Decreased Expression of Catalase mRNA in Thyroid Anaplastic Carcinoma

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Background: A decreased expression of glutathione peroxidase mRNA, an antioxidant enzyme, was previously observed in thyroid anaplastic carcinomas.

Methods: To clarify the expression of antioxidant-related enzymes in thyroid anaplastic carcinomas, the expression levels of catalase, copper and zinc superoxide dismutase and manganese superoxide dismutase mRNA in 85 benign and malignant thyroid tissues were measured by means of real-time quantitative reverse transcription-polymerase chain reaction.

Results: Decreased expression levels of catalase and copper and zinc superoxide dismutase mRNAs, but not manganese mRNA, were observed in five anaplastic carcinomas compared with normal thyroid tissues and differentiated tumors.

Conclusion: These results suggest the possibility that anaplastic carcinoma cells are more likely to suffer damage by oxygen free radicals than normal thyroid cells or differentiated tumor cells.

Key words: catalase – superoxide dismutase – mRNA – thyroid – anaplastic carcinoma

INTRODUCTION

We have reported a decreased expression of glutathione peroxidase (GPx) mRNA, an antioxidant enzyme, in thyroid anaplastic carcinomas (1,2). Other than GPx, many enzymes, e.g., catalase (CAT), thyroid peroxidase (TPO) and superoxide dismutase (SOD), take part in the catalysis of oxygen free radicals. These antioxidant enzymes protect cell constituents from damage by oxygen free radicals and play crucial roles in neoplastic disease (3). There are two main forms of SOD in mammalian cells: copper and zinc SOD (Cu/Zn-SOD) is found predominantly in the cytosol and manganese SOD (Mn-SOD) is localized in the mitochondria. In several studies, decreased or increased expression of CAT, SOD and GPx activities was observed in various kinds of tumors (4–9); however, except for GPx and TPO, decreased expression of which mRNAs in anaplastic carcinoma has already been reported (10), no intensive study has been carried out to clarify the expression of these enzymes in anaplastic carcinomas. In the present study, using 85 benign and malignant thyroid tissues, including five anaplastic carcinomas, we measured the expression levels of three antioxidant-related enzymes, CAT, Cu/Zn-SOD and Mn-SOD, by real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) (11).

PATIENTS AND METHODS

EXTRACTION OF RNA FROM THYROID TISSUES

Tissue samples from thyroid tumors or normal thyroid tissues in the opposite lobe of carcinomas were obtained by surgery. All tissues were frozen in liquid nitrogen immediately after resection. Total RNA was extracted according to the method of Chomczynski and Sacchi (12).

REAL-TIME QUANTITATIVE RT-PCR

RNAs from 20 normal thyroid tissues, 24 papillary carcinomas, 23 follicular adenomas, 13 follicular carcinomas and five anaplastic carcinomas were subjected to real-time quantitative RT-PCR analysis. Reverse transcription was performed using 1 μg of total RNA in an RT mixture containing 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 10 mM dithiothreitol, 3 mM MgCl₂, 0.5 mM dNTPs, 200 U M-MLV reverse transcriptase (Gibco), 2 U/μl RNase inhibitor (Takara, Shiga, Japan) and 2.5 μM...
oligo dT (Gibco, Gaithersburg, MD) in a total volume of 20 μl at 37°C for 60 min. Real-time quantitative PCR (TaqMan PCR) using an ABI PRISM 7700 Sequence Detection System and a TaqMan PCR Core Reagent Kit (Applied Biosystems, Foster City, CA) was performed according to the manufacturer’s protocol. A 1 μl volume of the first strand cDNA was used in the following assay. The two primers and one TaqMan probe used for the quantification of CAT (13), Cu/Zn-SOD (14), Mn-SOD (15) and β-actin (16) mRNAs were as follows: [CATF (0.5 μM): 5’-TTAATCCATTGCATCTCACC-3’ (bases 881–900)], [CATR (0.5 μM): 5’-GGCGGTGAGTGTCAGGATAG-3’ (bases 1071–1090)] and [CAT-TM (10 pmol): 5’-FAM-AGGCTATCTGTTCAACCTCAGCAAATGTAAT-TAMRA-3’ (bases 241–260)], [Cu/ZnSODR (0.5 μM): 5’-CCTGTTCTTTGTACCTTCTC-3’ (bases 461–480)] and [Cu/ZnSOD-TM (10 pmol): 5’-VIC-AGATCACAGAATCTTCAATAGACACATCGG-TAMRA-3’ (bases 351–380)], [MnSODR (0.5 μM): 5’-AGTGGTCAATACATACATAG-3’ (bases 521–540)] and [MnSOD-TM (10 pmol): 5’-FAM-CTGAGCCTTGGACACCACAGATGGACACAG-3’ (bases 901–920)], [ACF (0.5 μM): 5’-TGGACCATCCGCAAAGACCTG-3’ (bases 901–920)], [ACR (0.5 μM): 5’-CCGATCCACCGGAGTACTT-3’ (bases 1047–1066)] and [AC-TM (10 pmol): 5’-FAM-CACCACCATGACCTGGCATTGCC-TAMRA-3’ (bases 947–971)], respectively.

The conditions for the TaqMan PCR were as follows: 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 1 min. A recombinant pGEM T-vector (Promega, Tokyo, Japan) containing either CAT, Cu/Zn-SOD, Mn-SOD or β-actin cDNA was constructed by PCR cloning with the same set of primers as used in TaqMan PCR and were used as standard samples. The amplification plots of the PCR reaction were used to determine the threshold cycle (Cₜ). The Cₜ value represented the PCR cycle at which an increase in reporter fluorescence (ΔRn) above the line of the optimal value (optimal ΔRn) was first detected. The initial copy number of the target mRNA was calculated from a plot of Cₜ against the input target quantity.

**Statistical Analysis**

Statistical analysis of differences between the groups was carried out using the Mann–Whitney U test. P values of <0.05 were considered significant.

**RESULTS**

The expression levels of CAT, Cu/Zn-SOD and Mn-SOD mRNAs in thyroid tissues were measured by real-time quantitative RT-PCR. When compared with normal thyroids, the relative expression levels of CAT to β-actin mRNA were greatly and weakly decreased in anaplastic carcinomas and differentiated tumors, respectively (Fig. 1).

The expression levels of Cu/Zn-SOD to β-actin were decreased in all thyroid tumors compared with normal thyroids (Fig. 2), while the relative expression levels of Mn-SOD to β-actin were decreased only in differentiated tumors and not in anaplastic carcinomas compared with normal thyroids (Fig. 3).

**DISCUSSION**

In thyroid, SOD catalyses the removal reaction of superoxide anion with production of hydrogen peroxide, which is used as a substrate by TPO for thyroid hormone synthesis and the excess of superoxide which can be harmful for thyroid cells is then destroyed to H₂O and molecular oxygen by the reaction of GPx and CAT enzymes (17). Both Cu/Zn-SOD and Mn-SOD enzymes, two main forms of SOD, have the same enzymatic function but work in different subcellular compartments. Cu/Zn-SOD is a constitutively expressed enzyme, whereas Mn-SOD expression can be induced by cytokines and responds to the redox state of the cells (18–20). In several studies, the
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Activities of these enzymes were measured in various types of cancerous tissues and cells by the degree of tumor differentiation. In general, no agreement was obtained among the results of the studies. It has been reported that a decreased expression of CAT protein level was observed in human hepatocellular tumors (21) and expression of Mn-SOD mRNA was decreased in esophageal squamous cell carcinomas during poor differentiation (22), while expression of Cu/Zn-SOD mRNA was decreased in human monocytic and promyelotic leukemia cells during differentiation (23).

Very little is known about the tissue antioxidant defense in thyroid cancers. CAT activities have been reported to be significantly higher in follicular carcinomas, compared with those in normal thyroid tissues (24). In another study, Cu/Zn-SOD activities were reported to be significantly lower in follicular adenomas and papillary carcinomas compared with those of normal thyroid tissues (25). Furthermore, Mn-SOD activities were significantly higher in differentiated tumors compared with those of normal thyroid tissues (26).

No intensive study has been carried out on the expression of antioxidant enzymes in thyroid anaplastic carcinomas, because it is difficult to obtain fresh tissues since anaplastic carcinomas are rare and they usually undergo a rapid and aggressive clinical course. In this study, by using real-time quantitative RT-PCR, in which only a small quantity of materials is necessary, we examined the expression levels of antioxidant enzymes in anaplastic carcinomas and decreased expression of CAT and Cu/Zn-SOD mRNA, but not Mn-SOD mRNA was observed.

As summarized in Fig. 4, anaplastic carcinoma cells are likely to have no or weak resistance against oxygen free radicals, because the expression levels of most of the enzymes that are engaged in the defense mechanism were decreased. Hydrogen peroxide especially, which shows strong cytotoxicity, can be easily accumulated in anaplastic cells, since the expression of GPx and CAT enzymes that catalyse hydrogen peroxide was greatly decreased. It is assumed that decreased expression of CAT mRNA is critical for anaplastic carcinoma cells even though the expression of Mn-SOD mRNA is maintained, because the expression of mRNAs of two other enzymes, TPO and GPx, that catalyze hydrogen peroxide is much decreased. Therefore, a molecular-based treatment, which facilitates the production of excessive oxygen radicals inside the anaplastic carcinoma cells, may be one of the promising methods for the treatment of this most aggressive and lethal carcinoma.

The reason for the varied expression levels of Mn- and CuZn-SODs and CAT mRNAs in differentiated thyroid tumors is not clear. We were not able to find any differences in their expression levels among histological subtypes or tumor stages in the samples that we used in this study. This question seems to need further investigation.
Figure 4. Summary of the expression levels of antioxidant enzymes in anaplastic carcinoma.

Acknowledgments

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References


