miRNA expression analysis in HPV positive cervical scrapes as biomarkers for cervical disease detection.

Martha I. González[1], Samuel Agudelo[1], María C. Agudelo[1], Carlos Orozco[1], Li Li[2], Jone Garai[2], Jovanny Zabaleta[2], Gloria I. Sánchez[1]

[1] Infection and Cancer Group, School of Medicine, University of Antioquia, Medellin, Colombia, [2] Department of Integrative Oncology, Louisiana State University Health Sciences Center, New Orleans, LA USA

Cervical cancer (CC) is the fourth most common cancer among women globally, with an estimated 604 000 new cases and 342 000 deaths in 2020. More than 95% of CC is due to the human-papillomavirus (HPV) infection. Biomarkers to stratify HPV+ women with cervical lesions that may progress to CC are needed. miRNAs regulate gene expression and are differentially expressed between Cervical Intraepithelial Neoplasia Grade 2 or higher, (CIN2+), and ≤CIN1. We aimed to identify miRNAs differentially expressed between CIN2+ and ≤CIN1.

71 cervical scrapes of HPV+ women: 35 with low- (NEG= 26; CIN1= 9) and 36 with high-grade lesions (CIN3= 32; SCC= 4) collected through the ASCUS-COL-Trial in Medellin, Colombia, were proceeded to obtain miRNAs libraries which were sequenced in 2 pools on the NextSeq sequencer. The data was processed on GeneGlobe-Qiagen, using Bowtie for mapping to miRbaseV21. The DEG analysis was done using DESeq2 on GeneGlobe, and a cross-check using manual DESeq2 in RStudio program. We identified putative pathways using MetaCore.

An average of >9 million reads per sample and around 3.5 million reads mapped to miRBaseV21 were obtained. The PCA did not show factors that could introduce bias to differential gene expression analysis. DESeq2 default option on GeneGlobe identified 325 miRNAs differentially expressed and manual DESeq2 in RStudio identified 45 ($p<0.05$). We found 38 miRNAs in common, compared to ≤CIN1, 9 miRNAs were overexpressed and 29 underexpressed in CIN3+. Interestingly, 5 miRNAs underexpressed in CIN3+, target *VEGF*, a known angiogenic mediator linked to malignancy.

DEG analysis by manual DESeq2 allows us to exclude false discoveries and do filtration of miRNAs with low expression levels in the major of the samples, compared to GeneGlobe. Further validation on a larger cohort of cervical scrapes samples is needed to confirm the potential role of these miRNAs to triage HPV+ women.