miRNA profiling of primary lung and head and neck squamous cell carcinomas: Addressing a diagnostic dilemma

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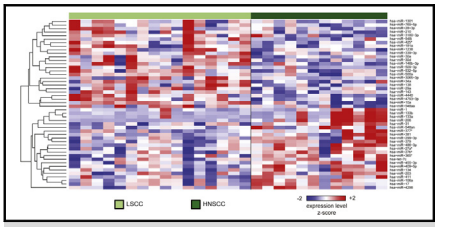
ABSTRACT

Objective: To determine whether microRNA (miRNA) profiling of primary lung and head and neck squamous cell carcinomas could be useful to identify a specific miRNA signature that can be used to further discriminate between primary lung squamous carcinomas and metastatic lesions in patients with a history of head and neck squamous cell cancer.

Methods: Specimens of resected primary head and neck and lung squamous cell carcinomas were obtained from formalin-fixed, paraffin-embedded blocks. Paraffin blocks were sectioned and deparaffinized, and total RNA was isolated and profiled. Quantitative polymerase chain reaction was performed to verify array results.

Results: Twelve head and neck and 16 lung squamous cell carcinoma samples met quality control metrics and were included for analysis. Forty-eight miRNAs were differentially expressed ($P < .05$) between the 2 groups. Of these, 30 were also significantly associated ($q < .25$) with tumor type in 2 independent sets of primary head and neck and lung squamous carcinomas profiled by The Cancer Genome Atlas consortium, including miR-34a and miR-10a. The ratio of miR-10a and miR-10b was especially predictive of primary cancer site in all 3 data sets, with area under the (receiver operating characteristics) curve values ranging from 0.922 to 0.982. Quantitative polymerase chain reaction confirmed the association of miR-34a expression and the miR-10:miR-10b ratio with tumor type.

Conclusions: MicroRNA expression may be useful for discriminating between head and neck and lung squamous cell carcinomas, including miR-34a and the miR-10a:miR-10b ratio. This differentiation has clinical importance because it could help determine the appropriate therapeutic approach. (J Thorac Cardiovasc Surg 2017;154:714-27)



Expression of 48 miRNAs associated with squamous cancer type, including miR-34a and miR-10a.

Central Message

MicroRNA profiling is useful for discriminating between head and neck and lung squamous cell carcinomas, which could help in the selection of the appropriate therapeutic approach.

Perspective

The distinction between a primary lung squamous cell carcinoma and a metastatic lesion in a patient with a history of head and neck squamous cancer is challenging but essential to determine the proper treatment. MicroRNA profiling can help discriminate tumors from different tissues of origin. MiR-34a and miR-10a:10b may be especially useful in differentiating between a primary and a metastatic lesion.

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Differentiating between a primary lung squamous cell carcinoma (LSCC) and a metastatic lesion in a patient with a history of head and neck squamous cell carcinoma (HNSCC) is challenging with the use of standard histopathology techniques. Both LSCC and HNSCC have several features in common, including similar histology,

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Abbreviations and Acronyms

AUC = area under the (receiver operating characteristics) curve
cDNA = complementary DNA
FDR = false discovery rate
FFPE = formalin-fixed, paraffin-embedded
GDC = National Cancer Institute Genomic Data Commons
HNSCC = head and neck squamous cell carcinoma
HPV = human papillomavirus
LSCC = lung squamous cell carcinoma
MCC = multiciliated cells
miRNA = microRNA
mRNA = messenger RNA
PCR = polymerase chain reaction
qPCR = quantitative polymerase real-time chain reaction
RLE = relative log expression
TCGA = The Cancer Genome Atlas

epithelial cells of origin, and association with tobacco exposure.[1,2] Even though the rate of metastasis from HNSCC is relatively low and may depend on locoregional control and lymph node status, the lungs frequently are involved, accounting for up to 70% to 85% of HNSCC metastatic cases.[3] Patients with a history of HNSCC who present with new lung lesions can therefore represent a challenge for surgeons who must plan the appropriate therapeutic approach. In patients with locoregional control of their HNSCC with metastasis to the lung, a complete, nonanatomical or wedge resection is indicated. However, the standard of care for primary LSCC is an anatomical resection, which includes a segmentectomy, lobectomy, or pneumonectomy, which have greater morbidity and perioperative mortality compared with nonanatomic resections.[4]

MicroRNAs (miRNAs) regulate gene expression and are involved in multiple biologic processes, including cell differentiation, proliferation, apoptosis, and metastasis.[5] Their role in the development and pathogenesis of cancer also has been described,[6] and it is known that miRNAs can act as tumor suppressors and oncogenes.[5] It also has been shown that miRNAs can be expressed differentially between different tissues and tumor types, and their expression can differentiate tumors with different developmental origin.[7] MiRNA profiling is a well-accepted method for the identification of specific miRNAs related to different diseases and types of cancer.[5,8] This could be useful in the diagnosis of metastatic cancer of unknown primary site[8] and in tumors with similar histopathologic findings in which a diagnosis under light microscopy can be challenging.

An accurate diagnosis of a new lung lesion in a patient with history of HNSCC is essential because this will help determine the appropriate therapeutic approach and surgical resection extent. In this study, we aimed to determine whether miRNA profiling is useful to differentiate between primary LSCC and HNSCC to identify characteristic features that are useful for differentiating between a primary LSCC and a metastatic lesion in a patient with history of HNSCC.

MATERIALS AND METHODS

Sample Identification, Formalin-Fixed, Paraffin-Embedded (FFPE) Processing, and RNA Isolation

Institutional review board approval was granted for this study (IRB ID #H-34408). LSCC and HNSCC specimens were obtained from FFPE blocks of surgically resected tissue from patients at Boston Medical Center. Of the 89 LSCC cases identified between September 2002 and April 2013 and the 44 HNSCC cases identified between March 2003 and May 2013, 18 LSCC and 17 HNSCC specimens from patients without a previous history of cancer or chemo/radiotherapy were selected for analysis. LSCC tissue was resected via segmentectomy, lobectomy, and pneumonectomy. Two patients with poor pulmonary function were not good candidates for anatomical resections and underwent wedge resection of their lung nodules. HNSCC were taken from the larynx, oropharynx, oral cavity, and floor of the mouth. Human papillomavirus (HPV) status was assessed by P16 staining in all HNSCC cases. The FFPE samples were sectioned with a microtome into four 10-μm sections and placed in a capped 1.5-mL centrifuge tube. Before sectioning, each specimen was examined with hematoxylin and eosin staining by a pathologist to confirm tumor histopathology and to ensure that the specimen contained at least 70% tumor. Sectioned FFPE samples were deparaffinized with QIAGEN Deparaffinization Solution (QIAGEN, Germantown, Md), and total RNA was isolated with the QIAGEN miRNeasy FFPE kit and protocol. RNA samples were quantified with a NanoDrop spectrophotometer (NanoDrop Products, Wilmington, Del), and RNA integrity examined with the Agilent Bioanalyzer (Agilent, Santa Clara, Calif).

Normalization and Quality Assessment

Total RNA was profiled with the Affymetrix GeneChip miRNA 3.0 array (Thermo Fisher Scientific, Waltham, Mass). Raw CEL files were normalized to produce probeset-level expression values and detection (Present/Absent) calls for all 5639 human and control probesets (including 1733 mature human miRNAs) with Affymetrix Expression Console (version 1.3.0.187), with the Robust Multiarray Average and Detection Above BackGround. Expression Console also was used to generate relative log expression (RLE) values as a measure of sample quality: a distribution of RLE values skewed upwards often indicates that the expression values were inflated by the normalization process. Samples with a mean RLE $\geq$0.5 or in which $\leq$20% of all miRNAs were called Present were excluded from analysis. Any miRNAs with spurious expression, ie, those that were not called Present in at least 25% of all samples, also were excluded from analysis.

The Cancer Genome Atlas (TCGA) Data

Preprocessed miRNA profiling data for primary tumors from 478 LSCC and 523 HNSCC TCGA samples, profiled by using Illumina Genome Analyzer (Illumina Inc, San Diego, Calif) or HiSeq instruments, were obtained from the National Cancer Institute's Genomic Data Commons (GDC) Portal at https://gdc-portal.nci.nih.gov. The data matrices used comprised counts that had been generated for 1881 human miRNAs in each sample via the use of miRBase version 21 and human genome build

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