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Heparanase, Galectin-3, and Tissue Factor mRNA Are Expressed in Benign Neoplasms of the Thyroid

Andreas E. Buchs,[1] Sergei Zehavi,[2] Osnat Sher,[1] Eyal Yeheskely,[3] Michael Muggia-Sulam,[4] Yoav Sherman,[5] and Micha J. Rapoport[1]

[1]Diabetes Unit and Internal Medicine "C"; [2]Department of Pathology; [3]Department of Ear Nose and Throat Assaf Harofeh Medical Center, Zerifin, Israel, affiliated to the Tel Aviv University; [4]Department of Surgery; and [5]Department of Pathology University Hospital Hadassah, Jerusalem, Israel

Objective: Heparanase, galectin-3, and tissue factor (TF) are overexpressed in solid malignant thyroid tumors. We studied their expression in multinodular goiters (MNGs).

Design and Methods: Thyroid tissue specimens from 15 MNGs were obtained during surgery. mRNA expression for galectin-3, heparanase, and TF was assessed by RT-PCR.

Results: Isolated expressions of heparanase and galectin-3 mRNA were expressed in 2 and 4 of the 15 MNGs, respectively; 8/15 MNGs were positive for both heparanase and galectin-3. TF mRNA was found in all MNG specimens.

Conclusion: Galectin-3, heparanase, and TF RNA expression is prevalent in MNGs. Further studies will be needed to determine the prognostic significance of these findings.

Key Words: Galectin-3; heparanase; tissue factor; thyroid neoplasms.

Introduction

Malignant thyroid tumors can be diagnosed easily by histology or cytology, and tumor markers such as galectin-3 have been contributing to the diagnostic certainty. The expression of the latter, playing a role in cell apoptosis, and of proteins associated with cell adhesion, cell matrix interaction, and angiogenesis, such as heparanase and tissue factor (TF), have been investigated in malignant tumors but little or no information is available regarding their expression in benign thyroid tumors *(1–4)*.

Heparanase, an endo-beta-glucoronidase, and TF, a transmembrane glycoprotein of the coagulation cascade, were detected in solid tumors of the gastrointestinal tract, breast, and urinary bladder as well as in precancerous lesions of the colon *(5–9)*. Their absence in normal colonic tissue and localization in areas with neovascularization indicates their importance for angiogenesis and possible hematogenous dissemination of tumors *(2)*. A role of the scavenger receptor galectin-3, a carbohydrate-binding protein for beta-galactosides, has been implicated in both malignant and benign thyroid tumors *(10–13)*.

It appears that these three proteins are not only important for tumor genesis per se but could also determine the biological behavior of benign thyroid nodules. We therefore investigated the expression of mRNA encoding for these three proteins in the most frequent benign thyroid tumors, multinodular goiters (MNGs).

Results

Specimens from 15 patients with MNGs were obtained during surgery. All tissues were positive for the housekeeping gene *GAPDH*, indicating successful RNA extraction and RT-PCR. All 15 specimens were also positive for TF RNA. Eight out of 15 MNGs were positive for heparanase and 10 for galectin-3. Seven patients with MNGs expressed both heparanase and galectin-3. One and three patients expressed only heparanase or galectin-3, respectively. Seven specimens were negative for heparanase and five for galectin-3. The results of three patients are ilustrated in Figs. 1 and 2.

These data reveal that TF is ubiquitously expressed in MNGs and that over half of the MNGs also express galectin-3 and/or heparanase mRNA.

Discussion

It was the purpose of this study to assess the expression of heparanase, galectin-3, and TF in MNGs. Half of the investigated specimens were positive for galectin-3 and/or heparanase at the mRNA level, whereas all of them expressed TF. To the best of our knowledge, this is the first report examining the expression of heparanase and TF in MNGs.

Although heparanase has been mainly detected in malignancies and, especially, their metastatic lesions, it has also been found in benign tissues such as in the endothelium of capillaries from normal ovaries *(14)*. Heparanase from mice fibroblasts also induces the secretion of angiogenetic factors into the extracellular matrix in vivo and in vitro *(15)*.

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Author to whom all correspondence and reprint requests should be addressed: Dr. Andreas E. Buchs, Department of Internal Medicine "C", Assaf Harofeh Zerifin 70300, Israel. E-mail: abuchs@netvision.net.il

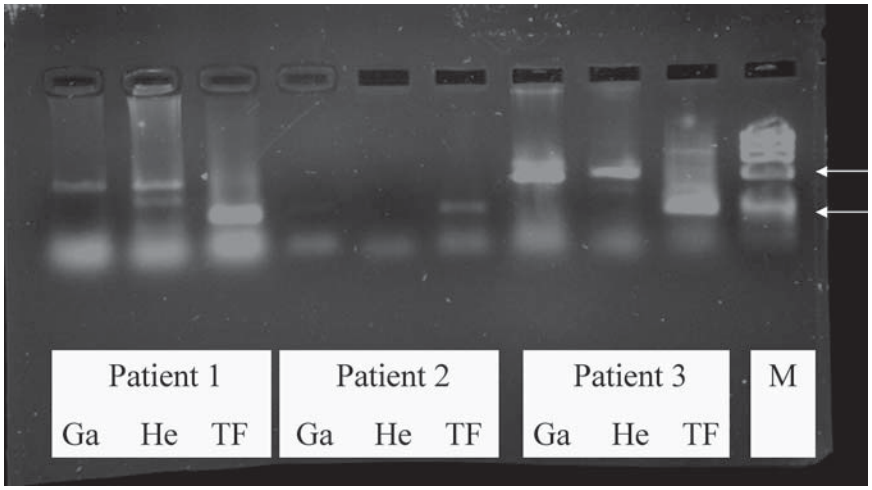

Fig. 1. A typical gel from three patients with MNGs. Patients 1 and 3 were positive for heparanase (he), galectin-3 (ga), and TF. Patient 2 was positive for TF only. RNA was extracted from thyroid specimens obtained from surgery and RT-PCR was performed as indicated in *Methods* and separated on an ethidium-stained 1.5% agarose gel. The upper arrow to the right points toward gal-3 and heparanase. The lower arrow points toward TF. M: molecular weight marker.

Thus, heparanase could also play a possible role in promoting the growth of benign thyroid neoplasms.

Most previous studies reported galectin-3 expression mainly in thyroid malignancies; but it was also found in benign thyroid tissues *(10,11,16)*. Bartolazzi et al. reported that galectin-3, checked by immunohistochemistry, was confined beneath the tumor capsule in 5 out of 132 follicular adenomas and in 11 out of 13 follicular neoplasms of indeterminate malignant behavior. More so, in a small number of cases with Hashimoto's thyroiditis, they found galectin-3 in areas intermingled with activated lymphoid follicles *(11)*. Saggiorato reported galectin-3 expression in the nucleus but not in the cytoplasm of benign follicular adenomas *(10)*. Recently, Martins using rtPCR like us reported galectin-3 in 45% of adenomas and 17% of MNGs *(13)*. Total RNA, as used for RT-PCR, originates from complete cells including the nuclei and allows detection at very low expression levels. This may partially explain the high prevalence of galectin-3 positive tissue in benign thyroid tissues in our and Martins' studies as compared to the reports by Bartolazzi and others *(11,13)*.

A substantial part of the MNGs investigated by us was negative for galectin-3 and/or heparanase expression. Similarly to this, the in vivo behavior of thyroid nodules is also nonuniform. Up to 50% shrink spontaneously over the period of the years, whereas others, though benign, grow continuously and need to be removed due to their size *(17,18)*. It is of interest that galectin-3 expression has been attributed to decreased cell apoptosis via enhanced adhesion to laminin, fibronectin, and vitronectin 4. Overexpression of galectin-3 could be causatively involved in "surviving" of thyroid nodules.

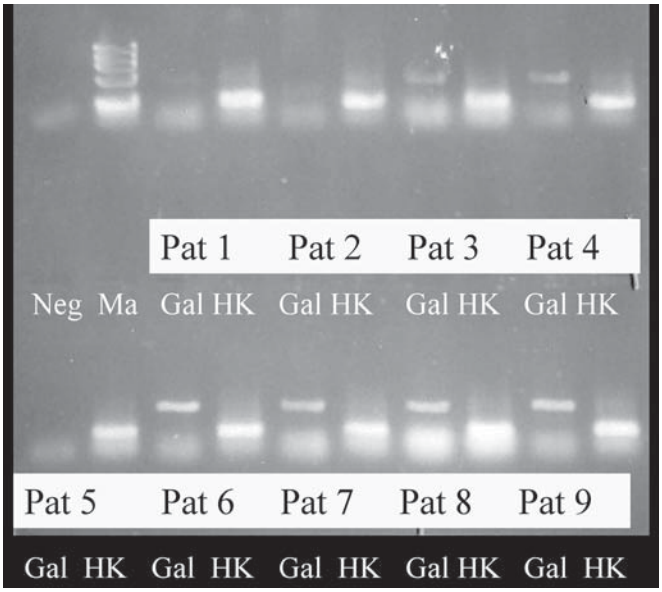

Fig. 2. A typical gel from nine patients with MNGs. Except for patients 2 and 7 all were positive for galectin-3 (gal). RNA was extracted from thyroid specimens obtained from surgery and RT-PCR was performed as indicated in methods and separated on an ethidium-stained 1.5% agarose gel. Ma: molecular weight marker, neg: negative control, HK: housekeeping gene *GAPDH*.

In summary, we report that heparanase, galectin-3, and TF are expressed at the mRNA level in some but not all MNGs. Future studies, correlating their expression to continuous growth or, respectively spontaneous shrinking of MNGs are indicated. This could help to clarify whether mRNA for

heparanase and galectin-3 expression can serve as a prognostic marker for the growth of MNGs.

Methods

Patients, who underwent thyroidectomy for benign tumors were eligible for this study. Removed thyroid glands were transferred to the pathology department and sections of single thyroid nodules from MNGs were handed over to us for investigation. The expert pathologists (Y.S. and S.Z.) carefully examined the remaining tissue. All pathology reports were made available to us to ensure that only benign tumors were studied. RNA was extracted from 100 mg thyroid tissue and lysed in TRI reagent (SIGMA) according to the manufacture's instructions and resuspended in 10 µL of DPEC water. Five micrograms of total RNA was reverse transcribed into cDNA by RT-PCR by commercially available kits (Epicentre and SIGMA) according to the manufacturer's instructions. Primers for GAPDH, galectin-3, TF, and heparanase were designed according to previous reports *(16, 19,20)*. The coding strand primer for GAPDH corresponds to position 562–582, the noncoding strand to position 807–827. The coding strand primer for TF corresponds to position 759–784 of the published DNA sequence, the noncoding strand to position 1016–1041. The coding strand primer for heparanase corresponds to position 171–189, the noncoding strand to position 565–583. The coding strand primer for galectin-3 corresponds to position 102–118 of the coding strand and 672–688 of the noncoding strand. PCR reactions were performed with Taq polymerase (MBI, Fermentas): 94ºC 3 min (hot start), 94ºC 1 min, 60ºC 30 s, 72ºC 1 min, 40 cycles for GAPDH and TF. For heparanase PCR conditions were as follows: 95ºC 3 min (hot start), 95ºC 15 s, 58ºC 1 min, 72ºC 1 min, 30 cycles and for galectin-3 94ºC 5 min (hot start), 94ºC 1 min, 56ºC 1 min, 72ºC 1 min, 30 cycles. PCR products were analyzed on a 1.5- % agarose gel containing ethidium bromide. Restriction endonuclease digestions verified heparanase and galectin-3 PCR products.

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