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miRNA-17-5p Target Prediction and its Role in Senescence Mechanism through p21 Interference

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Abstract

BACKGROUND: Cellular senescence is known to be correlated with the cessation of cell cycle. The progression of cell cycle is promoted by activities of various proteins, including cyclin-dependent kinase (CDK) and cyclin proteins, which work synergistically. CDK-cyclin complexes are influenced by other proteins, such as retinoblastoma (Rb) and E2F proteins. In cell cycle, both Rb and E2F proteins could be affected by one of the CDK inhibitors, that is, p21. MicroRNA (miRNA) is well known for its role in biological processes, including cell cycle. However, the contribution of miRNA in cell cycle is still poorly understood. Some miRNAs play a role in pro-proliferation and anti-proliferation.

AIM: This study was performed an *in silico* study analysis to reveal the relationship between miRNA-17-5p and p21 in the process of cellular senescence.

METHODS: The extensive data mining was conducted to determine the miRNA that contributes to the process of anti-aging prevention and the desired target genes through the Human Protein Atlas and cancer database. miRNA target prediction was performed using DIANA-microT-CDS. Gene function of the miRNA-17-5p target was annotated using DAVID GO.

RESULTS: The sequence of hsa-miRNA-17-5p (CAAAGUGCUUACAGUGCAGGUAG) has three attachment sites with binding types of 8 mer, 6 mer, and 8 mer at the transcription sites of 447–474, 485–513, and 1132–1154, respectively. The main profile of hsa-miRNA-17-5p showed that it bound to 3'-untranslated region and the coding region (exon).

CONCLUSIONS: The miRNA-17-5p was involved in cellular senescence by influencing the process of cell proliferation in the cell cycle pathway.

Introduction

The previous studies have provided evidence that microRNA (miRNA) plays a critical role in various cell cycle and cell proliferation processes. Proliferative diseases, such as cancer, may occur due to the deregulation of miRNA that elevates the levels of oncogenes or tumor-suppressor genes [1]. In cell cycle progression, both direct and indirect contribution, miRNA has target genes involved in cell cycle (including cyclins, cyclin-dependent kinases [CDKs], and CKI) [2]. The miRNA-17-92 group is located at the MIR17HG non-protein-coding gene locus on chromosome 13 (13q31.3) [3], [4]. The miRNA-17-92 group transcribes 800 nucleotides, which are composed of miRNA-17-5p (known as miRNA-17), miRNA-18a, miRNA-19a, miRNA-19b, miRNA-20a, and miRNA-92a. This miRNA group is critical in cell cycle progression that occurs through the mechanism of cellular reprogramming, tumorigenesis, and cell cycle [3], [5], [6].

The cell cycle presents a series of events in a cell that leads to division and duplication, which results in identical replicates. More importantly, the work of cell cycle and cell division is highly correlated to cell (cellular senescence) and organ aging [7], [8]. In general, eukaryotic cell cycle is divided into two main phases: (i) Interphase (S, G1, and G2 phases) and (ii) mitosis (M phase) [8]. The progression of all cell cycle is coordinated by activities of various proteins that work synergistically, such as CDK and cyclin proteins [9], [10]. Retinoblastoma (Rb) is the most important target of CDK-cyclin complex in transition phase of G1/s [11].

p21 is one of the CDK inhibitors that act as an inhibitor of the cell cycle because it can inhibit the cell cycle progression during the transition phases G1/S and G2/M. Cell progression is regulated by p21, which is modulated by E2F proteins [12]. Fortunately, many studies have been conducted to elucidate the regulation

of Rb protein Rb's regulation by p21 along with the related oncogenic function. Through the inhibition of CDK2/4 complex and other transcription factors, p21 contributes to cell survival, morphology, and gene expression. Phosphorylation by CDK2 and CDK4/5 on proliferating cells may inactivate Rb; therefore, induction of p21 may cause Rb to go through dephosphorylation, and the cell cycle may be terminated at G1 [13].

Some miRNAs are found to act as negative regulators of cell proliferation and potential suppressor of tumorigenesis process. miRNAs that target cell cycle proteins include miRNA-34, miRNA-449, miRNA-15a-16-1, miRNA-24, and miRNA-195 [1], [14]. Nevertheless, the biological relevance of miRNA in the cell cycle needs to be elucidated further by conducting *in vivo* studies [14]. The single expression of miRNA from the miRNA-17-92 and miRNA-17-5p groups is sufficient to trigger the proliferation signal in cultured cells [1]. This study aimed to evaluate the target of miRNA-17-5p and identify the pathway that is particularly involved in cellular senescence.

Materials and Methods

Data mining

Extensive data mining was conducted to determine the miRNA that contributes to the process of anti-aging prevention and the desired target genes through the Human Protein Atlas (https://www.proteinatlas.org/ENSG00000124762-CDKN1A/pathology#gene_information) and cancer database (https://www.cancer.gov/).

Prediction of miRNA-17-5p target

miRNA target prediction was performed using DIANA-microT-coding sequence (CDS) (http://www.microrna.gr/microT-CDS) [15]. This tool's algorithm specifically targets miRNA Recognition Element sites located at the 3'-untranslated region (UTR) and CDS region. DIANA-microT-CDS is capable of detecting target miRNA that has high sensitivity and is proficient enough to be compared with the experimental or proteomic data.

Gene ontology (GO) of the miRNA-17-5p target gene

Gene function of the miRNA-17-5p target was annotated using DAVID GO [16]. Deep analysis was performed using DIANA Tools mirPath (v.30) to elucidate the possible pathway after miRNA-17-5p interacts with the target gene [17]. This tool provides the information from miRNA functional annotation using GO combined with Kyoto Encyclopedia of Genes and Genomes pathway database.

Results

miRNA-17-5p target gene and p21-specific target

Target prediction of miRNA-17-5p had detected approximately 480 genes, including P21 (CDKN1A) with gene ID ENSG00000124762 and miTG score of 0.9479 (equivalent to 95%), which represented a high prediction accuracy.

The sequence of hsa-miRNA-17-5p (CAAAGUGCUUACAGUGCAGGUAG) has three attachment sites with binding types of 8 mer, 6 mer, and 8 mer at the transcription sites of 447–474, 485–513, and 1132–1154, respectively (Figure 1). The main profile of hsa-miRNA-17-5p showed that it bounds to 3'-UTR and the coding region (exon) (Figure 2).

According to the Human Protein Atlas database, p21 (CDKN1A) regulates the G1 cell cycle modulated by p53 protein. The p21 protein is able to interact with proliferating cell nuclear antigen and plays a role in DNA replication and DNA damage repair (Figure 3). A transcriptomic study through cancer database (https://www.cancer.gov/), which has been summarized by Human Protein Atlas, revealed that p21 is expressed in various cancers, such as glial cell, thyroid, lung, liver, pancreatic, head, and neck, stomach, colorectal, urothelial, renal, prostate, testis, breast, cervical, endometrial, and melanocyte cancer (Figure 4).

A total of 480 target genes were obtained and analyzed for their functional enrichment using DAVID GO. The results denoted the top 10 terms, which were detected to have contribution in transcription activity and regulation, which implied that miRNA-17-5p has potential target protein associated with transcription activity (Table 1).

Pathway analysis is required to elucidate the molecular mechanism in cells. A total of 480 genes obtained from the previous analysis were analyzed for all possible pathways related to cell cycle. The

Table 1: Top 10 functional target gene annotations of miRNA-17-5p detected by DAVID GO

No.	Terminology	p-value
1.	Transcription, DNA templated	4.66E+12
2.	Heart development	0.001688855
3.	Positive regulation of transcription, DNA templated	0.001735865
4.	Sodium ion transmembrane transport	0.001895283
5.	Regulation of transcription, DNA templated	0.002106672
6.	Vasodilation	0.004187626
7.	Regulation of cell motility	0.004353929
8.	Positive regulation of transcription from RNA polymerase II promoter	0.004932677
9.	Sphingomyelin biosynthetic process	0.005556164
10.	Long-term synaptic depression	0.006215772

98 ENSG00000124762 (CDKN1A) hsa-miR-17-5p 0.947908434429616

Gene details
miRNA details
pubMed links: miRNA | gene | both
UCSC graphic

Region	Binding Type	Transcript position	Score	Conservation
UTR3	8mer	447-474	0.012955646612841	3

Position on chromosome: 6:36654025-36654052
Conserved species: panTro2,rheMac2,monDom5
Binding area:

```
(Transcript)5'C      AGAA    AACAGAU        3'
             AUUUG     GUA      GGCACUUUG
             |..||     |||      .|||||||||
             UGGAC     CAU      UCGUGAAAC
(miRNA)   3'A      GUGA                    5'
```

Region	Binding Type	Transcript position	Score	Conservation
UTR3	6mer	485-513	0.00458927260018664	6

Position on chromosome: 6:36654063-36654091
Conserved species: panTro2,rheMac2,mm9,bosTau4,canFam2,loxAfr3
Binding area:

```
(Transcript)5'    GAGUGGGG    CAUCAAAA      3'
                    ACC         GCAU        ACUUUG
                    |||         |||.        ||||||
                    UGG         CGUG        UGAAAC
(miRNA)   3'A   A             ACAUUCG       5'
```

Region	Binding Type	Transcript position	Score	Conservation
UTR3	8mer	1132-1154	0.0405788225157225	8

Position on chromosome: 6:36654710-36654732
Conserved species: panTro2,rheMac2,oryCun2,bosTau4,canFam2,dasNov2,loxAfr3,echTel1
Binding area:

```
(Transcript)5'CCCAUC    C C A  UCAUU       3'
                    CCU C C GU     GCACUUUG
                    ||| | | ||     ||||||||
                    GGA G G CA     CGUGAAAC
(miRNA)   3'     U   C U A  UU             5'
```

Figure 1: Detailed information of mir-17-5p

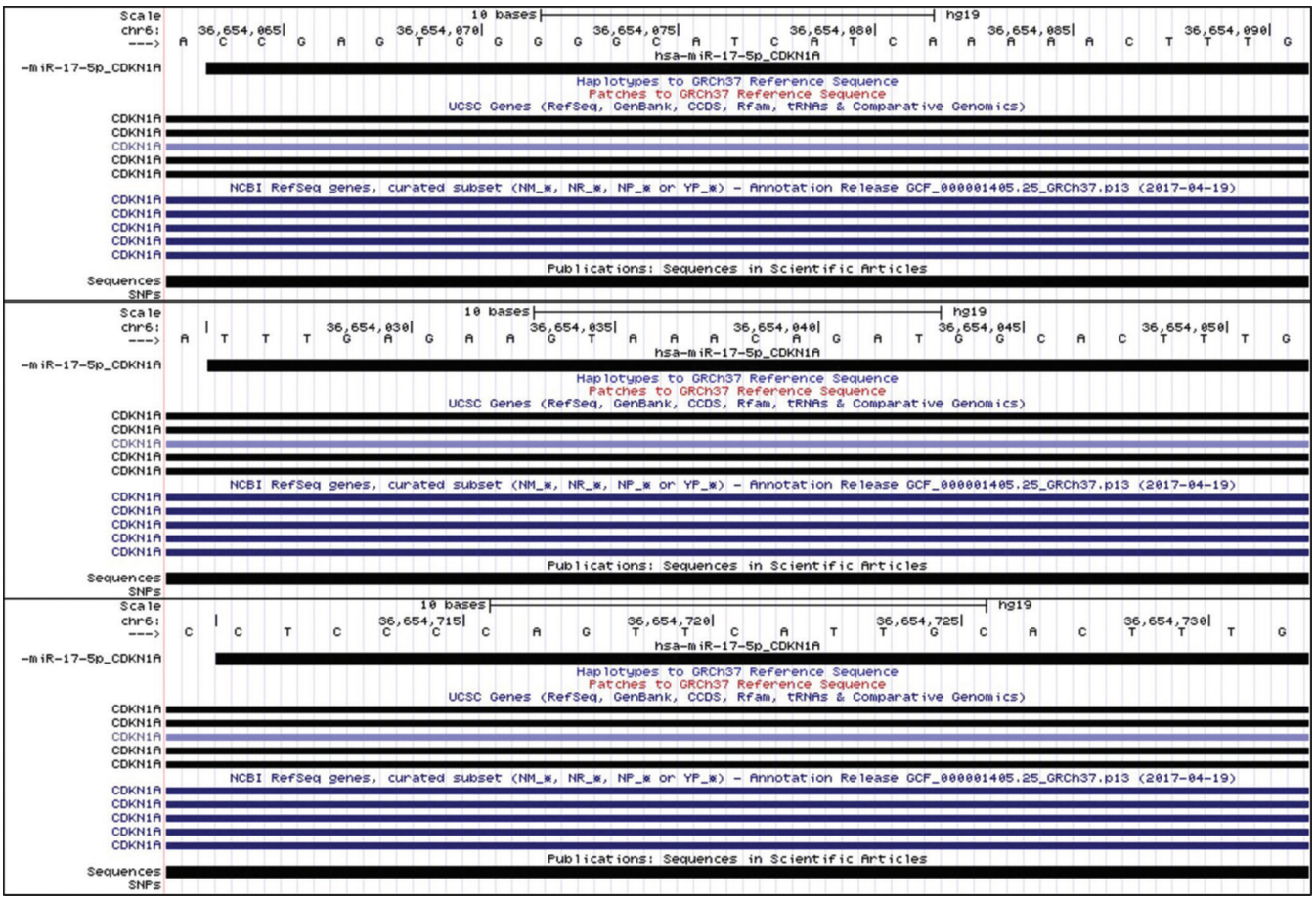

Figure 2: Visualization of the hsa-miR-17-5p position in the CDKN1A gene from several transcript positions using UCSC software; A. (6:36654025-36654052); B. (6:36654063-36654091); C. (6:36654710-36654732)

GENE INFORMATION[i]	
Gene name	CDKN1A (HGNC Symbol)
Synonyms	CAP20, CDKN1, CIP1, P21, p21CIP1, p21Cip1/Waf1, SDI1, WAF1
Description	Cyclin dependent kinase inhibitor 1A (HGNC Symbol)
Entrez gene summary	This gene encodes a potent cyclin-dependent kinase inhibitor. The encoded protein binds to and inhibits the activity of cyclin-cyclin-dependent kinase2 or -cyclin-dependent kinase4 complexes, and thus functions as a regulator of cell cycle progression at G1. The expression of this gene is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli. This protein can interact with proliferating cell nuclear antigen, a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair. This protein was reported to be specifically cleaved by CASP3-like caspases, which thus leads to a dramatic activation of cyclin-dependent kinase2, and may be instrumental in the execution of apoptosis following caspase activation. Mice that lack this gene have the ability to regenerate damaged or missing tissue. Multiple alternatively spliced variants have been found for this gene. [provided by RefSeq, Sep 2015]
Chromosome	6
Cytoband	p21.2
Chromosome location (bp)	36676460 - 36687339
Protein evidence	Evidence at protein level (all genes)
Ensembl	ENSG00000124762 (version 88.38)
Entrez gene	1026
UniProt	P38936 (UniProt - Evidence at protein level)
neXtProt	NX_P38936
Antibodypedia	CDKN1A antibodies

Figure 3: General information of p21 as a target gene of miRNA-17-5p

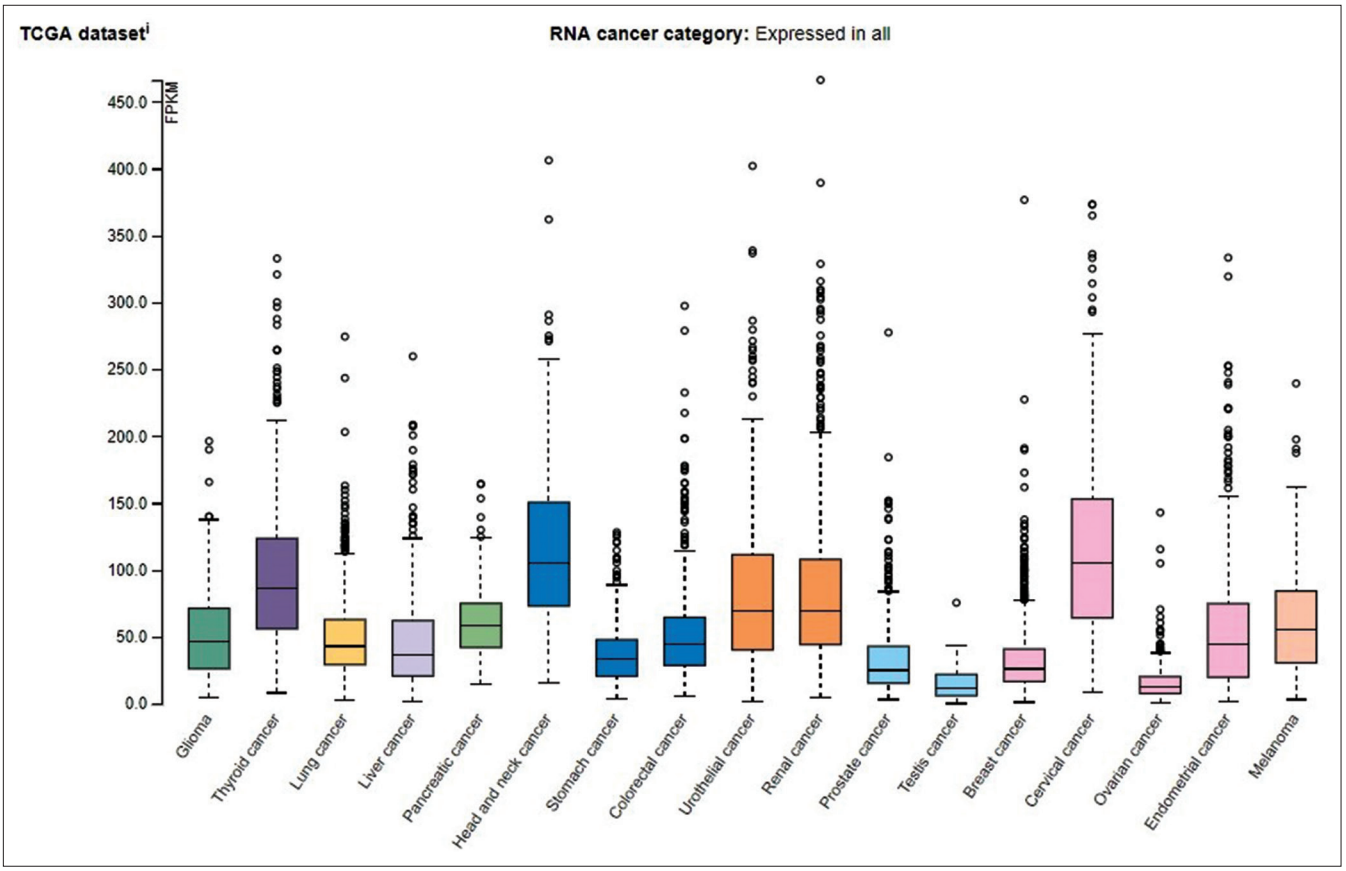

Figure 4: RNA dataset of P21 expression in various cases of cancersf

analysis results identified 28 pathways (Figure 5), and one of these pathways was related to cell cycle with p-value of 8.844459e-05. The predicted pathway was represented in the heat map. The red color indicates a high significance level based on Log score (p-value), which is getting smaller, while the yellow color indicates a low significance level based on Log score (p-value), which is getting bigger (color key; Figure 5). miRPath software implied the five possible target genes of miRNA-17-5p involved in cell cycle, namely, E2F1 (ENSG00000101412), CCND1 (ENSG00000110092), SMAD4 (ENSG00000141646), MYC (ENSG00000136997), and CDKN1A (ENSG00000124762). The pathway involving CDKN1A

is presented in Figure 5. The involvement of p21 in the cell cycle was emphasized by the presence of CDKN1A/P21 as one of the downstream in cell cycle, which is also a target of p53 (Figure 6).

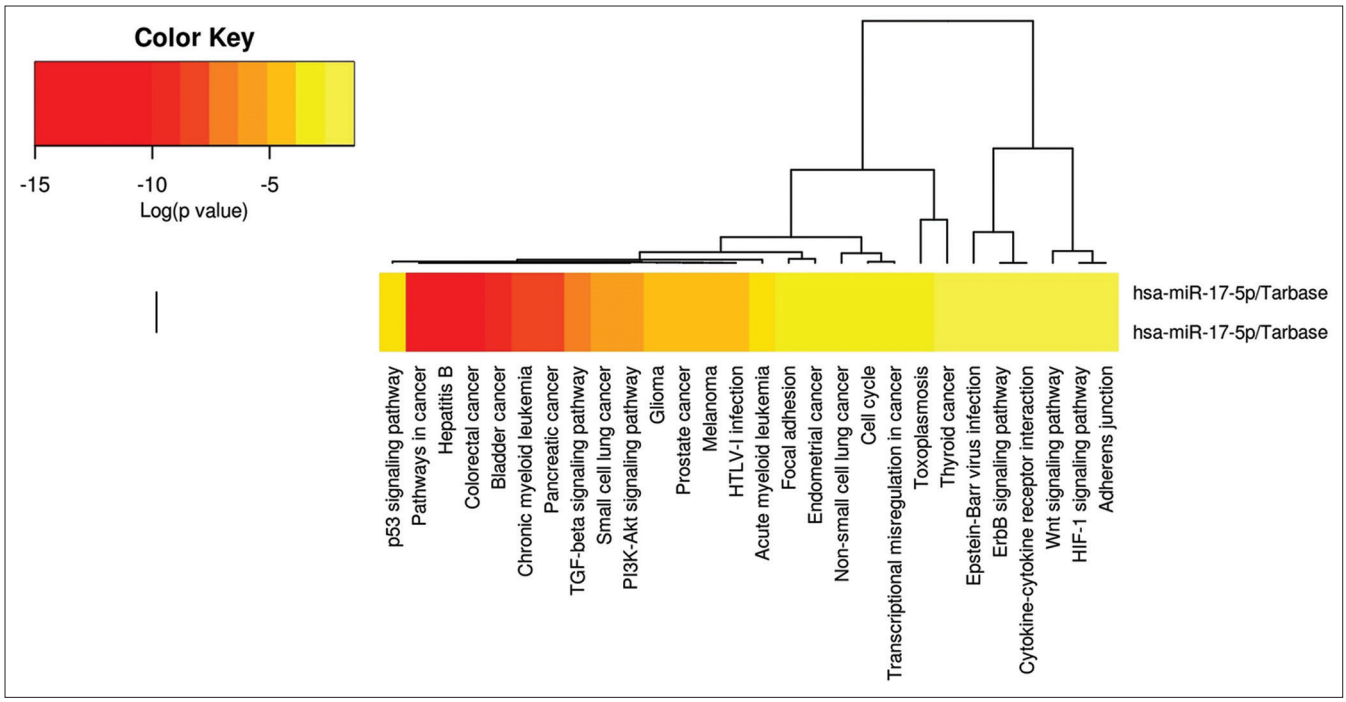

Figure 5: Several pathways involved in both miRNA-17-5p and its target gene, which were analyzed using miRPath

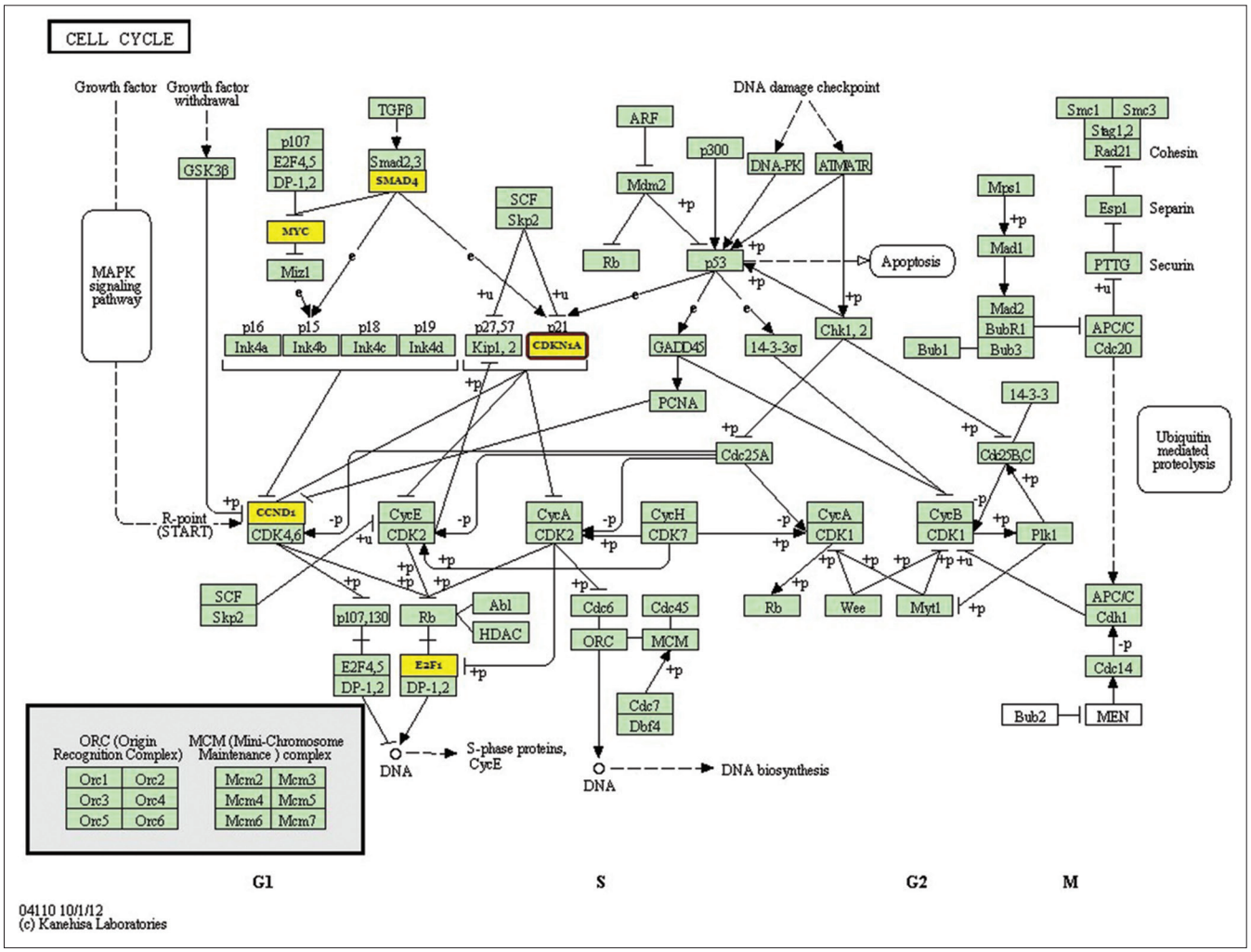

Figure 6: The cell cycle pathway in which P21 (CDKN1A) gene involved (analyzed using pathway viewer from miRPath)

Discussion

The type of miRNA target prediction has been widely performed computationally. Here, we show the finding of protein target of mir-17-5p using DIANA Lab database because it provides a comprehensive data and high accuracy (Figure 1). The identification of miRNA target protein is critical for further analysis to support the data validity [15]. Moreover, by deciphering the specific target location in CDKN1A genome browser, we able to determine mir-17-5p strength in cell senescence condition (Figure 2). GO related to the biological functional enrichment pointed out terms related to the cell cycle, supported by the pathway analysis involved in the mirPath database (Table 1 and Figure 5).

In our study, we identified 480 target proteins of miR-17-5p, and one of them is CDKN1A with miTG score 0.947. The miTG score represents probability of target protein, the higher miTG score or close to 1, the higher accuracy of the prediction. The protein target data were supported by the finding according to Bueno and Malumbres [1], which mentioned that the miRNA-17-5p also targets p21 gene (CDKN1A), which improves cell proliferation and transcription in cell cycle similar to anti-aging therapies. The finding leads to the hypothesis of mir-17-5p regulating the cell senescence by CDKN1A pathway. Since the binding of mir-17-5p was detected in the 3' UTR and CDS in the three different locations of CDKN1A (Figure 2), the mechanism action of mir-17-5p might be varied. Fang and Rajewsky [18] stated that both mRNA and protein expression of the gene on the 3'-UTR and coding region have a tendency to have a more significant and functional regulatory effect than that of mRNA, which is only in 3'-UTR. The distinct regulatory effect of miRNA binding to the CDS and to 3'UTR has been evaluated by Brümmer and Hausser (2014). The most well-known theory stated that the miRNA should be bind to 3'UTR region to induce the mRNA degradation. In fact, the difference was in the binding regulating specific action. The miRNA binding to the 3'UTR influences the protein abundance, notably by mRNA deadenylation. Besides, the miRNA targeting CDS has probability to accelerate the miRNA-mediated gene to form stable mRNAs (Brümmer and Hausser, 2014). Thus, the explanation leads to the hypothesis that mir-17-5p has two types of action toward CDKN1A: (1) Initiation of decaying mRNA of CDKN1A by binding to the 3'UTR (the poly (A) tail shortening process) and (2) expedite the recruitment of miRNA-mediated CDKN1A to silence the mRNA continuously and stably.

Further, to deepen the exploration of gene targets by mir-17-5p, the biological function enrichment analysis was performed. Potential screening results of functional enrichment by DAVID GO were ranked based on the p-values, indicating the corresponding significance of the predictive results. The smaller the p-value, the more significant the prediction results [16]. Our analysis showed that top 10 terms related to the regulation of transcription were detected in the enrichment. Based on the GeneCards database, the CDKN1A was surrounded by numerous transcription factors. The majority of GeneHancer types are enhancer (Supplementary Data 1). A total of 218.117 enhancer positions were detected in GeneCards, with the range score between 0.25 and 3.11. A report mentioned that CDKN1A is epigenetically regulated by TRF2, which is a telomere repeat binding factor, to repress the p21/CDKN1A. The pathway involved is through engagement of REST by changing the histone marks at CDKN1A promoter in TRF2. In addition, the G-quadruplex (G4 motif) complex formed as the results of TRF2 interaction to regulate the p21/CDKN1A promoter activity (Hussain *et al*., 2017).

Cellular senescence is defined as the cessation of cell cycle accompanied by phenotypic changes resulting in the loss of replicative ability [19], [20]. *In vivo* cellular senescence is considered to significantly contribute to the aging process [20], [21]. Cells experiencing cessation may exhibit particular aging phenotypes, such as cell enlargement and flatten, thereby increasing the activity of senescence-associated β-galactosidase [20], [22]. Senescent cells may get accumulated with increasing age, particularly in the areas of the body that is in pathological conditions due to age (age-related pathologies). This will influence the tissue that is physiologically normal to be progressively damaged [23].

In senescent cells, Rb protein may get accumulated in the hypophosphorylation state due to which the cell may be unable to enter S phase. Mao *et al*. [24] stated that cells undergo senescence possibly because of cessation of the cell cycle in G2 phase. The decreased expression of p21 mediated by RNAi may cause senescent cells to reenter the cell cycle [25]. Suppression of p16 pathway may cause cells to reenter the S phase, even though it may be unable to perform cell division [26], [27], [28], [29]. In conclusion, p21 and p16 in the mechanism ensure the cellular senescence to be irreversible [20].

Conclusions

P21 was the main target of miRNA-17-5p with high prediction accuracy. The miRNA-17-5p was involved in cellular senescence by influencing the process of cell proliferation in the cell cycle pathway. Further, laboratory experiments need to be performed to support the result of this study.

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