Cancer Metastasis-Related microRNAs: An Association study of A549 Human Lung Adenocarcinoma Cells in Migration and Invasion

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Introduction
Understanding the progression of tumor invasion and metastasis responsible for high mortality rates in most lung cancer patients, is one of the most challenging problems. Past research have indicated that dysregulation in the expression of microRNAs (miRNAs) contributes toward the initiation and progression of cancer metastasis through their capability to regulate multiple target genes. The aims of this study were to investigate the miRNAs contributed to lung cancer metastasis and gain an insight into the molecular mechanisms required for migration and invasion of A549 lung cancer cell line.

Materials and methods
Two sub-cell lines of A549 possessing different degrees of migration and invasion properties were established from heterogeneous parental A549 cells using serial transwell invasion assay. High and low invasiveness sub-cancer cell lines were designated as A549-I7 and A549-NI7 respectively. Invasion, migration and doubling time properties were validated using invasion assay, wound healing assay and cell proliferation assay. MiRNA microarray was conducted on both sub-cell lines and microarray data was validated using Real-Time PCR. Pathway enrichment analysis was then performed using DIANA-mirPath (-ln(p-value) score of ≥ 3) together with DIANA-microT 4.0 (miTG score of ≥ 0.20) and TargetScan 5.2 (total context score of ≤ -0.10) to evaluate the possible pathways network involved in lung cancer metastasis.

Results
A total of 11 miRNAs differentially expressed between A549-I7 and A549-NI7 were identified and validated by real-time RT-PCR. Through pathway enrichment analysis, a hypothetical pathway model was proposed describing lung cancer metastasis by showing the network interaction of miRNAs and their gene targets. All these miRNAs act in concert in modulating three main pathways which were the non-canonical Wnt/planar cell polarity, transforming growth factor-β and integrin-focal adhesion kinase-Src intracellular signaling cascade to promote lung cancer migration and invasion.

Conclusion
These results provided potential candidate metastatic markers for non-small cell lung cancer classification, prognosis and a potential therapeutic value through targeting these miRNAs to lung control tumor invasion and metastasis.

Key words
microRNA, lung cancer, invasion, migration, metastasis