Signature MiRNA Eksosom pada Adenokarsinoma Paru sebagai Kandidat Biomarker Diagnosis

Intisari

Kanker paru memiliki gejala yang tidak spesifik, heterogenitas yang tinggi, dan dipengaruhi oleh kondisi lingkungan. Sebagian besar pasien terdeteksi pada stadium lanjut metastasis, sehingga diagnosis dini, penanganan, dan terapi kanker paru belum menunjukkan hasil yang optimal. Penelitian ini bertujuan untuk mendapatkan *profil signature* miRNA eksosom pada pasien adenokarsinoma paru, serta fungsi biologisnya secara bioinformatik, serta validasi miRNA eksosom terpilih pada plasma pasien adenokarsinoma paru sehingga mendapatkan *biomarker* diagnosis.

Penelitian ini merupakan studi observasional analitik dengan desain *case-control* secara potong lintang yang meliputi penelitian eksploratif dan validasi miRNA eksosom. Jumlah spesimen untuk penelitian eksploratif sebanyak 12 sampel yang terdiri dari 4 pasien adenokarsinoma paru *locally advanced*, 4 metastasis, dan 4 kontrol sehat. Adapun pada tahap validasi, penelitian ini melibatkan 77 pasien adenokarsinoma paru dan 57 kontrol sehat. Prosedur penelitian meliputi, ekstraksi eksosom dan RNA total plasma darah, kuantifikasi eksosom menggunakan NTA, sedangkan RNA menggunakan nanodrop. Kemudian, *profiling* miRNA eksosom menggunakan NanoString *panel V3 Human*. Data hasil *profiling* miRNA eksosom dianalisis menggunakan Rosalind, dan analisis fungsi biologi secara komoputasional. MiRNA eksosom terpilih divalidasikan ke pasien adenokarsinoma paru dibandingkan kontrol sehat menggunakan qRT-PCR.

Signature miRNA eksosom pada adenokarsinoma paru *locally advanced* dibandingkan kontrol sehat yang mengalami kenaikan ekspresi secara signifikan yaitu miR-604, miR-1261, miR-648, dan miR-320a. MiRNA eksosom yang mengalami kenaikan ekspresi pada adenokarsinoma paru metastasis dibandingkan dengan kontrol sehat yaitu miR-185-5p, miR-4435, miR-1915-3p, miR-455-3p, miR-604, miR-648, miR-190a-5p, miR-423-3p, miR-1262, miR-3065-5p, miR-1976, miR-320a, dan miR-503-5p. MiRNA eksosom yang mengalami kenaikan ekspresi pada adenokarsinoma paru metastasis dibandingkan dengan *locally advanced* yaitu miR-190b, miR-320e, miR-1296-5p, miR-3150b, miR-1909-3p, miR-455-3p, miR-514a-5p, miR-2053, miR-876-5p, miR-376c-3p, miR-221-5p, miR-564, miR-942-5p, miR-302a-5p, miR-4421, miR-212-3p, miR-134-5p, dan miR-378i.

Validasi miR-320a pada pasien adenokarsinoma paru metastasis dibandingkan kontrol sehat menunjukkan kenaikan ekspresi yang signifikan (*p-value*= 0,00032) dengan nilai AUC 0,718. Sedangkan, validasi miR-185-5p mengalami kenaikan ekspresi yang signifikan pada adenokarsinoma paru *locally advanced* dibandingkan kontrol sehat (*p-value*= 0,00000013), maupun metastasis dibandingkan kontrol sehat (*p-value*= 0,00000000066) dengan nilai AUC 0,829. Sementara, validasi miR-190b mengalami penurunan ekspresi yang signifikan pada adenokarsinoma metastasis dibandingkan kontrol sehat (*p-value*= 0,0000043) dengan nilai AUC 0,701.

Penelitian ini berpotensi memberikan kontribusi yang kuat terhadap pengembangan *biomarker* diagnosis berbasis biopsi cairan tubuh pada adenokarsinoma paru. Hasil penelitian ini diharapkan dapat menjadi dasar bagi studi lanjutan untuk pengembangan panel *biomarker* multi-miRNA dan melakukan validasi multisenter.

Kata kunci : *profiling*, validasi, miRNA eksosom, adenokarsinoma paru, *biomarker* diagnosis.

Exosomal MiRNA Signatures in Lung Adenocarcinoma as Candidate Diagnostic Biomarkers

Abstract

Lung cancer has non-specific symptoms, high heterogeneity, and is influenced by environmental conditions. Most patients are detected at an advanced metastatic stage, so early diagnosis, management, and therapy of lung cancer have not shown optimal results. This study aims to obtain the signature profile of exosomal miRNA in lung adenocarcinoma patients, as well as its biological functions through bioinformatic analysis, and to validate selected exosomal miRNA in the plasma of lung adenocarcinoma patients to obtain diagnostic biomarkers.

This study is an analytical observational study with a cross-sectional case-control design, including exploratory research and validation of exosomal miRNA. The number of specimens in the exploratory study was 12 samples consisting of 4 locally advanced lung adenocarcinoma patients, 4 metastatic patients, and 4 healthy controls. In the validation stage, this study involved 77 lung adenocarcinoma patients and 57 healthy controls. The research procedures included extraction of exosomes and total RNA from blood plasma, quantification of exosomes using NTA, while RNA was measured using Nanodrop. Then, exosomal miRNA profiling was performed using the NanoString Human V3 panel. The exosomal miRNA profiling data were analyzed using Rosalind, and biological function analysis was conducted computationally. Selected exosomal miRNA were validated in lung adenocarcinoma patients compared to healthy controls using qRT-PCR.

The signature of exosomal miRNA in locally advanced lung adenocarcinoma compared to healthy controls that showed significantly increased expression were miR-604, miR-1261, miR-648, and miR-320a. Exosomal miRNA that showed increased expression in metastatic lung adenocarcinoma compared to healthy controls were miR-185-5p, miR-4435, miR-1915-3p, miR-455-3p, miR-604, miR-648, miR-190a-5p, miR-423-3p, miR-1262, miR-3065-5p, miR-1976, miR-320a, and miR-503-5p. Exosomal miRNA that showed increased expression in metastatic lung adenocarcinoma compared to locally advanced were miR-190b, miR-320e, miR-1296-5p, miR-3150b, miR-1909-3p, miR-455-3p, miR-514a-5p, miR-2053, miR-876-5p, miR-376c-3p, miR-221-5p, miR-564, miR-942-5p, miR-302a-5p, miR-4421, miR-212-3p, miR-134-5p, and miR-378i.

Validation of miR-320a in metastatic lung adenocarcinoma patients compared to healthy controls showed a significant increase in expression (p-value = 0.00032) with an AUC value of 0.718. Meanwhile, validation of miR-185-5p showed a significant increase in expression in locally advanced lung adenocarcinoma compared to healthy controls (p-value = 0.00000013), as well as in metastatic compared to healthy controls (p-value = 0.00000000066) with an AUC value of 0.829. Meanwhile, validation of miR-190b showed a significant decrease in expression in metastatic adenocarcinoma compared to healthy controls (p-value = 0.0000043) with an AUC value of 0.701.

This study has the potential to provide a strong contribution to the development of diagnostic biomarkers based on liquid biopsy in lung adenocarcinoma. The results of this study are expected to serve as a basis for further studies to develop multi-miRNA biomarker panels and to conduct multicenter validation

Keywords: profiling, validation, exosomal miRNA, lung adenocarcinoma, diagnostic biomarker