Abstract. Background: Various nuclear envelope derivatives, such as the annulate lamellae, the intranuclear tubules as well as the nuclear projections and pockets may be observed electron microscopically in tumour cells. Patients and Methods: In a series of eight gastric adenocarcinomas, ultrastructural features of nuclear envelope changes were analyzed and correlated to the biology of the tumours. Results: Histologically, three tumours were intestinal-type adenocarcinomas and showed annulate lamellae in the cytoplasm of some tumour cells. Five tumors were mixed-type adenocarcinomas, with a solid growth pattern; two of these tumours were characterized by the presence of intranuclear tubules, whereas the remaining three tumours exhibited nuclear pockets and projections. Seven out of eight patients died due to metastatic disease during the follow-up period (median 31 months). Conclusion: Ultrastructural evaluation of pleomorphism of the nuclear envelope may be an ancillary method for the pathologist in the study of nuclear grading of gastric carcinomas.

The grading of tumours is routinely performed during the work-up of most patients who have cancer. In general, the grading process includes light microscopy assessment of both the architectural and nuclear features of the tumours (1). However, grading of gastric carcinomas is based entirely on the architectural features. For example, this tumour may be classified using a 3-tiered system that distinguishes the degree of morphological resemblance to gastric or metastatic intestinal glands (1). Gastric adenocarcinomas are graded as well-differentiated, moderately differentiated, or poorly differentiated, but this classification has only a limited prognostic value (1). Recently, the study of nuclear envelope changes has been proposed as a supplement to the tumour grading system (2, 3). The deep biological significance of nuclear envelope pleomorphism has been confirmed by in vitro models. In thyroid cancer, for example, it has been shown that micro-injection of the \textit{RET/PTC} oncogene into thyroid cells is able to induce nuclear envelope irregularity (4, 5). Changes in the nuclear envelope may occur early in neoplastic processes (6, 7), are detectable even in the pre-cancerous stage (8), and mark a difference from the smooth, roundish nuclear shape of the normal cells of corresponding tissues and organs (9). Nuclear envelope proliferation may result in the formation of intranuclear tubular inclusion, annulate lamellae as well as nuclear projections and pockets (7).

In the current study, we describe the clinicopathological findings of eight patients with gastric carcinoma characterized ultrastructurally by nuclear envelope changes in tumour cells. The implications of our findings for nuclear grading of gastric carcinoma are discussed.

Patients and Methods

The eight patients with primary gastric carcinomas were identified from the surgical pathology files of the Department of Human Pathology (University Hospital), Messina, (Italy) from 1990 through 2001. Patients 1 and 3 have previously been studied and their data published elsewhere as case reports (10, 11). Follow-up information was available for all patients and lasted from 13 to 55 months (median, 31 months). The resected specimen was subjected to detailed pathological examination, which identified the depth of penetration of the stomach wall, whether the margins were free of tumour, and the presence of secondary deposits in 15 or more lymph nodes and tiers, according to the pTNM system (12). For each case, all available haematoxylin and eosin-stained sections were reviewed to evaluate the tumour type, according to Laurèn classification (13). The Laurèn classification (13) has the following categories: intestinal type, diffuse type, and mixed (e.g., tumours having equal proportions of intestinal and diffuse characteristics, and others with a solid growth pattern). Small pieces of the fresh tumour tissue from the 8 cases were routinely fixed for electron microscopy.

Results

This series included 5 male and 3 female patients, aged 55-80 years (median, 68 years) (Table I). All tumours were advanced and metastases involved perigastric lymph nodes.
Seven of the eight patients died because of metastatic disease. According to Laurèn classification (13), 3 cases were of intestinal type, 5 cases were of mixed type with a solid growth pattern, and no cases were found to be of diffuse type. Ultrastructural features of nuclear envelope proliferations in gastric carcinoma cells were variable. A prominent feature in two cases (patients 1 and 2) was the occurrence of intranuclear tubular inclusions in a large number of tumour cells. The intranuclear tubular inclusions were present as solitary tubules or closely packed circular profiles arranged in a honeycomb-like pattern (Figure 1). In three (patients 3, 4 and 5), annulate lamellae were found in the cytoplasm of some tumour cells (Figure 2). They consisted of parallel arrays of cisternae that had regular small pores. Annulate lamellae were usually found close to the nucleus. In four (Patients 1, 6, 7 and 8), the nuclear membranes showed nuclear projections and pockets (Figure 3).

**Discussion**

The current ultrastructural study showed an increased activity of the membranes of the nuclear envelope in tumour cells of eight cases of gastric carcinomas. The activity of the membranes was manifested as formation of various nuclear envelope derivatives, such as annulate lamellae, intranuclear tubules as well as nuclear projections and pockets.Annulate lamellae represent membranous cisterns that contain pore complexes (14). They are similar to the nuclear pore membrane complex, and it has been suggested that they are derived from the nuclear envelope (14, 15). Well-developed examples of annulate lamellae are most often seen in rapidly growing or differentiating cells, such as germ cells, embryonic cells, malignant cells, and cells in culture, and are seen infrequently or not at all in benign neoplasms and normal adult cells (14). Intranuclear tubular structures have been reported in a variety of cell types, including endometrial cells, hepatoma cells as well as lung and gastric adenocarcinoma cells (10, 16). Nuclear envelope irregularities and intranuclear tubules might be involved in or be reactive to defects in the nuclear-cyttoplasmic transport, reported as a characteristic feature of cancer cells (17). Nuclear projections and pockets are a common and fairly characteristic feature of malignant lymphomas, but many anaplastic carcinomas, and especially sarcomas, also exhibit nuclear pockets (7). Our

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Site</th>
<th>pTNM stage</th>
<th>Histology</th>
<th>Ultrastructural findings</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>68</td>
<td>Antrum</td>
<td>T2N1M0</td>
<td>Mixed</td>
<td>Intranuclear tubules; nuclear pockets and projections</td>
<td>DOD (36 months)</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>55</td>
<td>Antrum</td>
<td>T3N2M0</td>
<td>Mixed</td>
<td>Intranuclear tubules</td>
<td>DOD (9 months)</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>80</td>
<td>Antrum</td>
<td>T2N1M0</td>
<td>Mixed</td>
<td>Annulate lamellae</td>
<td>DOD (32 months)</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>67</td>
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<td>T2N1M0</td>
<td>Intestinal</td>
<td>Annulate lamellae</td>
<td>NED (60 months)</td>
</tr>
<tr>
<td>5</td>
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<td>65</td>
<td>Body</td>
<td>T2N1M0</td>
<td>Intestinal</td>
<td>Annulate lamellae</td>
<td>DOD (31 months)</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>68</td>
<td>Antrum</td>
<td>T2N2M0</td>
<td>Mixed</td>
<td>Nuclear pockets and projections</td>
<td>DOD (32 months)</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>70</td>
<td>Body</td>
<td>T2N2M0</td>
<td>Intestinal</td>
<td>Nuclear pockets and projections</td>
<td>DOD (15 months)</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>75</td>
<td>Antrum</td>
<td>T3N2M0</td>
<td>Mixed</td>
<td>Nuclear pockets and projections</td>
<td>DOD (12 months)</td>
</tr>
</tbody>
</table>

M, Male; F, female; NED, no evidence of disease; DOD, died of disease.
ultrastructural study extends these observations, showing their presence in four cases of mixed type gastric carcinoma characterized by a solid growth pattern (patients 1, 6, 7, 8). Light microscopy appreciation of foldings and indentations of the nuclear membrane is crude and indirect, being based on the staining of membrane-bound heterochromatin. However, electron microscopy provides a more detailed appreciation of the nuclear membranes. We believe that the ultrastructural findings of nuclear envelope changes may be of potential value in clarifying the prognosis of patients with gastric carcinoma. In fact, 7 out of 8 patients died due to metastatic disease during the follow-up period (median 31 months).

In summary, we report the results from a preliminary investigation of the potentially useful ultrastructural features of nuclear envelope changes in gastric carcinomas. We conclude that ultrastructural evaluation of pleomorphism of the nuclear envelope is a promising approach to achieve an improved nuclear grading of gastric carcinomas.

References