Galectin-3 and Cyclin D3 Immunohistochemistry and Tumor Dimensions Are Useful in Distinguishing Follicular Oncocytic Carcinomas from Oncocytic Adenomas of the Thyroid

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Aims. Oncocytic (Hurthle) follicular cell tumors (OTs) of the thyroid are both adenomas (OAs) and follicular carcinomas (OCs). The routine diagnosis of these tumors can be problematic even after an accurate sampling and histological examination. Beside preoperative evaluation due to the tumor's dimension several studies have been performed to find markers able to distinguish malignant from benign follicular tumors in the thyroid, with Galectin-3 being one of the most effective. Recently, some authors suggested cyclin D3 as adjunct to the diagnosis of the oncocytic lesions of the thyroid.

Methods and Results. In this paper we assess the role of Galectin-3 and cyclin D3 in a well-selected group of follicular oncocytic tumors (14 OCs and 26 OAs). The diameter of each lesion was also evaluated. The combination of Galectin-3 and cyclin D3 has a good specificity (81%) and sensitivity (100%). Moreover, the maximum diameter (in cm) of OCs is greater than OAs (4.1 versus 2.3).

Conclusions. We believe that the use of Galectin-3 and cyclin D3 in OTs of the thyroid can be a helpful panel in daily practice when histology is doubtful.

This paper is dedicated to the memory of Nicola Pacilio, a great scientist, and an excellent teacher, an example of human humility, who died in August 2010

1. Introduction

Hurthle cell thyroid tumors (also called oncocytic or oxyphilic thyroid tumors) are uncommon thyroid lesions. By definition, they are composed predominantly of follicular cells (75% or more) with deeply eosinophilic cytoplasm on haematoxylin and eosin (H&E) stained sections [1]. In the past [2], it has been proposed that all Hurthle cell neoplasms should be regarded as malignant or potentially malignant.

Recently, these tumors have been divided into two categories: the benign oncocytic adenoma (OA) and its malignant counterpart, follicular oncocytic carcinoma (OC), showing blood vessel invasion and/or capsular penetration. OC is an uncommon thyroid malignancy, representing about 2-3% of all thyroid carcinomas [3]. Some tumors may also have relatively small cells, high nucleus/cytoplasm ratios, and solid/trabecular architecture. For such lesions, having one or more of these features but lacking unequivocal sign of malignancy, Rosai and Tallini have suggested the following terms: atypical Hurthle cell adenomas/Hurthle cell tumors of uncertain malignant potential (HCT-UMP) [1], Rosai and the Chernobyl thyroid group of pathologists recommended, for tumors showing questionable capsular invasion, the term follicular tumor of uncertain malignant potential (FT-UMP) if papillary thyroid carcinoma- (PTC-) type nuclear changes are absent and well differentiated tumor of uncertain malignant potential (WDT-UMP) in case of questionable (incomplete) PTC-type nuclear changes [1]. Several attempts have been made to support the distinction between follicular adenomas and carcinomas, including the oncocytic type, using clinical characteristics and immunohistochemistry. Sippel et al. [4] found an association between tumor dimensions and risk
of nodal positivity in patients diagnosed with an oncocyti
thyroid tumor by preoperative fine needle aspiration (FNA)
biozy. Similarly, Erickson et al. [5] showed that the larger
the size of an oncocyti lesion, the higher the risk of malignancy.

Many markers have been used to classify oncocyti
neoplasms. Among these, Galectin-3 (Gal-3), a β-galactosidase-
bounding protein involved in regulating cell-cell and cell-
matrix interactions, is a very promising one and has been
shown to represent a very helpful adjunct to FNA biopsy of
thyroid nodules [6]. Regarding Hurthle cell thyroid lesions,
some authors [7] have demonstrated a very good sensitivity
(95.1%) and specificity (88%) for Gal-3 as a marker for
OCs. Furthermore, in combination with the marker HMBA-
1, they reported an excellent sensitivity for this panel (99%).
However, other papers [8–10] have suggested that Gal-3
immunoreactivity is not restricted to malignant neoplasms
but can also be detected in thyroid adenomas.

Some investigators [5–11] have performed immunohis-
tochemistry studies focusing their attention on proliferative
activity (Ki-67) and found lower activity in OAs compared
to OCs. Moreover, other results [12] have shown that high
proliferative activity is evident only in Hurthle cell carcino-
mas with clinically aggressive behaviour. Recently, studies
have been performed to assess the role and diagnostic value
of D-cyclins as diagnostic markers in cases of oncocyti
thyroid lesions. The cyclin D family plays a pivotal role
in the regulation of G1/S-phase cell transition [13], and
D-cyclins are overexpressed in different types of cancers
[13]. Cyclin D1, for example, was found to be absent in
normal thyroid tissue [12] and was as effective as cyclin
D3 (100% specificity) in determining the behaviour of FNA
specimens suspicious for Hurthle cell neoplasia [14]. Both
of the markers, however, showed a low sensitivity; thus, the
authors conclude that there should be increased suspicion
for malignancy in indeterminate oncocyti lesions of the
thyroid that overexpress cyclin D3. They concluded that
the risk of malignant behaviour increases with the rate of
cyclin D3-expressing cells. Its diagnostic value is improved
by combination with cyclin D1 evaluation.

Troncone et al. [15] demonstrated that, in OCs, the
accumulation of p27 (kip1) is associated with cyclin D3
overexpression, suggesting that cyclin D3 is a helpful marker
when the histology is unclear. The aim of our study was to
evaluate the hypothesis that a combination of tumor diameter
and immunohistochemical expression of cyclin D3 and Gal-
3 is helpful in distinguishing between benign and malignant
thyroid lesions.

2. Material and Methods

We retrieved all cases of thyroid specimens from 1995 to
2007 matching the key words: “Oncocyti OR Oxyphilic OR
Hurthle”. All cases of medullary and papillary carcinomas
(oncocytic variants) were excluded. After registering the
diameter of each specimen in the initial cohort of 96 cases,
we excluded cases of hyperplastic nodules and oncocyti
metaplasia and cases intermingled with poorly differenti-
ated carcinomas. All slides of the remaining 58 cases were
reevaluated by investigator Claudio Cacchi (CC). Only cases
that clearly had capsular and/or vascular invasion (OCs)
and cases that clearly did not have vascular and/or capsular
invasion (OAs) were chosen for further investigation. We
obtained a final group of 40 cases (14 OCs and 26 OAs).

Formalin-fixed, paraffin-embedded tissue specimens were
available from each case. A representative sample of each
lesion was selected for the successive immunohistochemical
investigation. 3-4 μm slices were cut and stained for Gal-
3 (Monoclonal Mouse Anti-Human; Clone 9C4, dilution
1:100, Novocastra) and for cyclin D3 (Monoclonal Mouse
Anti-Human; Clone DCS-22, dilution 1:40, Novocastra) to
assess the percentage of positive tumor cells. Immunostains
were developed according to an antigen retrieval treatment
(in citrate buffer at pH 6.0) using a biotin-free detection
system (En Vision, DakoCytomation).

Each antibody was tested with external positive and
negative control, and for Gal-3 we also considered foamy
macrophages to be an internal positive control. Both Gal-
3 and cyclin D3 expression were assessed/evaluated by two
independent investigators Claudio Cacchi (CC) and Bruno
Märkl (BM), without knowledge of the clinic pathological
status of the cases. Each slide was scanned at low power
magnification (x1.6 lens) to assess the average percentage
of tumor cells positive for each marker. We considered true
positive cells to be only those that showed nuclear reactivity
for cyclin D3 and concomitant/simultaneous nuclear and
cytoplasmic reactivity for Gal-3. For Gal-3 and cyclin D3,
cutoffs of ≥1% and ≥25% have been chosen to classify cases
as positive [7,14].

Results from investigator Claudio Cacchi (CC) were used
to evaluate the specificity and sensitivity of each antibody
tested alone or in combination.

This study was performed according to the national rule,
that is, law of hospitals in Bavaria (Bayerisches Krankenhaus-
gesetz). This study has been also examined by the internal
review board.

3. Statistics

For the comparison of the results, the paired t-test or Mann-
Whitney rank sum test was used, depending on the normality
test. P values < 0.05 were considered significant.

The averaged results are shown as mean values ±1 stan-
dard deviation (SD). All calculations were performed using
the Sigma Plot 11.0 software package (Systat, Richmond,
USA). A kappa value (κ) was also calculated to estimate the
interobserver agreement for both immunohistochemical
evaluations; an absolute difference of ≤10% in two measures
was considered agreement.

4. Results

The maximum diameter (in cm) of OCs was greater than in
OAs (4.1 ± 2.3 versus 2 ± 0.8) (P < 0.001).
The results of the investigators had good [16] agreement for both Gal-3 and cyclin D3 ($\kappa = 0.8$ and 0.7, resp.).

The mean values of Gal-3 in OCs versus OAs were (Claudio Cacchi (CC) and Bruno Märkl (BM)) 25% ± 25% and 24% ± 26% versus 3% ± 8% and 6% ± 17% (Figures 1 and 2). The examiners’ results (Claudio Cacchi (CC) and Bruno Märkl (BM)) showed a significant difference in percentage ($P$ value < 0.001 and 0.002) of positive tumor cells between OCs and OAs.

The results for cyclin D3 are the following (Claudio Cacchi (CC) and Bruno Märkl (BM)): 46% ± 37% and 47% ± 33% in OCs and 8% ± 13% and 13% ± 17% in OAs (Figures 3 and 4). The two evaluations also showed cyclin D3 overexpression in the OCs with respect to the OAs ($P < 0.001$ and $P = 0.001$, resp.).

Gal-3 as marker of malignancy showed a relatively good sensitivity (79%) and specificity (81%). Although the sensitivity of cyclin D3 as a single marker was low (50%), its
Table 1

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<tr>
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<th>Oncocytic carcinomas (14)</th>
<th>Oncocytic adenomas (26)</th>
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<tbody>
<tr>
<td>Diameter (cm)</td>
<td>4.11 ± 2.3</td>
<td>2 ± 0.8</td>
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<tr>
<td>Cyclin D3 (Claudio Cacchi (CC)) (% of tumor cells)</td>
<td>48 ± 37</td>
<td>8 ± 13</td>
</tr>
<tr>
<td>Cyclin D3 (Bruno Märl (BM)) (% of tumor cells)</td>
<td>47 ± 33</td>
<td>13 ± 17</td>
</tr>
<tr>
<td>Galectin-3 (Claudio Cacchi (CC)) (% of tumor cells)</td>
<td>25 ± 25</td>
<td>8 ± 13</td>
</tr>
<tr>
<td>Galectin-3 (Bruno Märl (BM)) (% of tumor cells)</td>
<td>24 ± 26</td>
<td>13 ± 17</td>
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Table 2

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<tr>
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<th>Cyclin D3</th>
<th>Galectin-3</th>
<th>Gal-3 OR cyclin D3</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>50%</td>
<td>79%</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96%</td>
<td>81%</td>
<td>81%</td>
</tr>
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speciﬁcity was very high (96%). A combination of the two markers (Gal-3+ OR cyclin D3+) demonstrated excellent sensitivity (100%) and a quite good speciﬁcity (81%). The results are summarized in Tables 1 and 2.

5. Discussion

For the FT-UMP or the HCT-UMP cases, Papotti et al. [17] demonstrated that there is no beneﬁt with Gal-3 and HBME-1 immunoproﬁling. Herein we studied only cases with either certain benignity or certain malignancy.

Regarding tumor dimensions, we described an important difference in the mean diameter of OCs (4.1 cm) in comparison to OAs (2 cm). These ﬁndings are similar to data found in the literature. In fact, Erickson et al. [5] reported a median diameter of 4.8 cm for OCs and 3.1 cm for OAs. Furthermore, Sippel et al. [4] demonstrated that OCs are larger than OAs (5.0 versus 2.7 cm), supporting the predictive role of neoplasm’s dimensions in cases of FNA biopsy with examination of oncocyte cytoplasm. In these cases [4], no malignancy was found for lesions smaller than 2 cm. In our study, only two out of fourteen cases (14%) in the OC group were <2 cm (1.5 and 1.7 cm).

Our results, in agreement with the literature [4, 5], underscore the importance of clinical information and/or macroscopic precision in such lesions. The correlation between tumor diameter and risk of malignancy of oncocytic tumors of the thyroid suggests the hypothesis of time-related carcinogenesis, in which an oncocytic adenoma represents a premalignant lesion, and the FT-UMP represents “formae frustrae” of carcinomas. The results published by Erickson et al. [5] seem to support this hypothesis, showing a diameter of 3.7 cm for neoplasms of uncertain malignant behaviour (UMB), which is larger than adenomas but smaller than carcinomas.

The use of Galectin-3 as an adjunct to routine diagnosis of follicular thyroid neoplasms has been previously demonstrated [6, 18, 19]. However, other studies have found reactivity for Gal-3 in adenomas [8-10]. A possible explanation for these discrepancies may be the presence of false positive reactivity for Gal-3 due to biotin dependent detection systems. For this reason, we used a biotin-free system (En Vision, DakoCyntomation).

Focusing on oncocytic cell tumors of the thyroid, Volante et al. showed a 94.3% rate of Gal-3 positive carcinomas and a 12% rate of Gal-3 positive adenomas. In this study, Gal-3 had a sensitivity of 95.1% and a speciﬁcity of 88%, and if used in combination with HBME-1, the sensitivity was 99%. Interestingly, another study [8] illustrated a signiﬁcant difference in immunostaining for Gal-3 between OCs and OAs (59% versus 71%). This diversity of percentage in Gal-3 immunostaining in our study is similar to the literature; however, probably due to the relatively small number of carcinomas in our study, the speciﬁcity and sensitivity for Gal-3 are lower than those previously reported [7].

Factors that affect cell cycle machinery, recently well elucidated, also have been tested in oncocytic follicular thyroid lesions.

For example, Müller-Höcker [11] showed a higher reactivity for p53 in oncocytic neoplasms of the thyroid (88% in OCs and 75% in OAs; eight of seventeen OCs but only three of twenty OAs with reactivity in more than 10% of the cells) and showed a higher cell proliferation in OCs than OAs (Ki-67: 76 and 12 cells per 10/HPF, resp.). Hoos et al. suggested, on the basis of a higher Ki-67 index in widely invasive Hurthle cell carcinoma, that this marker may have a role in the diagnosis of Hurthle cell thyroid tumors. In 2000 [5], Erickson et al. proposed Ki-67 and cyclin D1 as helpful indicators in distinguishing oncocytic adenomas from carcinomas.

Recently, a study performed on FNA samples with oncocytic features using cyclin D3 and cyclin D1 as predictors of malignancy illustrated a very good speciﬁcity (100%) but low sensitivity (32 and 79%, resp.). The authors of this study [14] adopted cutoffs of 6.5% and 7.5% to improve the predictive value of cyclin D1 and cyclin D3, respectively, and they recommend combining the two markers to enhance this capability. Interestingly, Troncone et al. demonstrated that increased p27 expression in oncocytic follicular carcinoma is a consequence of cyclin D3 overexpression and suggested that cyclin D3 was a valid immunohistochemical marker for distinguishing OCs from OAs in cases of unclear histology. Although translocation t(6;14)(p21.1;32.3) or ampliﬁcation of the cyclin D3 gene is observed in different neoplasms, the mechanism of the overexpression of cyclin D3 in OCs is still unclear and further investigation in this direction is needed.

Our study is the first that combines dimension, a nuclear marker (cyclin D3), and a nuclear-cytoplasmic marker (Gal-3) to differentiate oncocytic carcinomas and adenomas.
Because of the possibility of a focal reaction for Gal-3 in adenomas and to avoid the risk of using a very low threshold value, we did not use a cutoff in evaluation of this reaction.

On the other hand, we believe that adopting a cutoff of 25% for cyclin D3 is quite easy to perform and reduces the interobserver variability in routine practice.

Our results on the cyclin D3 expression in tumor cells are in accordance with those shown by Troncone et al., with a low interobserver variability (κ = 0.7).

In conclusion, our study, which was performed in a well-selected series of follicular oncyectic thyroid tumors, indicates that the combination of Gal-3 and cyclin D3 has excellent sensitivity (100%) and a relative good specificity (81%), it is easy to perform, and it demonstrates a good interobserver agreement.

Moreover, we believe that this panel also may be a useful combination in FNA cytology or cytological cell-block material, because oncyectic follicular lesions of the thyroid could represent a diagnostic challenge in the praxis. Moreover, it is important to improve the management of these patients, in order to avoid unnecessary operations [20]. Positive results of this panel in case of histological morphology of adenoma may indicate performing additional section to exclude a capsular or vascular invasion.

It also could be of interest to evaluate this same panel in oncyectic follicular tumors of uncertain malignant potential, after a proper follow-up, to understand better the behaviour of these lesions.

Conflict of Interests

The authors have no conflict of interests to declare.

Acknowledgment

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References
