Correlation analysis to identify DNA methylation and miRNA regulation toward IQGAP genes family

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Abstract. The aberrant expression of IQGAP genes family which consist of IQGAP1, IQGAP2 and IQGAP3 is related with various kinds of cancer where IQGAP can function as oncopgenes or tumor suppressor genes. The increasing number of the study suggested that upregulated or downregulated of IQGAP gene expression in cancer cell trigger the spreading of cancer. The model and function of the IQGAP1 gene have been investigating in many cancer types. However, study related IQGAP2 and IQGAP3 in cancer formation is still lack, and it needs to be further investigated. Moreover, epigenetic regulation related to the aberrant expression of IQGAP genes in cancer formation is not well studied yet. Therefore, this study conducted a correlation study to identify the interaction of IQGAP gene family expression with DNA methylation and miRNA expression in Ovarian cancer. This research was performed a correlation analysis which integrated miRNA, Gene and DNA methylation expression dataset. All the computation was calculated in MATLAB software. This study identified that IQGAP1 significantly negatively correlated with DNA methylation probe cg109784032 and forty-three miRNAs.

1. Introduction
Cancer formation in human has a very complex pathway. In molecular biology, there are two mechanisms which can cause the development of cancer. First, through the gene mutation which can cause a change in DNA sequence caused the function of a gene is not functioning normally, and the development becomes uncontrolled. Moreover, cancer also can be caused by epigenetic regulation where gene expression can be changed without a mutation. It can be caused by the regulation of DNA methylation, histone modification, miRNA regulation toward the aberrant expression of a gene in cancer. Epigenetic can regulate coding gene and non-coding gene such as microRNA. IQGAP genes family are the coding genes whose have the aberrant expression in many cancer types. Currently, cancer treatment is found not effective yet. Therefore, the innovation of cancer treatment is needed, one of the possible treatments can be conducted through epigenetic treatment by the modification of molecular biology such as DNA methylation and miRNA which known to targeting IQGAP genes expression in cancer formation. In the present, there is only research that related to the epigenetic regulation of IQGAP gene [1]. That research concluded that IQGAP2 functioning as a tumor suppressor gene in ovarian cancer where its expression is inhibited by hypermethylation. It suggested that IQGAP2 can become a biomarker in Ovarian cancer. Many researches has been conducted for IQGAP1 [2,3,4,5, 6], as the example, in the research performed by Jin Xin et al., 2017 in the pancreas cancer, it is found that the MAPK signaling pathway is an important factor for cancer cell proliferation,
migration and survival of cancer treatment and overexpression of IQGAP1 is significantly correlated with the metastases of pancreatic cancer. Therefore, it is suggested that IQGAP1 functioned as an oncogene in pancreatic cancer. In the other hand, the role of IQGAP2 and IQGAP3 in the cancer formation is not investigated well. Moreover, there is no clear pathway yet regarding IQGAP gene role in cancer formation. The research that conducted by Song et al., 2018 found that IQGAP3 plays an important role in the development of cancer. The upregulated expression of IQGAP3 is related to the clinical stage, T category, N category, locoregional recurrence, distant metastatic, and status vital [7]. Another example, the study which conducted by Naohide Oue et al., 2018 showed the upregulated of IQGAP3 expression in spheroid body-forming gastric cancer (GC) compared with parental cell [8].

Our research conducted a correlation study to identify the effect of miRNA and DNA methylation simultaneously toward the aberrant expression of IQGAP genes. In the previous research, our study has conducted a research a correlation study of miRNA regulated IQGAP genes in liver cancer [9]. It suggested that all the IQGAP genes were identified have a significant negatively correlated with miRNA. Furthermore, our previous study also identified DNA methylation regulated miRNA which target cancer genes related to ovarian cancer [10]. This research used the cancer human genome (TCGA) expression datasets of DNA methylation, miRNA and gene expression level 3. The aim of this study is to identify whether miRNA and DNA methylation can regulate IQGAP gene family simultaneously.

2. Datasets and methods
A dataset of gene expression, miRNA expression, and DNA methylation were derived from TCGA websites in July 2018 (https://portal.gdc.cancer.gov/). This study filtered the data by selected ovarian cancer as the initial study for this research and ignored the normal and the patient whose status is dead. The metadata from TCGA dataset was converted to the CSV format using an online converter. This study downloaded 77 cancer samples from ovarian cancer where each sample has DNA methylation, miRNA and gene expression datasets along with their genomic location information. We organized each dataset in the gene matrix, miRNA matrix and DNA methylation matrix where each matrix consists of the same order of the samples. For the patient ID matching, python programming was performed to match patient ID between miRNA-gene and DNA methylation-gene. And R programming was performed to obtain the expression dataset. Then, we used MATLAB software to compute miRNA-IQGAP matrix and DNA methylation-IQGAP matrix. And all the computation analysis to calculate correlation score using Spearman formula, retrieve the correlation score and p-value for each correlational dataset pair was performed in MATLAB software using the developed MATLAB function. The flowchart of the study is described in figure 1.

3. Results and discussion
This study identified IQGAP1 significantly negatively correlated with both DNA methylation and miRNA. This study found that IQGAP1 significantly negatively correlated with DNA methylation probe cg10978402 and forty-three miRNAs as described in table 1. For all the correlation results the cut off is the correlation score >/= -0.2 with p-value < 0.05. Moreover, both IQGAP2 and IQGAP3 were not found to have a negative interaction with both DNA methylation and miRNA expression. This study identified that both IQGAP 2 and IQGAP 3 have a significant negative interaction with forty-six and sixteen miRNAs respectively. As described in table 2 and table 3. Our results suggested that IQGAP1 is possibly regulated by miRNA and DNA methylation simultaneously. This result was not found by the previous study yet. Therefore, this study contribution can be used as the important information where IQGAP1 potentially can be targeted by both miRNA and DNA methylation. DNA methylation probe cg10978402 is located in the CPG island of the IQGAP1 promoter region. On the other hand, this study result does not support the previous study result that stated IQGAP2 could be regulated by epigenetic in ovarian cancer [11]. This different result can be caused by different sample number used and the platform of the DNA methylation datasets. This study is limited in used DNA methylation platform for Illumina 25 K instead of illumine 450 K. For further research, this study will include both platforms in the analysis.
**Figure 1** flowchart of the study

**Table 1** - Top 20 miRNAs and a DNA methylation probe significantly inversely correlated with IQGAP1

| miRNAs/DNA methylation | SRCC   | p-value   |
|-------------------------|--------|-----------|
| cg02387679              | -0.4   | 0.0008    |
| hsa-mir-6508            | -0.359556946 | 0.001319871 |
| hsa-mir-1290            | -0.335092422 | 0.002892225 |
| hsa-mir-6740            | -0.315053772 | 0.0052575   |
| hsa-mir-449c            | -0.309087482 | 0.00623443  |
| hsa-mir-449a            | -0.28047168  | 0.013485581 |
| hsa-mir-3960            | -0.275447828 | 0.015324687 |
| hsa-mir-3146            | -0.273579897 | 0.016061652 |
miRNAs/DNA methylation | SRCC  | p-value   
--------------------------|-------|-----------
hsa-mir-17               | -0.271649403 | 0.016855176   
hsa-mir-4322             | -0.268853222 | 0.01806421    
hsa-mir-20a              | -0.267653399 | 0.018605383   
hsa-mir-8077             | -0.26299308  | 0.020841235   
hsa-mir-449b             | -0.261075407 | 0.021825785   
hsa-mir-570              | -0.250259762 | 0.028152082   
hsa-mir-4686             | -0.249400978 | 0.028714807   
hsa-mir-5004             | -0.246942486 | 0.030378478   
hsa-mir-4735             | -0.245679999 | 0.031263868   
hsa-mir-92a-1            | -0.244176876 | 0.032346212   
hsa-mir-873              | -0.233592931 | 0.032584836   
hsa-mir-129              | -0.234081708 | 0.040459777   
hsa-mir-4478             | -0.233592931 | 0.040892057   

Abbreviations: SRCC denotes the Spearman rank correlation coefficient

### Table 2 - Top 20 miRNAs significantly inversely correlated with IQGAP2

| miRNAs      | SRCC  | p-value   |
|-------------|-------|-----------|
| hsa-mir-378c| -0.50471 | <0.0001  |
| hsa-mir-378a| -0.43175 | <0.0001  |
| hsa-mir-449b| -0.39574 | 0.000368 |
| hsa-mir-378d-1| -0.35769 | 0.001404 |
| hsa-mir-1266| -0.35438 | 0.001566 |
| hsa-mir-222 | -0.35041 | 0.001783 |
| hsa-mir-221 | -0.33771 | 0.002667 |
| hsa-mir-29a | -0.32695 | 0.003705 |
| hsa-mir-3622a| -0.32666 | 0.003737 |
| hsa-mir-320c-2| -0.32209 | 0.004282 |
| hsa-mir-449c| -0.32057 | 0.004477 |
| hsa-mir-625 | -0.31518 | 0.005238 |
| hsa-mir-449a| -0.30611 | 0.006779 |
| hsa-mir-7156| -0.29659 | 0.008814 |
| hsa-mir-582 | -0.28493 | 0.012018 |
| hsa-mir-6499| -0.28398 | 0.012318 |
| hsa-mir-1292| -0.28185 | 0.013016 |
| hsa-mir-4752| -0.27733 | 0.014612 |
| hsa-mir-1254-2| -0.27383 | 0.015961 |

Abbreviations: SRCC denotes the Spearman rank correlation coefficient

### Table 3 - 16 miRNAs significantly inversely correlated with IQGAP3

| miRNAs      | SRCC  | p-value   |
|-------------|-------|-----------|
| hsa-mir-664a| -0.50471 | <0.0001  |
| hsa-mir-6826| -0.43175 | <0.0001  |
### 4. Conclusion

This study is an immature study and needs to be further developed in more complex analysis. However, the idea of this study is novel and can be used as the initial steps to identify the treatment in epigenetic by DNA methylation and miRNA modification on IQGAP aberrant expression in cancer formation. For the future study, we will include differentially expression analysis where the normal samples should be used as the reference. We also will consider DNA methylation Illumina 450 K platform. Moreover, the validation on methyl-IQGAP and miRNA-IQGAP should be addressed through the web tools or literature study. Currently, TCGA were updated the dataset information for each patient, it included race, age, dead status, tumor grade and etc. Therefore, it is also interested to further investigate the role of epigenetic-based on a specific population or tumor grade.

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