Available online at www.sciencedirect.com
SCIENCE DIRECT®
Cancer Letters 224 (2005) 289–301
CANCER Letters
www.elsevier.com/locate/canlet

Diagnostic usefulness of PCR profiling of the differentially expressed marker genes in thyroid papillary carcinomas

Aiko Hamada[a], Svetlana Mankovskaya[b], Vladimir Saenko[c,*], Tatiana Rogounovitch[a], Mariko Mine[d], Hiroyuki Namba[a], Masahiro Nakashima[e], Yuri Demidchik[b], Eugeny Demidchik[b], Shunichi Yamashita[a,c]

[a]*Department of Molecular Medicine, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[b]*Belorussian State Medical University, Minsk 220116, Belarus*
[c]*Department of International Health and Radiation Research, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[d]*Department of Biomedical Statistics, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[e]*Tissue and Histopathology Section, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*

Received 27 September 2004; received in revised form 6 October 2004; accepted 6 October 2004

Abstract

The study was set out to determine whether characteristic changes in the gene expression profile in papillary thyroid carcinoma (PTC) discovered by microarray assays can be used for conventional molecular diagnosis. Expression levels of five reported to be overexpressed and three underexpressed genes were examined in PTC and normal human tissues by real-time PCR and semi-quantitative duplex PCR. Stepwise logistic regression analysis, duplex PCR data evaluation with recursive partition machine algorithm and hierarchical cluster analysis identified *SFTPB* (upregulated) and *TFF3* (downregulated) gene combination as most favorable for differential molecular diagnosis of PTC. Sensitivity, specificity and accuracy obtained in a series of histologically characterized thyroid tumor and normal tissue samples were 88.9, 96.7 and 94.9%, respectively. Applicability of the method to fine needle aspiration biopsy (FNAB) samples was demonstrated using a collection of needle washouts. In spite individual thyroid tumor and normal tissues as well as FNAB samples displayed a substantial degree of variability in the expression levels of analyzed genes, simultaneous molecular analysis of a panel of optimal markers allows making a high probability predictive estimate and may be considered as an informative method of preoperative PTC diagnosis.

Keywords: Papillary thyroid carcinoma; Preoperative diagnosis; Fine needle aspiration biopsy; Laboratory test

* Corresponding author. Tel.: +81 95 849 7122; fax: +81 95 849 7169.
E-mail address: saenko@net.nagasaki-u.ac.jp (V. Saenko).

0304-3835/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2004.10.012

1. Introduction

Incidence of palpable thyroid nodules is 4–7% of the population during the lifespan and 10 times more of the nodules are detectable by ultrasound examination [1]. Fine needle aspiration biopsy (FNAB) is the most appropriate procedure for the nodule evaluation. Although characteristic appearance of nuclei makes it facile to diagnose papillary thyroid carcinoma (PTC) by FNAB, sensitivity of the technique towards follicular variant of PTC (PTC-FV) and follicular thyroid carcinoma (FTC) remains insufficient due to an overlap in cytological features of benign and malignant tumor tissues, which sometimes are difficult to distinguish even if intraoperative pathological or cytological study was performed [2,3].

Tumor makers are the versatile tool for cancer screening and diagnosis. Nowadays, serum markers have proved usefulness for detecting disease recurrence and monitoring therapy in clinical practice, yet a few of them are suitable for case-finding [4]. Recently, novel molecular targets of potentially high diagnostic value have been proposed based on mRNA expression profiling and proteomic analyses [5,6]. Although the enormous data sets still need evaluation by the rigorous biostatistical methods to distill interpretable information and to bring it to consensual state [7,8], some of the revealed gene expression traits may be expected to yield routine laboratory tests.

Despite clinical heterogeneity, consistent gene expression patterns have been reported in PTC including PTC-FV [9] which is the second common histological subtype next to classical PTC. Using microarray profiling, expression levels have been shown to be increases in 8/8 tumors for 24 genes and in 7/8 tumors for 22 genes and decreased in 7/8 tumors for 8 genes and in 6/8 tumors for 19 genes. Additionally, differences in the expression of over than a hundred of genes have been found between malignant and benign thyroid follicular tumors [10,11]. Hence these studies provide a fundament for a purposeful selection of reliable markers to be detected by conventional methods. In this work, we attempted to establish a relatively simple and sensitive molecular diagnostic technique based on the combination of reported mRNA expression patterns.

2. Materials and methods

2.1. Tissues and FNAB samples

Collection and usage of biomaterials were performed according to the ethic guidelines effective in Nagasaki University Hospital. Eighteen postoperational PTC and matched normal thyroid tissues, 21 benign thyroid tumor specimens (19 cases of follicular adenoma (FA), 2 cases of nodular goiter (NG)) and 10 blood samples were available for the study. The tissues were kept frozen at −80 °C until use. Peripheral blood mononuclear cells were isolated from whole blood using a Lymhoprep density gradient medium (AXIS-SHIELD, Oslo, Norway).

Totally 42 FNAB samples were collected of which in nine cases histological diagnosis became available by the end of the project (diagnoses were kept undisclosed until the completion of molecular analysis) and 33 remaining patients are currently under follow-up. Ultrasound-guided FNAB was performed using a syringe–pistol manipulator equipped with a 20 ml syringe with 22-gauge needle. Aspirates were spread on a slide glass for cytological examination and leftover material inside the needle was washed out into 0.5 ml of an Isogen reagent (Nippon Gene, Tokyo, Japan) for RNA isolation.

RNA was extracted from all kinds of biological materials using Isogen according to manufacturers' protocol and reverse transcribed with MuLV Reverse Transcriptase in the presence of random hexamers (all reagents from Applied Biosystems, Foster City, CA, USA) for 1 h at 41 °C following by heat inactivation of the enzyme at 95 °C for 5 min.

2.2. Quantitative real-time PCR

In this study we analyzed five reported to be overexpressed genes (*CITED1, CHI3L1, FN1, RIL* and *SFTPB*) and 3 under-expressed ones (*ITPR1, TFF3* and *TPO*) [9]. The mRNA expression levels of each gene in PTC tumor tissue and matched normal tissues were quantified by real-time PCR using an ABI PRISM 7700 Sequence Detection system with SYBR Green PCR Core Reagent kit (Applied Biosystems). 0.5–1 μl of cDNA was amplified in 25 μl of reaction mixture in the presence of each primer pair. Primer sequences and