Detection of somatic oncogene alterations in FNA samples of cold nodules and 3 years follow-up of patients in Hungary

Bálint Tobiás[1], Bernadett Balla[1], János P. Kósa[1], János Horányi[2], István Takács[1], Zsolt Nagy[1], Péter Horváth[1], Balázs Járay[3], Eszter Székely[3], Roland Istók[3], Tamás Székely[3] and Péter Lakatos dr.[1]

[1] Semmelweis Egyetem I. sz. Belgyógyászati Klinika, Budapest, [2] Semmelweis Egyetem I. sz. Sebészeti Klinika, Budapest
[3] Semmelweis Egyetem II. sz. Patológiai Intézet, Budapest

Abstract

Cold nodules are one of the most common findings on scintigraphic examinations of the thyroid gland. About 5-10% of these nodules turn out to be histologically malignant. Our aim was to examine some somatogenetic alterations associated with thyroid cancer in FNA samples of the thyroid. These alterations included single nucleotide mutations (BRAF, HRAS, NRAS, KRAS) and genetic translocations (RET/PTC1, RET/PTC3, PAX8ex7/PPARgamma, PAX8ex9/PPARgamma). The SNPs were tested by real-time PCR with fluorescence melting curve analysis and the rearrangements were detected by Taqman probe-based quantitative real-time PCR.

We have analyzed 779 consecutive FNA samples and followed up the patients 3 years long. In the examined 779 samples, we found different genetic alterations (39 BRAF, 23 NRAS, 9 HRAS, 1 KRAS mutations and 1 RET/PTC3 rearrangement). After one year follow-up by histology, 52 cases (6.8%) were classified as malignant, from which we identified genetic alterations only in 40 (5.1%). (specificity 93.3%, sensitivity 46.2%, negative predictive value 96.0%, positive predictive value 32.9%) In two years follow-up group (n=504) by histology, 30 cases (6.0%) were classified as malignant, from which we indentified genetic alterations in 26 (5.2%). In three years follow-up group (n=250) by histology, 13 cases (5.2%) were classified as malignant, from which we found genetic alterations in 14 (5.6%).

No PAX8/PPARgamma rearrangements were demonstrated in the 779 samples. These data are not in complete accordance with published information. This fact might be due to several factors including the differences in iodine supply in different geographical areas.

Objectives and Materials

In the present study, we planned to investigate somatic mutations of the genes BRAF, HRAS, NRAS and KRAS as well as rearrangements of RET/PTC1, RET/PTC3 and PAX8/PPAR-gamma both in FNA samples of cold nodules of Hungarian subjects. The aim of this study was to examine somatic genetic alterations related to differentiated thyroid cancers in fine-needle aspiration biopsy samples.

We collected FNA samples at the 1st Department of Internal Medicine and the 1st Department of Surgery Semmelweis University.

The study protocol was reviewed and approved by the Ethic Committee (ETT-TUKEB 1160-0/2010-1018EKU).

Methods

Nucleic acid isolation

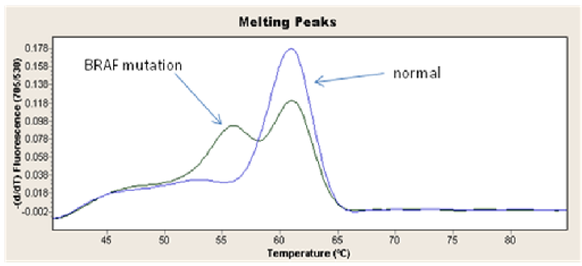
The cytological samples of thyroid were stored in -72 ºC after FNA until processing. The needle washer was phosphate buffered saline (PBS). Genomic DNA was isolated using Roche High Pure PCR template Preparation Kit (Roche, Indianapolis, IN, USA). Total RNA was separated by Roche High Pure RNA Isolation Kit (Roche). Quantification of isolated DNA and RNA was assessed by NanoDrop spectrophotometer (Nanodrop Technologies, Montchanin, DE, USA). DNA isolation was successful from 228 samples while RNA could be obtained from 142 samples.

Detection of mutations

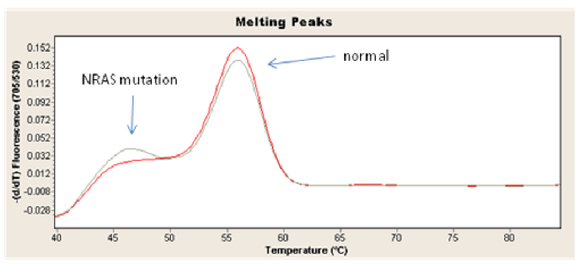
The genomic DNA was tested for *BRAF* codon 600 (rs113488022), *NRAS* codon 61 (rs79057879), *HRAS* codon 61 (rs28933406), *KRAS* codons 12 and 13 (rs121913535) point mutations using real-time PCR and fluorescence melting curve analysis (Roche Light Cycler 2.0 Instrument, Roche Instrument Center AG, Rotkreuz, Switzerland). Fluorescence melting peaks were built by plotting of the negative derivative of fluorescent signal corresponding to the temperature (-dF/dT). The sensitivity of mutation detection by melting curve analysis was 10% of cells with a mutant allele in the background of normal cells, as established by serial dilutions of the positive controls.

Detection of rearrangements

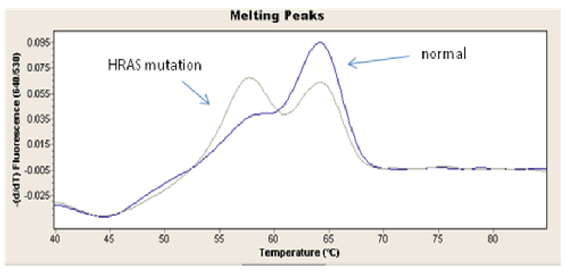
PAX8ex7 and PAX8ex9/PPAR-gamma, RET/PTC1 and RET/PTC3 rearrangements were detected on RNA by RT-PCR ABI Prism 7500 (LT, Foster City, CA, USA) with primers designed to flank the respective point.



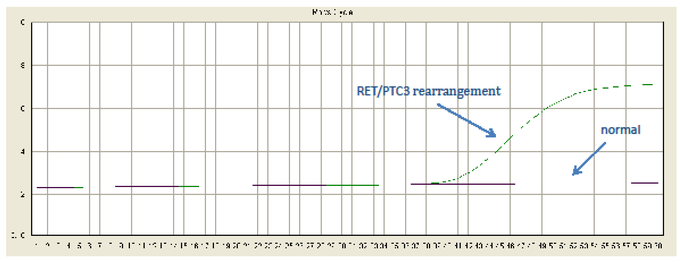
Melting curve of pathological BRAF mutation and mutation negative control (rs113488022)



Melting curve of pathological NRAS mutation and mutation negative control (rs79057879)



Melting curve of pathological HRAS mutation and mutation negative control (rs28933406)



Expression curve of RET and ELE1 (RET/PTC3) genes rearrangement in FNA samples of thyroid

Results

We analyzed 779 consecutive FNA samples from thyroid cold nodules. The DNAs of these samples were subjected to gene mutation analysis and RNAs to rearrangements analysis. In this time, 779 FNA samples finished the 1st year, 504 FNA samples the 2nd year and 250 samples the 3rd year follow-up.

1st year follow-up

After **one year follow-up** (n=779) by histology, **52 cases** (6.8%) were classified **as malignant** (40 PTC, 2 FTC, 10 other), **from which we identified genetic alterations only in 40** (5.1%). In this group was identified **73 genetic alteration (39 BRAF, 23 NRAS, 9 HRAS, 1 KRAS és 1 RET/PTC3).** We detected 22 BRAF, 1 NRAS mutations and 1 RET/PTC3 rearrangement in 40 PTC samples.
(specificity 93.3%, sensitivity 46.2%, negative predictive value 96.0%, positive predictive value 32.9%)

779 FNA samples		malignant	not malignant
		+	-
genetic alteration	+	24	49
	-	28	678

Table 1: All results of histological, cytological and genetic examination in this study after 1st year follow-up

2nd year follow-up

In **two years follow-up** group (n=504) by histology, **30 cases** (6.0%) were classified as malignant (26 PTC, 1 FTC, 3 other), from which we indentified **genetic alterations in 26** (5.2%). In this group we detected 26 genetic alterations (**12 BRAF, 5 NRAS, 7 HRAS, 1 KRAS mutations and 1 RET/PTC3 rearrangement**). In 26 histolgycal classified PTC were exhibited 8 BRAF mutations and 1 RET/PTC3 rearrangment. (**specificity 96,4%, positive predictive value 30,0%**)

504 FNA samples		malignant	not malignant
		+	-
genetic alteration	+	9	17
	-	21	457

Table 2: All results of histological, cytological and genetic examination in this study after 2nd year follow-up

3rd year follow-up

In three years follow-up group we found 14 different genetic alterations namely **4 BRAF mutations, 7 HRAS mutations, 1 NRAS mutations, 1 KRAS mutation** and **1 RET/PTC3 rearrangements** were detected. During the 3 years, we have identified **13** patients with **papillary cancer**. Out of these 13, genetic alteration was seen in 5 (**4 BRAF mutations** and **1 RET/PTC3 rearrangement**). In 9 cases carrying mutations, no sign of malignancy could be observed at present. (**specificity 96,2%, positive predictive value 38,5%**)

250 FNA samples		malignant	not malignant
		+	-
genetic alteration	+	5	9
	-	8	228

Table 3: All results of histological, cytological and genetic examination in this study after 3rd year follow-up

No PAX8/PPARgamma rearrangements were demonstrated in the 779 samples. These data are not in complete accordance with published information.

Conclusions

- **No PAX8/PPARgamma rearrangements were demonstrated in the 779 samples.**
- **The different frequency of genetic variants in our study compared to others might be due to the different iodine intake in Hungary. The US is a high iodine intake area in contrast to Europe including Hungary which is known of low dietary iodine intake of the population.**
- **Such a classification would be additional to the diagnostics, it would promote the personal therapeutics and the prediction of the outcome of the disease.**
- **The detection of mutations from FNA samples would be helpful in the future to guide targeted therapies can be initiated preoperatively or in those patients who are not surgical candidates.**

Bálint Tobiás – Semmelweis University 1st Department of Internal Medicine (1083 Budapest, Korányi S. u. 2/a, Hungary) – tobias.balint@med.semmelweis-univ.hu

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