Abnormal Nuclear Structures (Micronuclei, Nuclear Blebs, Strings, and Pockets) in a Case of Anaplastic Giant Cell Carcinoma of the Thyroid: An Immunohistochemical and Ultrastructural Study

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ABSTRACT

The authors report a case of a 70-year-old woman with an anaplastic giant cell thyroid carcinoma, along with immunohistochemical and electron microscopic findings. Histologically, the tumor is characterized by mononucleated and multinucleated giant cells, lack of architectural cohesion, atypical mitoses, and extensive areas of coagulative necrosis. Tumor cells showed AE1/AE3 positivity as well as nuclear overexpression of p53 and ki-67. Semithin sections revealed multiple nuclei with heterogeneous size ranging from micronuclei to large-size (giant) nuclei. Micronuclei were confirmed by electron microscopy that disclosed also the presence of nuclear blebs, strings, and pockets. Morphological findings of these abnormal nuclear structures in conjunction with p53 and Ki-67 nuclear overexpression suggested a faulty mitotic checkpoint/mitotic catastrophe in the progression of anaplastic giant cell thyroid carcinoma.

Keywords: abnormal nuclear structures, anaplastic giant cell-type, micronuclei, thyroid carcinoma

Anaplastic thyroid carcinomas, which account for less than 2% of all thyroid cancers, are among the most aggressive human malignancies and are nearly always fatal [13]. Microscopically, three histological patterns are commonly described: spindle, giant cell, and squamoid [1,2,14]. It is believed that anaplastic thyroid carcinoma is derived from follicular epithelial cells and represents a terminal dedifferentiation of preexisting differentiated carcinoma [12].

In an effort to bring additional information to the understanding of the morphologic characteristics of this rare tumor, we investigated light and electron microscopic characteristics of the nuclei in a case of anaplastic giant cell thyroid carcinoma associated with foci of differentiated carcinoma. These studies reveal previously unreported abnormal nuclear structures, including micronuclei as well as nuclear blebs, strings, and pockets, which were associated with an increased...
frequency of abnormal mitoses as assessed by Ki-67
immunolabeled paraffin sections of the same tumor.
Moreover, a p53 immunohistochemical overexpression
was also documented. The implications of nuclear
changes in the dedifferentiation progression of the
tumor reported here are discussed.

CLINICAL HISTORY
A 70-year-old woman presented with a growing,
painless anterior neck mass that had existed for 2
months and was associated with dysphagia and hoarse-
ness. There was no family history of thyroid disease and
the patient had no history of radiation exposure to the
neck region. Levels of serum-free thyroid hormones,
thyreotropin, and calcitonin were within the normal
range. A needle aspirate of the mass demonstrated a
noncohesive population of tumor cells with pleo-
morphic, vesicular nuclei containing prominent nucleoli
and abundant cytoplasm. The smears were blood-mixed
but necrosis or inflammation was not a conspicuous
feature. Total thyroidectomy was performed. The patient
rapidly developed local recurrence with massive medi-
astinal lymph node metastases, and died 2 months after
surgery. No autopsy was performed.

MATERIALS AND METHODS
For light microscopy, the specimens were fixed in 10%
formalin for 24 h at room temperature and embedded in
paraffin. Sections were stained with hematoxylin–eosin
(H & E). Immunohistochemistry was performed using
paraffin-embedded material and the avidin biotin
immunoperoxidase technique. Primary antibodies used
in immunohistochemistry were thyroglobulin (DAK-Tg6,
dilution 1/200), EMA (E29, dilution 1/200), cytokeratin
(AE1/AE3, dilution 1/50), calcitonin (CAL-3-F5, dilution
1/50), desmin (D33, dilution 1/100), smooth muscle actin
(1A4, dilution 1/50), CD68 (KP1, dilution 1/50), Ki-67
(MIB-1, dilution 1/50), and p53 (DO-7, dilution 1/50,
all from DakoCytomation (Glostrup, Denmark). As
controls, known positive tissue sections and negative
contROLS devoid of primary antibody were used. For
electron microscopy, fresh specimens were fixed in 3%
phosphate-buffered glutaraldehyde, pH 7.4, and postfixed
in 1% osmium tetroxide. Semi-thin araldite-embedded
sections were stained with toluidine blue for selection of
fields. Thin sections were double-stained with uranyl
acetate and lead citrate; they were then examined and
photographed with a Zeiss EM 902 electron microscope
(Carl Zeiss, Oberkochen, Germany).

RESULTS
Macroscopic findings revealed that the thyroid had a
lobular appearance and measured 7 × 6 × 5 cm. The
cut surface showed a multinodular aspect with nodule,
maximum of 2.5 cm in diameter. Microscopic findings
showed the tumor consisted of a solid sheet of poorly
cohesive mononucleated and multinucleated giant cells
(Figure 1). The stroma was scanty, and there were focal
areas of coagulative necrosis. H & E staining showed
foci of papillary tumor, organized in irregular follicles,
lined by cells with clear, overlapping nuclei (Figure 1).
Atypical mitotic figures were frequent, reaching 20
mitoses per 10 high-power fields. Araldite-embedded
semithin sections showed multiple micronuclei in giant
multinucleated cells (Figure 2). Micronuclei were round
or oval in shape with a diameter not exceeding one-
third of the diameter of the main nucleus. They had the

Figure 1. Follicular variant of papillary carcinoma, showing
clear, overlapping nuclei, is observed adjacent to anaplastic
tumor. H & E, ×100.

Figure 2. Large patches of giant tumor cells containing multi-
ple nuclei with a vesicular chromatin pattern and one or
more large nucleoli. The large giant tumor cell shows several
micronuclei (arrows). Semithin section; toluidine blue ×400.
same staining intensity and texture as the main nucleus and were distinctly observed within the cytoplasm of tumor cells (Figure 2).

Immunohistochemical results for epithelial markers (cytokeratin AE1/AE3 and EMA) showed only a few scattered, weakly positive cells. Cytokeratins and thyroglobulin stained in the more differentiated papillary tumor cells. Immunostaining for desmin, smooth muscle actin, thyroglobulin, and CD68 were negative in tumor cells, but stained normal components of surrounding tissues. The Ki-67 positive rate was 94% of the mononuclear cells and 85% of the multinucleated giant cells, whereas in the well-differentiated cell component was 8%. Furthermore, aberrant mitotic figures were decorated by Ki-67 immunostaining in multinucleated giant tumor cells (Figure 3). Intense nuclear staining of p53 was found in the anaplastic areas, but no positive cells were observed in either neighboring papillary thyroid tumor cells (Figure 4) or normal follicular thyroid epithelial cells (not shown).

Electron microscopy disclosed several abnormal nuclear structures, including nuclear strings and micronuclei, as well as nuclear blebs and pockets. Micronuclei were surrounded by a double membrane and contained a mixture of euchromatin and heterochromatin (Figure 5). Figure 6 shows a nuclear string connecting two lobes of a nucleus. Nuclear pockets are also present and are formed by apposed extensions of nuclear membranes, including their fibrous lamina and subjacent chromatin. Nuclear blebs are morphologically similar to micronuclei with the exception that they are joined to the nucleus by a thin nucleoplasmic connection (Figure 6). Furthermore, electron microscopy revealed a variable presence of large vesicular mitochondria and few microvilli, whereas endocrine granules were not found. The cells were joined by a few rudimentary cell junctions with broad intercellular spaces into which interdigitating, microvilli-like cell processes project partially. A basement membrane was absent (Figure 5).

Figure 3. Atypical mitoses of giant tumor cell. Note the nuclei with Ki-67+ chromosomes, resembling prophase. ×400.

Figure 4. Strong p53 staining is seen in the nuclei of the anaplastic tumor cells but lack of p53 immunoreactivity is seen in the neighboring papillary thyroid tumor cells. ×200.

Figure 5. Anaplastic carcinoma cell showing multiple nuclei, heterogeneous in size, and micronuclei (arrowhead). Nuclear projections (thick arrow) and blebs (thin arrow) are also present. Tumor cells are joined by a few rudimentary cell junctions. The cytoplasm of tumor cells contains a variable quantity of large vesicular mitochondria and few microvilli. ×8000.
DISCUSSION

We present a case of anaplastic thyroid carcinoma that coexisted with a follicular variant of papillary carcinoma. The preponderant pattern in anaplastic carcinoma was the pleomorphic giant cell that may be confused with sarcoma. In our case, focal immunoreactivity for cytokeratin AE1/AE3 and ultrastructural evidence of microvilli, often forming complex interdigitations, and rudimentary desmosomes, favour the diagnosis of an epithelial neoplasm. Medullary thyroid carcinoma rarely undergoes dedifferentiation/anaplastic transformation and loses immunoreactivity for calcitonin and CEA. Neurosecretory granules were not seen at the ultrastructural level, effectively excluding this consideration [1]. Anaplastic carcinoma with osteoclast-like giant cells is a rare variant and should also be considered in the differential diagnosis. Osteoclast-type giant cells are immunoreactive for CD68, but not for cytokeratin. They contain bland nuclei, which are different from the surrounding highly pleomorphic nuclei of carcinoma cells [6].

The nuclei of the cells of most solid tumors in histopathologic preparations vary in size, shape, and chromatin pattern, both from normal nuclei and from each other. Nevertheless, only a few attempts have been made to classify abnormalities in the morphology of interphase nuclei. The study of Gisselsson et al. [8], on primary cultures of solid tumors, suggested a rough classification into three types: nuclear strings, nuclear blebs, and micronuclei. In that study, a nuclear string was defined as a chromatin thread connected to the membrane(s) of one or two nuclei, a nuclear bleb as a round or oval protrusion of the nuclear membrane connected to the main part of the nucleus by a thinner chromatin segment, and a micronucleus as a rounded chromatin located adjacent to a nucleus, with a diameter not exceeding one-third of the diameter of that nucleus. Nuclear heterogeneity has been documented by Fenech et al. [5] in normal and folate-deprived cultures of human lymphocytes. In addition to micronuclei, they describe nuclear buds and nucleoplasmic bridges, which are synonymous with the nuclear blebs and strings of Gisselsson’s study [8], respectively. Thus, abnormal nuclear shape in solid tumors is an indicator of mitotic dysregulation, which may prompt further studies of mitotic instability [9].

Our case of pleomorphic giant cell carcinoma of the thyroid is characterized not only by abnormally large giant nuclei, but also by those that are abnormally small, in the form of micronuclei. In our study, araldite semithin sections permitted more precise evaluation of micronuclei than paraffin sections. Electron microscopy not only confirmed the presence of micronuclei, but also disclosed nuclear blebs, pockets, and strings, all morphological features suggesting the occurrence of an “amitotic nuclear division”. A Ki-67 immunohistochemical analysis revealed a mitotic ability of the multinucleated giant cells, thus confirming the occurrence of acytokinetic multinucleation.

Tissue necrosis is absent in differentiated thyroid carcinomas, while it is a common finding in poorly differentiated and anaplastic thyroid carcinomas [10]. Along the same lines, gene p53 mutations are restricted to aggressive tumors (17–38% of poorly differentiated thyroid carcinomas and 67–88% of undifferentiated thyroid carcinomas as opposed to 0–9% of well-differentiated thyroid carcinomas) [3,4,11]. In the anaplastic areas of our thyroid neoplasm a strong overexpression of p53 protein was detected with the DO-7 antibody, confirming previous data in literature.

Micronuclei and multinucleation (the presence of 2 or more nuclei with similar or heterogeneous size, resulting from deficient separation during cytokinesis) as well as abnormal mitoses are morphologic aspects of mitotic catastrophe, a common phenomenon occurring in tumor cells in vitro with impaired p53 function exposed to various cytotoxic and genotoxic agents [7,15]. Mitotic catastrophe often results in the generation of aneuploid and polyploid cell progeny, as also reported in anaplastic thyroid carcinoma [12]. The defective p53 checkpoint causes the improper segregation of chromosomes, resulting in aberrant mitosis, multiple micronuclei, and an eventual necrosis-like death [15]. In our case, necrosis occurs in an extensive
background of Ki-67-positive cycling tumor cells. Furthermore, Ki-67 immunohistochemistry decorated abnormal multipolar mitoses. Taken together, high mitotic activity, necrosis, multinucleation, abnormal nuclear structures (micronuclei, nuclear blebs, strings, and pockets), and p53 and Ki-67 overexpression suggest the hypothesis of a faulty mitotic checkpoint (mitotic catastrophe) in our case of anaplastic giant cell thyroid carcinoma.

In conclusion, our light and electron microscopy findings expand the nuclear morphology of anaplastic giant cell carcinoma of thyroid to include micronuclei, nuclear blebs (buds), and strings (nucleoplasmic bridges). These previously unreported abnormal nuclear structures were associated with p53 and Ki-67 nuclear overexpression. Therefore, our data suggest a faulty mitotic checkpoint (mitotic catastrophe), described mainly in experimental models in vitro and in vivo, and may be considered as expression of the dedifferentiation process in this unusual, but aggressive histological type of thyroid carcinoma.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES