

HISTOLOGICAL RECOGNITION OF DEGREES OF MALIGNANCY IN ADENOCARCINOMA OF THE OVARY

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In contrast to the considerable number of articles devoted to Cancer of the Cervix particularly its early detection and its therapy, little is being written about cancer of the ovary. This relative neglect is doubtless due to the inaccessibility of ovarian tumors in their early stages and to the failure of new ideas to appear which might improve its discouraging prognosis.

Carcinoma of the ovary is, however, of very great importance. Among the women of the United States at least, the disease ranks fifth among the causes of death from cancer. Furthermore the mortality rate from cancer of the ovary is steadily increasing and, since death rates from cancer of the uterus are declining, it may be that carcinoma of the ovary will some day be the gynecologist's most serious problem. Already it has been estimated that at any age in a woman's life, the chance of her eventually developing a malignant ovarian tumor is about one percent.

Not only has cancer of the ovary been accorded relatively little attention in general, but it is the rarer tumors which have received the most study. The granulosa cell tumors, dysgerminomas, mesonephromas, gonadoblastomas and many others have been examined in detail, partly because they afforded incidental insight into the embryology and hormonal functions of ovarian tissue cells, but partly perhaps because they stimulated the enthusiasm of medical collectors and taxonomists.

In the meantime, there has been a shortage of articles about the papillary serous cystadenocarcinomas. These to the clinician are the most important because they are commonest and, since they are usually highly malignant, result in more deaths than all the other kinds combined. A source of scientific interest in these tumors seems necessary if they are to receive the attention they deserve. This interest may be found in the rather clearly recognizable grades of malignancy in which different examples of these tumors manifest themselves. The use of this gradability for the study of the cancer problem is the subject of this lecture.

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THE PRINCIPLES OF GRADING IN CANCER

Interest in the phenomenon of different grades of malignancy, as manifested by individual examples of tumors developing in the same organ, arose in the latter part of the last century, particularly among the pathologists of Germany. According to views the prevalent, cancer was to be regarded as a return to a primitive pattern of growth, a regression from the differentiated state attained by the tissue after the embryologic period.

According to this concept a tumor was "differentiated" if its structure rather closely resembled that of the adult organ of its origin, and "undifferentiated" if it resembled its parent tissue only slightly. The undifferentiated tumor was termed "anaplastic" and regarded as of high malignancy, with a poor prognosis. The differentiated tumor was considered to be of low malignancy and to be more curable, or at least its host was expected to have a longer period of survival.

Efforts to devise a practical grading system for the squamous cancers of the cervix began early in this century in Vienna and have continued sporadically ever since. Disappointment in the usefulness of grading for squamous cancer of the cervix has tended to make some workers believe that grading will be useless in all tumors. Contrary to this conclusion, it will be shown in what is to follow, that grading is relatively dependable in the adenocarcinomas of the ovary.

The classical methods of determining grade or degree of differentiation were based upon an histological evaluation of tissue architecture in general, the relation of cells to each other. In particular, attention was given to the arrangement of cells along the surfaces or about gland spaces, the polarity of the cells and their occurrence in single or multiple layers.

Attention in grading systems has also been given to the characteristics of the cells themselves and such qualities as size of nucleus and its reactions to specific stains have been taken into consideration. The recent interest in cytology has naturally led to more detailed study of the cell itself and the actual count of the chromosomes as well as the size and shape of the nucleoli have been considered in relation to malignancy. In the future it may be predicted that biochemical and even immunologic qualities of the cells or tissues will be found that vary with the degree of malignancy.

ESSENTIALS FOR THE VALIDITY OF GRADING

Efforts to employ principles of grading require that the biological potentialities of the cancer cell in a given case be relatively constant. This consistency must exist in two respects

1. The grade must be the same throughout the primary tumor and its metastases, for otherwise the prognosis would be largely determined by the most malignant cells of the tumor and these might not be those reaching the microscopic field.
2. The same grade must persist throughout the life history of the disease in any given patient; in other words, there must be neither an increase nor a decrease in the biological malignancy of the individual cells as the disease progresses.

Investigations of the question of the constancy of grade of malignancy are difficult and somewhat unsatisfactory. Studies of multiple sections selected from different areas of the same tumor, graded by two independent observers, have led us to believe in the general consistency of grade, throughout the extent of the tumor as it exists at any given time. Furthermore, comparison of sections from a primary tumor with those from recurrent lesions biopsied many years later has also indicated an extraordinary consistency. It does appear then that the cells of a given adenocarcinoma of the ovary remain, at least as far as one can determine by present methods, the same wherever they may be growing and however long the disease may last.

HISTOLOGIC GRADING IN RELATION TO END RESULTS IN OVARIAN PAPILLARY SEROUS TUMOURS

The papillary serous cystadenomas and cystadeno-carcinomas offer very useful material for the studying of degrees of differentiation, or grades of malignancy, since there are several clearly definable types of clinical course against which one can test the accuracy of one's methods.

1. The Benign Cystadenoma presents relatively simple, low papillomas which on microscopic examination are found to consist of much fibrous stroma covered by a single layer of small epithelial cells.
2. The Borderline Type, is an almost unique form of papillary tumor in the ovary, which has histologic qualities which lead some pathologists to class it as benign, others as malignant. This tumor, which may be called intermediate or borderline, is capable of producing multiple implants on the peritoneal surface which may almost miraculously disappear when the primary tumor is surgically removed. There are in fact many reported instances of this occurrence in the literature. This peculiar biologic behaviour is seen

only with tumors whose structure is also "borderline". The papillae are complicated but well constructed, the epithelium single layered, the cells columnar and polarized toward a basement membrane. Sections of such tumors may be of great assistance to the pathologist in marking the histologic boundary between the benign and the malignant.

3. Malignant tumors of this cell type, the papillary cystadenocarcinomas, are all fatal unless completely excised. Nevertheless, it may be possible for the histologist to subdivide these into several groups with varying degrees of differentiation, from grade I in which tissue organization is still quite well maintained to grade III in which the cells are arranged in nearly solid sheets without evident structure.

That there is a clinical significance to grading of carcinomas of the ovary has been shown in several investigations. Our own studies of the results of therapy for papillary serous adenocarcinoma have been made with the aid of so-called survival curves. These show that there were only two deaths among the cases histologically classed as "borderline" and these occurred more than four years after surgery. The majority of patients with tumors graded II or III rapidly succumbed and only a few were alive at the five year period of observation. The cases with grade I malignant tumors had a course lying between those with grades II and III cancers. This correlation between histologic grading and subsequent course is at least some evidence of the validity of the method.

OTHER SIGNS OF DIFFERENTIATION OR GRADE OF MALIGNANCY

The method of determining grade of malignancy from a general histologic survey is relatively crude and better, perhaps quantitative, methods must be sought for. These would make prognosis more accurate and might also provide a new means for the study of the basic nature of malignancy.

In the series of forms, illustrated by the different grades of papillary serous tumors of the ovary, there is a gradual change in tissue structure, from the surely benign, through the so-called borderline types and the various grades to the really anaplastic examples of grade III. Through this series, the quality of malignancy itself steadily increased. If it could be shown that some measurable biochemical or biologic characteristic decreased or increased in a parallel manner, the investigator might properly assume that this characteristic was closely related to the malignant process itself.

With this concept in mind, we have been searching for nearly ten years in our Department for measurable characteristics of tumor tissues that could be shown to vary consistently with the degree of differentiation (1, 2, 3, 4, 5). With this purpose in mind, tissue respiration, radiophosphorous uptake, a variety of enzymes, and adaptability to heterotransplantation have been examined. The results of none of these studies have been very promising. There were, however, two investigations, both essentially cytologic in technique, which gave useful if perhaps not unexpected correlations. These will now be briefly described.

CORRELATION OF GRADE WITH THE DNA ("CHROMATIN") OF THE NUCLEUS

For several generations pathologists have associated dark hematoxylen staining of the nucleus, the so-called "hyperchromatism" with malignancy. Only in recent years, however, has scientific attention been directed to the counting of the chromosomes or to the measurements of the desoxyribonucleic acid (DNA), the chemical stuff of which the chromosomes are made.

Except during the phases of mitotic division itself, the DNA is distributed evenly throughout the nucleus and, accordingly, the cell's content may be determined by microspectrophotometric methods. The amount of DNA in the typical cell will correspond to that of the normal cell complement of chromosomes, the diploid number in man being forty-six. In a proliferating tissue there will be a certain proportion of cells, preparing for mitosis, in which new DNA is being synthesized to provide enough for the two new cells in prospect. The percentage of these darkly stained cells which have this high complement of DNA indicating impending division, will be proportional to the rate of cell multiplication. This in cancer is of course usually higher than in normal tissues. There is however, a second reason for "hyperchromatism" in hemataxylin stained tissues and that is the occurrence of cells with an abnormal number of chromosomes, a condition called "polyploidy".

Spectrophotometric studies of the DNA content of tissues from typical examples of different grades of malignancy were made by Dr. Saul Bader, one of the collaborators in our general project. Examining 100 cells, he was able to construct a "histogram" which showed the percentage of nuclei in which the DNA corresponded to the normal chromosome content and to various multiples of it. These histograms showed that polyploidy was specially common in the tumors of high malignancy.

CORRELATION OF GRADE WITH SIZE AND NUMBER OF NUCLEOLI

Another essential substance of the cell is ribonucleic acid, which is distributed diffusely in the cytoplasm as well as concentrated in the nucleolus

ar nucleoli. RNA is concerned with the complicated process of specific protein synthesis.

The relationship to the size of the nucleolus, as well as to the number of nucleoli, to the grade of malignancy seemed strikingly evident in the observation of our cases. Measurement of the diameter of the largest nucleoli of a series of cells from typical cases yielded figures showing a rather steady increase in size as the grade of malignancy increased. Indeed the difference between the size of the nucleolus in the borderline grade from that in a grade III tissue was so evident that precise measurements were scarcely needed. It seems evident, at least for the papillary serous tumors of the ovary, that the grade of malignancy is closely related with the average RNA content as measured by nucleolar size.

C O N C L U S I O N

The histologically determined grade of malignancy in the papillary serous cystadenomas and cystadenocarcinomas of the ovary is of some practical use in predicting the clinical course and ultimate outcome.

The diameter of the nucleolus in these tumors increases consistently with the grade of malignancy. The occurrence of cells with an abnormal number of chromosomes, "polyploidy", is characteristic of the tumors of high malignancy.

These measurable characteristics that vary in relation to the grade of malignancy must be regarded as in some way related to the essential nature of cancer itself.

S U M A R I O

El autor revisa brevemente los principios en que se basa la técnica histológica de clasificación de tumores según su grado de malignidad, que es útil al clínica para establecer un pronóstico basado en la correlación que se ha establecido entre la gradación histológica de grado de malignidad y el curso clínico ulterior de los tumores malignos de ovario.

Desde el punto de vista bioquímico, la determinación microespectrofotométrica del contenido tisular de DNA (ácido desoxiribonucleico) y la medición del diámetro nucleolar como expresión del contenido tisular de RNA (ácido ribonucleico), correlacionan bien con el grado de malignidad establecida con las técnicas histológicas y citológicas tradicionales.

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