Up-regulation of miR-196b and miR-205 and Down-regulation of miR-375 in Lung Squamous Cell Carcinoma and Lymph Node Metastasis

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Background

Squamous Cell Lung Carcinoma (SCC) is a type of non-small cell lung cancer (NSCLC). SCC accounts for 25-30% of all lung cancers. Active or passive exposure to smoking is the primary risk factor of SCC and it is more frequent in male than female. SCC is slow growing with poor prognosis in an advanced stage. Unlike lung adenocarcinoma, targeted therapies are not prevalent in SCC due to the lack of molecular markers and genetic characterization of SCC.

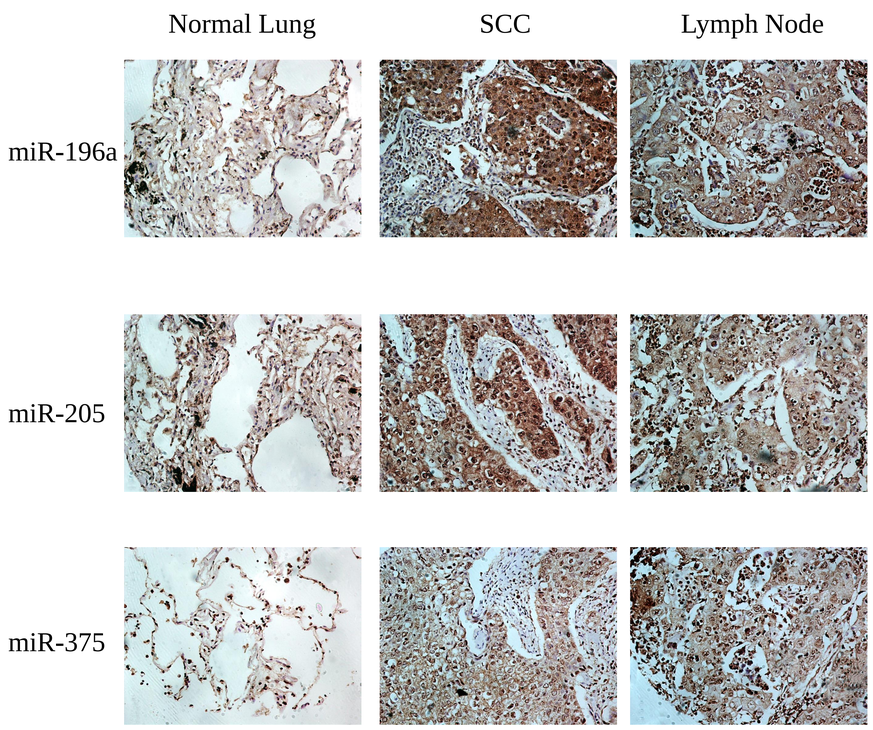
microRNAs (miRNAs) are 22 nucleotide long, small non-coding RNA molecules known as regulators of gene expression. Dysregulation of miRNA expression has been reported in different cancer types including lung cancer.

In the current study, we analyzed differential expression patterns of miR-196b, miR-205 and miR-375 in lung SCC paired with normal Lung and lymph node metastasis cases, in order to identify miRNA sequences as potential biomarkers for diagnosis of SCC.

Materials & Methods

miRNA expression profiling of miR-196b, miR-205 and miR-375 was evaluated in a total 107 FFPE archived cases of paired squamous cell carcinoma (P SCC, n= 34+13), paired lymph node metastasis (P Inv LN, n = 13) and normal lung, adjacent alveoli to tumor (N Lung, n= 34+13). *In situ* detection of miRNA was carried out using fluorescein labeled miRNA ISH probes and detection system (BioGenex, DF400-50KE). Following chromogenic *in situ* hybridization, stained slides were scored as negative, weak, moderate or strong .

Results



miR-196b and miR-205 were up-regulated (strong staining) in 88% (30/34) and 94% (32/34) of the cases of SCC respectively, a negative to weak staining pattern was observed in adjacent normal lung 62% (21/34) and 65% (22/34) cases, respectively. miR-375 was down-regulated in 88% (30/34) of the cases where weak to moderate staining was observed. 59% normal lung also showed weak to moderate staining with miR-375. miR-196b showed up-regulation in 77% (10/13) and 62% (8/13) of the cases of P SCC and P Inv LN, respectively. miR-205 was up-regulated in 92% of the cases of both P SCC and P Inv LN, whereas miR-375 was down-regulated in both 77 % (10/13) of the cases of P SCC and P Inv LN, where negative to moderate staining was observed .

Table 1. Differential expression of miR-196a, miR-205 and miR-375 in paired SCC (P SCC), normal lung tissue (N Lung), and lymph node metastasis (P Inv LN).

Cohort 1

miRNA	**N Lung**	**P SCC**
miR-196a	Negative/ Weak	Strong
miR-205	Negative/ Weak	Strong
miR-375	Negative/ Weak	Weak/ moderate

Cohort 2

	N Lung	**P SCC**	**P Inv LN**
miR-196a	Negative/ Weak	Strong	Strong
miR-205	Negative/ Weak	Strong	Strong
miR-375	Weak	Moderate/ Strong	Moderate/ Strong

Conclusion

In situ based detection provides visualization of miRNA in a histological context, which is helpful in determining tumor cell-type specific expression. Up-regulation miR-196b, miR-205 and down-regulation of miR-375 in SCC and invasive lymph node metastasis propose a potential diagnostic utility for these miRNAs in SCC, yet this should be further evaluated in light of the treatment response and a panel of miRNA markers. Lung cancer spreads to other organs though the lymphatic system, collaboration of SCC miRNA expression in lymph node may also be potential lead in diagnosis and prognosis of SCC.