Comprehensive Analysis of MicroRNA and mRNA Expression in Normal and Tumorous Human Esophageal Squamous Cell Lines Using Microarray Datasets

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1. Introduction

Esophageal cancer (EC) is the sixth most common cancer in the world that predominantly affects men and has a poor prognosis [1]. EC is classified mainly into two types—squamous cell carcinoma and adenocarcinoma—which begin as flat cells lining the esophagus and as cells that produce and release mucus and other fluids, respectively. Despite recent medical advances, EC associated with distant metastasis and local invasion still leads to a poor prognosis. A recent statistical study revealed that the numbers of new EC cases and EC-related deaths worldwide in 2008 were estimated to be 482,300 and 406,800, respectively [2]. This high mortality rate is largely due to poor subjective symptoms and difficulty with early diagnosis. Thus, EC is frequently diagnosed at late stages, leading to the unsatisfactory prognosis of affected patients, even though various therapeutic options such as surgery, chemotherapy, and radiotherapy are available. To date, endoscopic and radiologic examinations have been applied to detect EC at an early stage. Although the sensitivity and reliability of these diagnostic techniques have been improved, they do not always provide a satisfactory result. Furthermore, noninvasive diagnostic methods such as blood and urine tests for EC detection have not been established yet. Therefore, there is an urgent need to develop novel biomarkers for early detection and prognostic classification [3].

MicroRNAs (miRNAs) are small, noncoding RNAs of about 18–25 nucleotides in length that negatively regulate protein translation and/or mRNA stability by binding to target miRNAs. Although many miRNAs are expressed ubiquitously, a few are known to exhibit cell type/organ-dependent and/or developmental stage-dependent expression patterns...
Furthermore, aberrant miRNA expression has been found in various diseases, including many types of cancer [2]. Functionally, miRNAs act as either oncogenes or tumor suppressors via gene regulation and are thus called “oncomirs” [5, 6]. In silico target prediction revealed that individual miRNAs potentially target multiple mRNAs, indicating their crucial roles physiologically and pathologically. Thus, efforts to identify the actual target mRNAs of miRNAs experimentally are necessary to understand how miRNAs are associated with the onset and progression of various diseases, including cancer, possibly leading to improvements in current diagnostic and prognostic indicators. To achieve this goal, we used microarray analysis, which is a powerful tool because it can analyze the expression of a number of mRNAs and miRNAs in multiple samples at once.

Here, we present the datasets from our microarray experiments to analyze comprehensively and comparably mRNA and miRNA expression in normal and tumorous human esophageal squamous cell lines.

2. Methodology

Cell culture and RNA isolation were as follows. The ESCC-derived human cell lines used in this study were TE-1, TE-5, and TE-8, which are derived from well, poorly, and moderately differentiated tumors, respectively. These cell lines were purchased from RIKEN Cell Bank (Tsukuba, Japan) and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. Het-1A, an SV40 large T antigen-harboring normal human esophageal squamous cell line, was purchased from American Type Culture Collection (Manassas, VA, USA) and cultured using the BEGM kit (Lonza, Basel, Switzerland) but without the addition of the GA-1000 (gentamycin-amphotericin B mix) provided with the kit. Total RNA was isolated from these cells using ISOGEN reagent (Wako, Osaka, Japan) according to the manufacturer’s protocol. RNA purity was evaluated by the RNA integrity number (RIN), a representative index to assess RNA quality determined using the Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA, USA). We confirmed RINs of more than 9.9 for all RNA samples used in this study (data not shown).

Microarray study design for mRNA expression analysis was as follows. The RNA samples isolated from Het-1A and the ESCC cells were subjected to microarray-based expression analyses for mRNA using a SurePrint G3 Human Gene Expression 8 × 60 K v2 Array (Agilent). Cy3-labeled complementary RNA (cRNA) was prepared from 100 ng total RNA from each sample using the Low Input Quick Amp Labeling Kit, one color (Agilent). For each sample, 0.6 μg cRNA was hybridized using the Gene Expression Hybridization Kit (Agilent). The hybridized microarrays were subsequently washed using Gene Expression Wash Buffers Pack (Agilent) and subjected to fluorescent signal detection using a SureScan Microarray Scanner G4900DA (Agilent).

The intensities of the detected signals were quantified using Agilent Feature Extraction to generate raw data, which were registered as GEO accession number GSE61588. The quantified data were then normalized using Genespring GX12 (Agilent) to enable comparison of data from different microarrays.

Microarray study design for miRNA expression analysis was as follows. The RNA samples isolated from the normal and tumorous esophageal squamous cells were subjected to microarray-based expression analyses for miRNA using the miRCURY LNA microRNA Array Kit 7th generation—human, mouse, and rat (Exiqon, Vedbaek, Denmark). Total RNA (250 ng) was 3’-labeled with Hy3 using the miRCURY LNA microRNA Hi-Power Labeling Kit (Exiqon) according to the manufacturer’s instructions. For this labeling reaction, a synthesized miRNA mimic supplied in the Spike-in microRNA Kit v2 (Exiqon) was added to the reaction mixture to assess the quality of the obtained microarray data. The hybridized microarrays were subsequently washed according to the manufacturer’s instructions and subjected to fluorescent signal detection using a SureScan Microarray Scanner G4900DA (Agilent).

The intensities of the detected signals were quantified using Agilent Feature Extraction to generate raw data, which were registered as GEO accession number GSE61587. The quantified data were then normalized using GeneSpring GX12 (Agilent) to enable comparison of data from different microarrays.

Dataset Item 1 (Table). Normalized signal intensities from individual probes on SurePrint G3 Human Gene Expression 8 × 60 K v2 Arrays. The figures in the Signal Evaluation columns represent the significance of these signal intensities as follows: 2, detected; 1, difficult to judge; 0, not detected.

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Dataset Item 2 (Table). Normalized signal intensities from individual probes on miRCURY LNA microRNA Arrays.

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4. Concluding Remarks

Many recent studies have revealed that miRNAs could be promising biomarkers as well as therapeutic targets for cancer. This is particularly important for cancer types with a poor prognosis, including ESCC. In the present study, we conducted microarray analysis to analyze miRNA expression comprehensively in a normal human esophageal squamous cell line and in three types of human ESCC-derived cell lines to identify candidate miRNAs aberrantly expressed in ESCC. Furthermore, we performed microarray-based comprehensive gene expression analysis in these cell lines. Although it is generally believed that miRNAs suppress the translation of their target mRNAs into proteins, recent studies have revealed that miRNA-mediated gene silencing largely involves mRNA degradation [7, 8]. Thus, bioinformatic analyses of these microarray data, which include correlation analysis, miRNA target prediction, and ontology analysis, will provide an important clue to clarify how miRNAs are involved in the onset or progression of ESCC.

Dataset Availability

The dataset associated with this Dataset Paper is dedicated to the public domain using the CC0 waiver and is available at http://dx.doi.org/10.1155/2014/376541/dataset. In addition, the microarray data described herein (GSE61587 and GSE61588) were registered in GEO DataSets also as an integrated format as GSE61589. Deposited data are available at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61589.

Conflict of Interests

The authors declare that they have no conflict of interests.

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References


