Cytohistopathology of Cervical Cancer

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Summary

Proceeding from an account of the development, structure, and function of the uterine cervix, the cytology and histology of cancer of the cervix is presented by stage of the disease. The concept of biological progression in the pathogenesis of cancer of the cervix is utilized to consider a gradient of changes in the endocervical mucosa: reserve cell hyperplasia and squamous metaplasia as preliminary non-specific modifications; dysplasia as a cancer precursor; and the preinvasive and invasive stages of cancer.

The cervix is one of the two most frequent locations of cancer in the human female, lower in incidence in this country only to cancer of the breast. There are approximately 35,000 new cases and 10,000 deaths annually from cancer of the cervix.

In the past quarter century this disease became the 1st cancer to be diagnosed before the appearance of clinical signs or symptoms, the result of the discovery by Papanicolaou and Traut (20) that examination of a smear of exfoliated cells yielded criteria of sufficient reliability to correlate with the histological diagnosis of cancer. Because the uterine cervix is accessible for direct smearing and for biopsy confirmation as well, the use of the Papanicolaou (Pap) test as a method of detecting early cancer of the cervix was a natural extension of this discovery and the control or prevention of this disease by mass screening programs became a possibility. The Pap test also made it possible to investigate the epidemiology of cancer at an early stage of the disease, so that factors could be more directly related to etiology and onset (22–24).

In contrast is the difficulty of applying the concept of exfoliative cytology to early detection of cancer in less readily accessible locations; for example, a positive Pap test of a sputum sample in a patient with negative chest roentgenogram poses the problem of locating the origin of the cells somewhere in the bilateral ramifications of the bronchial tree.

Before going on to a discussion of the pathogenesis of cancer of the cervix, it is of interest to look at the structure and function of the organ from the perspective of its development.

Developmental and Comparative Features

From the oviduct system that served our primitive ancestors, fallopian tube, uterus, cervix, and vagina have evolved in response to the need to accommodate the more recently acquired functions of copulation, sperm transport, and gestation. The schematic representation of human internal genitalia shows the continuity of this modified system so that an ovum released from the ovary enters the funnel of the fallopian tube where fertilization occurs, moves into the lumen of the body of the uterus for implantation and fetal development, and then proceeds through the canal of the uterine cervix into the vaginal birth canal (Chart 1).

Reference to the embryology of these target organs is important because of possible harmful effects to the developing fetus of hormones or other drugs administered to pregnant women (19). There is general agreement that in human females the growing tips of the oviducts (Müllerian ducts) meet and fuse as they approach the dorsal wall of the urogenital sinus to form the uterovaginal rudiment which differentiates into uterus and vagina connected by a cervix. There is, however, a division of opinion about the origin and nature of the lining of these organs and as to whether the Müllerian epithelium disappears and is replaced prenatally by the endoderm of the urogenital sinus or by the ectoderm of the primitive cloaca (Chart 2). Witschi (28) argues that neither of these postulated origins can explain the results of studies in experimental embryology and concludes that the Müllerian epithelium is not replaced, thus retaining the potential of differentiating into columnar and squamous cell types.

The settlement of this problem will have important implications for the current concern with teratogenesis and cancer of the vagina and cervix in daughters of women treated with diethylstilbestrol during pregnancy (14). In the reported cases of cancer of the vagina with a history of in utero exposure to this synthetic estrogen, benign adenosis was described in association with the adenocarcinoma (15, 16), both benign and malignant lesions arising from columnar cells in an organ normally lined by squamous epithelium.

In the rodent the bipotential cell function of vaginal and cervical epithelium is normally expressed by an alternating production of keratin and acid mucopolysaccharide during each estrus cycle. For example, during the proestrus stage of the cycle, the superficial layers of epithelium are columnar, mucus-secreting cells. At this stage the deep layers are beginning to differentiate into squamous cells which will keratinize and reach the surface at estrus the following day.

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to be exfoliated as squames (25). These cells of a contrasted cytology are produced successively by the basal or germinal layer, a process which has been described as cyclic metaplasia. In castrated rats, the cycle is abolished, the lining is reduced to 2 layers of cells, but the full cycle of differentiation can be restored by estrogen (10).

In humans, there is an analogous rudiment of the alternating cycle of differentiation in the vagina and ectocervix. In the follicular phase there is an intraepithelial zone of keratinization as described by Dierks (9), the keratinizing layers reaching the surface at midcycle (1), while in the luteal phase neutral mucopolysaccharide is present in the superficial layers of epithelium (4).

Structure and Function

The human cervix, cylindrical in shape, is a continuation of the lower end of the corpus uteri, extending from the level of the internal os to the wall of the vagina. The endocervix extends from the internal to the external os and is lined by columnar mucus-secreting epithelium. The portio of the cervix or ectocervix projects into the vagina and, like the vagina, is covered by stratified squamous epithelium. The term squamocolumnar junction is used to describe the junction of the 2 types of epithelium which ideally coincides with the location of the external os (Chart 1).

However, the level of this histological junction may vary depending on whether the columnar epithelium has become everted onto the ectocervix where it is described as an erosion, whether the everted columnar epithelium has undergone metaplasia to squamous epithelium, or whether the columnar lining has retreated up into the canal as may occur after pregnancy (8) and the menopause (12). The squamocolumnar junction is of special interest to those studying cancer of the cervix since it is now recognized that squamous cancer of the cervix does not usually arise as expected in an area normally covered by squamous epithelium but rather in this zone of epithelial modulation.

If we look at the topography of the endocervix as reconstructed by Fluhmann (12) it seems evident that the endocervical mucosa is a continuous sheet forming ridges and clefts. With the demand for an expanded secretory surface as for example during pregnancy, there is an increased complexity of the clefts and folds. When involution occurs, remnants of the previous gestational proliferation may persist as tunnel structures resembling, on cross-section, tubular glands which may become distended by accumulated secretion. The erroneous description of glands in the wall of the endocervix is an example of the problem faced by the histologist in relating a slice through an object with its 3-dimensional reality (11).

Cellular alterations may thus involve the mucosal extensions into the wall of the endocervix arising independently or by direct spread from the surface. For example, the finding of cancer in the mucosa of a cleft deep in the wall is not regarded as invasion; in fact Fluhmann has illustrated direct continuity with the surface lesion (Chart 3). It is also important that true invasion of the stroma may begin from such an in situ lesion located deep in a cleft.

Since the pathogenesis of cancer of the cervix takes into account function as well as structure, it is of interest to look...
Chart 3. Above, cervical cleft with associated tunnels. The artist's drawing was based on tracings made from serial microscopic sections (left); below, the same cleft partly involved in carcinoma in situ (dark areas). Reprinted from Fluhmann (12) with the permission of W. B. Saunders and Company.

at some of the functional features of this organ (21). The cervix acts as a defense barrier between the relatively bacteria-free cavity of the corpus and the bacteria-laden vaginal canal. The mucus secretion of the cervix is under hormonal control, augmented by estrogen and inhibited by progesterone. Endocervical mucus is alkaline, in contrast to the acid environment of the vagina. Physical as well as biochemical properties of cervical mucus are factors in sperm transport. Pommerenke (21) concludes a review of the physiology of the cervix with the following statement, "Because of the broad categories in which its functions fall, namely those relating to barriers against bacteria, to the transport of spermatozoa, and to sphincter action in the birth canal, the cervix deserves the dignity of title as an organ, not merely the appendage of an organ."

Pathogenesis of Cancer of the Cervix

Cancer is a chronic disease of unknown etiology, prolonged latency, and relatively low incidence. Cancer is classified not by etiological agent but on the basis of location, cell type, grade of differentiation, and stage of the disease. The T.N.M. classification system for cancer of the cervix is based on extent of primary tumor, involvement of regional lymph nodes, and presence of distant metastases (18).

With the advent of the Pap smear test new concepts of the pathogenesis of cancer were introduced. Abnormalities noted in exfoliated cells of the Pap smear correlated in the biopsy specimen with histological changes consistent with neoplasia but confined to the mucosa. The intraepithelial lesions, dysplasia and preinvasive cancer, were thus identi-
fied and the concept was formulated that dysplasia is a precursor of cancer and that there is an *in situ* or preinvasive stage of the disease (17).

It is postulated that a gradient of changes at the site of origin in the endocervical mucosa—reserve cell hyperplasia, squamous metaplasia, dysplasia, preinvasive and invasive cancer—occurs in the pathogenesis of squamous cancer of the cervix (Fig. 1).

Reserve cell hyperplasia results from proliferation of the reserve cells normally present in patches and serving as depositories for regeneration of columnar lining cells (5). In the next change, squamous metaplasia, the cells become polygonal and resemble stratified squamous epithelium. These cells may begin to produce glycogen while cells at the surface may retain a columnar shape and continue to secrete mucus (6). Neither reserve cell hyperplasia nor squamous metaplasia, noted in from 40 to 90% of adult cervices, can be regarded as precursors of squamous cancer, but rather as nonspecific preliminary change of the epithelium.

In dysplasia, the cells are definitely abnormal and show criteria similar to cancer cells. Dysplasia is considered a transitional state in the pathogenesis of cancer of the cervix, with cellular attributes that permit progression to cancer as well as regression toward normality (22). For example, in a prospective study of women with dysplasia, we found that 12% progressed from dysplasia to cancer annually; approximately 34% per year regressed from dysplasia toward normality, and of these 30% per year showed a recurrence of dysplasia (27). Further, in following a population of women previously negative for cancer of the cervix, we found that almost all new cases of cancer were discovered in patients with dysplasia, while very few were found in the much larger fraction of the population which did not show dysplasia (26). In preinvasive (*in situ*) cancer the malignant process is confined to the epithelial layers. In the invasive stage, the disease spreads locally and to distant organs. There is evidence of a similar sequence of events in the pathogenesis of squamous cancer of the bronchus (2).

**Case Histories**

In the following examples, cytological and histological features of the disease process are illustrated. Cancer was detected during routine Papanicolaou screening at which time the patients were free of clinical symptoms or signs of the disease.

The 1st case is a Mexican-American woman, 22 years of age, gravida 3, whose routine Pap smear showed changes consistent with cancer *in situ*. The diagnosis was confirmed by sampling biopsy and the patient was referred for treatment. The cone biopsy demonstrates the gradient of changes already referred to. The circled areas on the low-power survey of the therapeutic cone biopsy specimen are magnified to show the detail at various levels of the endocervix. Squamous metaplasia is seen at the distal end of the specimen at or near the squamocolumnar junction. Proximal to this is a focus of dysplasia, then more metaplasia with atypism, and at a point proximal to this an area of cancer *in situ*, while at a level nearest the endometrial cavity there is an area of reserve cell hyperplasia. Thus, a number of lesser changes coexist with the preinvasive cancer, the most advanced stage of the disease in this patient (Fig. 2 to 4).

The 2nd example is of a Mexican woman, age 38, with a history of 10 pregnancies and is of interest because the Pap smears on 2 occasions, 2 weeks apart, are so different: in one the cancer cells are well differentiated, in the other they are poorly differentiated. The corresponding biopsy shows preinvasive cancer (Figs. 5 to 7). In the 3rd case, a 39-year-old woman, the disease has progressed to the stage of micro-invasion (Figs. 8, 9). In the last example there is a full-blown invasive process in a 52-year-old woman. The cancer, although widely invading, is confined to the endocervix, and seems to encounter a barrier of distended glands between it and the ectocervix (Fig. 10). The surface cancer is poorly differentiated while the invading cell groups are well differentiated (Figs. 11 to 13). In all the cases, the malignant change appears to originate well within the endocervical canal.

**Discussion**

The status of dysplasia as a cancer precursor and the relationship of preinvasive cancer to the invasive and clinical stages of the disease has not yet been fully established. However, it is expected that the relationship will be clarified by studies on the natural history of the disease in which the continuity of changes over time can be confirmed by repeated smear test on a population of women kept under observation.

In order to study the transition from normal through dysplasia to cancer, we have postulated a continuum from completely negative through a range of atypias and degrees of severity of dysplasia to cancer. Scores have been arbitrarily assigned ranging from zero (normal) to 100 (invasive cancer) with the score for minimal dysplasia set at about the midpoint. The diagnostic scale provides a semiquantitative estimate of the degree of change noted in the Pap test at entry into the study and at each follow-up visit. The photomicrographs are illustrative of the cytological criteria in the negative range as well as the dysplasia series. The use of such data will facilitate the testing of hypotheses about the progression rates of the various diagnostic categories (Fig. 14).

The steps of biological progression in carcinogenesis of the cervix are not inconsistent with the experimental model of initiation and promotion proposed by Berenblum (3). As a 1st step in this model, an irreversible but dormant malignant change is initiated in cells exposed to a single subthreshold dose of a chemical carcinogen, but tumors do not appear until after repeated applications of the promoter, a nonspecific agent. However, if the promoter is applied before exposure to the carcinogen, tumors do not develop. Initiation and promotion are not additive but represent different biological mechanisms. Progression to invasive cancer requires further changes in the promoted cells (Chart 4).

Spontaneous cancer of the cervix is rare in animals, but tumors of the cervix have been induced in rodents by repeated or continuous direct application of carcinogenic agents to the cervicovaginal area and progressive stages in
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A CONCEPT OF CARCINOGENESIS

Normal endocervical mucosa
Reversible epithelial modifications
Reserve cell hyperplasia
Squamous metaplasia

INITIATION
Dysplasia: a transitional state
Cancer of the cervix
The preinvasive stage

PROMOTION
Invasion
Metastasis

PROGRESSION

Chart 4. A concept of carcinogenesis.

the disease are described (13). Hormonal treatments have also been related to the development of these tumors (7).

As in the experimental model, the biological progression of this disease in women, which in its final stages we recognize as clinical cancer with its inevitably poor prognosis, may require a succession of heritable cellular changes. It may be that multiple etiological factors are involved in the multistep process.

Acknowledgments

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References

Fig. 2. Case 1. Preinvasive cancer, biopsy endocervix. H & E, × 250. Inset, cervical smear. Papanicolaou stain, × 500.

Fig. 3. Case 1. Section from cone biopsy. Orientation from A at approximately the squamocolumnar junction to E near the internal os. H & E, × 15.

Fig. 4. Case 1. High-power views from Fig. 3. All H & E reduced from × 250. A, squamous metaplasia, distal endocervix; B, dysplasia; C, squamous metaplasia with atypias; D, preinvasive cancer; E, reserve cell hyperplasia, proximal endocervix.

Fig. 5. Case 2. Preinvasive cancer. Cervical smear, well-differentiated cancer cells. Papanicolaou stain, × 500.

Fig. 6. Case 2. Cervical smear, poorly differentiated cancer cells, 2 weeks later. Papanicolaou stain, × 500.

Fig. 7. Case 2. Biopsy, preinvasive cancer, endocervix. H & E, × 250.
Fig. 8. Case 3. Microinvasive cancer; *arrow*, level of microinvasion, high in the endocervical canal. H & E, × 10.

Fig. 9. Case 3. Detail of microinvasion. H & E, × 25.
Fig. 10. Case 4. Invasive cancer, low-power view shows the intact squamous epithelial surface and distended endocervical crypts at the distal endocervix. There is widespread invasion of the proximal endocervix by trabeculae of cancer cells. H & E, × 6.

Fig. 11. Case 4. Cervical smear showing spindle-type cancer cells. Papanicolaou stain, × 500.

Fig. 12. Case 4. Cancer at surface, endocervix, at site of arrow in Fig. 10, poorly differentiated epithelium. H & E reduced from × 250.

Fig. 13. Case 4. Invading trabeculae of well-differentiated squamous cancer cells. H & E stain reduced from × 250.
Fig. 14. A to E, cytological features for a gradient of changes in the normal range. Papanicolaou stain reduced from × 500. A, negative, score 0. Normal squamous cells and a strip of columnar endocervical cells. B, metaplasia score 5. Normal squamous cells and metaplastic cells. C, atypia, score 15. Normal squamous cells, metaplastic cells, and atypical cells with slight variation in size and shape of nuclei. D, transitional, score 20. Cellular variation with multinucleation. E, intermediate, score 25. Sheet of cells with slight increase in nuclear-cytoplasmic ratio. F to J, cytological features for a gradient of severity of dysplasia. Papanicolaou stain, reduced from × 500. F, minimal dysplasia, score 35. Increase in size of nucleus, multiple nuclei, perinuclear halo, disparity in size and shape of adjacent cells. G, slight dysplasia, score 45. Increased nuclear size, increased disparity in adjacent cells. H, moderate dysplasia, score 55. Increased nuclear-cytoplasmic ratio; overall reduction in cell size. I, marked dysplasia, score 60. Further increase in nuclear-cytoplasmic ratio, reduction in cell size, coarse nuclear chromatin. J, cancer in situ, score 75. A cluster of malignant cells showing active nuclei with narrow rim of cytoplasm. Invasive cancer is assumed to have a score of 100 on this scale.
Score

F  Minimal  35
G  Slight   45
H  Moderate 55
I  Marked  60
J  Cancer in situ  75
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