Identification of MicroRNAs Targeting mTOR Gene Transcripts in Skin, Lung, Kidney, Uterus and Breast Cancer

Stefanus Satrio Hadi Wibowo¹, David Agustriawan¹*, Arli Aditya Parikesit¹, Rizky Nurdiansyah¹

¹Department of Bioinformatics, School of Life Sciences, Indonesia International Institute for Life Sciences, Jl. Pulomas Barat Kav.88 Jakarta 13210 Indonesia.

*Corresponding author: david.agustriawan@i3l.ac.id

Abstract. mTOR constitutively activated during tumorigenesis to stimulates mRNA translation through regulating cell energy metabolism. Using in silico approach based on miTS method, correlation analysis, and meta-analysis method, miRNA biomarker related to five cancer were researched. The input of the pipeline was THCA patients from GDC Data Porta ID and was processed using TCGA2STAT, dplyr, and metacor package in R studio, Excel, and MirTarBase. This study aims to discover the significance level of mTOR in five cancer: HNSC (Head and Neck Squamous Cell), lung adenocarcinoma (LUAD), Kidney Renal Clear Cell Carcinoma (KIRC), Uterine Corpus Endometrial Carcinoma (UCEC), and Breast Invasive Carcinoma (BRCA). Our study shows that in the cancer patient, mTOR tends to support cancer growth despite miRNA targeting it has low correlation rho value. The numerous microRNAs targeting mTOR have a specific role in each tissue, meaning it can be targeted as a precise medicine.

1. Introduction
MicroRNA (miRNA) is a short, around 22 nucleotides, non-coding RNA molecules, regulating gene expression at the post-transcriptional level, by inhibiting translation or initiating mRNA degradation [1]. Several studies have utilized miRNA profiles to indicate diagnostic and prognostic markers for numerous cancers, including skin, lung, kidney, uterus, and breast [2-4]. There are already 2000 miRNA in humans has been discovered, and so far, it has been found that they regulate 30% of all genes [5,6]. This characteristic in gene regulation is making them identified as a high-value target for cancer therapy [7].

In all eukaryotes, the mammalian target of rapamycin (mTOR) signaling pathway couples energy and nutrient uptake to support cell growth and division [8]. Consistent with its role in cell proliferation, the pathway is frequently hyperactivated in several human malignancies and is thus valued to be an attractive target for anti-cancer therapy [9]. As an example, in hepatocellular cancer, mTOR inhibitors (mTORIs) has shown a practical result in reducing cell growth and tumor vascularity [10]. Besides, several mTORs are already clinically available, e.g., sirolimus, temsirolimus, and everolimus [11]. Thus, mTOR biomarker can be utilized in cancer diagnostic, prognostic, and treatment.

There are numerous approaches in silico miRNA identification, i.e., genome-wide analysis (GWA) and miTS (miRNA data and tissue specificity of diseases). GWA utilize new genome sequencing
(NGS) data as the input while miTS use available data from bioinformatics database to be computed using statistical correlation \cite{1,12}. Since NGS data access to the public is mostly restricted and de novo data would cost 7-200$ per GB, utilizing a free to access tissue-specific disease database such as The Cancer Genome Atlas (TCGA) will be preferable since it is more efficient.

Since miTS method rely on TCGA repository and a statistical analysis require significant sample, the study was conducted only for five cancers containing more than 520 patients clinical information: head and neck squamous cell (HNSC), lung adenocarcinoma (LUAD), kidney renal clear cell carcinoma (KIRC), uterine corpus endometrial carcinoma (UCEC), and Breast Invasive Carcinoma (BRCA) \cite{13}. The research was conducted by performing data mining and correlation analysis from bioinformatics databases: TCGA and MiRTarbase by using TCGA2STAT, dplyr, and metacor package in R Studio and statistical analysis using Microsoft Excel. Compared to the previous study, this study aims to deliver a detailed correlation of each microRNA targeting mTOR in multiple sample tissue to study the efficacy of microRNA targeting mTOR as a biomarker for cancer treatment \cite{1}. The pipeline of the process and command line used was provided in an open-source package (https://github.com/stefanuswibowo/IQGAP_mTOR_biomarker).

2. Methods
The dataset was taken from The Cancer Genome Atlas project (https://portal.gdc.cancer.gov/) dataset and downloaded using R studio. R packages that used for the study are: (i) TCGA2STAT for downloaded all the datasets; (ii) dplyr for manipulated the dataset; and (iii) metacor for performed meta-analysis.

2.1. miRNAs Selection
The miRNA was collected from miRTarBase, a database for micro RNA and its targeted protein by applying keyword "mTOR" \cite{14}. The miRNA that hasn't been wholly validated through all strong evidence method: reporter assay, western blot, and qPCR were removed. The database was accessed on 20 Jan 2019.

2.2. Data Filtration
First, RNA and miRNA expression datasets were downloaded using TCGA2STAT package. TCGA2STAT is a simple tool to access the TCGA repository database based on the R environment \cite{15}. Each of the data objects will contain three different datasets, which are: (i) dat consists of miRNA or RNA expression dataset; (ii) clinical consist of clinical dataset and; (iii) merged.dat consist of the combination of expression and clinical dataset, stored as one data object, normal samples are not available within the dataset.

Next, mTOR and 11 miRNAs were extracted from merged.dat by using the dplyr package. Dplyr is a data manipulation tool based on the R environment \cite{16}. During the data extraction, two mTOR miRNAs need to be removed since it doesn't exist within the dataset. By default, the datasets were grouped based on cancer pathological stage from I to IV. Stage categorized as X and NA were excluded from the study. Stage X indicates that the tumor was detectable, but diagnostician can't confer the pathological stage of it, while NA suggests that the primary tumor wasn't detected. Since this study was focused on analyzing miRNAs targeting mTOR within each stage of breast cancer, the miRNA expression differences within each stage will be ignored. At the end of data preparation, each miRNA targeted mTOR from miRTarBase, and miRNA datasets from each tumor were matched and merged based on the sample barcode.

2.3. Correlation Analysis
Spearman correlation analysis was conducted for each cancer tissue for mTOR and nine selected miRNAs. Using R, the data was transposed to tidy up the outlook. Using Excel, sample size and table legend were added.
Meta-Analysis
The correlation dataset from each cancer tissue was combined and edited in Microsoft Excel. Using metacor in R, the meta-analysis was performed by using a combined dataset and metacor package [17]. Since each cancer tissue cannot be treated in the same way, then the random effect model was applied.

3. Result and Discussions

3.1. miRNAs Selection
From 29 miRNA targeted mTOR result, 18 was removed because it hasn't been fully validated through all strong evidence method.

3.2. Data Filtration
During the merging of datasets, two microRNAs targeting mTOR was removed since it wasn't listed within the dataset. From 528 HNSC patients, only 228 managed to undergo data filtration. From 522 LUAD patients, only 79 lead to suffered data filtration. From 537 KIRC patients, only 204 manage to undergo data filtration. From 548 UCEC patients, only 139 manage to undergo data filtration. While for BRCA, from 1097 patient, only 458 patients manage the data filtration process.

3.3. Correlation Analysis
Table 1 shows the correlation value of each microRNA targeting mTOR and cancer tissue. In general, each miRNA don't have a high correlation with the tissue cancer, and the value contains both positive and negative value, meaning that it may promote cancer or oncogene if it is positive and may suppress the tumor if it is negative. Uniquely, each miRNA act in a different way in a specific tissue, meaning it could be an oncogene or cancer suppressor. The previous study has labeled miR-520c and miR-373 as an oncogene since it upregulates MMP9 expression by targeting mTOR [18]. In contrast, mir-373 also has been marked both as oncogenes and tumor suppressors due to its variation in the role [19]. Therefore, miRNA targeting mTOR could work both as oncogenes and tumor suppressors.

The low rho value within each dataset shows that miRNA targeting mTOR work indirectly to promote the growth of cancer. Previous study has found that miR-99b work as an tumor suppressor in HNSC by targeting Insulin Growth Factor (IGF/IGFR), miR-193a as an oncogene in gastric cancer by targeting Phosphatase and Tensin Homolog (PTEN), miR-520c as an oncogene or tumor suppressor by targeting CD44 module, cadherin1 type 1E, nuclear factor I/B, and protein phosphatase 6 catalytic subunit [18,20].

Table 1. The result of the correlation analysis of mTOR within each cancer

| miRNAs     | HNSC   | LUAD   | KIRC  | UCEC  | BRCA  |
|------------|--------|--------|-------|-------|-------|
|            | n = 228| n = 79 | n = 204| n = 139| n = 458|
| hsa-mir-520c| -0.17  | 0.10   | 0.009 | 0.01  | -0.01 |
| hsa-mir-373 | -0.005 | 0.15   | -0.04 | -0.26 | -0.005|
| hsa-mir-99b | 0.03   | -0.09  | 0.16  | 0.16  | 0.03  |
| hsa-mir-144 | -0.002 | -0.08  | 0.11  | -0.01 | -0.0028|
| hsa-mir-100 | -0.002 | -0.05  | -0.23 | -0.05 | -0.0020|
| hsa-mir-224 | 0.021  | -0.09  | -0.05 | -0.13 | 0.02  |
| hsa-mir-193a| 0.07   | 0.26   | -0.17 | 0.05  | 0.07  |
| hsa-mir-497 | -0.06  | 0.03   | -0.09 | 0.29  | -0.06 |
| hsa-mir-3138| -0.06  | 0.17   | 0.05  | 0.19  | -0.04 |

Abbreviations: hsa-mir denotes homo sapiens microRNA
3.4. Meta-Analysis

Table 2 show z mean and p-value from the meta-analysis study of mTOR efficacy to the five cancer. Although insignificant, the positive z mean value show the tendency of mTOR to be upregulated as an oncogene. The value of meta-analysis study supports previous studies stating mTOR to be upregulated in cancer to aid proliferation, especially in metastasis state [8][9].

| Statistical Analysis | Results |
|----------------------|---------|
| z mean               | 0.02    |
| P                    | 0.05    |

4. Conclusion

The result of the study found that mTOR has a tendency to support cancer growth and has a value that closes to be called significant despite miRNA targeting it has low correlation rho value. For further analysis, the stage can be differentiated, and following the data growth, more sample and tissue can be gained.

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