Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

MicroRNAs influence the migratory ability of Human Umbilical Vein Endothelial Cells

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Various miRNAs have been demonstrated to influence angiogenesis in HUVECs. We here employed RNA sequencing and established a phenomorphological assay to identify miRNAs that are involved in cell migration in HUVECs.

HG stimulation significantly suppressed the migratory ability of HUVECs as compared to cells cultured in normal glucose. Based on this, miRNAs sequencing was performed. 19 up-regulated and 12 miRNAs down-regulated miRNAs were found as differently expressed miRNAs, respectively.

46 genes validated by other papers from PubMed were then imported into miRwalk. A miRNAs list was then acquired. After selecting 50 miRNAs based on the number of genes influenced by these miRNAs standardized wound healing assays were performed after transfection with these miRNAs to explore the effect of the miRNAs on the migrational ability of HUVECs. 29 miRNAs were found to lead to a significant change in cell migration.

Four miRNAs (miR-21, miR-107, miR-143, and miR-106b) were identified as overlapping hits between the phenomorphological screen and miRNA sequencing.

To explore potential pathways that may be regulated by genes related to the four miRNAs, the gene lists were analyzed by KOBAS. Nine shared pathways between genes influenced by the four miRNAs: “Metabolic pathways”, “Pathways in cancer”, “Herpes simplex virus 1 infection”, “PI3K-Akt signaling pathway”, “MAPK signaling pathway”, “Ras signaling pathway”, “Human papillomavirus infection”, “Rap1 signaling pathway”, and “Proteoglycans in cancer”. Amongst the nine shared pathways, “PI3K-Akt signaling pathway”, “Rap1 signaling pathway” and “MAPK signaling pathway” were had already demonstrated to be related to the angiogenesis of HUVECs by numerous papers and could thus be validated.

Protein-protein interaction (PPI) network analysis was then performed to predict the functionality of interacting genes or proteins. *TP53, BCL2L11, STAT3*, and *CYCS* were chosen as hub genes of miR-21 target genes. *PIK3R1, MAPK1, NRAS*, and *KRAS* were chosen as hub genes of miR-143 target genes. *FBXO21, FBXO11, FBXL22, TCEB1, FBXO10, FBXO31, FBXL5, FBXL7, ASB1,* and *FBXW2* were chosen as hub genes of miR-106b target genes. Besides, *BTRC, FBXO10, FBXL19, ZBTB16*, and *FBXL20* were chosen as hub genes of miR-107 target genes. Seven hub genes (*CYCS, FBXO21, FBXL22, FBXL7, ASB1, BTRC,* and *FBXL20*) were firstly predicted here to be related to HUVEC migration.

This project has three major findings. We firstly established a workflow using a standardized wound healing assay for high-throughput screening. A combination of ImageJ and KNIME in analyze wound closure was used as an efficient, accurate, and reproducible method for automated image analysis. These methods were then employed to perform a phenomorphological screen on 50 miRNAs. Based on this phenomorphological screen and miRNA sequencing, four miRNAs were identified to influence the migration of HUVECs. As shared pathways between genes influenced by the four miRNAs, “PI3K-Akt signaling pathway”, “Rap1 signaling pathway” and “MAPK signaling pathway” were identified among others.