Loss of CDKN2A and CDKN2B expression is associated with disease recurrence in oral cancer

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Abstract

Background: Loco-regional recurrence is one of the major reasons for poor prognosis of oral squamous cell carcinoma (OSCC). However, till date, no feasible molecular marker is available to predict the risk of recurrence in OSCC patients.

Aim: To evaluate the cell cycle regulatory genes expression and its association with the risk of recurrence in oral cancer patients.

Materials and Methods: Transcript level expressions of 47 cell cycle regulatory genes were analyzed in 73 OSCC tumors from buccal mucosa and tongue, 26 adjacent normal samples using real-time polymerase chain reaction. TaqMan low-density array data were analyzed using the DataAssist™ v 3.01. Significantly altered genes within the tumor samples and samples showing recurrence (re-appearance of disease during the follow-up in cases having complete response to initial treatment assessed after 3 months of the treatment) were identified. Further, Kyoto Encyclopedia of Genes and Genomes pathway analysis and The Cancer Genome Atlas (TCGA) online data analysis portal were used to analyze interacting protein and pathways significantly associated with the altered gene.

Results: CCNA1, CCNB2, CCND2, CCNE1, CCNF, CDC2, CDK6, CHEK1, and TGFA found to significantly alter in the tumor sample of oral cancer patients, and down-expression of CDKN2A and CDKN2B found to associate with the recurrence of disease in oral cancer patients. TCGA data also showed the loss of CDKN2A and CDKN2B significantly associated with recurrence in head and neck cancer patients.

Conclusion: CDKN2A and CDKN2B expression analysis can be used as the prognostic marker for the oral cancer patients. The present method of data analysis helps overcome the limitations and complications of high throughput techniques and thereby increases the opportunity of employing molecular markers in routine clinical management of OSCC.

Keywords: Biomarker, cell cycle, oral carcinoma, recurrence, TaqMan low-density array

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the major health burdens around the globe and India.1-3 Loco-regional recurrence is one of the major reason for the high mortality or poor prognosis of OSCC patients.4,5 Studies have showed that TNM status, depth of invasion, perineural involvement (PNI), and margin status of...
tumor are beneficial for predicting the prognosis of the OSCC patients.\(^8\)\(^{−}\)\(^{10}\) Despite the diagnostic and therapeutic advances, the 5-year survival rate of OSCC patients has been virtually unchanged during the past 30 years.\(^11\)\(^{,}\)\(^{10}\) Regardless of the large number of studies to unveil the molecular mechanisms of OSCC, currently, there is no molecular marker in clinical use that can predict the chances of recurrence. The complexity of cancer biology and the problems involved in the analysis of highly dimensional data have proven to be a formidable challenge to moving toward more predictive oncology with molecular markers.\(^11\)

Cellular programs such as proliferation, differentiation, senescence, and apoptosis are intimately linked to the cell cycle regulatory machinery.\(^13\) Dysregulation of the cell cycle machinery is a fundamental hallmark of cancer progression. A number of studies using whole transcriptome analysis to showed that among the dysregulated pathways, cell cycle regulation is significantly altered in many cancers.\(^13\)\(^{−}\)\(^{15}\) In the present study, we have evaluated the expression of 47 genes that are well known to be involved in cell cycle regulation by a real-time polymerase chain reaction (PCR)-based TaqMan low-density array (TLD).A number of studies using whole transcriptome analysis to showed that among the dysregulated pathways, cell cycle regulation is significantly altered in many cancers.\(^13\)\(^{−}\)\(^{15}\) In the present study, we have evaluated the expression of 47 genes that are well known to be involved in cell cycle regulation by a real-time polymerase chain reaction (PCR)-based TaqMan low-density array (TLD).

**MATERIALS AND METHODS**

**Study population**

A total of 73 oral cancer patients attending the head and neck clinic of the Regional Cancer Centre, Trivandrum, were included for the study. The study was IRB and IEC approved (HEC No 02/2013). Inclusion criteria: (1) Both sexes with age range from 18 to 80 were included in the study. (2) Patients planned for radical therapy only included. (3) Patients having histopathologically confirmed OSCC either at buccal mucosa or anterior tongue only included. (4) Patients who providing signed informed consent only included. Exclusion criteria: (1) Patients having clinical history for previous treatment for any form of cancer. (2) Patients having any chronic systemic diseases. The histopathological diagnosis was performed according to the criteria given by the WHO.\(^16\)\(^{,}\)\(^{17}\) Clinicopathological staging was determined as per the Union for International Cancer Control TNM staging system, version 7.\(^6\)\(^{,}\)\(^{18}\)

**Clinicopathological data**

The clinical variables considered were tumor size (T), node involvement (N), composite stage, histopathology of tumor, mode of treatment, and posttreatment disease status – nonresponding tumors, recurrence, and overall survival (OS). OS was defined as the period between the last day of first line of treatment and day of death or until the last follow-up (36 months), irrespective of disease status. Recurrence was defined as the re-appearance of disease after 3 months of treatment in cases that responded well to the first line of treatment. The cases with tumors not responding to treatment were excluded from the recurrence-free survival analysis as we have analyzed elsewhere.\(^19\)

**Treatment criteria**

Out of 73 study subjects, only 65 patients were completed the therapy and the remaining eight patients who did not complete the treatment or receive any treatment were not included in the further analysis. The treatment strategy for each patient was planned by a joint decision between the radiation oncologist, pathologist, and surgeon of the head and neck clinic.

**Preparation of sample**

All experimental workup of tissue samples were done at the molecular oncology laboratory of the Division Cancer Research, Regional Cancer Centre, Thiruvananthapuram. Total RNA was extracted from the frozen tumor and normal samples using TRI Reagent (Ambion, Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s protocol.\(^20\) Total RNA (1 ug) was converted to cDNA using the cDNA High Capacity Archive Kit (Applied Biosystems, Foster City, CA, USA) per the manufacturer’s protocol [Supplementary Figure 1a].\(^21\) All the laboratory procedure were done at Laboratory of Cell Cycle Regulation, Division of cancer research, Regional Cancer Centre, Trivandrum.

**TaqMan low-density array**

Gene expression analysis was performed by TaqMan® Low-Density Array (TLD) in a 384-well plate preloaded with TaqMan Gene Expression Assays. Assays for 47 cell cycle regulatory genes and one endogenous control gene (18S rRNA) were included in the TLD card [Supplementary Figure 1b]. The probe and primers were selected from Applied Biosystems Assays-on-DemandTM Gene expression products. User-defined primers loaded and dried down in the microfluidic card. Genes spotted in the TLD card are listed in Supplementary Table 1. The card was run in an ABI Prism 7900 HT real-time PCR machine (Applied Biosystems, Foster City, CA, USA).\(^22\)

**Data analysis**

TLD data were analyzed using the DataAssist™ (v 3.01, Life Technologies Corporation, CA, US). Genes significantly altered in tumors compared to normal tissue were termed as tumor-specific genes (TSGs), and similarly, genes altered in recurrent tumor samples...
compared to recurrence-free tumor samples were termed as recurrence-specific genes (RSGs). Cluster analysis was performed using a Pearson correlation method with the fold-change values of significant genes in tumor samples and recurrence samples (TSGs and RSGs). Further, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis system was used to significantly altered checkpoint cell cycle. The Cancer Genome Atlas (TCGA) online data analysis portal used to analyze the altered gene in the TCGA data set.

Recurrence score (RS) is calculated as shown below:

\[
\text{RS} = \frac{\Delta Ct \text{ of RSGs}}{\Delta Ct \text{ of TSGs}}
\]

\[\Delta Ct = \text{Ct test gene} - \text{Ct control gene (18sRNA)} \]

\[\text{Ct} = \text{Threshold cycle}.\]

The ability of RS to predict recurrence in oral cancer patients was represented by a receiver operating characteristic (ROC) curve and the corresponding area under the curve (AUC).[24] The RS was dichotomized based their median values and considered for Chi-square analysis, Cox’s hazard model, and Kaplan–Meier survival analysis.

RESULTS

Study population characteristics

The details of patients included in the study, such as tumor site, histopathology, and clinical stage, are shown in Table 1. Thirteen patients (20%) showed persistence of the disease (nonresponding tumor) even after the completion of the first line of treatment, and another 14 patients (22%) developed loco-regional recurrence during the follow-up. A Chi-square analysis showed that nonresponding tumors is significantly associated with tumor size \((P = 0.002)\), nodal status \((P = 0.0001)\), composite staging \((P = 0.004)\), site of disease \((P = 0.003)\), and PNI \((P = 0.003)\), but disease recurrence is not associated with any of the above clinicopathological features [Table 1]. Thus, these results reveal that clinicopathological features are sufficient to predict the initial responsiveness of the tumors and are insufficient to predict the recurrence.

Alterations in cell cycle regulatory genes

Initially, the study compared the expression pattern of 47 cell cycle regulatory genes between tumor and normal samples to find out the gene(s) that are commonly altered in the tumor (TSGs). t-test with a 1% cut-off false discovery rate was used for the analysis, which found that 11 (fold change <2.0) genes were significantly deregulated in tumor samples compared to normal tissue [Table 1]. The results showed that CCNA1, CCNB1, CCNB2, CCND2, CCNE1, CCNF, CDC2, CDK6, CHEK1, and TGFA were altered in OSCC tumor samples. With increased stringency in cutoff criteria \((P < 0.005 \text{ and fold change} <3.0)\), CCNB1 and TGFA were found significant [Supplementary Table 2]. Comparing the expression of these 47 genes between cases that had recurred and not recurred during posttreatment follow-up revealed that CDKN2B and CDKN2A were significantly downregulated. The finding of the present analysis clearly indicates that cell cycle machinery is altered in tumor samples and that there is a molecular heterogeneity in the cell cycle regulatory machinery between recurrent and recurrence-free cases.

Classification of the samples using alterations in cell cycle regulatory genes

Cluster analysis using 10 TSGs revealed that all samples clustered into two major subgroups. Out of 99 total samples, 54 samples grouped into one cluster, all of which were tumors with the exception of five samples and second cluster had 21/45 samples were normal [Figure 1a]. Cluster analysis using the expression of CCNB1 and TGFA genes grouped 99 samples into two independent groups, 50 samples (49 tumors and 1 normal sample) in one group and another group with 25 normal samples and 24 tumor samples \((P = 0.001)\) [Figure 1b]. The results of a Pearson correlation cluster analysis using CDKN2B and CDKN2A revealed that all samples were grouped into two different subgroups, and Chi-square analysis between clusters versus the recurrence status of samples [Figure 1c] showed that 9/14 recurred samples clustered in Cluster II \((P = 0.001)\).

Evaluation of CDKN2B and CDKN2A in the Cancer Genome Atlas sample sets

To evaluate the effect of loss of CDKN2A and CDKN2B in other set of samples, we used TCGA data set. Using head and neck cancer data from the TCGA data set, we analyzed the effect of CDKN2A and CDKN2B on disease-free survival and OS. The results showed that the loss of CDKN2A and CDKN2B was significantly associated with the OS \((P = 0.002)\). Similarly, the loss of CDKN2A and CDKN2B is associated with poor disease-free survival \((P = 0.074)\) [Figure 2a and b].

Pathway and protein network analysis

KEGG color pathway analysis is used to identify the most affected pathway and cell cycle stages. KEGG analysis revealed that in the recurrent sample the most affected phase of the cell cycle was G1 phase [Figure 2c]. Interaction analysis for CDKN2A and CDKN2B proteins showed that they are associated directly with proteins that involved in the cell proliferation, DNA repair, and senescence [Figure 2d].
Association of biomarkers with prognostic endpoints of the oral squamous cell carcinoma patients

CDKN2A ($P = 0.001$) and CDKN2B ($P = 0.005$) showed significant association between the recurrence and recurrence-free samples. However, fold-change calculation using normal tissue for the clinical application is practically difficult. Hence, TSGs and RSGs in combination were evaluated. The average ratios of $\Delta Ct$ values for RSGs to TSGs have been considered to assess the risk of recurrence; this ratio has been termed the RS. The RS was found to be significantly associated with recurrence in oral cancer cases ($P = 0.02$).

Survival analysis

The Kaplan–Meier survival curve with log-rank analysis also showed that higher values of RS (above median), values $>1.0$ were associated with poor recurrence-free survival [Figure 3a]. ROC curve and the corresponding AUC analysis showed that the AUC value for RS was 0.714 ($P = 0.002$) [Figure 3b]. Univariate Chi-square analysis showed that patients with an RS $>1.0$ have the highest relative risk of 8.32 ($P = 0.001$) for recurrence of disease. This analysis clearly showed that RS is better and has greater potential than any other marker we analyzed for predicting the risk of recurrence in OSCC patients.

DISCUSSION AND CONCLUSION

In the present study, the expression of 47 cell cycle regulatory genes at the RNA level was analyzed using a real-time PCR-based TLDA system. The association between deregulations of cell cycle regulatory gene expression and different clinical outcome of oral cancer was analyzed. Studies have shown significant link between the disease-free survival with both depth of invasion and PNI, but in the present study, no such correlation was identified. Similar results were observed in the study by Fakih et al.,[24] Access in-depth of invasion and PNI are subjective and also there are difficulties in measuring depth of invasions such as identification of the deepest invading tumor cell and a series of subjective assessments are required.[8] In this context, the quantitative biomarker can provide a greater advantage for predicting prognosis. Hence, quantitative gene expression method was used for developing the biomarker for predicting loco-regional recurrence in OSCC patients. Studies have also shown that real-time PCR-based strategies for detecting the RNA-based molecular marker effective method.[25,26]

It is noted that all the major cell cycle-positive regulator genes (CCNA1, CCNB1, CCNB2, CCND2, CCNE1, CCNF, CDC2, CDK6, CHEK1, and TGFA) were upregulated in the OSCC tumor compared to the normal. However, tumor sample with low expression of cell cycle inhibitors such as CDKN2B and CDKN2A showed poor recurrence-free survival. The efficiency of the significantly altered genes in tumor sample and recurrence sample was also proved in our cluster analysis. In cluster analysis, discrepancy was observed in few samples that may be due to the tumor heterogeneity. None of clinical parameters such as T status, N status, and PNI were found to be significantly associated with the recurrence of disease in our data set. The results also clearly indicate that the prognostic difference for patients with clinically similar tumors may be due to the molecular heterogeneity between these tumors.

The observation of the present study compelled us to believe that RS would be a much more feasible and...
promising biomarker for detecting the risk of recurrence than the average of CDKN2B or CDKN2A alone or even the average of RSGs. The results in Figure 2 clearly show that determining RS using the ratio of RSGs to TSGs separated the recurrent and recurrence-free samples to different entities more clearly. These increased variations provide the advantage of ensuring the detection of any sample with a high recurrence risk with greater sensitivity and specificity. There are plenty of reports showing that use of combination of markers is better than a single gene maker in predicting treatment outcome (like prognosis) in cancer patients. This advantage may be because cancer is a multifactorial disease. The RS developed in the present study is a multi-gene marker that can screen for alterations in different pathways, and this provides the advantage of screening a wide range of heterogeneity in cancer. However, the present result also provides a lead for developing a new methodology for a more convenient and feasible biomarker. The ROC curve analysis also emphasized that RS has a higher AUC value (AUC = 0.788) and significance \( P = 0.001 \) than the ΔCt of CDKN2A (AUC = 0.724, \( P = 0.014 \)) alone. To our knowledge, no prior reports show the use of a ΔCt ratio of two different sets of genes for detecting the risk of recurrence. However, a few reports showed the usefulness of gene expression ratios in different ways. It was demonstrated that the ratio of gene expression in cancer to its paired normal could predict relapse of esophageal cancer. Another report showed that the CCND1/CDKN2A mRNA expression ratio gives the RB1 status in cell lines. It was shown that the gene expression ratio in combination with FNA biopsy provides a better tool for predicting patient’s outcome in malignant pleural mesothelioma. In Hodgkin’s lymphoma, it was shown that the outcome of treatment can be predicted from the logarithmic mean of ΔCt. Taking the ratio of the average RSG to TSG ΔCt values to calculate RS provided a greater advantage by enhancing the sensitivity and specificity of the biomarker, as the ratio increased the variation between the recurrent and recurrence-free samples. Thus, our

| Clinicopathological factors | Recurrence | Nonresponding tumor |
|----------------------------|------------|---------------------|
| **T**                      |            |                     |
| T1                         | 10         | 5                   | 0.826 |
| T2                         | 16         | 6                   | 22    |
| T3                         | 6          | 2                   | 8     |
| T4                         | 6          | 1                   | 7     |
| **N**                      |            |                     |
| N0                         | 19         | 6                   | 0.424 |
| N1                         | 16         | 8                   | 24    |
| N2                         | 3          | 0                   | 3     |
| **CS**                     |            |                     |
| CS1                        | 8          | 3                   | 0.663 |
| CS2                        | 8          | 3                   | 11    |
| CS3                        | 14         | 7                   | 21    |
| CS4                        | 8          | 1                   | 9     |
| **Site**                   |            |                     |
| Tongue                     | 21         | 10                  | 0.223 |
| Buccal mucosa              | 17         | 4                   | 21    |
| **Histopathology**         |            |                     |
| SCC (nongradable)          | 5          | 2                   | 0.58  |
| PDSCC                      | 1          | 0                   | 1     |
| MDSCC                      | 21         | 10                  | 31    |
| WDSCC                      | 11         | 2                   | 13    |
| **Treatment**              |            |                     |
| Surgery                    | 8          | 5                   | 0.523 |
| Radiotherapy               | 10         | 2                   | 12    |
| Surgery+chemo/radiotherapy | 20         | 7                   | 27    |
| **Depth of invasion >4 mm**|            |                     |
| No                         | 4          | 2                   | 0.65  |
| Yes                        | 17         | 8                   | 25    |
| **Perineural involvement** |            |                     |
| No                         | 17         | 7                   | 0.401 |
| Yes                        | 4          | 3                   | 3     |
| **Margin status**          |            |                     |
| Negative                   | 19         | 10                  | 0.734 |
| Positive                   | 2          | 1                   | 0     |

*Significant. PDSCC: Poorly differentiated squamous-cell carcinoma, MDSCC: Moderately differentiated squamous-cell carcinoma, WDSCC: Well-differentiated squamous cell carcinoma, CS: Clinical stage
Figure 2: (a) Kaplan–Meier recurrence-free survival analysis of the HNCC patients of The Cancer Genome Atlas dataset stratified with status of (a) CDKN2A; (b) CDKN2B; (c) KEGG pathway using CDKN2A and CDKN2B; (d) protein interaction map using CDKN2A and CDKN2B

Figure 3: (a) Receiver operator curve of ΔCt of CDKN2A, ΔCt of TGFA, recurrence-specific genes, tumor-specific genes and recurrence score; (b) Kaplan–Meier recurrence-free survival analysis of the oral squamous cell carcinoma patients stratified with status of recurrence score using a cutoff at 1.0
study strongly suggests that the RS can more precisely differentiate tumors that have the potential for recurrence. Kaplan–Meier and multivariate Cox’s regression analyses further supported our multi-gene expression ratio as a better option for predicting recurrence than the use of CDKN2AΔCt or CDKN2BΔCt alone. Real-time PCR-based approaches have unparalleled sensitivity and specificity and could lead to a more immediate clinical adoption when compared with microarray- or proteome-based techniques. The innovative design of the TLDA card addresses a major problem by detecting the expression of multiple genes simultaneously. Along with this detection, possibility of isolating RNA from formalin-fixed paraffin-embedded samples could allow for the testing of a wide range of samples. On the other hand, using the RS value for diagnostic purposes provides a way to overcome the fundamental challenges of complex analyses such as cluster analysis and the requirement of control samples (normal or recurrence-free) in the clinical application of biomarkers. Taken together, these data show the feasibility of employing a TLDA-based array system in any clinic for diagnosis.

Acknowledgments
V. G. Deepak Roshan is a recipient of a Senior Research Fellowship of the Indian Council of Medical Research, Government of India. The authors declare that there are no conflicts of interest.

Financial support and sponsorship
The present study was financially supported by the FIST grant of the Kerala State Council for Science, Technology and Environment, Government of Kerala.

Conflicts of interest
There are no conflicts of interest.

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Supplementary Figure 1: (a) Schematic diagram showing the complete workflow of the study. (b) Image showing the TaqMan low-density array card experiment setup.
## Supplementary Table 1: List of genes studied, its function

| Genes   | Function of gene                                                                 |
|---------|----------------------------------------------------------------------------------|
| *CCND2  | Cell cycle regulator - G1/S transition                                          |
| TGFA    | Activate EGFR signaling - regulate G1 phase                                     |
| *CDK6   | Cell cycle regulator - G1/S transition                                          |
| *CCNB1  | Cell cycle regulator - G2/M transition                                          |
| *CCNE1  | Cell cycle regulator - G1/S transition                                          |
| *CHEK1  | Component of the G2/M transition                                                |
| CCNC    | G1 phase of cell cycle                                                          |
| *CDC2   | G1/S and G2/M phase transition                                                  |
| *CCNF   | E3 ubiquitin-protein ligase complex - G2 phase                                   |
| *CCNB2  | Cell cycle regulator - G2/M transition                                          |
| BCL2    | Apoptosis, G1 phase                                                             |
| MKI67   | Enhances in mitosis                                                             |
| CCND3   | Cell cycle regulator - G1/S transition                                          |
| CDKN1A  | Cyclin inhibitor                                                                |
| CDKN1C  | Cyclin inhibitor                                                                |
| CDC20   | Cell cycle regulator - G2/M transition                                          |
| CDK2    | G1-S transition to promote the E2F transcriptional                              |
| CDK4    | Cell cycle regulator - G1/S transition                                          |
| CCNE2   | Cell cycle regulator - G1/S transition                                          |
| CDC25C  | Regulate M Phase of cell cycle                                                 |
| CCNG2   | Cell cycle regulation mid/late-S phase                                          |
| CCND1   | Cell cycle regulator - G1/S transition                                          |
| EGFR    | Cell signaling - G1 regulation                                                  |
| CDK5    | Interacts with D1 and D3-type G1 cyclins                                        |
| E2F1    | Transcription factor - G1/S transition                                          |
| *CCNA1  | Control of the cell cycle at the G1/S (start) and G2/M transitions              |
| CCNK    | May play a role in transcriptional regulation                                   |
| *CDKN2B | Inhibit CDK4 and CDK6                                                           |
| *CDKN2A | Inhibit CDK4 and CDK6                                                           |
| RB1     | Inhibit E2F                                                                      |
| CDC34   | Involved in the regulation of the cell cycle G2/M phase                         |
| CDKN2C  | Inhibit strongly CDK6, weakly CDK4                                             |
| TP53BP2 | Plays a central role in regulation of apoptosis                                 |
| CDK8    | A coactivator involved with RNA polymerase II-dependent genes                   |
| CDKN1B  | Involved in G1 arrest - inhibitor of cyclin E- and cyclin A-CDK2 complexes       |
| CDK7    | Cell cycle control and in RNA polymerase II-mediated RNA transcription          |
| CDKN2D  | Involved in G1 arrest                                                           |
| PIN1    | Essential PPIase that regulates mitosis                                          |
| ATR     | Activates checkpoint signaling upon genotoxic stresses                          |
| TP53    | Tumor suppressor                                                                |
| RBL2    | Key regulator of entry into cell division                                       |
| RBL1    | Key regulator of entry into cell division                                       |
| E2F2    | Transcription factor - G1/S transition                                          |
| CCNH    | Regulates CDK7                                                                  |
| MDM2    | Regulate p53, oncogene                                                          |
| ATM     | Regulates checkpoint signaling upon double strand breaks                        |
| GADD45A | Inhibits entry of cells into S phase                                            |

*Significant tumor specific genes; #Significant recurrence specific genes

## Supplementary Table 2: Gene Expression in Fold change for Tumor compared to normal

| Gene name | Fold change | Significant (P) |
|-----------|-------------|-----------------|
| ATM       | 1.0685      | 0.7497          |
| ATR       | 1.1288      | 0.5842          |
| BCL2      | 0.8531      | 0.7497          |
| CCNA1     | 5.7351      | 0.0215          |
| CCNB1     | 3.5952      | 0             |
| CCNB2     | 2.6597      | 0.0001          |
| CCNC      | 1.6416      | 0.0001          |
| CCND1     | 0.8117      | 0.5824          |
| CCND2     | 2.4841      | 0              |
| CCND3     | 0.1354      | 0.5435          |
| CCNE1     | 2.1937      | 0.0125          |
| CCNE2     | 1.5631      | 0.1236          |
| CCNF      | 2.3956      | 0              |
| CCNG2     | 1.6742      | 0.0071          |
| CCNH      | 1.1135      | 0.5696          |
| CCNK      | 1.4611      | 0.0327          |
| CCNC      | 2.572       | 0.0001          |
| CDC20     | 1.6851      | 0.4387          |
| CDC25C    | 3.5461      | 0.4387          |
| CCND4     | 1.1195      | 0.7497          |
| CDK2      | 1.7079      | 0.0011          |
| CDK4      | 1.6668      | 0.0148          |
| CDK5      | 1.5899      | 0.0215          |
| CDK6      | 2.5043      | 0              |
| CDK7      | 1.3001      | 0.4016          |
| CDK8      | 1.2653      | 0.3565          |
| CDKN1A    | 1.7173      | 0.0327          |
| CDKN1B    | 0.9185      | 0.7497          |
| CDKN2A    | 0.6608      | 0.5045          |
| CDKN2C    | 1.5261      | 0.5488          |
| CDC34     | 1.8384      | 0.0293          |
| CDKN2B    | 0.9416      | 0.8064          |
| CDKN2C    | 1.2422      | 0.3756          |
| CHEK1     | 2.7221      | 0.0001          |
| E2F1      | 1.5595      | 0.0213          |
| E2F2      | 1.0942      | 0.7497          |
| EGFR      | 1.9012      | 0.0183          |
| GADD45A   | 1.0612      | 0.7497          |
| MDM2      | 1.0668      | 0.8179          |
| MKI67     | 0.6925      | 0.7497          |
| PIN1      | 0.3224      | 0.5488          |
| TP53      | 1.0705      | 0.7497          |
| TP53BP2   | 1.3133      | 0.252           |