Integrins in thyroid tissue: upregulation of \( \alpha 2\beta 1 \) in anaplastic thyroid carcinoma

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Abstract

Objective: To evaluate the integrin pattern in the normal thyroid gland and in different pathological disorders including malignant tumors, because the aggressiveness of several malignant tumors correlates with alterations in the expression of one or more integrins.

Design: We examined the expression of integrins and E-cadherin immunohistochemically in a large and well-defined sample of normal and pathological human thyroid tissue.

Methods: Cryosections of 58 thyroid tissue specimens from normal tissue, thyrotoxicosis, nodular goiter, oxyphilic adenoma, follicular adenoma, follicular carcinoma, papillary carcinoma and anaplastic carcinoma, and three lymph node metastases were investigated immunohistochemically using monoclonal antibodies specifically recognizing the integrin \( \beta 1-\), \( \beta 4-\), \( \alpha 1-\), \( \alpha 2-\), \( \alpha 3-\), \( \alpha 5-\) and \( \alpha 6\)-subunits, or E-cadherin.

Results: All thyroid epithelial cells expressed integrin \( \beta 1-\) and \( \alpha 3\)-subunits. Immunostaining of the \( \beta 4\)-subunit and the \( \alpha 6\)-subunits was found only in tumors. The staining pattern in the three lymph node metastases from papillary carcinomas did not differ from that in their primaries. Anaplastic carcinomas demonstrated neoexpression of the integrin \( \alpha 2\)-subunit. E-cadherin was detected in all tissues except anaplastic carcinomas.

Conclusions: Neoexpression of \( \alpha 6\beta 4 \) was seen in most malignant tumors, whereas \( \alpha 2 \) was exclusively found in anaplastic carcinomas. In the latter, a loss of E-cadherin expression was also seen. These changes in cell adhesion molecule expression strongly suggest an association with the acquisition of proliferative and invasive properties.

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Introduction

To date, four major families of adhesion receptors have been identified: the immunoglobulin superfamily (1, 2), the cadherins (3, 4), the integrins (5–10) and the selectins (11–13). Adhesion receptors have vital roles in epithelial morphogenesis, in the maintenance of epithelial integrity and in epithelial cell migration. Changes in expression and function of these receptors occur during development and during progression of various cancers.

Integrins constitute a large family of adhesion receptors that mediate both cell–cell and cell–matrix adhesion. Structurally, integrins are heterodimeric transmembrane glycoproteins composed of non-cova-

lently associated \( \alpha \)- and \( \beta \)-subunits. At least eight \( \beta \)-subunits and 16 \( \alpha \)-subunits have been isolated, these associate in various combinations to form the 20 or so different integrins that have been described to date. Available data suggest that all nucleated cells express one or more integrins. The aggressiveness of several malignant tumors correlates with alterations in the expression of one or more integrins. In particular, decreases in the \( \alpha 2\)- and \( \alpha 3\)- or the \( \alpha 6\)- and \( \beta 4\)-subunits, or in all of them, have been consistently noted in certain kinds of tumors (14–29).

Cadherins are \( \text{Ca}^{2+}\)-dependent homophilic adhe-
sion receptors mediating cell–cell adhesion. The expression of cadherins, as detected by immunohisto-
chemistry, is reduced in many invasive carcinomas (30–32).

Thyroid neoplasms account for about 1% of all malignant tumors. Papillary carcinomas are the most common, comprising 50–70% of all thyroid malignan-
cies, followed by follicular carcinoma (10–15%) and anaplastic carcinoma (10%); medullary thyroid carci-

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In this study, we have examined the distribution of E-cadherin and integrin β1, β4, α1–3, α5 and α6 chains in a series of well-defined normal and abnormal human thyroid glands and lymph node metastases from papillary carcinoma. By using immunohistochemistry, it was possible to distinguish the pattern of expression of integrins by the epithelial tumor cells from that of the surrounding vessels.

Materials and methods

Antibodies

Monoclonal antibodies (mAbs) against the integrin β1, α1–3, α5 and α6 chains were kindly donated as follows: P4C10, anti-β1 by Dr Kurt Gehlsen (San Diego, CA, USA); TS2/7, anti-α1 by Dr Timothy Springer (Boston, MA, USA); PIB5, anti-α2, PIB5, anti-α3 and PID6, anti-α5 by Dr William Carter (Seattle, WA, USA); GOH3, anti-α6 by Dr Arnold Sonnenberg (Utrecht, Holland). All antibodies were of mouse origin except for the rat mAb, GOH3. Antibodies were purchased from Telios, San Diego, CA, USA (3E1, anti-β4), Beckton Dickinson, San José, CA, USA (PIE6, anti-α2), British Biotechnology Products Ltd, Abingdon, UK (HECD-1, anti-E-cadherin), Sigma Immunochimicals, St Louis, MO, USA (L-9393, anti-laminin), Sanbio, Arndton, UK (HECD-1, anti-E-cadherin). Sigma Immunochimicals, St Louis, MO, USA (L-9393, anti-laminin), Sanbio, Amuden, Holland (61001–1, anti-Pal-E) and Dakopatts, Glostrup, Denmark (61601, anti-vWF). An additional anti-β1 antibody (mAb 13) purchased from Beckton Dickinson was used in double immunofluorescence staining together with the anti-α2 antibody, PIE6 (see above), to demonstrate co-localisation of β1 and α2 in anaplastic carcinoma. All antibodies were mouse monoclonals except for the rabbit polyclonal L-9393. Because one of the main findings in this study was the expression of the integrin α2-subunit in anaplastic carcinoma but not in any other thyroid tissue investigated, we chose to use two different anti-α2 antibodies to ensure that our data were valid.

Human tissue samples

Tissue specimens from 58 normal and abnormal glands and three lymph node metastases from papillary carcinomas from a total of 52 patients were included in the present study. All the specimens were obtained at surgery at the Karolinska Hospital, Stockholm, Sweden. The tissue specimens were fixed immediately in 10% buffered formalin for 18–24 h or snap frozen in liquid nitrogen and stored at −70°C until required for staining. Paraflin sections 4 μm thick and cryosections 6 μm thick were cut and attached to glass slides. Routine histopathological diagnosis was performed in hematoxylin–eosin stained deparaffinized sections according to the criteria of the WHO histological classification of thyroid tumors (34); 13 specimens showed normal histology; seven of these were taken from normal glandular tissue adjacent to adenomas (three) or papillary carcinomas (four). Seven specimens showed thyrotoxicosis, seven nodular goiter, four oxyphilic adenoma, six follicular adenoma, seven follicular carcinoma, ten papillary carcinoma and four anaplastic carcinoma.

Immunohistochemistry

Cryosections 6 μm thick were fixed in ice-cold acetone for 10 min and then stained with hematoxylin–eosin to confirm that they were representative of the histopathological diagnosis or immunostained to investigate expression of integrin subunits or E-cadherin. The peroxidase–anti-peroxidase method was used for immunostaining with 3-amin-9-ethylcarbazole as chromogen, followed by a short counterstaining in hematoxylin. In addition, immunostaining with antibodies to laminin was used to demonstrate basement membranes, and antibodies to PAL-E and Factor VIII were used to get an overview of the distribution of blood vessels. The cryosections were pretreated with 0.3% hydrogen peroxide in phosphate buffer for 15 min and then incubated in normal rabbit serum (for mAbs) or normal swine serum (for polyclonal antibodies, pAbs) for 5 min at room temperature. The primary mouse mAbs were applied overnight at +4°C. After washing in PBS, rabbit anti-mouse conjugate (for mAbs) or swine anti-rabbit conjugate (for pAbs) was applied for 30 min at room temperature, followed by peroxidase–anti-peroxidase conjugate (Dakopatts) for 30 min at room temperature. Every tumor was stained several times with the same antibodies, and showed the same results. Negative controls were obtained by omitting the primary antibody or replacing it with non-immune sera. Sections from human skin and colon were used as positive controls.

Immunofluorescence

Double immunofluorescence staining against the β1- and α2-subunits was performed with a technique that enables the sequential use of two mouse mAbs as primary antibodies. After being blocked with avidin and biotin for 15 min each, sections were incubated with normal horse serum for 5 min. Cryosections were then incubated with the first primary antibody (PIE6, anti-α2) for 30 min, rinsed and incubated with biotinylated horse anti-mouse IgG diluted 1:200 for 30 min. Sections were rinsed and incubated with Texas red avidin diluted 1:100 for 30 min, rinsed again and blocked with normal rabbit serum for 5 min. The sections were then incubated with the second primary antibody (mAb 13, anti-β1) for 30 min, rinsed and incubated with rabbit anti-mouse IgG fluorescein isothiocyanate (FITC) conjugate diluted 1:20 for 30 min. The mounting medium was fluorescein. The same technique was used for staining against the...
β4- and α6-subunits. The antibody against the α6-subunit (GOH3) was used as the first primary antibody and the antibody 3E1 (anti-β4) was used as the second primary antibody. In this case the sections were then investigated with an image analyzer in order to establish whether β4 and α6 were co-localized.

Double immunofluorescence used one mouse mAb and one rabbit pAb as primary antibodies. The cryosections (6 μm) were incubated in normal rabbit serum for 10 min at room temperature. The first primary antibody (3E1, anti-β4) was then repeated, but using normal swine serum, another primary antibody (L-9393, anti-laminin) and a swine anti-rabbit rhodamine-conjugated secondary antibody. Sections were examined by Zeiss 410 confocal laser scanning microscopy and microphotographs were taken in RGB-mode, simultaneously showing laminin in the red channel, β4 in the green channel and co-localized signals as white.

Table 1 Expression of β1, β4, α2, α3, α6 and E-cadherin (Ec) in human thyroid epithelium. No expression of α1 or α5 was seen in any of the patients.

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† The anti-α2-antibody PIH5 was routinely used and the results are shown here.

± α6 immunostaining co-localized with the β4-subunit could be detected with double immunofluorescence staining and examination by image analyzer. Immunostaining scored as follows: (+) very weak, ++ weak, ++ moderate, +++ strong, ++++/+, staining varied within the section from ++ to +; —, no immunostaining; 0, section not representative.

N, normal histology; TT, thyrotoxicosis; NG, nodular goiter; OA, oxyphilic adenoma; FA, follicular adenoma; FC, follicular carcinoma; PC, papillary carcinoma; AC, anaplastic carcinoma.

met, metastasis.
Results

Expression of integrins in human thyroid follicular cells

The epithelial cells in all thyroid tissue specimens examined, with a few exceptions, displayed a basally localized \( \beta_1 \) immunostaining (Table 1). In normal thyroid glands and in benign lesions, the immunostaining for the \( \beta_1 \) integrin was, in most cases, stronger in columnar than in cuboidal cells. In adenomas, the immunostaining was predominantly seen in areas with preserved follicular arrangement, but was less strong in more solid areas. In the differentiated carcinomas, immunostaining appeared focally. Tumor cells from anaplastic carcinomas showed immunoreactive cytoplasm.

Positive \( \beta_4 \) immunostaining was present only in tumors and was localized basally in the neoplastic cells, for example against the fibrovascular stroma (Fig. 1b–d). This immunoreactivity, however, did not show a linear distribution and was often interrupted. In the anaplastic carcinomas, the immunostaining was seen along the border of the tumor cells and also in the cytoplasm of some neoplastic cells (Fig. 1d).

In normal, hyperfunctioning and goitrous glands, a basal \( \alpha_3 \) immunostaining of the epithelial cells was seen. In columnar epithelium from hyperplastic and goitrous glands, additional immunostaining between the cells was detected. Adenomas and differentiated tumors also displayed immunoreactivity at their lateral (intercellular) cell border. In anaplastic carcinomas, the immunostaining appeared around the tumor cells.

Immunostaining of the \( \alpha_2 \)-subunit was seen exclusively in anaplastic carcinoma (Fig. 1g) and was detected with two different anti-\( \alpha_2 \) antibodies (PIH5 and PIE6). The anti-\( \alpha_2 \) antibody, PH15, was routinely used (Table 1), whereas PIE6 was used on two representative sections from each diagnostic group. Both antibodies gave the same result: positive immunostaining could be detected only in tumor cells from anaplastic carcinoma. Immunostaining with PIE6 gave a somewhat stronger immunostaining than PH15. The \( \alpha_2 \) immunostaining was seen between the cells, whereas the \( \alpha_6 \) immunostaining appeared along the tumor cell surfaces facing the matrix; however, some tumor cells also showed cytoplasmatic staining.

The integrin \( \alpha_1 \)- and \( \alpha_5 \)-subunits were not detected immunohistochemically in epithelia of any of the thyroid tissues investigated.

E-cadherin displayed a strong staining of the lateral cell borders in all tissues examined, except in anaplastic carcinomas, in which the staining was weak and in some specimens absent. In tissues from oxyphilic...
adenomas, the immunostaining showed a similar pattern, but varied from very strong to negative.

The three lymph node metastases investigated displayed the same pattern of staining as the primary tumor of the papillary carcinomas (Fig. 1b,c).

**Human thyroid tissue capillaries**

The results are summarized in Table 2.

Immunostaining with β1-, β4- and α1-subunits was seen both in small capillaries localized between the follicles and in large capillaries in the interlobular septa (Fig. 1a–c). In addition, positive staining for α3- and α6-subunits was seen in capillaries of the interlobular and interfollicular types respectively.

A weak, but focal staining of the α2-subunit (PIH5) was seen in some abnormal glands of all diagnostic categories. In all normal, and in the majority of the abnormal glands, α2 immunostaining with the anti-α2 antibody, PIH5, was lacking. The other anti-α2 antibody, PIE6, however, gave moderate to strong staining in capillaries throughout (Fig. 1e,f).

The immunostaining pattern of vessels in three lymph node metastases from papillary carcinomas did not differ from that of the corresponding primary tumor, except for a greater variation in the staining intensity in the primary tumors (Fig. 1b,c). In vessels of the metastases, positive staining for α2 seemed to be more frequent compared with that in the primary tumor.

**Immunofluorescence**

Using double immunofluorescence staining, we were able to demonstrate co-localisation of the integrin β1- and α2-subunits in anaplastic carcinoma (Fig. 2). The staining pattern for the neoexpressed integrin α2-subunit in anaplastic tumour cells closely resembled that of the integrin β1-subunit, suggesting that these cells express α2β1.

With the use of double immunofluorescence staining, together with image analysis, it was evident that the basal staining of β4 found in some of the differentiated carcinomas was co-localized with α6 (data not shown).

Confocal laser scanning microscopy was used to analyze anaplastic carcinoma double stained with antibodies against the integrin β4-subunit and laminin, using the indirect immunofluorescence technique. Immunostaining with the anti-β4 antibody (pseudocolored green) was partly co-localized with staining from the anti-laminin antibody (pseudocolored red) at the border between tumor cells and the surrounding matrix; this co-localization gave a white signal (Fig. 1h).

**Discussion**

The availability of specific monoclonal antibodies against individual integrin subunits has allowed investigation of the localization of integrins in tissues and changes in their expression during development and in specific disease conditions (14–21, 34–46). We have now presented data on integrin expression in a large and well-defined sample of normal and diseased human thyroid glands. In agreement with what has been reported for most epithelial cells, we found that thyroid follicle cells expressed the integrin β1- and α3-subunits. Among thyroid follicle cells studied previously using colony formation, subpopulations of cells were found also to express α1-, α5- and α6-subunits (47). By using immunohistochemistry, we could clearly distinguish

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**Table 2 Integrin expression in capillaries of interlobular (a) and interfollicular (b) types.**

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For scoring and explanation of abbreviations, see Table 1.
those integrins expressed by normal and tumor cells from those seen in the surrounding vessels, and found that the α1- and α6-subunits were expressed by the capillaries. One major finding of our study was that the integrin α2-subunit was detected only in tumor cells in anaplastic carcinoma and not in follicle or tumor cells of any of the other thyroid tissues investigated. However, α2 was expressed in capillaries in most tissues investigated. We also detected an upregulation of the integrin α2-subunit in capillaries of adenomas and carcinomas. The fact that, in contrast to other types of carcinomas, tumor cells in anaplastic thyroid carcinomas display an upregulation of the integrin α2β1, suggests that this integrin has diverse functions that are of importance for the biology of a highly malignant tumor. In contrast to the present findings, it has previously been reported (48) that the integrin α2-subunit was expressed by tumor cells in both adenomas and carcinomas of the thyroid gland. It could be speculated that part of the α2 signal recorded in the previous studies (47, 48) reflected the presence of α2-positive vascular cells, and not glandular epithelial cells. In the present study we used an immunohistochemical staining technique with two different integrin anti-α2 antibodies. Both of these detected α2 in epidermis of normal skin and in colonic epithelium (data not shown). Serini et al. (49) reported a lack of α2 expression in thyroid tissue, but they used an α2 antibody distinct from those we used and did not report the use of positive controls. Neoexpression of the integrin α2-subunit in normal thyrocytes cultured as monolayers has also been reported (50) and, furthermore, the increase in α2 preceded cell proliferation and its expression was downregulated when cell cultures reached confluence, that is, when cell–cell contacts had re-established. Thus it seems reasonable to suggest that the integrin α2β1 is upregulated on thyrocytes when these cells proliferate and/or migrate. Such a hypothesis seems likely in the light of the upregulation of α2 in anaplastic thyroid carcinoma that we have reported here.

Several studies have indicated that tumor cell variants expressing the collagen–laminin receptor α2β1 are more tumorigenic, invasive or metastatic than cell variants that do not express this integrin (51–54). This is exemplified by melanoma cells, in which the upregulation of α2β1 coincides with more aggressive behavior and the ability to reorganize collagen matrices (55). In contrast, decreases in the α2- and α3- or α6- and β4-subunits, or all of them, have been consistently noted in many types of carcinomas. This pattern is exemplified in carcinoma of the breast (15, 19, 22–24), lung (16), colon (14, 17, 18, 25), kidney (20, 21), pancreas (26), bladder (27), basal cell carcinoma of the skin (28) and oral squamous cell carcinoma (29). Re-expression of the α2β1 integrin in a poorly differentiated mammary carcinoma resulted in an alteration in phenotype from a fibroblastoid, invasive cell to an epithelioid, less motile and less invasive type of cell (56). Expression of α2β1 is reduced or lost in most invasive (Duke’s stage C/D) human colon carcinomas (14, 17, 18, 25). Furthermore, in colon carcinoma, the loss of continuity of α2β1 and α3β1 expression at the basolateral surface of tumor glands correlates with tumor malignancy (42). In the present study, we have demonstrated that expression of α2β1 in thyroid carcinoma correlated positively with malignancy. Papillary and follicular carcinomas grow unencapsulated in papillary structures of columnar epithelial cells or encapsulated in follicle arrangements respectively. Anaplastic thyroid cancers, however, display a totally dedifferentiated growth pattern, extending widely into the gland and its surrounding tissues and forming distant metastases at an early stage of the disease. The expression of α2β1 by tumor cells in all four investigated cases of anaplastic carcinomas is thus in closer agreement with those experimental studies that showed that this integrin would favour tumour invasiveness (51–54).

The α6β4 integrin is selectively present in hemidesmosomes of several epithelia, where it anchors the intermediate filament system to the basement membrane (57–60). To our knowledge, there are no reports on hemidesmosomes in epithelia of the normal thyroid gland, and the present data support this, as α6β4 integrin was not detected in acinar epithelia. However, α6β4 was detected in capillaries of the interfollicular type (Table 2) and in epidermis and in colonic epithelium (data not shown), using the same reagents and staining methods. Apart from being expressed in blood vessels, the α6- and β4-subunits were also detected in most of the well-differentiated tumors, and in all the anaplastic carcinomas. The neoexpression of α6β4 in most of the carcinomas investigated is consistent with previously published data on integrin expression in thyroid tumors (49). The biological significance of the observed expression of α6- and β4-subunits remains unknown. In many carcinomas, such as oral squamous carcinoma (29, 61) and basal cell carcinoma of the skin (62–64), and in prostate carcinoma (65, 66) expression of α6β4 is reduced or lost, whereas it is enhanced in the neoplastic progression of cervical epithelia (67) and squamous cell carcinoma of the skin (62–64). In some types of tumor, a decrease in expression and polarization of α6β4 coincides with a decrease in basement membrane components, as has been observed in carcinoma of the breast (22) and bladder (27), and oral squamous cell carcinoma (29). The distribution of both integrin α6- and β4-subunits at the margins of tumor cell masses in anaplastic carcinomas demonstrated in the present study suggests the presence of basement membrane components at these sites. Using double immunofluorescence staining, we were able to demonstrate at these sites a presence of laminin that coincided mainly with the integrin β4-subunit in anaplastic carcinomas. It is noteworthy that anaplastic carcinoma, being the most
dedifferentiated thyroid tumor, showed neoexpression of α6β4, which is an integrin usually associated with a polarized cell structure.

Little is known concerning changes in integrin expression in metastases compared with that in their primary tumors. It is possible that such changes reflect a change in phenotype as tumor cells acquire the ability to invade and metastasize. The change of environment may also affect the expression of integrins. It is also possible that the presence of a particular set of integrins gives an indication of the metastatic potential of a tumor cell. A study of changes in the expression of α6β4 in primary and metastatic breast cancer showed that α6β4 was maintained in lymph node metastasis, but was less frequent in extranodal metastatic foci such as the pleural cavity and parenchymal tissues (22). This is consistent with our data, as no change in the expression of a set of integrins was observed in metastases from lymph nodes compared with that in the primary tumor (papillary carcinoma) in the thyroid gland.

The anaplastic carcinomas, but not the other types of thyroid tumor investigated, displayed a loss of detectable expression of E-cadherins. Such a loss has been detected in many types of cancers and in many cases does correlate with the incidence of metastasis (reviewed by Takeichi (32)). E-cadherin expression also correlates with invasiveness as measured in experimental in vitro systems. In colonic epithelium, involvement of E-cadherin-containing cell–cell junctions has also been indirectly implicated in the regulation of cell proliferation (68). These data are thus in good agreement with the biological behavior of anaplastic carcinomas—namely, their marked invasive and proliferative potential.

In the present study we have shown that, among a series of well-defined thyroid cancers, anaplastic carcinoma segregates with regard to tumor cell expression of certain adhesion receptors. Anaplastic carcinomas are also the most malignant of the thyroid tumors investigated. Expression of the integrin α2-β4 and α6-β4 subunits is in contrast with findings in other carcinomas, but in good agreement with previously reported roles of these adhesion receptors in experimental tumor models and the in vitro behavior of tumor cells.

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