE 1** NSCLC samples based on RYR2 mutation/wild grouping

|              | No. of pts. | RYR2 MUT | RYR2 WT |
|--------------|-------------|----------|---------|
| LUAD         | 491         | 171      | 320     |
| LUSC         | 480         | 166      | 314     |

Abbreviations: NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cancer; pts, patients; MUT, mutation; WT, wild type.
**FIGURE 2** Overall survival and DEGs of RYR2 mutant group and wild group. (a) Kaplan-Meier curve comparison of survival times between RYR2 wild-type and mutant groups. (b, c) volcano plot (b) and heat map (c) of DEmRNAs obtained by “edgeR” analysis. (d, e): volcano plot (d) and heat map (e) of DElncRNAs obtained by “edgeR” analysis. DEGs, differentially expressed genes; DEmRNAs, differentially expressed mRNAs; DElncRNAs, differentially expressed lncRNAs; FC, fold change; FDR, false discovery rate; RYR2 WT, RYR2 wild-type; RYR2 MUT, RYR2 mutant.
3.4 | Univariate regression analysis to screen prognosis-related genes in blue, light green, and red modules

For DEGs, univariate regression analysis with $p < 0.05$ as the filtering condition was used to screen a total of 777 prognosis-related genes from blue, light green and red modules, including 57 mRNAs and 20 lncRNAs (Table S7), and the significance was selected for forest plotting from the top 15 (Figure 5a). With the median expression of prognosis-related genes in all samples considered, the samples were grouped into high and low expression cohorts, and survival conditions were analysed using R package “survival”. Among them, 13 genes were differentially expressed with statistically significant differences in OS, and the Kaplan-Meier curves were shown (Figure 5b–e and Figure S1).

3.5 | Risk prognostic model of mRNA

The 57 prognosis-related mRNAs obtained by univariate regression analysis were put into a Lasso regression model, and 12 mRNAs were selected according to the parameter Lambda value, with Lambda.min. The minimum value was used as the threshold (Figures S2a–b), and then the Cox penalty regression model was used to reduce the dimension and finally screened a linear risk score model consisting of 5 mRNA genes associated with survival: risk score $1 = \sum (\text{coefficient} \times \text{expression level of mRNA}) = (-4.27e-3*RAB44) + (5.11e-4*GNG7) + (1.61e-4*RA5A3) + (-1.26e-3*CD200R1) + (3.69e-5*DKK1)$.

All samples were assigned a score and defined as high- and low-risk based on the median risk score 1 value as the cutoff. Figure 6a shows the expression of 5 prognosis-related mRNAs in two cohorts. Figure 6b shows the survival time in two groups, and Figure 6c shows the heat map which reflects the expression of the 5 genes in the risk model in different risk score 1, diagnoses, and genders. The Kaplan-Meier curve shows the lower OS of the high-risk patients compared with the low-risk group (Figure 6d); meanwhile, the area under the ROC curve (AUC) for the survival rate at 3 years was 0.622, indicating the favourable predictive ability of the risk model (Figure 6e).

Risk score 1 was combined with the age, gender, tumour stage, presence of EGFR mutation, type of diagnosed disease, smoking history, tumour location, and clinical factors of radiation therapy in a multivariate Cox regression model. Age, tumour stage, radiation therapy and risk score 1 were determined as independent predictive factors for OS, while risk score 1 possessed a stronger impact on survival (Figure 6f). In order to facilitate the utilisation of risk score 1, a nomogram

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**Table 2** Differentially expressed mRNAs and lncRNAs

| DEGs | Up | Down |
|------|----|------|
| mRNA | 5346 | 2702 | 2644 |
| lncRNA | 3925 | 2168 | 1757 |

Abbreviation: DEGs, differentially expressed genes.

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**Figure 3** The most enriched GO terms and KEGG pathways of DEmRNA. (a, b, c) GO functional enrichment analysis annotated DEmRNA in terms of BP (a), CC (b), and MF (c), respectively. (d) KEGG pathway analysis. The X-axis displays the number of genes activated in each pathway. BP, biological process; CC, cellular component; KEGG, Kyoto encyclopedia of genes and genomes; MF, molecular function.
was plotted (Figure 6g). The results of the external validation showed that the area under the ROC curve for 3 year survival had an AUC of 0.595 and this model had acceptable predictive power (Figure S3).

### 3.6 Risk prognostic model of lncRNA

The 20 lncRNAs with prognostic related genes obtained by univariate regression analysis were first analysed by Lasso
regression analysis (Figures S2c–d), and 10 lncRNAs were selected by the threshold parameter, Lambda.min (Figure 7a), which constitutes a linear risk assessment model associated with survival. The lncRNA risk score model is risk score 2 = \[ \sum (\text{coefficient} \times \text{expression level of lncRNA}) = (-3.86e-4*LI\ NCO0042) + (-7.83e-3*AC007880.1) + (-1.74e-3*LINC01352) + (-7.58e-4*RP11-354E11.2) + (-3.28e-3*RP11-374F3.5) + (-6.46e-4*AC018647.3) + (-5.75e-4*HLA-DQB1-AS1) + (-3.45e-2*GS1-115G20.1) + (-4.34e-3*SMCR5) + (-2.00e-3*RP11-357N13.6). \]

Similarly, all samples were assigned into high- and low-risk cohorts (cutoff: risk score 2). Figure 7b demonstrates the change in risk values; Figure 7c demonstrates the survival difference; Figure 7d carries out the expression of 10 genes in the two cohorts, the diagnostic outcome, and the gender subgroup. Survival curves (Figure 7e) indicated that the OS rate of patients with high-risk was lower; meanwhile, the AUC value was 0.565, suggesting that the model had certain predictive accuracy in the prognosis of NSCLC patients (Figure 7f).

Risk score 2 was combined with the age, gender, tumour stage, presence of EGFR mutation, types of diagnosed disease, smoking history, tumour location, and clinical factors of radiation therapy in a multivariate Cox regression analysis. Age, tumour stage, radiation therapy and risk score two were observed to be the predictive factors for OS in an independent manner, while the prognostic performance of risk score two was much greater (Figure 7g). Similarly, the nomogram was plotted (Figure 7h).

### 3.7 | Crucial prognosis-related mRNA gene

**DKK1** is an independent prognostic factor and correlated with the expression of TP53, MTOR, VEGF

The mRNA genes included in the risk score 2 were analysed. There were five mRNA genes in the mRNA-based risk prognostic model, and only one gene, **DKK1**, was carried out with survival significance between high and low expression groups (Figure 5b). **DKK1**, therefore, was selected for the study and then observed to present increased expression in the TP53 mutation group compared with the wild group with a statistically significant difference (Figure 8a). The AUC value was 0.607 using high and low DKK1 expressions to predict survival at 3 years of NSCLC cases (Figure 8b). With clinical information considered, multivariate analysis by Cox regression identified **DKK1** as an independent prognostic factor (Figure 8c). Additionally, GSEA analysis found that the differential genes in the high and the low **DKK1** expression groups were related to the expression of MTOR, VEGF and TP53 (Figure 8d).

With regard to the regulation network of mRNA-TFs, 911 DEGs were of decreased expression and 574 DEGs were of increased expression (Table S8). The DEGs which were enriched in these TFs were: CHX10, E47, LIHX3, CREL, AP1 and HNF3B. The top 18 TFs associated mRNAs with greater \[|\log_{2}(FC)|\] were selected for the regulatory network mapping (Figure 9).

### 3.8 | Crucial prognosis-related lncRNA

**GS1-115G20.1** is an independent prognostic factor, associated with the expression of C1ORF21, multiple target miRNAs, and DKK1

The lncRNA genes in the risk score 2 were analysed, and the patients were assigned into high and low expression groups on the basis of median lncRNA expression values. **GS1-115G20.1** was identified to be of survival significance at a higher degree (Figure 5e); **GS1-115G20.1**, therefore, was selected for the study. TP53 mutation group witnessed a downward trend of GS1-115G20.1 expression compared with the wild group characterised by a statistically significant difference (Figure 10a). The expression level of **GS1-115G20.1** was used to predict the 3 year survival rate of NSCLC cases with AUC = 0.560 (Figure 10b). Combined with the clinical information, multivariate Cox regression analysis showed that **GS1-115G20.1** was a prognostic factor independent of other variables (Figure 10c).

The correlation analysis by C1ORF21, GS1-115G20.1 and ceRNAs of C1ORF21 expression showed that the correlation coefficients were between −0.033 and 0.423 (Figure 10d), and the correlation coefficient between GS1-115G20.1 and C1ORF21 was 0.365, \( p = 2.2E-16 \), which is much higher in LUAD, \( r = 0.468 \). The “custom prediction” function of miRDB database was used to predict miRNA targets of lncRNA GS1-115G20.1, and there were 27 miRNAs that might interact with GS1-115G20.1 (Table S9). In addition, we also found low GS1-115G20.1 expression in the high DKK1 expression group (Figure 10e,f). However, we did not find any evidence of their direct interaction in public databases.
FIGURE 5  Univariate regression analysis for screening prognostic genes. (a) Top 15 prognostic relevant genes in blue, light green and red modules of significance. (b, c, d, e): prognostic relevant genes were assigned into high and low expression groups based on median expression level, and the final Kaplan-Meier curves of four genes with p < 0.05 were demonstrated. Kaplan-Meier curves of the GS1-115G20.1 curve (c) divergence is obvious. CD1E curve (d) has a significant crossover and therefore is not significant.
**FIGURE 6**  Construction of the mRNA risk prognostic model. (a, b): Distribution of samples in high- and low-risk groups using median risk score one, with vertical coordinates of risk score one is shown. (c) Heat map shows the expression of five mRNA genes in the risk model in different risk score 1, different diagnoses, and different genders. (d) Kaplan-Meier curves for survival time of patients with high- and low risk. (e) ROC curves for predicting 3 year survival. (f) The forest plots of multivariate Cox regression. Black squares on the horizontal line indicate the hazard ratio and the horizontal lines show the 95% confidence interval. (g) Nomogram for predicting survival in NSCLC.

4 | DISCUSSION

It has been proven that RYR mutations are frequently found in most cancer genomic studies with somatic mutations [6]. Nevertheless, the role and mechanism of RYR2 mutations in NSCLC pathogenesis and progression have not been confirmed. It is important to further clarify the potential genes related to the prognosis of NSCLC with RYR2 mutation. Extracting transcriptomic data from the UCSC Xena dataset can help identify prognostic factors that may be involved in cancer development or evolution. In this study, we used genomic and transcriptomic data of LUAD and LUSC from the UCSC Xena database to identify lncRNAs and mRNAs, which are differentially expressed in RYR2 mutant and RYR2 wild-type NSCLC. By survival analysis, we found that patients in the RYR2 mutant group have better survival, and the somatic mutation of RYR2 may be protective.
To further investigate the molecular mechanism by which RYR2 mutation improves the prognosis of NSCLC patients, we constructed co-expression networks for DEmRNAs and DElncRNAs, selected modules related to NSCLC prognosis for analysis, and constructed risk prognosis models for the prognosis-related mRNA and lncRNA genes. The important DEmRNA genes included were RAB44, GNG7, RASA3, CD200R1 and DKK1. KEGG enrichment analysis showed that the DEmRNA gene was mainly related to neuroactive ligand-receptor interaction, complement and coagulation cascades, cAMP signalling pathway, adrenergic signalling in cardiomyocytes, cell cycle and other pathways. Among them, the cell cycle and cAMP signalling pathway are pivotal in tumour development in the study of NSCLC [33, 34]. Meanwhile, based on the above results, we constructed the mRNA risk prognostic model with reliable results for both internal and external validation. Although the transcriptome-based prognostic model has not yet reached a very satisfactory level for the prediction of survival in NSCLC patients, the attempt still has far-reaching implications.
As the crucial DEmRNA gene in this study, the Dickkopf Wnt signalling pathway inhibitor-1 (DKK1) is a well-established classical Wnt signalling inhibitor [35], which is essential in the proliferation and migration of multiple tumour cell types [36]. Overexpressed DKK1 promotes bony metastasis of breast cancer while inhibiting its lung metastasis, and even in the same tumours, an organ-specific role of DKK1 has been noted [37]. Several studies have indicated associations of DKK1 overexpression with cancer malignant progression and adverse prognosis in a raft of human cancers, suggesting a potential oncogenic function of DKK1 [38–41]. Yamabuki et al. showed that high DKK1 expression indicates adverse outcomes of NSCLC patients, and its exogenous expression improves migration and invasion of cells [40]. Notably, significant correlations between elevated serum DKK1 protein concentrations and tumour progression as well as lowered survival were identified in lung cancer patients [42]. In the results of this study, high DKK1 mRNA expression in the RYR2 wild type suggested a worse prognosis for lung cancer by analysing the UCSC xena database. In the present study, E47, CREL, and AP1 were important cancer-related TFs enriched to DKK1 high- and low-expressing DEmRNAs [43–45].

Recent advances have suggested that non-coding genes may also be new participants in the cancer paradigm [19]. In this study, a predictive model of IncRNAs was constructed, and in these IncRNAs, GSI-115G20.1 may play a role in the development of NSCLC. GSI-115G20.1 (also called ENSG00000230470.1; OTTHUMG0000035468.1; AL078645.1) is located on chromosome CHR1:184408336-184412360 (Grc h38), which is exactly located on the protein-coding gene
C1ORF21 (chr1:184387057-184629020) that encodes this protein gene [46]. The general regulatory effect of lncRNAs on adjacent mRNAs, coupled with expression correlation, leads to the speculation that GS1-115G20.1 regulates the expression of C1ORF21, but the correlation is not strong. We found that the lncRNA’s high expression in NSCLC indicated better survival and may play a protective function in NSCLC.

Due to the lack of experimental studies for GS1-115G20.1, we could only use predictive databases. As a result, several target miRNAs that may interact with GS1-11520.1 were identified (Table S8). hsa-miR-608 has been confirmed to play a significant part in the apoptosis of NSCLC cells via the regulation of migration inhibitor factor (MIF), Akt serine/threonine kinase 2 (AKT2) and transcription factor activation enhancer binding protein 4 (TFAP4) [47–50]; Dong et al. found that hsa-miR-105-5p could be a biomarker for early diagnosis of NSCLC [51]; Zheng and other researchers found that hsa-miR-4651 elicited a negative effect on the progression of NSCLC via targeting bromodomain-containing protein 4 (BRD4) [52]; from a study by Wang’s group, lncRNA LIFR-AS1 could inhibit NSCLC cell invasion and migration by serving as a sponge for hsa-miR-942-5p [53]; in patients

**FIGURE 9** The transcript factors regulatory network for DEmRNAs between high and low DKK1 expression groups. The red genes are up-regulated, the green genes are down-regulated, and the blue boxes indicate the enriched transcript factors.
suffering from anaplastic lymphoma kinase (ALK)-positive NSCLC, decreased hsa-miR-362-5p was accompanied by longer progression-free survival [54]. Therefore, we speculate that GSI-115G20.1 may interact with the above miRNAs to have an influence on the phenotype, treatment, and prognosis of NSCLC, but further validation of molecular experiments is still needed.

In conclusion, using survival analysis, we found that RYR2 mutations may have a protective effect on NSCLC. Through comprehensive bioinformatic analysis, two risk prognostic models of mRNA and lncRNA were established in this study, and prognostic risk models have some degree of predictive ability. The OS of high DKK1 expression group and low GSI-115G20.1 expression group was worse. Overall, our findings may extend our understanding on the protective mechanisms of RYR2 mutations on the prognosis of NSCLC and identify new targets for prognostic assessment and treatment.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interests.

**PATIENT CONSENT STATEMENT**

Not applicable.

**DATA AVAILABILITY STATEMENT**

All data, models, or codes that are generated in this study could be available upon reasonable request to the corresponding author.

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SUPPORTING INFORMATION
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