The Expression of the High Mobility Group I(Y) mRNA in Thyroid Cancers: Useful Tool of Differential Diagnosis of Thyroid Nodules.

Sang-Jin Kim, M.D., JIN-Woo Ryu*, M.D., Dong-Seop Choi, M.D.

Department of Internal Medicine, Korea University, Seoul, Korea
*Department of General Surgery, Dankook University, Cheonan, Korea

Objective : *Thyroid nodule is frequent and occurs in about 5% of the general population. In contrast, thyroid cancer is much less frequent and occurs in about 5-10% of thyroid nodules. Distinguishing between benign and malignant lesions is an important task that is best accomplished by fine needle aspiration. Recently, Chiappetta et al. reported that the expression of the high mobility group (HMG) I(Y) proteins correlates with the malignant phenotype of human thyroid neoplasia, and suggested that the detection of the HMG I(Y) proteins might be a valid tool for an easy and sensitive discrimination assay between benign and malignant neoplastic thyroid disease.*

Methods : *we evaluated the expression of the HMG I(Y) mRNA in 39 frozen thyroid tissues from patients with thyroid nodule by semiquantitative RT-PCR.*

Results : *The expression of the HMG I(Y) mRNA was low in all of 10 normal thyroid tissues. In all of 3 adenomatous goiters, 6 follicular adenomas and 2 Hurthle cell adenomas, the HMG I(Y) mRNA expression level was low. In 11 of 13 papillary carcinomas and all of 5 follicular carcinomas, the HMG I(Y) mRNA expression level was high.*

Conclusion : *These results indicate that there is a correlation between the expression of HMG I(Y) and the malignant phenotype of thyroid cancer, suggesting that these proteins may be useful as a marker in thyroid cancer.*

Key Words : *HMG I(Y), thyroid cancer*

INTRODUCTION

Thyroid tumor in a human is one of the most common endocrinologic diseases and occurs in about 5% of *the* population[1]. Thyroid cancer occurs in about 5-10% of thyroid nodules[2]. Distinguishing between benign and malignant lesions is an important task that is best accomplished by fine needle aspiration. But the reported accuracy of cytologic diagnosis ranges from 70-90%[3], largely depending on the experience of the person performing the biopsy and that of the cytopathologist interpreting it. In addition, follicular carcinoma cannot be differentiated with follicular adenoma by fine needle aspiration. Therefore, a new diagnostic technique is warranted.

Investigations regarding the activation and/or inactivation of oncogenes, tumor suppressor genes and growth factors have not yet revealed consistent differences between the benign and malignant tumors[4,5].

High mobility group (HMG) I(Y) proteins are a class of low-molecular mass, non-histone nuclear proteins characterized by their high content of basic and acid amino acids and binding to the DNA minor groove at A/T rich sequences[6,7]. HMG I(Y) proteins are required for induction of the human IFN- gene by viruses[8] and for the regulation of the TNF-[9] and rRNA genes[10]. The

Address reprint requests to : Sang Jin Kim, M.D., Department of Internal Medicine, College of Medicine, Korea University Hospital, 126-1, 5ka Anam-Dong, Seongbuk-Ku, Seoul, 136-075, Korea

expression of these proteins correlates with the neoplastic transformation in several systems. There is a correlation between an elevated expression of the HMG I(Y) proteins and the appearance of a highly malignant phenotype in rat thyroid differentiated cells, as well as in thyroid and skin experimental tumors[11,12]. Moreover, a correlation was found between the ability of rat prostatic cell lines to metastasize and the expression of the HMG I(Y)[13], and between the elevated expression of the same protein and progressive transformation of mouse mammary epithelial cells[14]. Furthermore, expression of HMG I(Y) in high grade human prostate cancer was found by in situ hybridization[15]. Recently, Chiappetta et al.[16] demonstrated that HMG I(Y) proteins are expressed in human thyroid carcinomas and thyroid carcinoma cell lines, but not in adenomas, goiters and normal thyroid tissues and cells.

Our study evaluated the expression of HMG I(Y) mRNA and its clinical implication in 39 frozen thyroid tissues from patients with thyroid nodule by use of semiquantitative RT-PCR.

MATERIALS AND METHODS

1. Subjects

The study involved 39 thyroid tissues that were obtained from Dankook University Hospital from March 1995 to March 1997. The thyroid tissues that we used were as follows: normal thyroid tissue (n=10), adenomatous goiter (n=3), follicular adenoma (n=6), Hurthle cell adenoma (n=2), papillary carcinoma (n=13) and follicular carcinoma (n=5). The normal tissues were obtained from operations on patients with thyroid nodules. The tissues were frozen in liquid nitrogen and stored frozen until RNA extraction was performed.

2. RNA isolation and RT-PCR

Total RNA was extracted from *the* thyroid tissue using Ultraspec RNA Isolation System (Biotex Lab. INC. USA) according to the manufacturer's recommendations. 4μ l of total RNA was mixed with 3.0μ l 10X Buffer, 3.0μ l 2mM/ μ l dNTP, 3.0μ l 10pmol/μ l HMG I(Y) primer, 1.5μ l 10pmol/μ l β-actin, 1.5μ l 100mM DTT, 0.3μ l 40 units RNase inhibitor, 0.3μ l 10 units AMV-reverse transcriptase, 0.1μ l 5 units/μ l Taq polymerase and 13.3 μ l DEPC-treated distilled water and simultaneously performed reverse transcription and PCR with Perkin

Elmer DNA Thermal Cycler(Model 9600). The condition of RT-PCR was as follows: reverse transcription-42 for 45 minutes. PCR(24 cycles)-denaturation, 95 for 30 seconds, annealing, 58 for 30 seconds, extension, 72 for 45 seconds. Sequences of the sense and antisense HMG I(Y) were as follows: 5'-TGC AAC ACC TAA GA GAC CTC G-3' (sense), 5'-AAA GCT GTC CAG TCC CAG AAG C-3' (antisense). These primers were designed on the basis of the cDNA sequence of the HMG I(Y) gene exon 6 and exon 8. The expected size of the amplified product was 234bp. As a control, β-actin was used. Primer sequences of the β-actin were as follows: 5'-CAC TGT GTT GGC GTA CAG GGT-3' (sense), 5'-TCA TCA CCA TTG GCA ATG AG-3'. The expected size of the amplified product was 154bp. After amplification, the PCR products were separated by electrophoresis on a 2.0% agarose gel containing ethidium bromide. Negative controls included the substitution of RNA with distilled water. The HMG I(Y) mRNA was quantified to the amount relative to β-actin with Vilber Lourmat Darkroom CN-UV/WL image analyser(C.B.S. Scientific Co., USA) and Bio-1D software.

3. Nucleotide Sequencing

PCR products were sequenced using an ABI Prism Automated DNA Sequencer(model 310, PE Applied Biosystems, USA)and the ABI Prism BigDye Terminator cycle Sequencing Ready Reaction Kit(Perkin-Elmer Co., USA).

4. Statistical analysis

The difference of HMG I(Y) expression between benign and malignant tumors was analyzed using Student t-test. P value less than 0.05 was considered significant. All statistical analyses were performed using a commercially available personal computer program SPSS.

RESULTS

Table 1 and Figure 1 show the distribution of the HMG I(Y) mRNA expression in various thyroid tissues. The ratio of HMG I(Y) to β-actin is high in papillary and follicular adenoma and Hurthle cell adenoma. If we take *the* cutoff value that differentiates between benign and malignant tumors as 1.5, the expression of HMG I(Y) was low in all of 10 normal thyroid tissues, 3 adenomatous goiters, 6

follicular adenomas and 2 Hurthle cell adenomas. On the other hand, in 11 of 13 papillary carcinomas and all of 5 follicular carcinomas, the HMG I(Y) expression level was high(Fig. 1). There was a strong association between HMG I(Y) expression and a diagnosis of carcinoma($p < 0.0001$).

Table 1. HMG I(Y) expression in various thyroid tissues

Histology	samples	expression levels* (range)
Normal thyroid	10	0.90± 0.46(0.28- 1.46)
Adenomatous goiter	3	0.61± 0.22(0.35- 0.75)
Folliculalr adenoma	6	0.85± 0.18(0.70- 1.12)
Hurthle cell adenoma	2	1.10± 0.10(1.03- 1.17)
Papillary carcinoma	13	2.19± 0.54(1.44- 3.14)
Follicular carcinoma	5	2.78± 0.69(2.26- 3.94)

*; expression levels mean ratio of HMG I(Y)/ -actin

Figure 2 shows an example of RT-PCR. In normal tissues, lane 6 shows low expression of HMG I(Y), but lane 9 shows a considerable amount of HMG I(Y) expression which is similar to papillary carcinoma(the ratio of HMG I(Y)/ -actin is 1.32). The expression of HMG I(Y) relative to -actin is low in adenomatous goiter(lane 4) and follicular adenoma(lane 3), but high in papillary carcinomas(lanes 1, 2, 7, 8) and follicular carcinomas(lanes 5, 10). The products of HMG I(Y) and -actin were confirmed by sequencing(data not shown). There was a nonspecific band between -actin and HMG I(Y), because -actin and HMG I(Y) were amplified in *the* same tube.

DISCUSSION

Because of previous reports that no presence of HMG I(Y) proteins is detected in normal thyroid cells[16], we firstly performed RT-PCR with only HMG I(Y), but there was abundant expression of HMG I(Y) mRNA in not only malignant tumors but also in normal and benign tumor tissues. So, we performed semiquantitative RT-PCR that -actin was coamplified as a control. At first, we separately performed RT-PCR with each protein, and confirmed the amplified bands. Then we simultaneously amplified both proteins and compared the relative amount of expression. As a result, we confirmed that malignant tumor had a higher expression of HMG I(Y) than normal or benign tumor tissues. This result is compatible with a

previous report[16]. The mechanism by which HMG I(Y) can influence transcription is still incompletely understood. HMG I(Y) has previously been shown to facilitate the binding of certain transcription factors to DNA[8,17-19]. This feature of HMG I(Y) could be explained by at least two mechanism. First, since HMG I(Y) can bend DNA, this could promote binding transcription factor to A/T-rich DNA sites[20]. Second, direct protein-protein interactions between HMG I(Y) and transcription factors may promote the binding of the latter to their cognate DNA-binding sites[21,22]. But the HMG I(Y) gene does not behave like a classical transforming oncogene since, when transfected in normal thyroid cells, it did not cause their transformation, thus suggesting that its expression is necessary but not sufficient to achieve the transformed phenotype[23].

In contrast to previous results by Northern blotting, Western blotting, immunohistochemistry[16] or in situ hybridization[15], normal tissues and benign tumors had a considerable amount of HMG I(Y) mRNA. This result means that HMG I(Y) may regulate the development and differentiation of normal thyroid cells in *the* cell cycle and its increased expression is correlated with malignant phenotype.

The expression of HMG I(Y) mRNA in two cases of papillary carcinoma was low. But even in these cases, the expression of HMG I(Y) mRNA was higher than normal thyroid tissues from the same patients (ratio of HMG I(Y)/ -actin in normal tissues: papillary carcinoma; 0.728:0.926, 0.631:1.465). In this study, although the cases were too small, all follicular carcinomas had a higher expression of HMG I(Y) than normal or benign tumor tissues. So, detection of HMG I(Y) might be extremely useful in the differential diagnosis between follicular adenoma and follicular carcinoma, but further evaluation with more cases is warranted.

In conclusion, HMG I(Y) mRNA expression level was high in thyroid carcinomas, but not in normal and benign tumor tissues. These results indicate that increased HMG I(Y) expression is correlated with thyroid carcinogenesis and these proteins may be useful as a marker in thyroid cancer.

REFERENCES

1. Vander JB, Gaston EA, Dawber TR. *The significance of non-toxic thyroid nodules: final report of a 15-year study of the incidence of thyroid malignancy. Ann Intern Med*

1968; 69:537-540.

2. Werk EE Jr, Vernon BM, Gonazalez JJ. *Cancer in thyroid nodules: a community hospital survey. Arch Intern Med 1984; 144:474-476.*

3. Caruso D, Mazzaferri EL. *Fine needle aspiration biopsy in the management of thyroid nodules. Endocrinol 1991; 1:194-202.*

4. Farid NR, Shi Y, Zou M. *Molecular basis of thyroid cancer. Endocr Rev 1994; 15:202-232.*

5. Fagin JA. *Molecular defects in thyroid gland neoplasia. J Clin Endocrinol Metabol 1992; 6:1398-1400.*

6. Solomon MJ, Strauss F, Varshavsky A. *A mammalian high mobility group protein recognizes any stretch of six A.T base pairs in duplex DNA. Proc Natl Acad Sci USA 1986; 83:1276-1280.*

7. Winter E, Varshavsky A. *A DNA binding protein that recognizes oligo(dA). oligo(dT) tracts. EMBO 1989; 8:1867-1877.*

8. Thanos D, Maniatis T. *The high mobility group protein HMGI(Y) is required for NF- B-dependent virus induction of the human IFN- gene. Cell 1992; 71:777-789.*

9. Fashena SJ, Reeves R, Ruddle NH. *A poly(dA-dT) upstream activating sequence binds high-mobility-group I protein and contributes to lymphotoxin (tumor necrosis factor-) gene regulation. Mol Cell Biol 1992; 12:894-903.*

10. Yang-Yen HF, Rothblum LI. *Purification and characterization of a high-mobility-group-like DNA-binding protein that stimulates rRNA synthesis in vitro. Mol Cell Biol 1988; 8:3406-3414.*

11. Giancotti V, Berlingieri MT, Di Fiore PP, Fusco A, Vecchio G, Crane-Robinson C. *Changes in nuclear proteins following transformation of rat thyroid epithelial cells by a murine sarcoma retrovirus. Cancer Res 1985; 45:6051-6057.*

12. Goodwin G. *Elevated levels of a specific class of nuclear phosphoproteins in cells transformed with v-ras and v-mos oncogenes and by co-transfection with c-myc and polyoma middle T genes. EMBO 1987 6:1981-1987.*

13. Bussemakers MJG, van de Ven WJM, Debruyne FMJ, Schalken JA. *Identification of high mobility group protein I(Y) as potential progression marker for prostate cancer by differential hybridization analysis. Cancer Res 1991; 51:606-611.*

14. Ram TG, Reeves R, Hosick HL. *Elevated high mobility group-I(Y) gene expression is associated with progressive transformation of mouse mammary epithelial cells. Cancer Res 1993; 53:2655-2660.*

15. Tamimi Y, Poel HG, Denyn MM, Umbas R, Karthaus HFM, Debruyne FMJ, Schalken JA. *Increased expression of high mobility group protein I(Y) in high grade prostatic cancer determined by in situ hybridization. Cancer Res 1993; 53:5512-5516.*

16. Chiappetta G, Bandiera A, Berlingieri MT, Bisconti R, Manfioletti G, Battista S, Martinez-Tello FJ, Santoro M, Giancotti B, Fusco A. *The expression of the high mobility group HMG I(Y) proteins correlates with the malignant phenotype of human thyroid neoplasias. Oncogene 1995; 10:1307-1314.*

17. Du W, Thanos D, Maniatis T. *Mechanisms of transcriptional synergism between distinct virus-inducible enhancer elements. Cell 1993; 74:887-898.*

18. Falvo J, Thanos D, Maniatis T. *Reversal of intrinsic DNA bends in the IFN gene enhancer by transcription factors and the architectural protein HMG I(Y). Cell 1995; 83:1101-1111.*

19. Wolffe AP. *Architectural transcription factors. Science 1994; 264:1100-1101.*

20. Zhao K, Kas E, Gonzales E, Laemmli UK. *SAR-dependent mobilization of histone H1 by HMG-I/Y in vitro: HMG-I/Y is enriched in H1-depleted chromatin. EMBO 1993; 12:3237-3247.*

21. Ginse K, Kingsley C, Kirshner JR, Grosschedl R. *Assembly and function of a TCR enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein-protein interactions. Genes Dev 1995; 9:995-1008.*

22. Grosschdl R, Ginse K, Pagel J. *HMG domain proteins: architectural elements in the assembly of nucleoprotein complexes. Trends Genet 1994; 10:94-100.*

23. Berlingieri MT, Manfioletti G, Santoro M, Bandiera A, Visconti R, Giancotti V, Fusco A. *Inhibition of HMGI-C protein synthesis suppresses retrovirally induced neoplastic transformation of rat thyroid cells. Mol Cell Biol 1995; 15:1545-1553.*

Fig. 1. The distribution of the HMG I(Y) mRNA expression in normal tissues and thyroid tumors. The dotted line is a cutoff value that differentiates between benign and malignant tumors.

Fig. 2. RT-PCR analysis of the HMG I(Y) gene expression in normal and neoplastic thyroid tissues. M; PhiX174/Hae III marker, Lanes 6, 9; normal tissues, Lane 4; adenomatous goiter, Lane 3; follicular adenoma, Lanes 1, 2, 7, 8; papillary carcinoma, Lanes 5, 10; follicular carcinoma

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