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Title: Independent validation of prognostic value of 22 microRNAs (miRs) in stage I-II lung adenocarcinoma (AC)

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Body: Background: About 50% of NSCLC patients (pts) will develop distant metastases following pulmonary resection. Currently, apart from clinical stage at diagnosis, there are no reliable factors to select the high risk pts for adjuvant chemotherapy. We previously demonstrated prognostic value of 22 miRs in frozen tissue samples of early stage SqCLC, and the feasibility of their expression assessment in formalin fixed paraffin embedded (FFPE) samples (Skrzypski et. al. J Clin Oncol 2010; 28;15s). In this study, we validated the prognostic value of these miRs in an independent cohort of early AC pts. Methods: FFPE tumor samples were obtained from 82 stage I-II AC pts who underwent radical pulmonary resection, 44% of whom developed distant metastases. Median follow-up of pts who did not develop metastases was 5.53 years (range, 3,01-8.9 years). miRs were isolated from tumor tissue with RecoverAll kit (Ambion). Expression of 22 miRs previously found to be related to the risk of metastases was analyzed by RT-PCR assay (Appliedbiosystems). Raw data were normalized vs. the expression of U6. Individual miRs were correlated with distant metastases-free survival (MFS). Results: MiR-222* (p=0,0003) and miR-222 (p=0,002) were significantly related to MFS. Using the median of the miR-222* expression as a cut-off value, the median MFS was 2.12 years in the high risk group, and not reached in the low risk group (HR=1.95). Using the median of the miR-222 expression as a cut-off value, the median MFS was 2.56 years in the high risk group, and not reached in the low risk group (HR=1.78). Conclusions: MiR-222* and miR-222 are strong predictors of distant relapse in operable early AC.