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MicroRNA (miRNA) PROFILING IN PROSTATE CANCER CARCINOGENESIS: EXPLORATORY RESEARCH

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ABSTRAK

Penelitian di masa depan akan terus dilakukan dalam hal mencari penanda molekuler yang terlibat dalam proses awal karsinogenesis, metastasis dan target terapi pada pasien kanker prostat. MicroRNAs (miRNAs) adalah RNA kecil tanpa kode yang berkontribusi terhadap perkembangan kanker prostat dengan mengendalikan gen yang terlibat dalam jalur pensinyalan reseptor androgen (AR), ekspresi ektopik protein yang terlibat dalam siklus sel dan apoptosis, transisi epitel-mesenkimal (EMT) dan metastasis sel induk kanker (CSC). Penelitian ini merupakan penelitian eksploratif, tujuannya adalah untuk menilai ekspresi miRNA pada kanker prostat derajat histopatologis rendah dibandingkan dengan kanker prostat derajat histopatologis tinggi. Kebaruan dari penelitian ini adalah menilai 25 miRNA yang paling meningkat (up-regulasi) dan 25 miRNA yang paling rendah (down-regulasi) dari 800 jenis miRNA. Selanjutnya, kami akan menganalisis mekanisme dan memastikan karsinogenesis kanker prostat. Hasil penelitian dapat digunakan untuk data penelitian selanjutnya khususnya untuk terapi target pada pasien kanker prostat.

ABSTRACT

MicroRNA (miRNA) Profiling In Prostate Cancer Carcinogenesis: Exploratory Research. *Future research will continue to be carried out in terms of looking for molecular markers involved in the early processes of carcinogenesis, metastasis and therapeutic targets in prostate cancer patients. MicroRNAs (miRNAs) are small noncoding RNAs that contribute to prostate cancer progression by controlling genes involved in the androgen receptor (AR) signaling pathway, ectopic expression of proteins involved in the cell cycle and apoptosis, epithelial-mesenchymal transition (EMT) and metastasis of cancer stem cells (CSCs). This study was an exploratory study, the aim was to assess miRNA expression in low histopathological grade prostate cancer compared with high histopathological grade prostate cancer. The novelty of this research is to assess the 25 most increased (up-regulated) and 25 lowest (down-regulated) miRNAs from 800 types of miRNA. Next, we will analyze the mechanisms and confirm the carcinogenesis of prostate cancer. The research results can be used for further research data, especially for targeted therapy in prostate cancer patients.*

INTRODUCTION

Prostate cancer is the second most common cancer in men worldwide and the seventh leading cause of cancer death.[1,2] Epidemiological data records prostate cancer as a global health issue, due to its high incidence and impact on patients' quality of life.[3] Prostate cancer is a very heterogeneous cancer, in the early stages patients are often asymptomatic, and optimal clinical management of each individual is still a challenge.[4,5] Despite improvements in the diagnosis and therapy of prostate cancer in recent years, histopathological features or profiles have not been included as biomarkers or marker.[2] Prostate cancer has different molecular markers, including chromosomal translocations, gene deletions, and defects in DNA repair mechanisms.[6] Almost all prostate cancers begin in a state of androgen dependence, so androgen deprivation therapy is given and provide good clinical results. However, as time goes by, some cancer cells are able to survive and grow during this treatment, caused by prostate cancer cell that is not dependent on androgens (androgen independent). Most prostate cancers require androgen receptor (AR) signaling to survive. During progression toward androgen-independence, these signaling cascades change at multiple levels in prostate cancer. Mechanisms that increase AR signaling during androgen deprivation include amplification of the AR gene, mutation of the AR gene, changes in the expression of AR co-regulatory proteins, changes in the expression of steroid-producing enzymes, ligand-independent activation of AR via the outlaw pathway, and the AR-independent pathway that becomes active, called a 'bypass' pathway. One or more of the changes mentioned above may cause prostate cancer cells to acquire androgen-independent properties.[7]

Interestingly, most of the miRNAs found to be altered in prostate cancer are related to the androgen receptor (AR) pathway. miRNAs can be regulated by AR while others act upstream by regulating AR at the mRNA or protein level. MiRNAs can be regulated by androgens through direct binding to androgen-responsive elements in promoters (e.g., miR-21, where overexpression causes inhibition of the transforming growth factor β pathway associated with chemoresistance). Direct regulation of AR transcripts by miRNAs has also been demonstrated. In particular, among several potential miRNAs, miR-135b and miR-185 emerged as key AR regulators, both of which are capable of regulating AR by targeting its 3’ UTR. Downregulation of miR-135b and miR-185 in prostate cancer tissue has been widely demonstrated and partly due to increased AR expression. In contrast, indirect regulation has also been observed for miR-141, whereas other miRNAs are deregulated through reprogramming of downstream AR signals (miR-221/miR-222).[8]

Epithelial-mesenchymal transition (EMT), a process characterized by decreased expression of epithelial genes and increased expression of mesenchymal genes, plays an important role in tumor invasion, metastasis, and recurrence. In prostate cancer, EMT is involved especially in cases of metastasis and miRNAs act as important post-transcriptional regulators of prostate cancer EMT. The study results show that miRNAs mediate efficient and reversible control of prostate cancer EMT through various mechanisms, including directly suppresses single or multiple EMT-TFs (EMT-Transcription Factors) or regulates cytoskeletal components (epithelial/mesenchymal genes), regulating the main signaling pathways involved in EMT. Oncogenic miRNAs act as EMT promoters by suppressing epithelial characteristics and tumor suppressive miRNAs act by inhibiting mesenchymal development. Furthermore, EMT is mechanistically linked to stem cells in prostate cancer and several miRNAs involved in EMT have been reported to influence stem cells in prostate cancer. Loss of EMT-inhibiting miRNAs and/or increase in EMT-promoting miRNAs leads to induction of prostate cancer EMT, leading to tumor progression, metastasis, and recurrence.[9] From research

it is reported that miRNAs are involved in carcinogenesis and show promising results in prostate cancer diagnosis. The study of miRNAs is currently growing rapidly, especially in cancer diagnosis.[10] Restoring the expression of tumor-suppressing miRNAs and inhibiting oncogenic miRNAs is a potential therapeutic opportunity to prevent cancer metastasis and recurrence.[9]

METHOD

This research is an exploratory study, with quantitative cross-sectional methods, the expression of miRNAs in low histopathological grade prostate cancer compared with high histopathological grade prostate cancer. The histopathological grade of prostate cancer is determined based on histopathological examination with Hematoxylin-Eosin (HE) staining using microscope with 100-400x magnification. The Gleason score is determined based on WHO regulations. miRNA expression will be analyzed using the nano String profiling method to identify 827 types of miRNA. The field of view (FOV) value is set for barcode calculations with a minimum POV value of 75%. MiRNA expression will be assessed from the *fold change* and *p value*, the calculation of the p value is determined using the parametric t-test statistical test. The results of the miRNA comparison are said to be significantly different if the significance value (p) is <0.05. The novelty in the research is the discovery of 25 types of miRNA that increased significantly with the highest fold change (FC) value (up-regulation) and 25 types of miRNA with the lowest FC (down-regulation). Another novelty is the analysis of the mechanism and role of miRNA in carcinogenesis through bioinformatics and journal reviews. The aim of this study is to assess the expression of miRNAs involved in the process of prostate cancer carcinogenesis and what miRNAs are up-regulated and down-regulated in high and low histopathological grade prostate cancer.[10]

Population and Sample

The target population in this study was prostate cancer patients in Palembang, South Sumatra. The accessible population is prostate cancer patients in Palembang with a histopathological diagnosis of Prostate Carcinoma, in 2019-2023 whose preparations were stored in the form of paraffin blocks. A purposive sampling method will be performed for this study with the total sample size was 12 cases which included 6 cases of prostate carcinoma with low histopathological grade, 6 cases of prostate carcinoma with high histopathological grade (Table 1)

Table 1. Sample Data Design

Sample	Sample Type	Sample Code	Group
1	FFPE	1	Low Histopathological Grade Prostate Cancer (Gleason score 7 or less)
2	FFPE	2	
3	FFPE	3	
4	FFPE	4	
5	FFPE	5	
6	FFPE	6	
7	FFPE	7	High Histopathological Grade Prostate Cancer (Gleason score more than 7)
8	FFPE	8	
9	FFPE	9	
10	FFPE	10	
11	FFPE	11	
12	FFPE	12	

Research variable

The dependent variable is low and high histopathological grade prostate carcinoma. The independent variable is miRNA expression. In addition, patient characteristic variables (age, family history, body weight, BMI) and PSA levels were also assessed for their correlation with histopathological grade and miRNA expression**.**

RESULTS

Data analysis

miRNA profiling measurements will use NanoString technology, with the Human V3 Code miRNA Assay Kit panel. The miRNA expression panel will analyze 800 human miRNAs. The profiling data will be analyzed using the nSolver 4.0 Analysis Software System and ROSALIND platform. The In-Silico approach was used to analyze the results of miRNA profiling. Stage 1 analysis is a miRNA profiling analysis, then obtained data on up-regulated and down-regulated miRNAs, 25 each of the miRNAs expressed in significant up-regulation and down-regulation will be presented in table form (Tables 2 and Table 3).

Table 2. Up-Regulated miRNAs Expression (25 highest)

	miRNA	Fold change	p-value	miRBase Accession
1	miR-141	-3,43755	0,002201	MIMAT0x
2	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
3	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
4	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
5	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
6	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
7	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
8	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
9	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
10	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
11	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
12	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
13	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
14	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
15	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
16	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
17	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
18	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
19	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
20	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
21	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
22	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
23	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
24	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
25	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x

Differences in miRNA expression from the two groups will be analyzed and displayed in the form of a heatmap as well as plot analysis, calculation of fold change (FC), log2 FC, p value. Stage 2

analysis is Bioinformatics analysis, with purpose to determine the mRNA or genes that can be targeted by miRNA. Genes targeted by miRNA will be grouped based on validated genes and predicted genes. Validated genes will be searched using the Rosalind platform, TarBase (link), miRTarbase (link) or manual search with PubMed. The strength of the relationship between the miRNA and the target gene will be selected which shows "strong validation" (direct) based on PCR, blotting and luciferase reporter assay compared to less strong (indirect) based on microarray and NGS. Predicted genes can be determined from the Rosalind and TargetScan platforms (link). Binding position (3'UTR mRNA, 8mer/7mer) (using TargetScan, DIANA-Tools microT-CDS), binding affinity score /kD (RNA22, TargetScan), miTG Score prediction score (DIANA-Tools), Conserved Region (TargetScan, DIANA-Tools).

Table 3. Down-Regulated miRNA Expression (25 highest)

	miRNA	Fold change	p-value	miRBase Accession
1	miR-222	-3,42443	0,00272 1	MIMAT0x
2	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
3	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
4	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
5	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
6	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
7	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
8	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
9	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
10	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
11	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
12	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
13	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
14	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
15	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
16	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
17	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
18	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
19	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
20	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
21	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
22	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
23	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
24	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x
25	miR-xxx	-x,xxxxx	0,00xxxx	MIMAT0x

DISCUSSION

MicroRNA (miRNA) is a small noncoding RNA that epigenetically regulates gene expression, playing an important role in many tumorigenesis processes, including apoptosis, cell growth and proliferation, autophagy, epithelial to mesenchymal transition, invasion and metastasis.[4] In the last 2 decades, the concept of noncoding RNA changing very quickly, it is thought to play a role as an important regulator that regulates 2/3 of the transcription process in humans, previously only considered "black matter".[11,12]

Role of miRNAs in Prostate Cancer

miRNAs contribute to prostate cancer progression by controlling genes involved in the androgen receptor (AR) signaling pathway, ectopic expression of proteins involved in the cell cycle and apoptosis, epithelial-mesenchymal transition (EMT), and metastasis of cancer stem cells (CSCs) which are largely is a hallmark of cancer (Hallmarks of Cancer).[4,10,13] RNA polymerase II transcribes miRNA into ~ 80 nucleotide long pre-miRNA in the nucleus, which is then cleaved by Drosha RNase III and DiGeorge Syndrome Critical Region 8 (DGCR8) into more fragments. short ones known as pre-miRNAs. Mobility of pre-miRNA from the nucleus into the cytoplasm is triggered by Exportin 5. Dicer RNase cleaves pre-miRNA into small 22-bp long dsRNA in the cytoplasm. One strand is integrated into the RNA induced silencing complex (RISC) and usually targets the 3' UTR of the mRNA. The targeted mRNA undergoes degeneration and results in gene silencing. (Figure 1)[10]

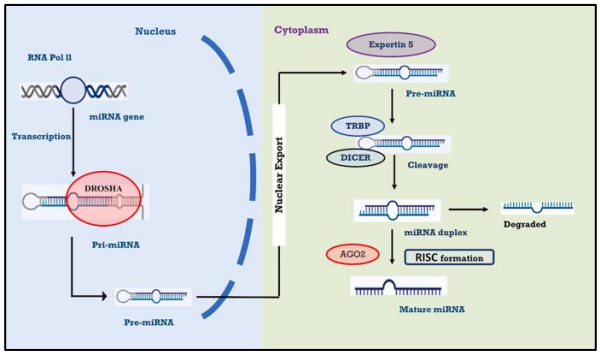

Figure 1. Biogenesis of miRNA.[10]

miRNAs play an important role in gene expression by repressing transcription and translation. On the other hand, abnormal miRNA expression is associated with several cancer-like diseases, including prostate cancer. The different signaling pathways involved in prostate cancer development are apoptosis avoidance, angiogenesis, cell growth, and cell differentiation. miRNAs interfere with the cell cycle and apoptosis by targeting cyclin proteins and pro-apoptotic genes. Recent studies report that miRNAs have a dual function, namely as oncomiR and tsmiR (tumor suppressor miR) in tumor development. miRNAs contribute to cancer progression by upregulating oncogene expression and downregulating tumor suppressor genes. The following miRNA; miR-204–5p, miR-329–3p, miR-127–3p are tumor suppressor miRNAs while miR-454–3p, miR-20a-5p, and miR-32–5p are oncomiRs. The miRNA expression profile clarifies the line of development, cancer stage, cancer grade, and the history behind the cancer. Previous studies reported that the expression of miR-21 and miR-75 was increased in prostate cancer patients in the early stages, while the aggressive state was characterized by increased expression of miRNA-1246.[10] Both in physiological and pathological conditions, various types of cells secrete miRNAs. Changes in miRNA expression profiles are used as potential indicators of pathological conditions.[13]

CONCLUSION

This study aims to assess the expression of miRNAs involved in the process of prostate cancer carcinogenesis using explanatory methods. The results will be assessed and determine the 25 miRNAs with the lowest expression values and the 25 miRNAs with the lowest expression values. The novelty of this research is a database of miRNAs involved in prostate cancer carcinogenesis in

prostate cancer patients in Palembang, as well as obtaining new carcinogenesis pathways through miRNA regulation. The type of miRNA obtained will be reviewed for its role in carcinogenesis. The research results can be used for further prostate cancer research data, especially to predict patient prognosis.

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