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The 3-D Microstructure of Cancer and Its Topological Properties

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The form of cancer (adenocarcinoma) deviates from its parent structures not only on cellular but on architectural levels, while the previous studies focussed on the former aspect alone. However, the form of cellular assemblies is of equal fundamental significance as that of a single cell in microscopically diagnosing cancer and studying its morphogenesis. Reconstruction disclosed that according to whether or how much the cells are maturated, they are assembled into a form ranging from continuous network of tubes to partial disintegration into isolated cells, and these varieties were shown accessible to geometric description combining the topological parameters 0-th and 1-st Betti numbers. A computer system was introduced by which to reproduce 3-D structures graphically from serial 2-D images, a device that proved to be quite helpful in studying the way organ structures are subject to transformation in variously diseased states.

Introduction — The Form of Assembled Cancer Cells

One of the most important tasks for pathologists may be, from both clinical and social viewpoints, to make microscopic diagnosis on biopsy specimens taken from patients who are suspected of harboring malignant disease. For cancer patients this is the final diagnosis on which physicians depend in selecting all therapeutic details. Shown as an example in Fig. 1 is mucosa of a stomach excised from a patient and, somehow, one may recognize a cancer growing in the left half. However, the basis for this recognition has to be established in more definite terms, since there are many what we call borderline lesions or quasi-cancers where pathologists' verdicts often disagree.

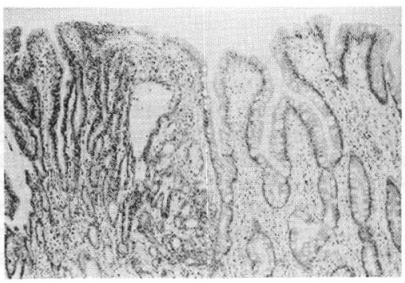


Fig. 1 Mucosa of a human stomach with cancer (adenocarcinoma) growing in the left half. In the right, pre-formed glands are seen branching downward.

The microscopic diagnosis of cancer involves different aspects of pattern recognition. Of primary interest have always been the changes of individual cells, mainly on account of the common understanding that the cancer is essentially a disease of cell. Indeed, one can see for instance in Fig. 2 that in the carcinoma cells the nuclei are apparently larger, more irregular in shape and more darkly stained than in the non-carcinomatous cells. These are all well studied features which, comprizing the so-called "cellular atypism" of cancer, have lead to the establishment of diagnostic techniques for various types of cancers, now generalized into the system of clinical (exfoliative) cytology. It seems however that these cellular aspects have been stressed too much, leaving another, equally important feature of cancer much behind, that is the spatial arrangement of cell.

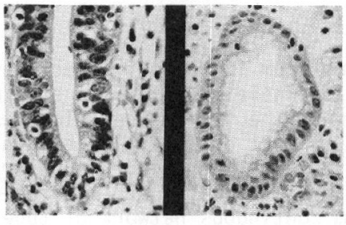


Fig. 2 Right: An ordinary gland of the stomach forming a tube lined by mature epithelial cells. Left: Gland-type cancer forming a tube as well. Note the irregularly shaped, large nuclei.

This aspect, the "structural atypism" (Jap. Res. Soc. for Gastric Cancer 1974), is to be shown in carcinoma of the stomach. Usually, this cancer arises from the gastric glands through transformation of some constituent cells into malignant cells. In a normal individual, a gastric gland comprizes epithelial cells that are assembled to form a tree-like system of continuous

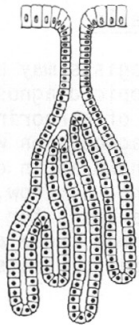


Fig. 3 The basic design of gland; a tree-like system composed of continuous tubes.

tubes (Fig. 3). The cells produce the juice of stomach which is then secreted into the lumen of the tube and carried to the exterior through the dendritic canals. It is a general rule that a cancer originating from these cells also retain, more or less, the basically tubular structure of gland penetrated by a central lumen, hence, the term "adenocarcinoma"(adeno=gland). However, in contrast to the regular tree-like pattern of normal glands branching toward the bottom of mucosa (Fig. 1 right) cancer cells are assembled into a complex netty structure (Fig. 1 left), and this deviation serves not only to the recognition of cancer as one of the markers of malignancy, but also

to the study of cancer morphogenesis.

These two aspects of cancer morphology are tabulated in some textbooks with the implication that one, while making microscopic examination, should pay attention not only on the aberration of cells but equally on that of architecture. However, studies have always been focussed on the cellular aspect alone, partially because single cells are much more accessible to pattern recognition. In these circumstances, and based on the significant fact that cancer is a disease not only of a single cell but also of assembled cells, we have undertaken to study the form of cancer, thereby focussing on what architectural principles are underlying the perverted microscopic form.

The Spatial Form of Cancers

Some examples are to be shown. Fig. 4 is from an adenoma, or one may call a quasi-cancer of the stomach, 3-dimensionally reproduced from serial sections. The tree-like glands branching downward have a continuous lumen that is lined by cells which, today, are still open to debate as to whether they really behave as cancerous cells (Nagayo 1971); therefore, a "quasi" cancer. From a point of 3-D structural view, the only difference from normal

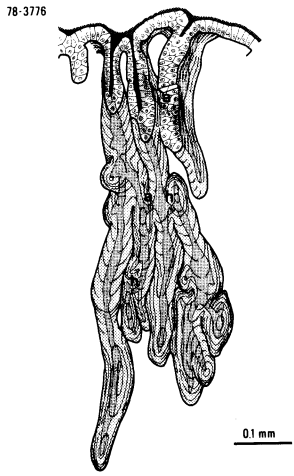


Fig. 4 Three-D microstructure from part of an adenoma. Ordinary trees of glands are transformed into a loose network on account of connections at two places (a).

glands is that there are connections emerging so as to bridge adjacent trees, forming a loose network. Fig. 5 is from another stomach having an overt, well differentiated adenocarcinoma, and in this case the structure is more typical of carcinoma than the quasi-cancer in that, there are many connections and loops forming a dense network. With regard to the connectivity of these, however, the form has not yet gone very far from normal, seeing that both the branches and their lumina are continuous throughout the system.

In the next type, the moderately differentiated adenocarcinoma of Fig. 6, the structure is more aberrant. There are a series of loops, showing that the framework is again a network. However, the lumina have already lost continuity, and are separated into many small vesicles that are dispersed in the system. Seeing that the branches are no longer a tube, we express this condition as a porous state or porosity. The last type, the poorly differ-

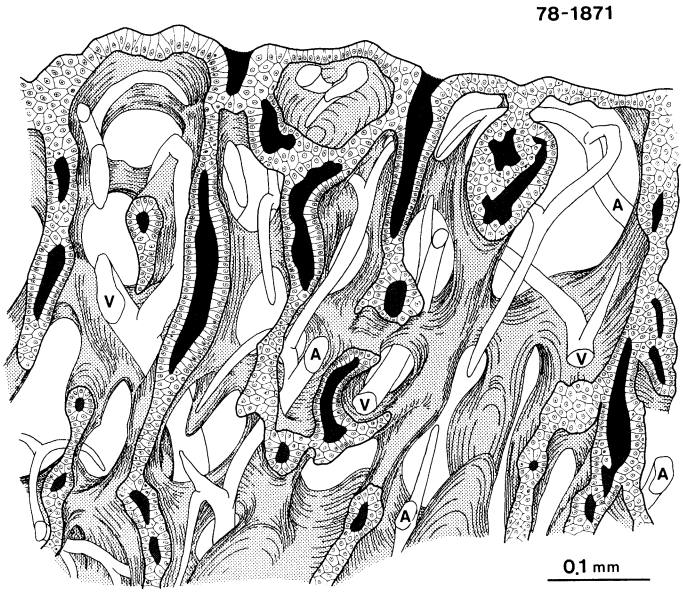


Fig. 5

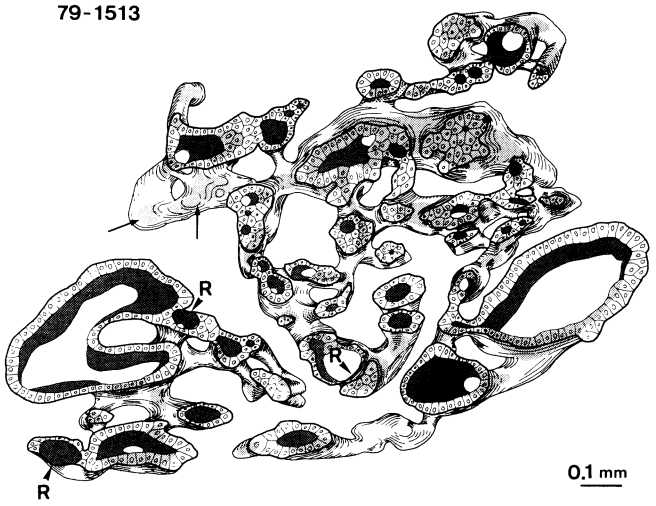


Fig. 6

Fig. 5 (upper) Reconstruction from a well differentiated adenocarcinoma of stomach without visualizing lumina. Tubes of cancer cell aggregates are seen forming a network that closely intertwines with small arteries (A) and veins (V).

Fig. 6 (lower) Moderately differentiated adenocarcinoma. The framework is again a network, however with lumina separated into many small vesicles.

entiated adenocarcinoma, is the most intractable gastric tumor (Fig. 7). Here again a porous network, but at places the cells are moving away from the network as small clusters. The disunited cells are apt to slip away to distant places and grow, showing that the structure is beginning to disintegrate; a state that is most remote from the tightly organized form of an ordinary gland of the stomach.

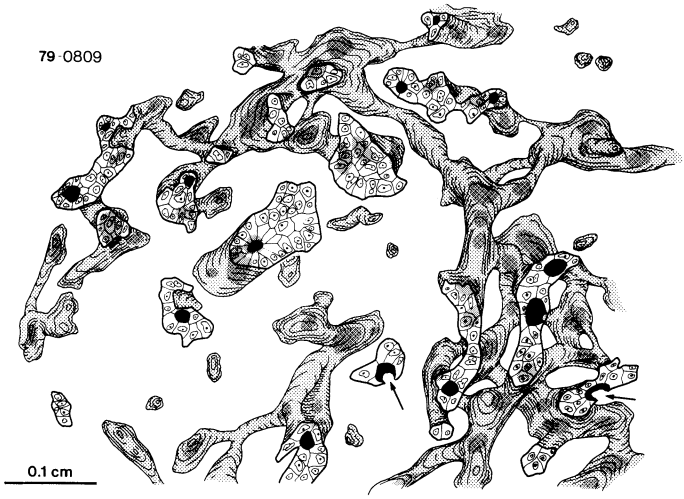


Fig. 7 Poorly differentiated adenocarcinoma. Disintegration of architecture is shown by clumps of cells moving away from porous network.

Topological Expression for the Skeleton of Cancer Microstructure

We now understand that the structure of cancer not only deviates from the ordinary glands, but varies according to the grade of differentiation, in other words, according to malignancy. The basic skeleton of cancer is a variously connected network, and the lumina harbored in it form a second network. This state makes it appropriate to describe the framework of different cancers by reducing the connectivity into a pair of topological invariables, p_0 and p_1. p_0, the 0-th Betti number, is the number of separate parts, and p_1, the 1st Betti number, is that of inner connections a network contains. We performed measurement of these on serial sections of various types of adenocarcinomas, including "quasi" cases. For instance, in the "quasi" cancer (adenoma) the 1st Betti number per cubic millimeter tumor tissue is much lower than in a well differentiated cancer for both branches and lumina, showing that the network is much looser (Fig. 8). Thus, a "quasi" cancer corresponds to an intermediate state between a well differentiated cancer and the normal tree, an information quite important from a diagnostic viewpoint. Another example is found in the porous state of the moderately differentiated type as expressed by the 0-th Betti number of the lumen as large as 439 per cubic millimeter, suggesting that the lumen is split into so many separate parts. The expression of topological properties with a pair of values p_0 and p_1 was introduced by the senior author while

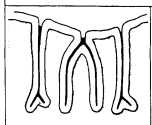
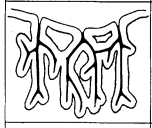
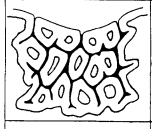
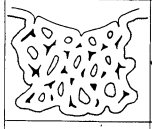
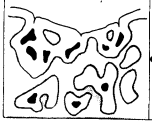
		p_0 (Number of separate parts/mm³)		p_1 (Number of anastomosis/mm³)	
		Tubule	Lumen	Tuble	Lumen
	Normal	–	–	0	0
	ATP				
	Case 1	(1)	(1)	103	24
	Case 2	(1)	(1)	129	29
	Case 3	(1)	(1)	192	48
	Adeno-carcinoma (tub_1)	(1)	(1)	1640	384
	Adeno-carcinoma (tub_2)	(1)	439	287	0
	Adeno-carcinoma (por)	871	1133	653	0

Fig. 8 The gland pattern in ordinary mucosa, "quasi" cancer (ATP) and three types of adenocarcinomas was analyzed by the topological parameters p_0 and p_1.

dealing with the morphogenesis of liver cirrhosis (Takahashi 1978; Takahashi and Suwa 1978), and proved to be quite effective in describing the genetic processes of tissue changes in various diseases, which in fact often involve transformation of skeleton originally framed in the form of network.

Factors Involved in the Configuration of Cancer

Why an adenocarcinoma forms a network instead of a tree may be induced from its spatial relationship with blood vessels. While it is a common behavior of cancer to grow in such a way as to fill the space uniformly, the growth is limited in the presence of blood vessels whose formation precedes the development of cancer. As a matter of fact, cancer always co-exists and shares the space with capillaries which, supplying blood to cancer cells, are in themselves a network. In this situation, inevitably, cancer tissues are made to intertwine with the network of capillaries, which moldes the cancer into another network (Figs. 5 and 9).

Here we assume that the differentiation of the cancer cells constituting the network vary from one case to another. The differentiation of adenocarcinoma cells implies that they are specialized as a secretory element, and the secretory function of a cell finds its morphological expression in its polarization into the basal and apical poles. If entire cells are well differentiated and accordingly are polarized, and if they are aligned side by side on the basement membrane, then the overall archi-

tecture must be a network with continuous lumina (Fig. 10B), a pattern exactly we find in "quasi" or well differentiated cancer. In contrast, the porous state of moderately or poorly differentiated type cannot be explained without assuming that there are incompletely polarized or non-polarized cells (Fig. 10C). The lumina are obstructed at several places by stack of such poorly differentiated cells.

CA-Reconstruction as a Means of Visualizing Microstructure

The tissue skeleton is so characteristic of each morphological variety of cancer that, from a clinical viewpoint, it is of fundamental importance to define its topological properties in actual cases. However, the methods

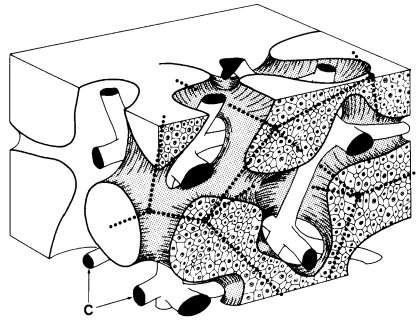


Fig. 9 The origin of netowrk of adenocarcinoma. A mass of carcinoma cells is molded into a network as it intertwines with capillaries (C).

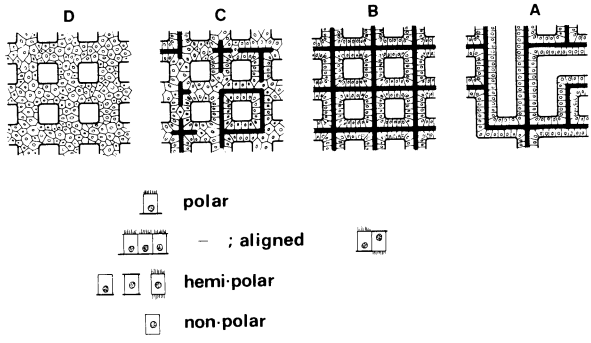


Fig. 10 Morphogenesis of adenocarcinoma assumed for well differentiated (B) and moderately or poorly differentiated types (C).

of stereology are of course not available in this context so long as they are based on geometric probability. Therefore, in the present status, we cannot avoid scanning the space with serial sections of tissue specimens. Serial images transcribed from serial microscopic sections of adenocarcinoma are placed one upon another, on which we count the Betti numbers. A tessellation is set in this, and the counting rule we applied has much in common with the unbiased sampling of particles introduced by Sterio (1984).

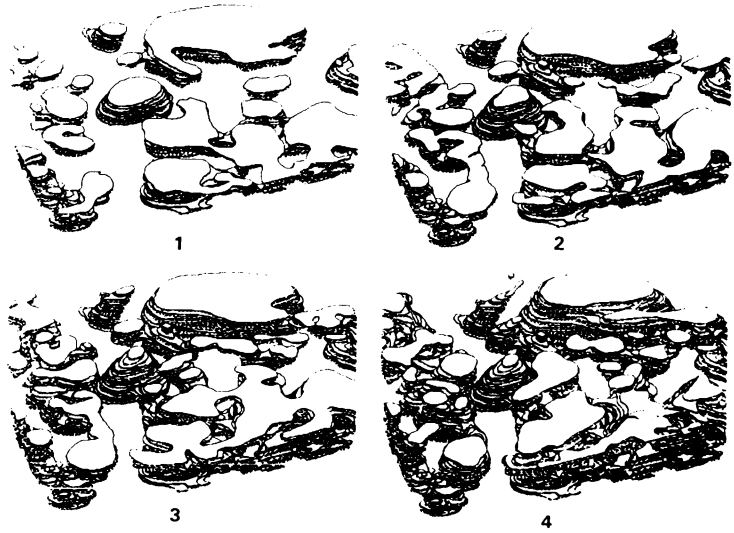


Fig. 11 Steps of reconstruction for multiply connected cancer (moderately differentiated), with the aid of a microcomputer system we have developed.

It is worth special note that in establishing the framework of cancer tissues, we undertake laborious steps of reconstruction only to abstract some principles of structure. We can now resort to the aid of a microcomputer system in graphically visualizing 3-D pictures (Fig. 11); we have also developed one such system in cooperation with Olympus Co. Ltd. However, if one is not certain of what to abstract from such 3-D pictures, he has to content himself only with getting a view that is somewhat novel. A picture like this only presents a surface of a stack, giving us little insight into the solid mass that continues behind the surface. The principles to be established include, for instance, the connecting relation of branches that cannot be visualized unless the picture is reduced into a network somehow.

Conclusion

Above described are the topological properties of the form we encounter in diseased human tissues. In cancer, it is of fundamental importance not only from diagnostic but also from biological viewpoint to establish these properties for each morphological variety. Any indirect method of analysis like stereology does not seem available in this task, and so

we are looking forward to attaining such a progress of image analysis and computational geometry that would serve as a new tool of morphology.

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Q: This is a very interesting study. Recent studies on other tumor types have shown that not only the atypia within indivisual cells matters, but that also the architectural changes and the volume fraction are prognostically important. Here you demonstrate an architectural pattern which seems to have diagnostic and possibly also prognostic value. Could you suggest a simple method which could reveal features which correlate with 3-dimensional architecture that you describe? Such a method would be valuable for further evaluation of the prognostic value of the architectural change. (Y. Collan)

A: We think what we have shown today is a basis on which further investigations should be made, especially on the relationship between the architectural changes of tumors and their prognostic as well as diagnostic implications. At this stage of development, we have formed some 2-D features of architectural aberrations. For instance, the so-called back-to-back pattern has been confirmed exactly to correspond to a sectional pattern of porosity, and therefore we can predict malignancy when multiple, rounded lumens are found on a 2-D section.

C: Your results are interesting because they offer a way to estimate the proportional influence of factor associated with concentric and essentric hypertrophy. The application of the principles you presented have a positive influence on the research in cardiac pathology. (Y. Collan)