Reduced Expression of Interleukin 6 in Undifferentiated Thyroid Carcinoma: In Vitro and in Vivo Studies

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ABSTRACT
Cytokines appear to play an important role in the development and progression of epithelial tumors. Cultured normal human thyroid follicular cells constitutively release high levels of interleukin-6 (IL-6) and IL-8, together with low to moderate levels of transforming growth factor-α (TGF-α) and TGF-β. IL-6 appears to play multiple functions in thyroid physiology and disease. Because certain data indicate an inverse relationship between IL-6 production and epithelial tumor aggressiveness, we used both tissue culture methods and histochemical techniques to search for possible alterations of cytokine expression in thyroid carcinomas. As compared to cultures from normal tissue and well-differentiated carcinoma, production of IL-6 was strongly down-regulated in cultures derived from undifferentiated carcinoma. In contrast, levels of IL-8, TGF-α, and TGF-β produced by neoplastic TFC were similar to those produced by normal cells. Actually, production of TGF-α was slightly enhanced in cultures from well-differentiated carcinoma. Immunoassay results were confirmed by reverse transcriptase-PCR analysis. Immunohistochemistry of human thyroid carcinomas (n = 99) and normal thyroid tissue (n = 85) showed that immunoreactive IL-6 was strongly diminished in undifferentiated forms (n = 34) and slightly reduced in well-differentiated carcinoma (n = 65). In agreement with the in vitro results, TGF-α expression was significantly increased in neoplastic thyrocytes, as compared to their normal counterpart. The results indicate that, as in the mammary and salivary glands, down-regulation of IL-6 expression may represent a marker of undifferentiated thyroid carcinoma.

INTRODUCTION
Normal human mammary epithelial cells constitutively produce IL-61 and IL-8 (1, 2). Transformation of these cells by several different genes (e.g., c-Ha-ras, c-erb-B2, and SV40 T large antigen) is associated with loss of IL-6 production and responsiveness (3, 4). Immunohistochemical studies showed that, as compared to normal tissue and to in situ lesions, the in vivo expression of immunoreactive IL-6 is significantly reduced in invasive mammary carcinoma (5). We also observed that, in neoplastic mammary cells, the in vitro response to IL-6 was greatly diminished. Similar but less pronounced alterations were found in the apparently normal breast tissue adjacent to carcinoma (5). Taken together, these data suggested that alterations of IL-6 pathways are frequently found in mammary neoplasia.

IL-6 is mainly a multifunctional regulator of the immune response, hematopoiesis, megakaryocyte maturation, and acute phase reactions (6). A few data indicate that IL-6 can also promote differentiation of cells that are not derived from the hemolymphopoietic system, e.g., regulation of gene expression in hepatocytes (7), conversion of pheochromocytoma cells and PC12 cells into neuron-like cells (8, 9), induction of alkaline phosphatase expression in a lung carcinoma cell line (10), and enhancement of corneal cell adhesion and migration (11). Although it is well established that IL-6 deregulation is involved in a variety of diseases, including lymphatic malignancies (12), there is little information on its role in solid tumors (5, 13–15).

Growth and functions of epithelial TFCs are positively regulated by TSH, EGF, TGF-α, and insulin-like growth factors. TGF-β has an inhibitory role (16). Some of the above regulators are directly produced by thyrocytes and may have an autocrine function. In fact, cultured TFCs constitutively express several different cytokines, including IL-1α, IL-6, and IL-8 (17).

Although some information is available on the role of cytokines in both thyroid physiology and autoimmune disease (18), data on the possible relationship between cytokines and thyroid neoplasia are extremely scarce (19, 20).

Different types of epithelial cells have been shown to produce IL-6. Among them, cells from the mammary and salivary glands (1, 2, 15), retinal pigment cells (21), and renal tubular cells. Studies of salivary and mammary cancers suggest an association between increased tumorigenicity and loss of IL-6 expression (2, 5, 15). Pilot experiments with cultured TFCs prompted us to investigate if alterations of cytokine pathways could be detected also in a series of thyroid carcinomas. Immu-

1 The abbreviations used are: IL, interleukin; TFC, thyroid follicular cell; TSH, thyroid-stimulating hormone; EGF, epidermal growth factor; TGF, transforming growth factor; WDC, well-differentiated carcinoma; UC, undifferentiated carcinoma; IUDR, iodo-deoxyuridine; RT-PCR, reverse transcriptase-PCR; IL-6R, IL-6 receptor.

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2 This work was supported by the Italian Association for Cancer Research (Milan, Italy) and by Istituto Superiore di Sanità (Rome, Italy) Grant 9405-12.

3 G. Conaldi, unpublished observations.
nohistochemical studies have been carried out in parallel with in vitro investigations of TFC cultures prepared from both non-neoplastic and neoplastic thyroid tissue. This approach allowed us to compare the in vivo findings with the results obtained by studying cytokine release in pure TFC cultures.

MATERIALS AND METHODS

Thyroid Tissue Collection. Thyroid tissue was obtained for diagnosis at the Institute of Pathology, University of Pisa, (Pisa, Italy). Primary cultures were obtained from cancer patients undergoing total thyroidectomy. Unaffected (normal) thyroid tissue was obtained from the lobe opposite to the neoplastic one (five cases). Thirty-nine tissue samples of thyroid carcinoma were derived from previously untreated patients. This group included 32 WDCs (papillary histotype, 29 cases; follicular histotype, 3 cases) and 7 UCs. Immunohistochemical studies were performed on archival material that included 85 samples of normal tissue, 65 cases of WDC, and 34 cases of UC.

Primary Cultures of Normal and Neoplastic Thyroid Tissue. Unless otherwise specified, chemicals, media, and serum were from Sigma Chemical Co. (St. Louis, MO), whereas tissue culture plasticware was from Costar (Cambridge, MA). Sterile samples of thyroid tissue were obtained at surgery and immediately subjected to mechanic and collagenase (CLSII, 150 units/ml; Worthington Biochemical Co., Freehold, NJ) dissociation. Cell aggregates and isolated cells were cultured in DMEM supplemented with 10% fetal bovine serum (low-endotoxin type) but no exogenous hormones/growth factors (22). Primary cultures were used for experiments between 30 and 50 days after plating, i.e., at a time when contaminant nonepithelial cells were <5%, as judged by morphology and immunocytochemistry. Cultures from “normal” thyroid tissue and the undifferentiated ARO human thyroid carcinoma cell line were used as controls.

For characterization, samples of each culture were plated in chamber slides, acetone/methanol-fixed and stained by indirect immunofluorescence with the following antibodies: thyroglobulin (murine clone TG03; Cis Bio International, Paris, France); calcitonin (rabbit polyclonal; Dako Ltd., Bucks, United Kingdom); pancytokeratin (murine clone MNF 116; Sigma); chromogranin (murine clone A-3; Dako); human leukocytes and human granulocyte/monocyte (murine clones MB1 and CLB302; ICN Pharmaceuticals, Costa Mesa, CA).

Matrigel Colonization Assay. As previously described (23), 500 μl of liquid Matrigel (10 mg/ml) was placed in 16-mm wells of 24-well plates and polymerized at 37°C for 30 min. Cells (5 x 10⁵) in complete DMEM were seeded in each well on the top of the gel and incubated at 37°C with 5% CO₂. Cultures were examined daily and photographed after 7–10 days.

Immunohistochemistry. After formalin fixation, processing, and paraffin embedding, 3–5-μm-thick sections were prepared. The following antibodies, capable of staining paraffin-embedded sections, were used: IL-6 (rabbit polyclonal antibody and mouse IgG1 monoclonal antibody, LP-716 and 1618-01; Genzyme, Cambridge, MA); TGF-α mouse IgG1 monoclonal antibody (Oncogene Science, Manhasset, NY). As reported (5), sections were incubated with primary antibody and stained with the avidin-biotin immunoperoxidase complex technique (Vector Laboratories, Burlingame, CA). Sections were scored independently by two pathologists (F. B. and L. P.) using the following method: the intensity of staining for each antigen was scored from 0 to 3 (0, absent; 1, weak; 2, moderate and 3 strong); the proportion of malignant cells positively stained was scored from 0 to 4 (0, no positive cells; 1, <10% positive cells; 2, 11–50%; 3, 51–75%; and 4, 76–100%). The two scores were then added to yield the total score (0–7). Immunoreactivity was defined as negative when the score was 0, weak when it was 1–3, and strong when it was ≥4.

Measurement of Cytokine Levels and IL-6 Responsiveness. Conditioned medium of primary cultures in T25 flasks from either normal or neoplastic thyroid tissue was used to measure the release of IL-4, IL-6, IL-8, TGF-α, and TGF-B1. Immunoenzyme dosage kits were from: R&D Systems, Minneapolis, MN (IL-4, IL-6, and IL-8), Oncogene Science (TGF-α), and Genzyme (TGF-B1). Medium samples were taken at 3, 6, and 9 days from plating; determinations were made in quadruplicate. Reported data refer to day 6.

The responsiveness of cultured TFCs to IL-6 was studied under serum-free conditions, as reported previously (4). Recombinant human IL-6 (Genzyme) and IL-6-neutralizing monoclonal antibody (anti-IL-6, clone 8; Collaborative Research, Becton-Dickinson, Milan, Italy) were used. DNA synthesis was measured as incorporation of [³²P]-UdR (Amersham, Milan, Italy) 4 days after stimulation with IL-6 (10 and 100 ng/ml) or treatment with IL-6-neutralizing antibody (50 μg/ml).

Analysis of Cytokine Transcripts. Cytokine-specific mRNAs were detected as described (23) in total RNA extracted from 10⁶ cells by the guanidinium thiocyanate method. After treatment with RNase-free DNase for 1 h at 37°C, cDNA was produced with Moloney murine leukemia virus reverse transcriptase in conjunction with random hexamer primers (Clontech, Palo Alto, CA). cDNA was then amplified by PCR using Taq polymerase and cytokine-specific primer pairs (Cytokine MAPping Amplifiers; Clontech). Amplification was carried out for the following human transcripts: IL-4 (amplified product, 344 bp), IL-6 (amplified product, 628 bp), IL-6-receptor-α (amplified product, 251 bp), IL-8 (amplified product, 289 bp), TGF-α (amplified product, 297 bp), TGF-β (amplified product, 161 bp). As a control reaction, RT-PCR of glyceraldehyde-3-phosphate dehydrogenase mRNA (amplified product, 983 bp) was carried out with all samples. For detecting IL-6 transcripts, the following primers were used (25): downstream primer, 5'-GAAGAGCCCTCAGGCTTACTG-3'; upstream primer, 5'-ATGAACCTCCTCTCCAAAGGCGC-3'. Amplification products were separated by electrophoresis on 2.5% agarose gels and visualized under UV light by staining with 0.5 mg/ml ethidium bromide. PK174/HaeIII digest (72–1353 bp) was used as a size marker.

RESULTS

Characterization of Cultured Cells. Primary cultures from 5 samples of normal thyroid tissue and 39 thyroid carcinomas of different histotypes (32 WDCs and 7 UCs) were studied. By day 10–15 postplating, TFC monolayers reached confluence. Primary cultures were analyzed between 30 and 50 days after plating, i.e., at a time when contaminant nonepithelial cells were not detected by immunostaining. As shown in Fig. 1,
primary cultures from normal and neoplastic tissue had similar morphology: TFCs were oval or spindle-shaped, without any significant difference between normal and neoplastic tissue. By immunocytochemistry, over 80% of cells from normal tissue and WDCs were thyroglobulin positive, and over 95% were positive for cytokeratin. All cells were calcitonin and chromogranin negative. In cultures derived from UC, only a few cells were stained by thyroglobulin antibody, but all cells had an epithelium-like morphology. The ability of TFCs to form colonies on reconstituted basement membrane was also evaluated. The morphological appearance of cells cultured in Matrigel for 5 days is shown in Fig. 2. Normal TFCs remained as single cells or small clusters in Matrigel (Fig. 2A), whereas cells obtained from WDCs and UCs formed either large colonies or branching structures (Fig. 2, B–D). These results are in agreement with observations of Bond et al. (26).

Cytokine Production by Cultured Epithelial Thyroid Cells and Response to IL-6. Table I shows the levels of IL-4, IL-6, IL-8, TGF-α, and TGF-β1 in the supernatant of cultures derived from both normal and neoplastic thyroid tissue. IL-4 was not produced by TFCs. Primary cultures from both normal tissue and WDC released high amounts of IL-6 and IL-8, together with very low levels of TGF-α (i.e., <100 pg/ml) and medium amounts of TGF-β1. As compared to normal cells, IL-6 production was significantly reduced in cells from UC (P < 0.001). The undifferentiated ARO cell line did not release IL-6. Production of TGF-α and TGF-β1 was not significantly different in cells from either normal or neoplastic tissue. The above results were confirmed by analysis of cytokine transcripts in cultured cells. Fig. 3 shows representative results of RT-PCR analysis of cytokine transcripts. IL-6 mRNA was expressed in 5 of 5 normal cultures, 7 of 11 cultures from WDC, and 1 of 6 cultures from UC. ARO cells failed to express IL-6 mRNA. Comparable expression of IL-8, TGF-α, and TGF-β mRNA was found in cultures from both normal and neoplastic tissues. With the exception of TGF-β, the ARO cell line showed a reduced expression of cytokine transcripts. Transcripts for IL-4 and IL-6-Rx were not detected in any of the thyroid cultures examined (data not shown).

DNA synthesis in TFCs was analyzed, under serum-free conditions, as [125I]IUDR incorporation after treatment with either exogenous IL-6 (10 and 100 ng/ml) or high amounts of IL-6-neutralizing antibody (10 and 50 µg/ml). As compared to serum-free medium, no significant modulation of DNA synthe-
Table 1  Release of IL-4, IL-6, IL-8, TGF-α, and TGF-β in cultures of epithelial thyroid cells from normal and neoplastic tissue (pg/ml)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Normal (n = 5)</th>
<th>WDC (n = 32)</th>
<th>UC (n = 7)</th>
<th>ARO cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td></td>
<td>&gt;2100</td>
<td>433 ± 84</td>
<td></td>
</tr>
<tr>
<td>IL-6*</td>
<td></td>
<td>&gt;2100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>3600 ± 1800</td>
<td>4600 ± 2100</td>
<td>6100 ± 1050</td>
<td>480</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>650 ± 200</td>
<td>500 ± 200</td>
<td>455 ± 7</td>
<td>1730</td>
</tr>
<tr>
<td>TGF-α</td>
<td>45 ± 18</td>
<td>96 ± 64</td>
<td>20 ± 3</td>
<td>430</td>
</tr>
</tbody>
</table>

*IL-6 group: normal tissue vs. WDC, not significant; normal tissue vs. UC, P < 0.001. (No significant differences were found among levels of IL-8, TGF-α, or TGF-β1 secreted by cultures of normal tissue, WDC, or UC.)

Fig. 3  RT-PCR amplification of total cell RNA extracted from pure cultures of thyroid follicular cells. Lane 1, molecular size markers. Cultures were obtained from: normal tissue (Lanes 2–4), WDC (Lanes 5–7), UC (Lanes 8–10), and undifferentiated thyroid ARO cell line used as control (Lane 11). Lane 12, positive control. PCR was carried out with primers specific for IL-4, IL-6, IL-8, TGF-α, TGF-β, and glyceraldehyde-3-phosphate dehydrogenase. Ethidium bromide-stained agarose gel.

The role of cytokines in cancer is the subject of intense investigation. As seen in other systems, proteins of the TGF-β family have a potent antiproliferative effect on TFC growth (27). TFCs from multinodular goiter were shown to be frequently unresponsive to the growth-inhibiting activity of TGF-β (28), and it has been proposed that escape from TGF-β control may favor growth of thyroid tumors (29). Besides TGF-β, other cytokines/growth factors appear to act on thyrocytes as regulators of cell growth, differentiation, and hormone production. IL-1 has been shown to stimulate proliferation of the human thyroid cell line NIM 1 (30) and to enhance the release of both IL-6 and IL-8 by the human thyroid cell line Htori3 (17). IFN-γ reduced significantly both insulin- and TSH-stimulated growth of human fetal TFC, as well as thyroglobulin accumulation (31). IL-8 is constitutively produced by TFCs (32), but so far, no evidence has been produced for a direct effect of this cytokine on thyrocytes (17). Because IL-8 release is up-regulated by IL-1, an abundant cytokine in the inflammatory infiltrate, IL-8 might play a role in the induction and maintenance of autoimmune thyroid disease.

The differentiated thyroid cell lines WRO and NPA were shown to produce mesenchymal growth factors (basic fibroblast growth factor or platelet-derived growth factor-b) that did not appear to act in an autocrine manner but rather by stimulating stromal cells (33). Recent immunocytochemical studies indicated that TGF-α is expressed in the cytoplasm of normal and neoplastic human thyrocytes and that EGF-R is expressed on the surface of TFCs (34). Experiments in vitro also showed that exogenous TGF-α stimulates growth and invasivity of human thyroid cancer cells (35). Growth stimulation has been confirmed in cultured porcine TFCs, and in this model, TGF-α appeared also to inhibit the TSH-induced iodide uptake (36).

As is IL-8, even IL-6 is constitutively produced by TFCs (17, 37) and appears to influence differentiated thyrocyte functions (18). In cultures of human TFCs, IL-6 inhibits TSH-

Thus, IL-6 appeared to have neither an autocrine nor a paracrine effect on pure TFC cultures.

Immunohistochemical Studies. Sections of normal and neoplastic thyroid tissue were studied for immunoreactivity with antibodies to IL-6 and TGF-α. Eighty-five samples of normal thyroid tissue, 65 cases of WDC, and 34 cases of UC were evaluated (Table 2). As compared to normal tissue, IL-6 reactivity was slightly diminished in WDC (P < 0.001) and severely reduced in undifferentiated histotypes (P < 0.001). IL-6 anti-bodies produced a defined cytoplasmic staining in WDC but failed to stain UC (Fig. 4). In our hands, normal thyroid tissue was not stained by antibody to TGF-α. Expression of immunoreactive TGF-α was, however, evident in most cases of WDC and UC (P < 0.001; Table 2). No significant differences of TGF-α expression were noticed between WDC and UC forms.
Table 2  IL-6 and TGF-α immunoreactivity in normal and neoplastic thyroid tissues

Statistical analysis was performed by $x^2$-test with Yates' correction.

<table>
<thead>
<tr>
<th></th>
<th>Normal tissue, no. of cases (%)</th>
<th>WDC, no. of cases (%)</th>
<th>UC, no. of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of cases</td>
<td>85</td>
<td>65</td>
<td>34</td>
</tr>
<tr>
<td>IL-6 immunoreactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1 (1)</td>
<td>40 (62)</td>
<td>32 (94)</td>
</tr>
<tr>
<td>Weak</td>
<td>78 (92)</td>
<td>14 (21)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Strong</td>
<td>6 (7)</td>
<td>11 (17)</td>
<td></td>
</tr>
<tr>
<td>TGF-α immunoreactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>83 (98)</td>
<td>17 (26)</td>
<td>12 (35)</td>
</tr>
<tr>
<td>Weak</td>
<td>1 (1)</td>
<td>10 (16)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Strong</td>
<td>1 (1)</td>
<td>38 (58)</td>
<td>19 (56)</td>
</tr>
</tbody>
</table>

* IL-6 group: normal tissue vs. WDC, $P < 0.001$; normal tissue vs. UC, $P < 0.001$.
* TGF-α group: normal tissue vs. WDC, $P < 0.001$; normal tissue vs. UC, $P < 0.001$.

induced peroxidase gene expression and reduces T3 secretion (38), a finding confirmed in rats (39). In contrast, production of thyroglobulin and DNA synthesis were not influenced by exogenous IL-6 (40). Here, transcripts relative to the α-chain of IL-6-R could not be detected by RT-PCR in either normal or neoplastic thyrocytes. This finding would imply that IL-6 cannot act in an autocrine way on thyrocytes. However, several tissues that do not produce IL6-Rα are known to express an incomplete IL6-R consisting only of the β chain (IL6-Rβ; Ref. 41). In principle, the latter type of cells could respond to IL-6 when this cytokine is given not by itself but together with soluble forms of IL6-Rα (6). The above concept could help us to understand the conflicting results obtained by different authors studying IL-6 responsiveness of cultured thyrocytes. In vivo, a more complex situation undoubtedly exists because mutual interactions with stromal/vascular cells may strongly influence TFC differentiation and receptor functions (17, 33). In our hands, primary cultures of both normal and neoplastic TFC were not responsive to exogenous IL-6. In addition, DNA synthesis was not modulated by neutralizing antibody to this cytokine, indicating that IL-6 does not influence TFC proliferation in vitro.

Although the influence of IL-6 on the thyroid has been the subject of intense investigation both in culture and in vivo (normal tissue, multinodular goiter, autoimmune pathology, and systemic disease; Ref. 18), there are no data on the role of this cytokine in thyroid neoplasia.

Our experiments confirmed that pure cultures of normal TFCs release high amounts of IL-6 and IL-8, together with medium levels of TGF-β1. In addition, we report that human TFCs accumulate in the supernatant measurable levels of TGF-α that are close to detection limits. Production of IL-4, a regulator of the immune response that also has a potent effect against solid tumors (42), was not detected. The above findings were confirmed by RT-PCR analysis of cytokine-specific transcripts in primary TFC cultures.

As compared to cultures derived from normal tissue, IL-6 release was significantly down-regulated in cultures derived from undifferentiated forms of thyroid carcinoma and essentially nonexistent in the ARO cell line that derives from an anaplastic thyroid cancer. In contrast, levels of other test cytokines in TFC supernatants were not significantly reduced in cultures of neoplastic cells.

The concept that a selective reduction of IL-6 expression occurs in neoplastic TFCs has been confirmed by in vivo observations. Immunohistochemical studies of a large series of thyroid carcinomas ($n = 99$) in comparison to normal thyroid tissue ($n = 85$) revealed that IL-6 expression was frequently normal in well-differentiated forms of thyroid carcinoma but strongly suppressed in undifferentiated forms (only 2 of 34 cases were IL-6 positive). This is consistent with previous findings on mammary tumors (5). Thus, even in thyroid pathol-

Fig. 4 Immunohistochemical staining for IL-6 of formalin-fixed, paraffin-embedded thyroid carcinoma; Harris hematoxylin counterstaining. WDC shows clear cytoplasmic immunoreactivity (A; ×250); there is an absence of immunoreactivity in UC (B; ×100).
ogy, down-regulation of IL-6 production appears to be associated with advanced stages of tumorigenesis. The results suggest that IL-6 can have a differentiative role in thyroid epithelial cells; definition of this role awaits further investigation.

In vitro, TGF-α production occurred at low and not significantly different levels in TFC cultures derived from both normal and neoplastic tissue. In our hands, immunostaining revealed that follicular cells of normal thyroid tissue were TGF-α negative, whereas approximately 65% of samples from both WDC and UC were TGF-α-positive. Thus, TGF-α expression is apparently induced or enhanced in cancerous thyroid cells. In partial contrast with our findings, van der Laan et al. (34) reported that immunoreactive TGF-α and EGF-receptor are widely expressed both in normal tissue and in a wide spectrum of thyroid diseases (i.e., multinodular goiter, follicular adenoma, and carcinomas originating from follicular or parafollicular cells).

The observation that many cases of thyroid cancer are TGF-α immunoreactive suggests that this growth factor may play a role in TFC transformation. Supporting this suggestion is a report by Holting et al. (35): in a TFC line, blockade of EGF-receptor by either monoclonal antibody or genistein prevents TGF-α-induced growth and invasion. TGF-α expression has been also documented in the medullary forms of thyroid carcinoma (43).

To our knowledge, this is the first study of IL-6 expression in thyroid cancer. As in other epithelial tumors (2, 5, 15), loss of IL-6 production in undifferentiated thyroid carcinoma might play different roles: removal of an immunopotentiating factor (6); removal of a growth-inhibiting signal (12); and removal of a putative differentation signal. Investigation of the above mechanisms will require innovative experimental systems for studying thyroid cell differentiation and will benefit from an in-depth analysis of IL-6 receptor expression in thyroid tissue.

REFERENCES


Reduced expression of interleukin 6 in undifferentiated thyroid carcinoma: in vitro and in vivo studies.

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