Restricted Patterns of CD44 Variant Exon Expression in Human Papillary Thyroid Carcinoma

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ABSTRACT

CD44 is a polymorphic family of cell surface proteoglycans and glycoproteins implicated in cell-cell and cell-matrix adhesion interactions, cell migration, and tumor metastasis. CD44 exists as a standard form and as multiple isoforms arising from alternative splicing of variant exons (termed v1–v10) encoding parts of the extracellular domain. We demonstrated previously that papillary thyroid carcinomas exhibit aberrant patterns of alternative CD44 mRNA splicing (G. Ermak et al., Cancer Res., 55: 4594–4598, 1995). In the present report, we use reverse transcription-PCR using a new high-performance polymerase formulation (Ex Tag; Takara Shuzo Co., Ltd., Otsu, Japan), followed by Southern hybridization, and demonstrate that alternative exon usage in papillary thyroid carcinomas is restricted primarily to exons v6, v7, v8, v9, and v10, with weak expression of v3. Expression of v8 is tightly linked to v9 and closely related to v10 expression. Also, v6 and v7 expression are closely related. Papillary thyroid cancers exhibit a marked increase in specific mRNA species containing combinations of exons v6 to v10. Several isoforms found in papillary cancers are not detectable in histologically normal tissue derived from the corresponding contralateral thyroid lobes. Examples include a 750-bp v6- and v7-containing PCR product and a 650-bp v8- and v9-containing PCR product. Finally, a novel 530-bp PCR product was discovered and shown to contain a subsegment from exon 4 joined to a subsegment of exon 13 (v8), followed by the complete sequence of exons 14 (v9) and 15 (v10). This novel isoform was present in both the papillary cancers and contralateral tissues. In conclusion, papillary thyroid cancers exhibit specific patterns of aberrant alternative CD44 splicing, distinguishing them from histologically normal thyroid tissue.

INTRODUCTION

Papillary thyroid cancer is the most common of the endocrine malignancies, comprising about 70% of thyroid cancers in iodine-replete areas. Of all solid cancers presenting in adults, papillary thyroid cancer generally carries the best long-term prognosis with a 20-year cause-specific mortality rate of only 5.5% (1). The cancer, although usually indolent, often presents clinically with metastases to regional lymph nodes and occasionally with invasion of local neck structures; blood vessel invasion and distant metastases are uncommon (2). Regional lymph node metastases appear to have at most a small impact on long-term mortality from the tumor (1, 3). Despite these interesting biological properties, very little is understood about the molecular factors (4) involved in the pathogenesis and metastatic properties of this neoplasm.

Recently, Figge et al. (5) reported that significant levels of CD44 protein are expressed on the plasma membrane of papillary thyroid cancer cells. CD44 is a heterogeneous family of integral membrane proteoglycans and glycoproteins implicated in lymphocyte activation and homing, cell-cell and cell-matrix adhesion, and tumor metastasis

(Reviewed in Refs. 6–9). CD44 is thought to be a major receptor for hyaluronate (10). CD44 protein isoforms range in size from M<sub>r</sub> 80,000 to 250,000 (11). The heterogeneity of CD44 results from posttranslational modifications (11) as well as alternative mRNA splicing of variant exons (termed v1 to v10) that encode parts of the extracellular domain (Refs. 12–13; Fig. 1). The standard M<sub>r</sub> 85,000–95,000 CD44 form lacks all 10 variant exons in its mRNA, whereas the larger variant protein isoforms contain a segment encoded by one or more of exons v1 through v10. Variant CD44 molecules are expressed on a variety of epithelial cells in a tissue-specific pattern (14, 15), suggesting that the process of alternative splicing is normally tightly regulated. Papillary cancers express CD44 isoforms containing v6 on their plasma membranes (5). Ermak et al. (16) showed that alternative CD44 mRNA splicing is deregulated in papillary thyroid carcinomas and that CD44v6-containing mRNA isoforms are expressed in these tumors.

Little is known about the function of variant CD44 molecules. Tumor cells that express certain CD44 isoforms have enhanced cell growth and metastatic potential in vivo. In one rat cancer cell line, expression of an isoform containing CD44v6 was found to be both necessary and sufficient to confer metastatic potential (17–21). Similarly, expression of an isoform containing v8 to v10 enhanced the metastatic capability of murine fibrosarcoma cells (22). Therefore, it is likely that specific CD44 isoforms on the surface of tumor cells have a direct impact on the growth and metastatic behavior of the cells. Given the unusual biological behavior of papillary thyroid cancers, it is important to characterize the CD44 isoforms in these tumors. In the present report, using semiquantitative RT-PCR and Southern hybridization with probes against each of the variant exons v1–v10, we demonstrate that alternative exon usage in papillary carcinomas is restricted primarily to exons v6, v7, v8, v9, and v10, with weak expression of v3. Several of the isoforms present in papillary cancers are not detectable in histologically normal tissue derived from the corresponding contralateral thyroid lobes.

MATERIALS AND METHODS

Specimens

Samples of papillary thyroid cancer and histologically normal thyroid tissues were obtained fresh from the operating room and flash frozen in an isopentane bath (−65°C). Frozen sections were cut and stained immediately to confirm the histology and were later verified on permanent sections. The specimens were stored in the Albany Medical Center tumor bank (−85°C) prior to processing.

RNA Extraction and cDNA Preparation

Total RNA was extracted immediately after thawing the specimens using the RNAzol B isolation kit (Cinna/Biotex Laboratories, Houston, TX) as described previously by Ermak et al. (16). The synthesis of first-strand cDNA was performed using the Superscript preamplification system (GIBCO-BRL, Gaithersburg, MD). A 5-µg aliquot of RNA per reaction was reverse transcribed using oligo(dT) as the primer.

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3 The abbreviations used are: RT-PCR, reverse transcription-PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
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Fig. 1. Schematic diagram of the CD44 genomic DNA showing variant exons v1—v10 in black. Primers P1 and P2 were described by Matsumura and Tarin (23). The S1 probe is directed against exon 4, which encodes part of the standard CD44 sequence. This probe will, in theory, detect all isoform products unless the exon 4 sequence is disrupted. Probes v1—v10 will detect isoform products containing the corresponding exon sequence.

PCR Primers and Southern Hybridization Probes

A schematic diagram of the CD44 genomic DNA is presented in Fig. 1. The gene contains a total of 20 exons (13). The standard CD44 mRNA lacks exons 6 through 15, which are known as variant exons and are designated as v1 through v10. PCR primers P1 and P2 (23) were selected to flank the insertion site where variant exons (v1 to v10) can be included in the mRNA transcript to produce alternate forms of CD44. Samples containing mRNA encoding the standard CD44 isoform (lacking variant exons) are expected to exhibit a PCR amplification product of 482 bp (16). Samples containing alternatively spliced transcripts (containing one or more variant exons) would exhibit larger amplification products, up to a maximum size near 1650 bp. It is anticipated that all CD44 amplification products will hybridize to the S1 probe, which is directed against exon 4, a region included within the standard part of the CD44 sequence. Probes against each of the variant exons v1 through v10 were synthesized as: v1, 5'-TCA AAC AGC CAC ACA GCA GCT CAG GAG CAA-3'; v2, 5'-GCA ACT GAG ACA GCA ACG AAG AGG CAA GAA-3'; v3, 5'-ATC TCA GCA GGC TGG GAG CCA AAT GAA GAA-3'; v4, 5'-GCC AAG CCA TTC AAA TCC GGA AGT GCT ACT-3'; v5, 5'-CCC TCA TTC ACC ATG AGC ATG AGG AAG-3'; v6, 5'-TGG CAT GAG GTA TGC CAA ACA CCC AGA-3'; v7, 5'-CTT CAA CCC AAT CTC ACA CCC CAT GGG ACG-3'; v8, 5'-GGT GGA AGA TCG GGA CAG GAC AGG ACC TCT-3'; v9, 5'-CAG AGC TTC TCT TCA GAC GTA GAG TTC-3'; and v10, 5'-CCT CTC ATT ACC CAC ACA CGA AGG AAA GCA-3'.

CD44 Gene. The reaction mixture contained 2.5 mM MgCl₂, 0.2 mM of each deoxyribonucleoside triphosphate, 2 μM of each primer, and 2.5 units of Ex Taq polymerase (TaKaRa Shuzo Co., Ltd.). The primers were primer P1 and P2 as described by Matsumura and Tarin (23). The mixture was amplified for 32 cycles: 98°C for 20 s (denaturation) and 68°C for 15 min (annealing and extension). The Ex Taq polymerase formulation consists of a combination of thermostable DNA polymerase exhibiting a 3'-exonuclease activity (24). Polymersomes of this type have been shown to be capable of amplifying long targets with high fidelity (25, 26).

GAPDH Gene. Primers described by Robbins and McKinney (27) were used. Ex Taq was used with the following PCR conditions for 28 cycles: 98°C for 20 s and 68°C for 10 min. Because these primers can amplify the GAPDH target sequence from genomic DNA as well as from cDNA, it is necessary to exclude the presence of significant genomic DNA contamination in each sample. Such contamination, if present, would distort the quantitation of results. Therefore, control reactions for GAPDH were run in which RNA samples were input directly into the PCR mixture without prior treatment with reverse transcriptase. These controls were negative in all cases, proving that the RNA samples used in these studies did not contain significant amounts of contaminating genomic DNA.

Southern Hybridization

Southern hybridization was carried out exactly as described previously by Ermak et al. (16). The probe for the standard part of the CD44 gene was probe S1, as described by Matsumura and Tarin (23). The probes for v1 through v10 were synthesized as described above. Under the hybridization conditions used in this study, these probes exhibited specific hybridization to their target sequences.

Cloning and Sequencing of a 530-bp PCR Product

A selected 530-bp PCR product was visualized in an ethidium-stained agarose gel and excised and eluted. The DNA segment was ligated into a pCR II vector (Invitrogen, San Diego, CA). Sequencing of the insert was produced by the PCR process using an ABI automated sequencing system.

RESULTS

Confirmation That Papillary Thyroid Cancers Can Overexpress CD44 Isoforms in the Range of 750 to 1000 bp. Samples of five primary papillary thyroid carcinomas which had been studied previously (16) were selected for further analysis in the present study. Paired tissues from the contralateral thyroid lobe of each patient were also studied for comparison. In two cases (PC6 and PC8), the cancer was confined to one lobe, and the tissue from the contralateral lobe was histologically normal by light microscopy with no diagnostic abnormalities. The contralateral tissue in these cases was designated as PC6(N) and PC8(N), respectively. In the remaining cases (PC2, PC5, and PC7), there was focal involvement of the contralateral tissue with cancer. These contralateral tissues were designated as PC2(C), PC5(C), and PC7(C), respectively. The paired samples from each patient were processed in parallel under identical conditions, with extreme care to avoid cross-contamination. A sample of histologically normal thyroid tissue from a patient with no history of any thyroid disease (N2) was included as a control. A semiquantitative RT-PCR technique (16) was used using the GAPDH gene as an internal control to ensure that closely matched amounts of total cDNA were introduced into the CD44 assays. In this experiment, 32 cycles of PCR amplification were used for CD44, and 28 cycles for GAPDH. We used a new high-performance, heat-stable polymerase formulation (Ex Taq, TaKaRa) in the present experiments, whereas in the previous study (16), we used the conventional Taq polymerase. The Ex Taq enzyme formulation has the advantage of being capable of amplifying much longer target sequences with higher fidelity than Taq polymerase (Refs. 24—26; see “Materials and Methods”). The amplified CD44 PCR products were separated by electrophoresis through an agarose gel, transferred to a filter, and cross-linked. The filter containing the bound DNA species was incubated with one of the probes, washed, and subjected to autoradiography. The filter was then stripped and probed with the next probe in the series. The same filter was used to generate all of the data in this report; therefore, all of the autoradiographs shown in the figures are directly comparable.

Auroradiographs showing the specific PCR products are given in Figs. 2—4. Fig. 2A shows the results of probing the filter with the S1 probe. The first lane in this figure (MW) contains molecular weight markers. The second lane contains input RNA from the normal thyroid tissue (N2), but the reverse transcriptase enzyme was omitted during cDNA preparation. As expected, there are no signals detected in the first two lanes. The third lane is derived from the histologically normal thyroid gland (N2) and exhibits a major band near 800 bp and a band corresponding to the standard isoform at 482 bp. There is also a faint band near 700 bp, but 35 PCR cycles are required to readily demonstrate this product from sample N2, as shown previously (16).
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The possibility of cross-reactivity between the v8 and v9 probes was ruled out in control experiments in which the two probes were shown to have absolute specificity against known antisense v8 and antisense v9 target sequences (data not shown). The pattern observed for v10 is close to that of v8 and v9. The major.

Fig. 2. A, autoradiograph of various RT-PCR products after Southern hybridization with the S1 probe. Thirty-two PCR cycles were used. Lanes: MW, molecular weight markers; N2, histologically normal thyroid tissue sample; (-RT), control PCR in which reverse transcriptase was omitted from the cDNA synthesis reaction; PC, papillary carcinomas; PC (N), histologically normal tissue from the contralateral lobe of a thyroid gland harboring a papillary carcinoma; PC (C), tissue from the contralateral lobe with focal involvement with papillary cancer. Molecular sizes are shown in bp. The standard CD44 isoform RT-PCR product is present at 482 bp. B, ethidium bromide-stained gel demonstrating RT-PCR products for the housekeeping gene GAPDH, indicating that equal amounts of total cDNA were loaded in the CD44 PCR samples in A. Lanes are aligned with the corresponding lanes in A. The MW and N2(-RT) lanes are not represented.

Fig. 2B documents the internal control gene (GAPDH), showing that all PCR reactions had equal input of total cDNA.

The remaining lanes in Fig. 2A show the PCR products from the paired papillary cancers and corresponding contralateral tissues. Note that the histologically normal tissues PC8(N) and PC6(N) exhibit prominent bands at 482 bp and near 700 and 800 bp, in agreement with previous results (16). The cancers PC8, PC7, PC6, and PC5 show prominent overexpression of isoforms in the range of 750 to 1000 bp, in comparison with the histologically normal tissues PC8(N), PC6(N), and N2. In contrast, cancer PC2 does not exhibit such a marked increase in these isoforms. There is also a reduction in the intensity of the 482-bp standard isoform in cancers PC8 and PC6, compared with that of the histologically normal tissue on the contralateral side, PC8(N) and PC6(N), respectively. These results using Ex Taq are in accord with those obtained previously with Taq polymerase (16) and confirm the observation that CD44 alternate mRNA splicing can be deregulated in papillary carcinomas, with an increase in certain alternate isoforms coupled with a decrease in the standard isoform.

Of note, the contralateral tissues PC7(C), PC5(C), and PC2(C), all of which are known to contain metastatic foci of cancer, exhibit marked variability in the expression of CD44 isoforms (Fig. 2). This may be due to heterogeneity in the distribution of metastatic foci, both within each tissue specimen and between specimens, so that the particular aliquots of tissue used in the experiment contained more tumor cells in some cases, such as PC2(C), than in others, such as PC7(C) (see “Discussion”).

Papillary Thyroid Carcinomas Exhibit a Highly Restricted Repertory of Variant Exon Usage. As shown in Fig. 3, the papillary carcinomas exhibit isoforms containing alternate exons v8, v9, and v10. The pattern observed with the v8 probe is identical to that observed with v9, indicating that the expression of these two exons is tightly linked. The corresponding autoradiographs are shown. Lane designations are as given in Fig. 2.
difference results from the appearance of an isofom at 650 bp that reacts with the v8 and v9 probes but not with the v10 probe. Nearly all of the other identifiable isoforms in Fig. 3 react with each of the probes v8, v9, and v10, suggesting that these three exons are coexpressed in most cases.

Fig. 4 demonstrates that papillary carcinomas also express isoforms containing alternate exons v6 and v7. The autoradiograph shows a different distribution of isoforms for v6 and v7 than that observed for v8–v10 in Fig. 3, although a subset of the isoforms may be common to both groups. The v6 and v7 blots are nearly identical to each other, with the exception of minor differences in the relative intensity of several bands. This suggests that v6 and v7 are coexpressed in most cases.

Fig. 4 also demonstrates low intensity hybridization to the v3 probe, indicating weak expression of v3-containing mRNA. The pattern observed here is different from that seen in the v8 to v10 group or in the v6 and v7 group.

No reactivity was observed against probes v1, v2, v4, and v5 (data not shown). The v1 exon is not expected to be expressed in humans. Normal thyroid tissue and papillary cancers apparently do not use exons v2, v4, and v5 to any appreciable degree. These results imply that there are restricted patterns of alternative CD44 exon usage in papillary thyroid cancers and that there is coordinated expression of two groups of exons: v8 through v10, and v6 to v7.

**Papillary Cancers Overexpress Specific Isoforms in Coordinated Patterns.** Papillary cancers can overexpress an isofom at approximately 650 bp containing variant exons v8 and v9 that is found only in trace amounts in histologically normal thyroid tissue. This can be readily seen in Fig. 3 for cases PC8, PC7, PC6, PC5, and PC2, and also PC2(C). As noted above, this band does not react with the v10 probe. The histologically normal tissues N2, PC8(N), and PC6(N) have significantly less intense bands in this region. The cancers PC8, PC7, PC6, and PC5 also have marked overexpression of several isoforms in the range of 750 to 1000 bp that react against probes v8, v9, and v10.

Similarly, as reported previously by Ermak et al., (16), papillary cancers can overexpress an isofom at approximately 750 bp that contains exon v6. This product also contains exon v7 (Fig. 4). This mRNA isofom is seen clearly in cases PC8, PC6, and PC5, in keeping with our previous results for these cases (16). These results, taken together with those reported above, suggest that deregulation of mRNA processing in papillary thyroid cancers specifically affects only selected isoforms and that it occurs in a coordinated fashion. That is, v8 and v9 are affected as a coordinated group, as are v6 and v7. Deregulation of v10 processing is clearly linked to v8 and v9, but deregulation involving v8 and v9 can be independent of v10. Therefore, deregulation of alternative mRNA splicing in papillary cancers is a coordinated and selective process, as opposed to a generalized or random process. This suggests the hypothesis that there must exist separate groups of RNA splicing machinery with differing specificities that are independently regulated, and certain of these are deregulated in papillary cancers.

**Expression of a Novel CD44 mRNA Isoform Containing a Subsegment of Exon 4 Joined to a Subsegment of v8, Followed by v9 and v10.** In most known cases, alternative splicing within the CD44 mRNA transcript involves variant exons v1 to v10, corresponding to exons 6 through 15 (Fig. 1). Several novel splice patterns have been described involving exons 5 and 16, which have, therefore, been called v0 and v11, respectively (13). In this case, this involves a cryptic splice donor within exon 5 (28). In Fig. 3, it is evident that an isofom is present at 530 bp, which is reactive against probes v8, v9, and v10. This appears to be a paradox because such an isofom containing v8 through v10 would be expected to far exceed 530 bp in size. However, on inspection of Fig. 2, it is evident that there is no detectable signal with the S1 probe at 530 bp, indicating that at least a significant portion of the exon 4 sequence is not represented in this 530-bp isofom. In fact, an exact visual alignment of the actual
autoradiographs (data not shown) conclusively proves that the 530-bp band seen with v8-v10 is not represented on the autoradiograph obtained with the S1 probe. Therefore, we cloned and sequenced the 530-bp fragment to determine its exact composition. The results are shown in Fig. 5 and indicate that a subsegment of exon 4 has joined to an internal site within exon 13 (v8). The nucleotides within the box, ACAG, are present in both exons 4 and 13 (v8). The double-underlined C is a T in the wild-type sequence (12, 13).

### DISCUSSION

In a previous report (16), we demonstrated that alternative CD44 mRNA splicing is deregulated in papillary thyroid carcinomas and that CD44v6-containing mRNA isoforms are expressed in these tumors. In the present study, we have extended the previous observations by further characterizing the CD44 mRNA isoforms expressed in papillary thyroid cancers. Our aim was to identify CD44 isoforms that are either uniquely expressed or overexpressed in papillary thyroid cancer as opposed to histologically normal thyroid tissue. We have used a panel of papillary thyroid cancers together with the paired control tissue from the contralateral thyroid lobe. This type of control tissue was histologically normal in the remaining two cases: PC7(C), PC5(C), and PC2(C). The contralateral tissue was histologically normal in the remaining two cases, PC8(N) and PC6(N); however, we cannot rule out the possibility that these tissues contained metastatic cancer cells that eluded discovery by light microscopy.

Our data must be interpreted in view of the above caveat. Nevertheless, it is clear that there are several definite conclusions that can be reached regarding isoforms that are expressed in papillary cancers but not in histologically normal thyroid tissue: (a) it is clear that v6 and v7 are coexpressed in a tightly coupled manner in these cancers (Fig. 4); (b) a 750-bp isoform containing v6 and v7 sequences can be expressed in the papillary cancers (e.g., PC8, PC6, and PC5) but is not found in the histologically normal tissues (Fig. 4). The estimated size of this isoform is in accord with the expected size (743 bp) of a CD44 variant isoform harboring v6 and v7 sequences. From previous work, it is known that this isoform is also expressed in some adenomas and goiters (16). Thus, this 750-bp v6- and v7-containing isoform appears to be a marker for a disorganized growth state of thyroid follicular cells (16); (c) it is clear that v8 and v9 expression are tightly coupled in the papillary cancers (Fig. 3); and (d) there is an isoform at approximately 650 bp containing v8 and v9 sequences that is expressed in all five cancers but not in histologically normal tissues (Fig. 4). The specificity of this isoform for papillary cancers versus other disordered thyroid growth states is not known.

It is also possible to make several definite conclusions about overexpression and underexpression of certain isoforms: (a) it is clear that the standard 482-bp isoform is underexpressed in at least two of the cancers (PC8 and PC6) compared with the contralateral histologically normal tissue (Fig. 3). The estimated size of this isoform is in accord with the expected size (743 bp) of a CD44 variant isoform harboring v6 and v7 sequences. From previous work, it is known that this isoform is also expressed in some adenomas and goiters (16). Thus, this 750-bp v6- and v7-containing isoform appears to be a marker for a disorganized growth state of thyroid follicular cells (16); (c) it is clear that v8 and v9 expression are tightly coupled in the papillary cancers (Fig. 3); and (d) there is an isoform at approximately 650 bp containing v8 and v9 sequences that is expressed in all five cancers but not in histologically normal tissues (Fig. 4). The specificity of this isoform for papillary cancers versus other disordered thyroid growth states is not known.

It is also possible to make several definite conclusions about overexpression and underexpression of certain isoforms: (a) it is clear that the standard 482-bp isoform is underexpressed in at least two of the cancers (PC8 and PC6) compared with the contralateral histologically normal tissue (Fig. 3); (b) there are isoforms in the range of 750–1000 bp containing v8, v9, and v10 sequences that can be overexpressed in the papillary cancers (e.g., PC8, PC7, PC6, and PC5;
and goiters is not known. However, it was shown previously that the specificity of this finding for cancers versus adenomas is not identical with N2 in each of the blots, suggesting that the particular isoform (Fig. 5) that arises via an unknown mechanism in thyroid tissue differentiation. There is also a band near 480 bp seen in Fig. 3 (especially for the v10 probe) that might arise due to an unexpected splicing mechanism. Alternatively, this band might be due to nonspecific hybridization between the probe and the standard 482-bp isoform.

The contralateral tissues PC7(C), PC5(C), and PC2(C), each of which is known to contain foci of metastatic cancer, exhibit marked heterogeneity in CD44 isoform expression. PC7(C) appears nearly identical with N2 in each of the blots, suggesting that the particular fragment of tissue used in the PCR had no contaminating cancer cells, despite the fact that some microscopic foci of papillary cancer were seen in other sections of this sample. In contrast, PC2(C) exhibits features suggesting heavy contamination with cancer cells in many of the blots. These results suggest that there is significant heterogeneity in the distribution of cancer cells within these contralateral tissues, and the results obtained in any given PCR reaction will likely depend on the relative proportion of normal versus cancer cells in the specific sample.

The histologically normal contralateral tissues PC8(N) and PC6(N) were similar to each other in most of the blots but showed some striking differences from the N2 sample. The reason for the heterogeneity between the histologically normal samples is not known but could be due to contamination of PC8(N) and PC6(N) by metastasizing cancer cells that were not identified on light microscopy. It is also possible that this heterogeneity arises from differences in genetic background between patients or is due to the release of soluble factors that regulate RNA splicing from the cancers or invading immune cells.

In conclusion, the deregulated expression of CD44 isoforms in papillary cancers appears to follow certain restricted patterns. Since CD44 is involved in cell-cell and cell-matrix interactions, it is likely that specific patterns of deregulation of CD44 isoform expression are related to the characteristic biological properties of papillary thyroid cancer. We believe that the deregulation of CD44 mRNA splicing is possibly an early step in the pathogenesis of papillary thyroid cancer (16). The specific changes in tissue architecture and metastatic potential that are characteristic of this cancer may be related at least in part to the expression of the specific isoforms that are characterized in this paper. We would also expect that more aggressive thyroid cancers, such as anaplastic cancers, might exhibit different patterns of CD44 isoform expression. Further experiments are required to test this hypothesis and also to define the exact biological function of individual CD44 isoforms in normal and malignant thyroid tissues.

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