The Changes in the Mitotic Mechanism of Human Cancer Cells

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Many of the mitotic irregularities characteristic of cancer cells had already been described by the end of the last century. Good reviews of the older literature on this subject are found, for instance, in Politzer (18) and Caspersson and Santesson (2). Our knowledge of these phenomena has, however, greatly increased during recent years. This is to a large extent due to the development of new staining methods and of the squash-technic in making preparations, which have brought the human chromosomes within the reach of much more exact observation than was possible earlier.

The most important work on chromosomal conditions in human cancer cells has been carried out by Koller (11-13), who has described and explained a great variety of mitotic abnormalities in them. Our observations agree in their main features with those made by Koller. Now, however, such a wealth of data on this subject has accumulated that the next important task seems to be the establishment of a common ground for their interpretation. The present observations seem also to have an important bearing on the much discussed question of the origin of cancer.

MATERIALS AND METHODS

The present study is based on 174 cases of carcinoma of the female genital tract diagnosed and/or treated in the Women's Clinic (I and III) of the University of Helsinki during a period from the end of 1948 to the end of 1949. This material falls into the following groups: vaginal carcinoma, 4 cases; cervical carcinoma, 92 cases; corpus carcinoma, 53 cases; ovarian carcinoma, 24 cases; and one case of carcinoma of the fallopian tubes. All the biopsies were taken from untreated cases. The chromosomes of normal tissue were examined from 35 cases of endometrium in the proliferation stage. Since earlier observations, as well as our own, show that, as regards the chromosomal conditions, the different types of carcinoma agree in all their pertinent features, we have in this connection considered the different cases together.

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The chromosomes in normal and cancer tissue were studied both from squash and sectioned preparations. The squashes were made according to the technic described in 5, 7, and 10. The material was fixed in acetic-alcohol (1:3) for 1 hour. The Feulgen method was mainly used for staining, i.e., a hydrolysis of 8 minutes in 60° C. HCl; this was followed by staining in Feulgen's leuco-basic fuchsin for 2-3 hours. Thereafter, the biopsies were squashed and the preparations made permanent (5). This method gave the best results with human chromosomes and was, therefore, mainly used. Some minor modifications were, however, tried. Thus, a slight staining in acetic-lacmoid after Feulgen often improved the staining of the chromosomes. Light green was also applied as a counterstain for Feulgen with good results (7).

To obtain a histological picture of the cases in question, paraffin sections (4 and 10 μ) were examined. The material for these was fixed in 10 per cent formol and stained with Feulgen, crystal violet, or van Gieson's triple stain (19). With the two latter methods, the spindle structure, which is completely invisible in squash preparations, is beautifully revealed.

The figures have been drawn with the aid of camera lucida with a ×25 ocular and ×90 objective, and the final magnitude is ca. ×2000. The photographs have been taken with a Zeiss camera using a ×18 ocular and ×90 oil immersion objective, the final magnification being ×1500.

The diagram in Figure 1 is based on a count of 200 dividing cells in each case. The shading illustrates the percentage of irregular divisions in which chromosomes were seen lagging outside the plates, irrespective of whether the cells were diploid or polyploid.

OBSERVATIONS

Chromosomes in normal cells.—To obtain a basis for the interpretation of the cytological phenomena characteristic of cancer, material from normal endometrium in the proliferation stage was examined for comparison. The most obvious feature of the normal chromosomes, as compared with
those in cancer cells, was their much better fixability. The stickiness so often described in cancer chromosomes is absent, and the chromosomes fix and stain excellently. Even with the rather crude method of fixation in 10 per cent formalin combined with van Gieson's staining, this difference is discernible. The divisions appear regular as a rule; no lagging chromosomes are visible either at metaphase or anaphase.

A normal metaphase plate containing 48 chromosomes is seen in Figure 2, illustrating the different chromosome types described in man (15). In accordance with earlier observations, we expected this chromosome number to prevail exclusively in the normal somatic cells of man. Our expectations were, however, not fulfilled. In a great number of endometrium cells the chromosome number was considerably lower than 48. In Figure 3, an example of a prometaphase stage with 18 chromosomes is seen. In addition to hypoploid cells, polyploid cells, which have presumably arisen through endomitosis, may also be observed in the normal endometrium.

It has usually been thought that chromosome numbers deviating from the normal are not found in somatic cells of an individual; and Koller (12)—to take one example out of many—especially stresses that aneuploid cells characterize malignant tissues. It may, however, be pointed out that grave doubts concerning the presumed constancy of the chromosome number in somatic cells of a given organism have been raised lately (cf., especially, Huskins [8]).

The present finding will be considered in more detail in another paper (Timonen and Therman, unpublished). In this connection it seems worth pointing out, however, what interesting implications this phenomenon has for cytology as well as ontogeny.

Cytology of cancer cells.—A characteristic feature of neoplastic tissue is that many more cells are seen dividing, as a rule, than in the corresponding normal tissue. In the normal endometrium in the proliferation stage, when the division rate is at its height, the frequency of mitosis is, however, about the same as in cancer tissue. Whether the frequency of divisions in cancer tissue depends on a greater number of dividing cells or on a change in the length of the mitotic cycle, or on both, could naturally be decided only from an examination of living cells.

A fact which is evident from fixed material is that the relative duration of the different mitotic stages in cancer cells is considerably changed. In Figure 1 we see the relative frequencies of prophase, metaphase, and anaphase stages, as determined from five cases of normal endometrium and five cases of cancer. In each case, 200 dividing cells have been counted. In the normal endometrium, the number of prophases slightly exceeds that of the metaphases, as a rule. In the neoplastic tissues, the number of prophases is greatly reduced relative to the metaphase stages. The relative number of anaphase stages shows also a tendency to decrease. Case 1149, in which the number was somewhat higher than the number of prophases, suffered from metropathia haemorrhagica. On the basis of the present observations, the ratio of prophases/metaphases would seem to be a fairly reliable indicator of malignancy—which might possibly even have practical importance.

The relative frequencies of the mitotic stages may be interpreted as indicating their relative duration. The relative duration of prophase stages is thus greatly reduced in cancer cells (cf. 19), and it seems fairly safe to conclude that their absolute length is also shortened. This would mean that the formation of the spindle is more rapid in cancer cells. Other facts are in good agreement with this assumption. In many cases half the metaphases displayed lagging chromosomes outside the plate (Figs. 4, 5, 23). The frequency of divisions exhibiting this kind of irregularity are indicated by the shaded columns in Figure 1. In case 1773, the divisions display so many kinds of irregularity that the metaphases and anaphases containing lagging chromosomes cannot be scored. Instead, the stippled parts of the columns show the frequency of multipolar divisions which constitute about one-half of the total.
FIGS. 2-3.—Cells of normal endometrium.
Figs. 4-10, cancer cells.
Fig. 2.—Normal metaphase stage with 48 chromosomes.
Fig. 3.—Hypoploid cell with 18 chromosomes.
Fig. 4.—Metaphase with lagging chromosomes.
Fig. 5.—Polyploid metaphase with lagging chromosomes.
Fig. 6.—Hollow metaphase plate.
Fig. 7.—A cell with 43 chromosomes showing the “colchicine-effect.”
Fig. 8.—Small tripolar metaphase.
Fig. 9.—Tripolar metaphase.
Fig. 10.—Tripolar anaphase displaying stickiness.
(Figs. 8 and 10, Fo, F; the others, AA, F; AA = acetic alcohol; Fo = formol; F = Feulgen.)
Figs. 11-17.—Cancer cells.
Fig. 11.—Pentapolar metaphase.
Fig. 12.—Quadripolar metaphase.
Fig. 13.—Quadripolar anaphase with lagging chromosomes.
Fig. 14.—Highly multipolar metaphase.
Fig. 15.—Degenerating nucleus, the chromatin in droplets.
Fig. 16.—Many-lobed giant nucleus.
Fig. 17.—Octopolar anaphase with lagging chromosomes.
(Figs. 11-13 AA, F; the others, Fo, F.)
In anaphase, the laggard chromosomes (Fig. 21) are either included in the separating chromosome groups or divide afterward. Even when no true laggards are seen outside the metaphase plates, the chromosomes show a much less strict orientation than in normal cells. This is especially clear in the polyploid plates in which the chromosomes often lie in several layers.

The failing orientation of the chromosomes naturally gives rise to the extremely variable chromosome numbers found in tumor cells (18, 12). The same variation in the chromosome number has been observed by us (e.g., Fig. 18). As described above, the lowered aneuploid chromosome numbers are not, however, restricted to malignant cells. The same is true of polyploid cells, except perhaps the very high polyploids. In Figure 24, a giant prophase stage is illustrated; it seems as if the chromosomes were in part in a state of fragmentation.

A further feature observed in the mitoses of cancer tissue is the occurrence of hollow metaphase plates (Figs. 6, 25), which are absent in the normal endometrium. We have observed all intermediate configurations between normal metaphase plates and completely hollow plates in which the center is without chromosomes. The hollow ring is often broken, also. The degree of hollowness is correlated neither with a small chromosome number nor with greater stickiness of the chromosomes. It may tentatively be suggested that even this phenomenon is ultimately caused by the rapidity of the spindle mechanism. Hollow spindles have been observed in man in the spermatogonial mitoses (9) and in the human pre-myelocytes as a result of pernicious anaemia (14).

The stickiness of the chromosomes in cancer cells has often been described. As stressed by Koller (12, 13), different tumors, and even neighboring cells in the same tumor, vary greatly in this respect. The stickiness of the chromosomes has been attributed to an excess of deficiently polymerized nucleic acid (11). As seen in the figures, the same variation in the stickiness of the chromosomes is evident in our material, too. It seems to us that no decisive role can be ascribed to the stickiness of the chromosomes—although often present in cancer cells—in the origin of cancer, since it is often absent and the chromosomes in certain cases may even display the contrary appearance of undercharging with thymonucleic acid. In this connection, the studies of Biesele (1) on the chromosomes in the lymphocytes of leukemic mice may be mentioned. These chromosomes, which showed an increased size as compared with normal lymphocyte chromosomes, shrank with pepsin digestion more than normal chromosomes. This indicates, in Biesele’s opinion, that the greater size of the former depends on an increase of nucleic acid as well as of pepsin-digestible protein.

A number of cancer cells exhibit features which closely resemble the effects induced by colchicine and other drugs. The chromosomes lie scattered around the cell, being at the same time more contracted than usual. In their division, they show failing synchronization. It is seen in many cells that a number of chromosomes have already divided, others are just dividing, forming typical “c-pairs,” while the rest show no signs of division (Figs. 7, 22). In these cells the spindle mechanism has evidently broken down, and the failing coordination leads to defective synchronization. Some of the neighboring cells in a tissue may show this “colchicine-effect,” while others appear unaffected. It might be thought that this phenomenon is a result of the abnormal metabolism in neoplastic tissues (6). The same may be the cause of the fragmentation of the chromosomes in cancer cells (14).

Favorable material for the investigation of multipolar mitoses was provided by a case of ovarian carcinoma (1773) in which one-half of all the mitoses seemed to be multipolar. In this carcinoma, all cell types from actively dividing diploid or hypoploid cells to giant multipolar cells evidently containing up to one thousand chromosomes were encountered. Although the giant cells often had several poles, multipolarity by no means always coincides with polyploidy. We have found very small tripolar metaphases (Fig. 8) as well as polyploid plates which are regularly bipolar (Figs. 5, 23). Regarding Figure 8, it may be mentioned that this cell was situated in a tissue strand which consisted entirely of very small, evidently hypoploid cells. This shows, in our opinion, that Koller’s idea (12, 13) that the hypoploid cells in cancer tissue are able to live only when supported by cells with higher chromosome number cannot be accepted as such.

The most usual type of multipolar divisions are triplolar divisions; examples are seen in Figures 8 and 20. A modification of this basic shape is seen in Figures 9 and 19. In this case, chromosome plates have been formed only between two of the three poles. Quadrilopar metaphases are illustrated in Figures 12 and 25, while Figure 11 provides an example of a pentapolar metaphase. In Figure 14 a highly multipolar metaphase stage is depicted. It is, however, too complicated to be analyzed in detail. In Figure 10 we see a triplolar anaphase in which the chromosomes are very
FIGS. 18-25.—Microphotos of cancer cells.
Fig. 18.—Hypoploid cell with 22 chromosomes.
Fig. 19.—The same as Fig. 9.
Fig. 20.—Tripolar metaphase.
Fig. 21.—Bipolar anaphase with laggards.
Fig. 22.—Cancer cell showing the "colchicine-effect"; some chromosomes are divided, others not.
Fig. 23.—The same as Fig. 5.
Fig. 24.—Giant prophase stage.
Fig. 25.—Quadripolar metaphase, at the left a similar tripolar configuration to that in Fig. 20, the fourth plate at the upper right hand corner is hollow. (AA, F.)
sticky. Between the separating chromosome groups, three chromatid bridges have been formed, of which two have broken and one has persisted. Figure 13 again provides an example of a quadrupolar anaphase, in which the chromosomes are slender and well fixed. Figure 17 again illustrates an octopolar anaphase. All the multipolar divisions, at least in anaphase, contain lagging chromosomes which evidently will fail to be included in the daughter nuclei. It may be mentioned, in passing, that Koller (12, Fig. 8) has depicted a stage which he interprets as a multinucleate cell, in which the chromosomes have formed metaphase plates synchronously. In our opinion, this figure represents a multipolar anaphase analogous to our Figure 17. In our material (especially case 1773) which is rich in multipolar configurations, the course of the divisions could well be followed. In addition, the synchronously dividing multinucleate cells—which are also found in our material—have thicker chromosomes, clearly differing from the more slender anaphasic chromosomes.

The multipolar anaphases form either several nuclei, in which case a multinucleate cell is often formed, or a many-lobed restitution nucleus (Fig. 16). It seems, however, that when the cell has reached a certain size it becomes inviable. In the giant multipolar metaphases it is often seen how the chromosomes begin to disintegrate, forming amorphous chromosome masses. A final stage in this series is seen when the chromatin collects into droplets (Fig. 15). The same phenomenon is reflected by Figure 1 (case 1773), which shows that the percentage of multipolar metaphases in the total is considerably higher than the percentage of multipolar anaphases. This suggests that a number of multipolar metaphases never reach the anaphase stage.

DISCUSSION

The precocity of the spindle mechanism.—The normal mitosis consists of two main processes: the reproduction and division of the chromosomes, and the functioning of the spindle mechanism. These may also be called the intrachromosomal changes as opposed to the extrachromosomal changes in the cell. In normal mitosis, these processes act in step with each other. The reproduction of the chromosomes in prophase (in this connection the much discussed problem of the actual time of reproduction of chromosomes is irrelevant) is regularly followed by spindle formation and the congregation of the chromosomes in the metaphase plate, where the “double” chromosomes divide.

Various deviations from the normal course of mitosis have shown that the spindle mechanism and the intrachromosomal processes are to a great extent independent of each other (3, 20). Of the regulated processes in which the spindle cycle and the chromosomes cycle are out of step, the most important is naturally meiosis. Regarding this interpretation of the meiotic phenomena, we might cite Darlington (3, p. 48): “This difference can be expressed by saying that in meiosis the changes outside the chromosomes are advanced in relation to the changes inside the chromosomes. In meiosis, as compared with mitosis, the external changes are precocious.” This precocity theory of meiosis has been further developed by Oksala (16) in his study concerning the premeiotic spermatogonial divisions in the dragonflies of the genus Aeschna. He comes to the important conclusions that precocity is a phenomenon which develops slowly during the last premeiotic divisions and that it implies more the premature beginning of the polarized stage in the cell, i.e., metaphase, than the premature beginning of prophase, as the precocity theory so often has been interpreted.

As pointed out by Oksala (16), not only meiosis, but a great variety of other cytological phenomena are explicable on the basis of changed timing relations between the intrachromosomal and extrachromosomal processes. We have, thus, all intermediates between endomitosis, which implies the division of the chromosomes without any division of the cell, and somatic reduction, in which the cells divide without any division of the chromosomes (8).

In our opinion, the cytological phenomena characteristic of cancer cells would best be explained along the same lines. Cancer tissue, as is well known, is characterized by an increased rate of cell division. This increased rate seems especially to be brought about by an acceleration in the cycle of the spindle. This, again, is shown by the greatly reduced duration of the prophase stage. The extrachromosomal processes in the cell are, however, not only absolutely accelerated. They also seem to be more rapid as compared with the intrachromosomal changes. In other words, as in meiosis, the spindle mechanism in cancer cells is precocious as compared with the chromosomal mechanism.

Naturally, the regularity governing the meiotic phenomena is absent in cancer, but the same tendency toward a precocity of the extrachromosomal changes is clearly noticeable. The spindle mechanism needs to be, in the beginning, only a little in advance of the chromosomal processes. This desynchronization grows, however, in the following divisions, leading finally to the gross abnormalities observed in cancer cells.

The precocity of the spindle mechanism is reflected also by the behavior of the chromosomes.
In most cases, a considerable proportion of the metaphase stages exhibits lagging chromosomes outside the plate. Obviously, the spindle has been too rapid for them. This happens in polyploid as well as hypoploid cells. The tendency of the metaphase plate to become more or less hollow may also be the result of the acceleration of the spindle.

The multipolarity of the divisions might be interpreted in the same terms. The occurrence of the very small tripolar and quadripolar divisions shows that these by no means always need to be the result of the formation of restitution nuclei. A small quadripolar division would arise when the precocity of the spindle mechanism has reached such a degree that the centrosomes divide twice while the chromosomes divide only once, and the occurrence of higher multipolars could be explained similarly.

As pointed out above, the irregular desynchronization of the different processes in the division of cancer cells leads to numerous cytological abnormalities. Thus, not even the centromeres divide simultaneously, and this results in the formation of the commonly observed tripolar divisions as well as the higher multipolar cells with an uneven number of poles. Comparable phenomena have been observed by Peters (17) in the cornea of Triturila during recovery from colchicine treatment. He ascribes the occurrence of multiple star-like configurations (p. 48) as “due to an abnormal increase in the number of centrioles or to a lack of coordination between the chromosomal cycle and the division of the centrioles.”

It was noticed quite early that certain cytological features in cancer cells resembled meiosis (11, 18). In the light of the present observations, this resemblance need not be so superficial as has been supposed (13). The common features might be brought about by the precocity of the spindle mechanism shared by cancer cells. The stickiness of the chromosomes in cancer cells might be caused by the same factors which give rise to the somewhat similar appearance of the meiotic chromosomes. The behavior of lagging chromosomes at metaphase and anaphase exhibits features similar to univalent chromosomes in meiosis.

It cannot be denied that a number of the cytological phenomena observed in cancer are seemingly in contradiction to the precocity of the spindle postulated by us. Thus, for instance, the formation of polyploid cells through endomitosis is a process pointing in the opposite direction. It must, however, be remembered that polyploidy due to endomitosis is not uncommonly found in normal tissues of various organisms. Certain other phenomena, e.g., the “colchicine-effect” and the fragmentation of chromosomes, we ascribe to secondary changes in the tumor tissue due to abnormal metabolism.

Theories concerning the origin of cancer.—The various theories concerning the origin of cancer agree in that they all postulate a somatic mutation in the widest sense of the word. Such a mutation might be: (a) genic, depending on a gene mutation; (b) chromosomal, being the result of a change in the number or structure of the chromosomes; (c) heterochromatic, being brought about by a change in the amount of heterochromatin; or (d) plasmatic, implying that the change has taken place in the so-called plasmagenes. According to the first three hypotheses, the change which leads to the development of cancer takes place in the chromosomes, whereas according to the fourth it is to be sought in the cytoplasm.

The chromosomal-mutation theories of the origin of cancer may be traced back as far as Boveri (references to the older literature in 18 and 21), who assumed that the varying chromosome numbers observed in cancer cells were the cause of their malignancy. A serious objection may be raised to all the theories involving the chromosomes that, since actively dividing cancer cells display chromosome numbers ranging from 12 to very high polyploids, a chromosomal change can hardly be thought to be the ultimate cause of their malignancy (4). This argument gains further important support from our present observation that the chromosome numbers also vary greatly in cells of normal tissue.

According to the more recent hypothesis advanced by Caspersson and Santesson (2), the origin of cancer is to be sought in a change of the heterochromatic portions of the chromosomes. This hypothesis is based on spectrographic determinations of the various cell components which have shown that malignant cells contain more nucleic acids than normal cells, and that the rate of protein formation is also increased in them. Since, according to Caspersson and his associates, the nucleic acid metabolism and protein formation in the cell are governed by the heterochromatic portions of the chromosomes, Caspersson and Santesson have come to the above conclusion. This hypothesis is, however, open to severe criticism. The whole concept of heterochromatin seems to be so diffuse and poorly defined, being used by different authors in different senses, that such a hypothesis cannot be based on it. Especially as regards the role of heterochromatin in human cells, it is cytologically as well as physiologically all but unknown. The most important reason for rejecting this hypothesis is, however, the same as that presented above.
in regard to the other theories of the origin of cancer based on chromosomal changes.

If we consider the cytological observations made on cancer cells, we find that the most constant feature characterizing all the cases studied is the general acceleration in the division rate. This is connected with the accelerated rate of the spindle mechanism. The other cytological peculiarities seem to be more or less directly caused by this primary change. All these data best agree with the plasmagene theory of the origin of cancer proposed by Darlington (4). According to this theory, the origin of cancer is to be sought in a mutation of the plasmagenes. This would bring it into relation with genes, plasmagenes, proviruses, and viruses, which form an integrated system from heritable particles, the genes, to viruses capable of infection. It is noteworthy how well our observations fit in with Darlington’s conclusions arrived at from quite a different angle. Indeed, we might say that our data furnish the necessary illustration and proof for the following assumption presented by Darlington on theoretical grounds (4, p. 124): “In cancer I am supposing a cytoplasmic change which favours a high growth rate. In these circumstances both the nucleus and certain cytoplasmic constituents might well be unable to stand the pace.”

SUMMARY

1. The chromosomes in 35 cases of normal endometrium in the proliferation stage have been studied. In addition to the normal chromosome number, 48, both polyploid and hypoploid cells have been found.

2. The chromosome conditions in 174 cases of carcinoma of the female genital tract have been examined. Here also, cells from low hypoploids to very high polyploids have been observed. Other phenomena characterizing cancer cells are the occurrence of lagging chromosomes in the divisions, multipolar spindles, stickiness of the chromosomes, and the “colchicine-effect.”

3. The most constant features noticed in cancer cells are, however, the increased division rate of the cells and the changed relative duration of the different mitotic phases, as determined from their relative frequencies. In normal cells the prophase is usually a somewhat longer stage than the metaphase. In cancer tissue, it is much shortened—the metaphase, in this case, being of considerably longer duration.

4. On the above facts we present the following theory: The primary cytological change in cancer cells consists in an accelerated rate of the spindle mechanism. The intrachromosomal changes do not stand the pace, and thus the extrachromosomal changes become precocious in relation to them. From this original change, most of the other chromosomal aberrations can be derived.

5. Of the various theories concerning the origin of cancer our observations agree best with Darlington’s plasmagene theory.

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