Altered Golgi Apparatus Architecture in Animal and Plant Tumors[1]

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Abstract

Measurements from electron micrographs of normal and transformed cells of rat liver, mouse epidermis, mouse mammary gland and bean leaf show Golgi apparatus of tumor cells to have dictyosomes with a width/height ratio significantly greater than those of normal cells along with a reduced or near normal number of cisternae per stack. The change in dictyosome height is due to an increase in the thickness of the intercisternal region rather than to an increase in thickness of cisternal membranes or cisternal lumens. This is the first report of a consistent change in Golgi apparatus morphology associated with transformed cells.

In spite of numerous studies of the ultrastructure of neoplastic cells and tissues, no systematic examination of Golgi apparatus morphology has been carried out comparing normal and transformed cells. It is generally recognized that cancer cells have less rough endoplasmic reticulum, a predominance of free rather than bound polysomes, and large or unusually-shaped nuclei and mitochondria (1, 15, 31). Additionally, cell surfaces of cancer cells show altered immunological, contact, electrophoretic, and chemical properties suggesting an altered biogenesis of the cell surface (16). Because of the central role of the Golgi apparatus in the formation of plasma membranes and surface coats (29, 30), this study was initiated to seek morphological changes in Golgi apparatus architecture characteristic of cancer cells.

Materials and Methods

Electron micrographs from a variety of sources were analyzed. Measurements were made of dictyosome width and height and number of cisternae per dictyosome; frequency, size and characteristics of secretory vesicles were noted. Dictyosome width was recorded as the average cisternal diameter exclusive of secretory vesicles or peripheral tubules (Fig. 1). Dictyosome height was measured orthogonally to width and is a measure of the height of the stacked cisternae (Fig. 1). Only micrographs judged to represent near median cross sections were analyzed.

Tumor systems examined were as follows:

a) Rat hepatocytes and hepatoma induced by administration of 2-

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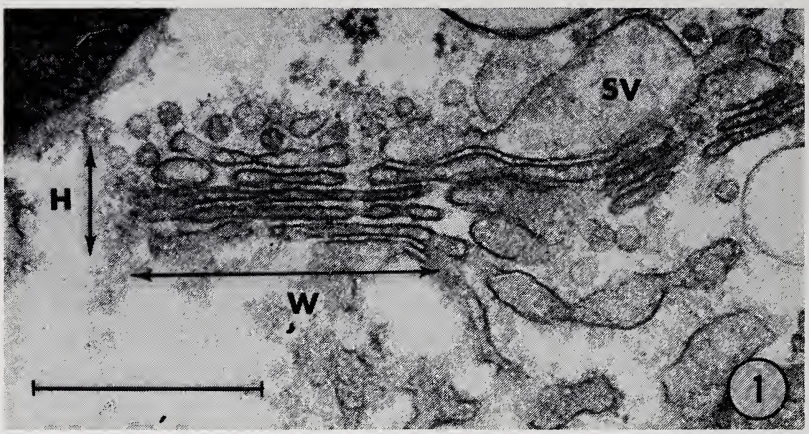

FIGURE 1. *Electron micrograph illustrating a near median transverse section of a dictyosome of rat liver consisting of 5 stacked cisternae and a partial 6th cisterna near the bottom of the stack. Secretory vesicles (SV), tubules and small vesicular profiles of cross sections through tubules surround the dictyosome periphery. The dimensional parameters that reveal differences in comparisons of normal and transformed cells (H = dictyosome height and W = dictyosome width) are illustrated. Scale bar = 0.5 μ.*

fluorenyl-acetamide (FAA) according to the method of Farber (23), and from the literature (1, 4, 5, 6, 8, 10, 11, 12, 13, 18, 26, 27, 35).

b) Mouse epidermis (8) and spontaneous carcinoma (31, 32, 33).

c) Mouse mammary epithelium (17, 24, 36) and mammary carcinoma (2, 3, 7).

d) Bean leaf and leaf tumors induced by *Agrobacterium tumefaciens* as described by Lippencott and Heberlein (21).

Bean tissue was fixed for election microscopy with 2% glutaraldehyde for 60 hours at 4°C followed by 1% osmium tetroxide post fixation for two hours. Liver tissue was fixed in 2% osmium tetroxide for 2 hours at 4°C. Dehydration was through an acetone series with embedment in Epon according to Spurr (34) for bean or Luft (22) for liver. Normal and tumor cells were prepared for electron microscopy in parallel to minimize differences in specimen preparation. Thin sections were viewed and photographed with a Philips EM 200. Measurements were from published electron micrographs (*b*, *c*) or from prints at a final magnification of 35,000 (*a*, *d*). A total of 103 Golgi apparatus from cancer cells and 41 Golgi apparatus from normal cells were analyzed. Golgi apparatus terminology follows that of Morré, Mollenhauer and Bracker (28, 29, 30).

Results

There was no consistent pattern in the number or arrangement of dictyosomes of Golgi apparatus comparing normal and transformed cells or in the frequency, size, or characteristics of secretory vesicles. However, an alteration in the size and shape of individual dictyosomes did present a significant pattern.

Among all four tumor systems analyzed (Table 1), there was a tendency for the dictyosomes to be of lesser or equal diameter and greater height in tumor cells compared to their respective controls. This is most noticeable in the height to width ratios. Dictyosome height was increased in the tumors even though, on the average, the number of cisternae per dictyosome was reduced by approximately 0.5 cisterna. Since less cisternae per stack were occupying the same or more height (compare Figs. 1 and 2; Fig. 3) either the cisternal thickness, the space between individual cisternae (intercisternal regions), or both were increased.

TABLE 1. *Diameter and height of dictyosomes and average number of cisternae comparing normal and transformed cells.*

Tissue	Cell type	Dictyosomes			
		No. cisternae	Width (μ)	Height (μ)	Ratio (H/W)
Rat liver	Parenchyma	3.17	1.80	0.146	0.08
	Hepatoma	3.26	1.49	0.166	0.11
	Hepatoma HND[1]	3.11	0.93	0.164	0.17*
Mouse epidermis	Normal	3.75	0.95	0.173	0.18
	Carcinoma	3.14	1.02	0.245	0.24
Mouse mammary	Normal	3.54	1.30	0.219	0.17
	Carcinoma	3.05	1.10	0.223	0.20
Bean leaf	Normal	5.4	0.75	0.156	0.21
	Tumor	4.74	0.64	0.205	0.32*

[1] From a single tumor—highly non-differentiated, highly malignant and rapidly growing.

* Difference from control significant at the 95% confidence level.

To determine the morphological basis for the greater height to width ratios of dictyosomes from tumor cells, the cisternal thickness (membranes + lumens) and the space occupied by the intercisternal region were determined from dictyosomes from rat liver and rat hepatomas and from bean leaves and crown gall tumors of bean leaves (Table 2). In the crown gall system, both cisternae and intercisternal regions were increased in tumors relative to normal cells (Fig. 3); in rat liver, only the intercisternal region was increased (Figs. 1 and 2).

TABLE 2. *Thickness of cisternae (membrane ± lumen) and intercisternal region of dictyosomes comparing normal and transformed cells and tissues.*

Tissue of origin	Cell type	Thickness (A)	
		Cisternae	Intercisternal regions
Rat liver	Parenchyma	408	264
	Hepatoma	372	306*
Bean leaf	Normal	166	133
	Crown Gall Tumor	208	262*

* Difference from control significant at the 95% confidence level.

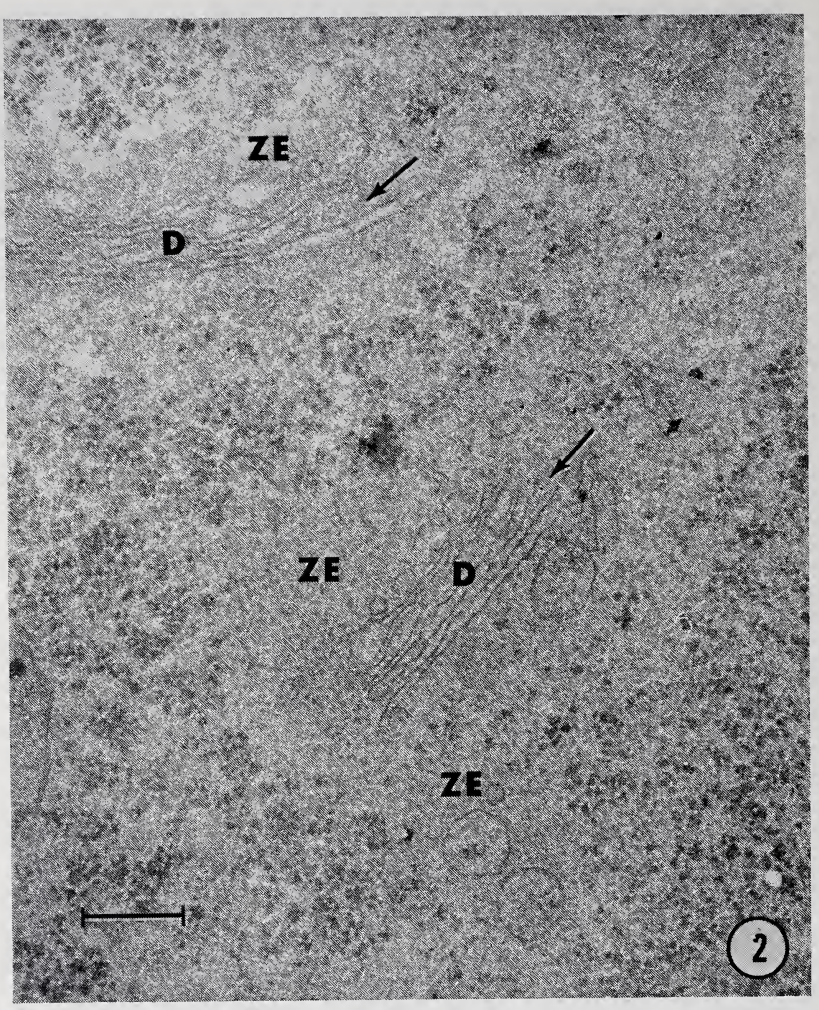

FIGURE 2. *Electron micrograph of a portion of a rat hepatoma cell showing two dictyosomes (D) of the Golgi apparatus surrounded by a zone of exclusion (ZE) of unusual electron density. The material of the zone of exclusion is continuous with the material of the intercisternal regions (arrows). Scale bar = 0.5μ.*

The consistent change was in the space of the intercisternal region (Table 2).

Discussion

Neoplastic transformation involves changes in chemical properties of the cell surface, specifically but perhaps not exclusively glycolipid simplification (16, 19); the enzymes of the latter are localized in Golgi apparatus of hepatocytes (20). Our findings provide preliminary evidence for a morphological alteration in Golgi apparatus associated with tumor cells. The change is expressed as an increase in the height to width

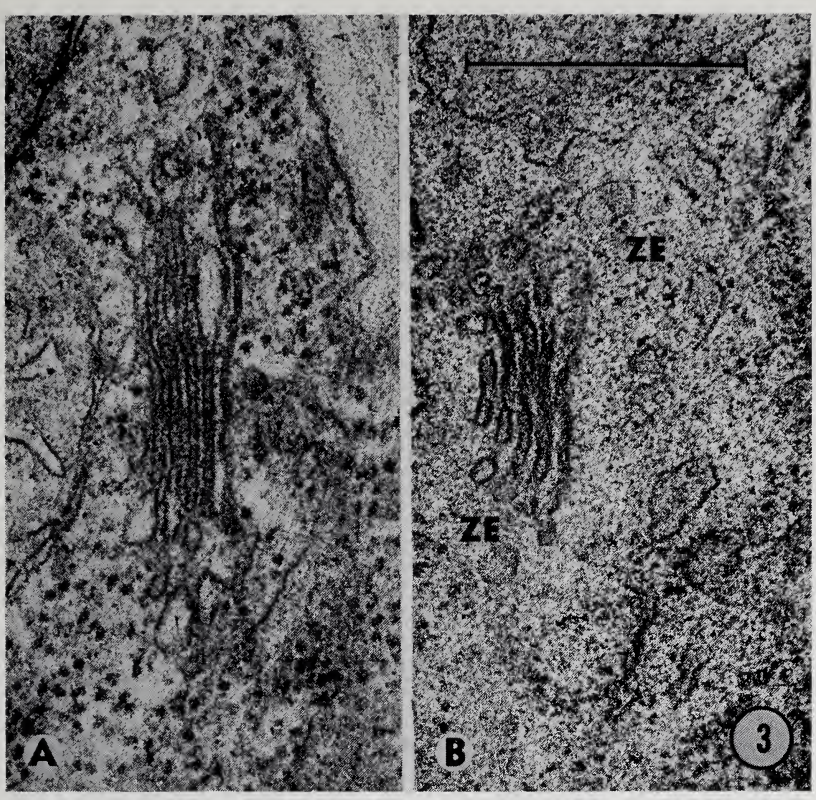

FIGURE 3. *Electron micrographs of portions of bean leaves illustrating A. A dictyosome with 6 stacked cisternae of a control leaf, and B. A dictyosome with 5 stacked cisternae from a crown gall tumor cell. Note that in B the dictyosome of the tumor cell is surrounded by a prominent zone of exclusion (ZE) while in the control dictyosome the zone of exclusion is less distinct (A). The dictyosome from the control cell (B) is wider and, even though the dictyosome from the tumor has one less cisterna than the control, they are of nearly equal height. The increase in height/cisternae of the tumor dictyosome is due principally to an increase in thickness of the intercisternal regions. Scale bar = 0.5 μ.*

ratio of dictyosomes resulting in total or in part from an increase in the thickness of the intercisternal regions.

Little is known about the intercisternal regions of Golgi apparatus. In special circumstances, the material of the intercisternal region appears to be continuous with the Golgi apparatus ground substance or zone of exclusion (29). The latter surrounds the Golgi apparatus and represents a region from which ribosomes, glycogen, rough-surfaced membranes or organelles are largely excluded (Fig. 2). In plants, the intercisternal region may contain special fibers known as intercisternal elements (14, 25). Intercisternal elements have not been reported for animal cells but dictyosomes of rat hepatomas show indications of some kind of fibrous element within the enlarged intercisternal regions (results unpublished). Whether the increase in size of the intercisternal

region and the appearance of filaments are related remains to be investigated.

The principal significance of the observation may lie in its potential application to biopsy material. A 50% increase in the thickness of the intercisternal regions of the stacked cisternae of Golgi apparatus is easily detected and is beyond the range of normal variation. Since this is the first observation that intercisternal regions are capable of modification and change, the findings add new impetus to the need to understand the nature of the intercisternal region of Golgi apparatus, and its possible role in the tumorigenic process.

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