Experimental and Molecular Pathology 96 (2014) 195–199

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

microRNA-146 up-regulation predicts the prognosis of non-small cell lung cancer by miRNA in situ hybridization

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ARTICLE INFO

Article history:
Received 11 June 2013
and in revised form 1 November 2013
Available online 18 January 2014

Keywords:
microRNA-146
NSCLC
ISH
Prognosis

ABSTRACT

Non-small cell lung cancer (NSCLC) accounts for approximately 70% of all lung cancer-related deaths worldwide. Prognostic markers are essential for the early detection of lung cancer in patients. In this study, we first identified microRNA146 (miR-146) expression in cancer cell lines using miRNA in situ hybridization (MISH) and confirmed the accuracy of MISH using q-RT-PCR. In addition, two different systems, BCIP/NBT and ELF, were used to detect the signal for a comparative analysis of the specificity of MISH. Compared to the BCIP/NBT system, the ELF detection system was more effective for MISH. Furthermore we detected the expression of miR-146 in NSCLC tissues (43 cases) and normal tissues (32 cases). Based on our results, we can conclude that miR-146 is more highly expressed in cancer tissue than normal tissue (t-test, $P < 0.05$) and that miR-146 can predict the prognosis of NSCLC by MISH. Our findings preliminary demonstrate that MISH can be applied as a molecular diagnostic tool to determine the expression and localization of miRNAs in cancer tissues and that miR-146, determined by MISH, predicts the prognosis of NSCLC patients.

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Introduction

Non-small cell lung cancers (NSCLCs) are the leading cause of cancer mortality, and the overall 5-year survival of NSCLC patients is not more than 15% (Jemal et al., 2009). If one can diagnose NSCLC at an early stage, the survival rate of patients can be effectively improved. Therefore, some specific early molecular markers are urgently needed for the detection of lung tumors. microRNAs (miRNAs) may open the door to the early molecular diagnosis of NSCLC and allow risk stratification based on miRNA profiles (Cho, 2011; Mendell, 2005; Xing et al.).

miRNAs are small non-coding RNAs of approximately 20–23 nucleotides that can regulate cancer cell processes by annealing to the 3′ UTRs of target genes (Griffiths-Jones et al., 2006). Some miRNAs are important as oncogenes or anti-oncogenes (Cho et al., 2011; He et al., 2005); many of these can facilitate the development of lung cancer as has been reported in previous studies (Bishop et al.). Meanwhile, lung cancer-related miRNA profiles have been established (Gee et al.; Hayashita et al., 2005; Vaporidi et al., 2012). For example, the increased expression of miR-146 was correlated with poor prognosis in lung cancer patients.

Currently, analyses of miRNA expression are usually performed by q-RT-PCR, Northern blotting using miRNA probes and microchip array technology (Hu et al.; van Rooij, 2011). However, these techniques cannot detect the localization of miRNAs in a tissue or single cell; miRNA in situ hybridization (MISH) can accomplish this (Neely et al., 2006). Perhaps more importantly, there are great differences between the expression profiles of miRNAs in cancer cells and non-cancer cells. Our previous study demonstrated that MISH can be applied to detect the expression of miRNA375 in paraffin-embedded esophageal cancer tissue (J. Li et al.; Y. Li et al.). In this work, we overcame the technical difficulty that the melting temperature (Tm) of the miRNA probe hybridization complex is too low to allow for its detection. Additionally, we further analyzed miR-146 to confirm its value. Several studies have reported that miR-146 is associated with lung cancer (Perry et al., 2009), but further details on the specific subcellular compartmentalization of miR-146 have not been reported. It is not possible to use RT-PCR or other methods to detect the expression of a miRNA in single cells or at low copy expression (Lu and Tsourkas, 2009).

Therefore, our study focuses on MISH and verifies the accuracy of this technique using q-RT-PCR. Briefly, we describe the MISH method to determine miR-146 expression in cultured cells and paraffin-embedded tissues and use it to detect the expression of miR-146 in NSCLC. The primary objective of this work is to enrich the molecular detection of miRNAs in cancer tissues.

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http://dx.doi.org/10.1016/j.yexmp.2013.11.004

Materials and methods

Cell culture and tissue treatment

Non-small-cell lung cancer cell lines ACC212102 and SCC211441 (established by our lab) and KYSE140, KYSE180, KYSE510, and HKESC1 cell lines were cultured in DMEM with 10% FBS supplemented with L-glutamine. The cells were cultured on glass cover slides at 37 °C in a 5% CO_2 environment. When the cells reached 70%–80% confluence, they were immediately fixed in 10% formalin for 2 h and MISH was performed on glass cover slides as described below.

Clinical specimen collection

The expression of miR-146 was evaluated in a total of 43 non-small cell lung cancer and 32 matched normal adjacent lung tissue samples by miRNA in situ hybridization (MISH). The samples included 20 lung adenocarcinoma samples, 17 squamous cell carcinoma samples, 6 adeno-squamous carcinomas, and no large-cell carcinomas. All the specimens were collected from patients in the Department of Thoracic Surgery, Sun Yat-Sen University Cancer Center, Guangzhou, China from October 2009 to July 2012 with patient consent and institutional review board approval.

ISH probes

An oligonucleotide probe, complementary to the hsa-miR-146b-5p (miR-146) probe, was purchased from Exonbio Lab (Guangzhou, China). The sequence of the probe is 5′-AGCCTATGGAATTCAGTTCTCA-3′; the 5′ and 3′ ends were modified with digoxigenin (DIG). Some of these bases were modified with 2-fluorine. A probe with the sequence5′-AGCGTATGGAATTCAGATCTCA-3′ served as control probe.

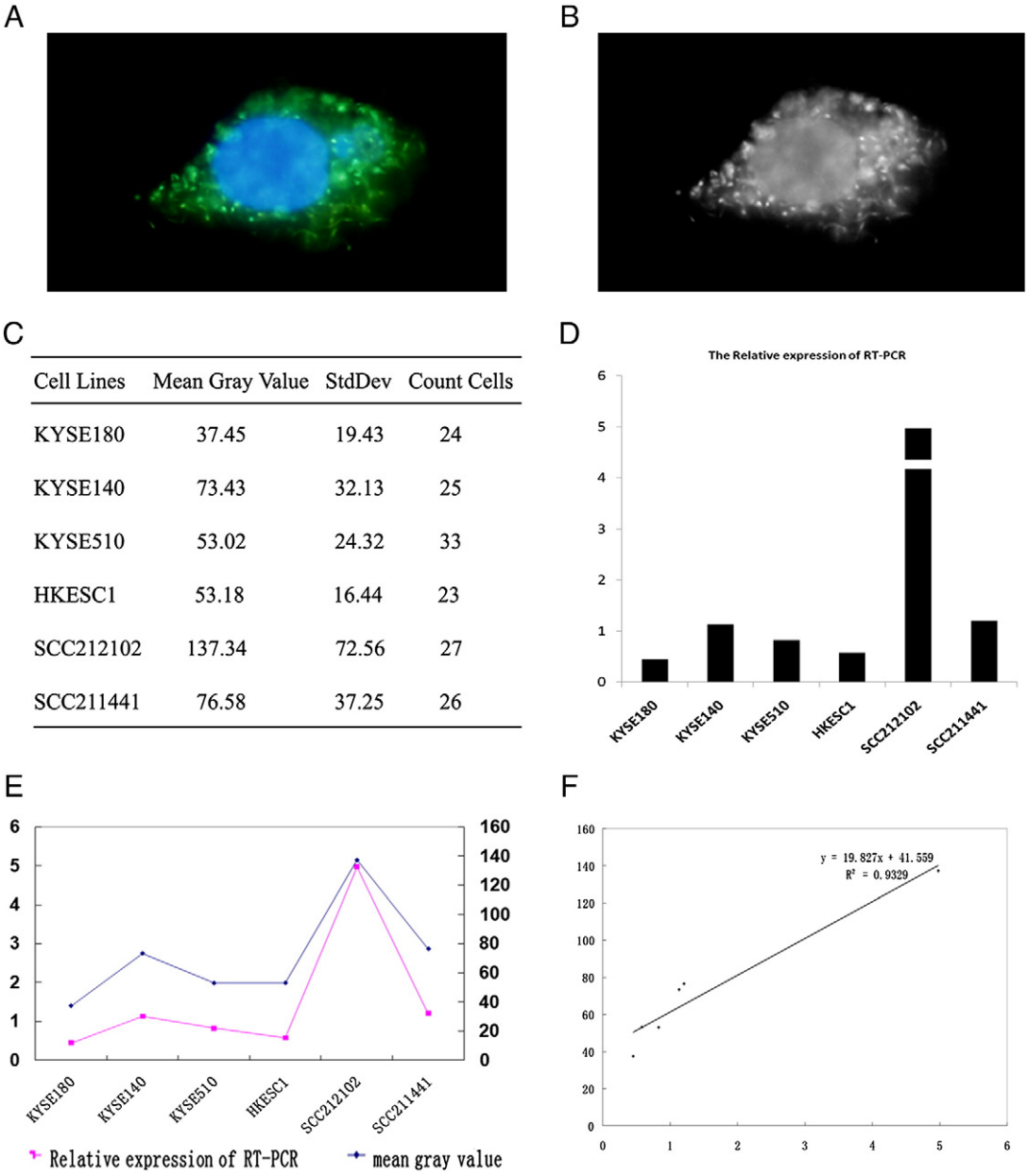

Fig. 1. MISH results are consistent with RT-PCR. (1) miRNA expression was analyzed in a series of cell lines by MISH, according to the data transformation as shown in A and B, as described above. The gray mean is shown in C. (2) In addition, the expression of miR-146 in these cell lines was quantified using RT-PCR, shown in a histogram (D). The expression of miR-146 by MISH detection is positively correlated with the data obtained from RT-PCR. The correlation coefficient ($R^2 = 0.93$) of the two methods is significant ($P < 0.05$, E and F).

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