Mitotic Cell Studies Based on *in vivo* Observations VII. The intrinsic nature of cancer cells and of carcinogenesis as interpreted from the viewpoint in reference to cell biology

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Introduction

The cause of carcinogenesis still remains as one of most difficult unsolved problems, but all cancer investigators recognize the fact that cancer cells unrestrictedly can increase by cell division without entering into cell differentiation. However, they know very little about the recent advances in knowledge of the mechanism of cell division. The concept of mitotic spindles established on the data of fixed preparations had led biologists into the misconception that nuclear membranes break down at the end of prophase and that spindles appear in the cytoplasm at metaphase with or without the aid of centrioles. This idea cannot hold true for mitosis in eukaryotes to-day (Wada 1950, 1966, 1969a, 1970b).

The nuclear origin of the spindle and its independency from the cytoplasm in higher organisms were first proposed by Fujii (1931) as the theory of atractoplasm. Much evidence corroborative of this theory has been reported from *in vivo* observations in plant and animal cells, and also in tumor cells (Wada 1941, 1955, 1969, Wada and Fukunaga 1957, Wada and Kusunoki 1964a, b, Wada, Hanaoka and Niitsu 1964). Recently, Sprey and Hasche (1972) has demonstrated by freeze-etching electron microscopy the fact that the spindle is enveloped in a biological membrane transformed from the nuclear membrane in *Lepidium* root-tip cell mitosis. This spindle envelope maintains the fine pore membrane structure generally demonstrated in the eukaryote resting nuclear membrane.

Unfortunately, the atractoplasm theory has been held at a distance by editors of current cytology books, because of their unyielding adherence to the old concept of the breakdown of the nuclear membrane before the spindle appearance. No cytology books have dealt with spindles as independent intracellular organs, nevertheless the term 'spindle body' is used commonly in scientific papers.

There are at least two fundamental reasons for which the nature of cancer cells has been left as continuing puzzle for mankind at present. One is the misunderstanding of the mitotic spindles and the second, a servile adherence to what may be termed the authorized concepts in medical fields.

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Detailed exposition

1. *Disregard for the independent spindle body renders the cause of carcinogenesis inaccessible*

In spite of conspicuous advances in biology accomplished in this century, the explanation of the mechanism of mitosis has been left incomplete. Current cytology books state no consolidated principle about the mechanism of mitosis in all the eukaryotes. This deficiency of knowledge about the morphology and the function of the spindle body has brought about great confusion concerning the intrinsic nature of cancer cells and of the cause of carcinogenesis.

Fig. 1. Schematic drawings of anaphase spindles in eukaryote mitotic cells. The upper, anaphase spindles appearing after the break down of nuclear membrane described in current cytology books. a and b, exceptional cases of spindles in protists because of a maintenance of the nuclear membrane during mitosis. c, spindle in animal cell called a mitotic apparatus. The spindle is covered with centrioles and asters. d, spindle in a higher plant cell in which no centrioles and asters appear. The lower, the fine structure of anaphase spindles based on the attractoplasm theory. a, b, c, and d, showing spindles in all eukaryote cells which are tactoids composed of parallel dispersed fibrils of nuclear sap colloidal sol. Each spindle is a system independent of the cytoplasm and is enveloped in a nuclear membrane (a and b) and in a biological membrane derived from the nuclear membrane (c and d).

According to the attractoplasm theory, metaphase spindles in plant and animal cells irrespective of higher and lower organisms appear as a tactoid composed of parallel arranged dispersed fibrils of a protein colloidal sol of nuclear sap, and are enveloped in a biological membrane derived from the nuclear membrane (Wada

1950, 1966, 1970 a, b). The fundamental reason for the fusi-form condition of all eukaryote mitotic spindles may be attributed to the tactoid formation of nuclear sap colloidal sol (Fig. 1). No consolidated principle applying to spindle formation in all eukaryotes has ever been described in current cytology books.

In living cells, it is obvious that the spindle is an independent organ of nuclear origin and behaves as a system independent of the cytoplasm. It is an absolutely necessary organ for transporting the separated daughter chromosomes to each spindle pole and for making two independent daughter cells. In other words, no cell proliferation would occur without the aid of spindle activity. The doubling of the DNA content in the mitotic cycle is one of the necessary conditions, but not an absolute one, to induce cell proliferation, e.g. appearance of endomitosis, chromosome doubling by inactivation of the spindle treated with colchicine. On the contrary, mitosis is repeated at the beginning of fertilized egg cells by aid of spindle activity without increasing the DNA contents in each cell (Hoff-Jørgensen 1954, Hinegardner *et al.* 1964). In short, to inquire into cancer cell proliferation without an understanding of the entity of the mitotic spindles is like fishing in the air.

Paradoxically speaking, all cancer research hitherto promoted by medical investigators, biologists or biochemists, without their taking the spindle activity into consideration has proved to be a failure in achieving any significant solution to carcinogenesis in this century.

2. *The relationship of spindles between normal cells and cancer cells is compared to that between atoms and their isotopes*

The chemical properties of atoms and their isotopes are similar but their nuclear constitutions are different from each other. In biology, the spindle activity of normal cells and of cancer cells is similar but their genic constitutions are different from each other.

After the repetition of the mitotic cycle several times, normal cells cease mitosis and enter into cell differentiation, but after the repetition of the same number of mitosis, cancer cells can continue the mitosis further, without entering into cell differentiation. *In all eukaryotes, both the cell proliferation and the cell differentiation take place antagonistically.* Therefore, in normal development of tissues or organs, at least two kinds of genes are necessary to control the spindle formation: the one is a gene or genes to induce spindle formation and the other to repress spindle formation.

In the process of tissue development, beyond the repetition of the mitotic cycles several times, there are no more repetition of mitosis: then mitosis ceases and the cells enter into differentiation. Accordingly, in normal growth and differentiation of tissues or organs, if an absence of the control power governing the cessation of the mitosis would occur, such tissue cells would continue mitosis without entering into cell differentiation. The growth of such tissue cells may show a well known cancer specific character. Therefore, it becomes clear that the way to find out the cause of carcinogenesis may be reduced to an inquiry into those genes which give information to continue the spindle formation without bringing mitosis to a halt. In other words, it may be to delete the activity of the spindle

formation repressor genes from normal tissue development.

According to the atractoplasm theory, the most important genes which participate in mitosis are those giving information for spindle formation. In cell divisions, chromosomes without aid of spindle activity in anaphase can neither migrate toward spindle poles nor form independent daughter nuclei. We call the genes which give information to form the spindle body, for convenience, as spindle formation inducer genes (SFI genes). On the other hand, in the case of tissue development, genes which give information to repress the spindle formation may become the starting point to bring the meristematic cells into cell differentiation. These genes are designated as spindle formation repressor genes (SFR genes).

In normal tissue cell mitosis, both the genes, SFI and SFR, may act in harmony for cell proliferation and cell differentiation. In the case of cancer cells, it is assumed that the cells exclusively continue mitosis without pulling the SFR genes into action. Starting the conclusion first, cancer cells are put into a state of deficiency with reference to SFR genes. This is a feature of cell biology in cancer cells. The role of SFR genes in mitosis is clear but the entity of the SFR substance, its locality in a cell, and also the products induced as a result of the deficiency of the SFR genes are unknown features at present.

Ultimately, it may be concluded that the difference of genic characters between normal cells and cancer cells is attributed to the presence or absence of SFR gene activity (Wada 1966, 1970b). Accordingly, the cause of carcinogenesis is by no means an exceptional case different from the biological doctrine of autonomous morphogenesis under control of genes. This clear and logical explanation for carcinogenesis has been concealed beneath the misinterpretations on the spindle body described in current cytology books. The above is the first reason that the cause of carcinogenesis is still to-day a problem unsolved.

It is the author's long-cherished conviction that any fundamental solution of cancers is impossible without an elucidation of the mechanism of mitosis. On the basis of the new blue-print on the mechanism of mitosis, the author has proposed a new interpretation of cancer cell development from the result of studies on *Stizolobium* tumor induced artificially (Wada 1966). In this paper, the author's interpretation on carcinogenesis is proposed with a revision of the prevailing insufficient explanations and with taking recent findings on cancer cell problems into consideration.

3. *Somatic cell mutation theories about carcinogenesis*

Already several reports have indicated that cancers are induced by somatic cell mutations. An incipient cancer cell, a stem cell (Makino 1956), removed from and cultured outside of a cancer-bearing animal can repeat mitosis and transmit its genetic characters to the descendant cells from the incipient cell. The grouping of such descendant cells develop into a tumor. Also, by transplanting a cancer cell to a new animal or by metastasis in a human body, a new cancer appears. From the results of these experiments, not a few investigators have concluded that cancers appear as a result of somatic cell mutation. However, they have not yet succeeded convincingly to point out any gene which participate in carcinogenesis.

Comparing the somatic cell mutation theories mentioned above with Huebner's viral oncogene theory (1972), the latter is too abstract in spite of the massive data on the virus cancers investigated. Viruses, as president in various organ tissue cells, have no relation to unrestricted repetition of mitosis without cell differentiation. From the viewpoint of cell biology, the viral oncogene theory is not acceptable. That the viral oncogene of RNA tumor virus genomes inherited as part of the natural DNA component in vertebrate cells is still maintained in the chromosomes of humans is biologically unthinkable, because there is no reason that humankind which stand on the summit of the evolution of organisms would cherish such a malignant gene. The evolution in biology is nothing but improvement toward the teleonomy.

All kinds of hereditary diseases may happen to appear by the mutation of benign gene or genes, e.g. hemophilia, phenylketonuria, color blindness, etc. Diseases of this group occur rather rarely. The genes mutated participate in a very important role of life. Another group of diseases related to gene mutations are mongolism, Klinefelter's syndrome, Turner's syndrome, etc. In this second group, mutations occur as a result of chromosome aberrations due to failure of somatic cell mitosis probably during embryogenesis. These diseases appear more frequently than those of the first group.

The third group of diseases related to gene mutations are represented by all kinds of cancers. Mitoses take place in all kinds of tissues and organs not only in the embryonal state but also in a grown differentiated tissue cell state for renewing the tissue. Accordingly, it is natural that cancers appear in almost all tissues and organs as a result of somatic cell mutations. Moreover, the unusually frequent occurrence of mitosis is expected in such organ tissues suffering from endogenous or exogenous injuries, from aging, artificial treatments or from intemperances. Viruses detected in various organ tissue cells may have a *raison d'être* for themselves but may have some injurious effects on the nucleic acid synthesis of host cells.

4. *Considerations concerning the mechanism of carcinogenesis due to somatic cell mutation*

In the previous chapter, the author has pointed out the genes which give information to bring cells into proliferation and differentiation and concluded that the deficiency of SFR genes is the immediate cause for all kinds of cancers. In this chapter the author explains the processes by which this deficiency may be induced as a result of recombination errors at the time of DNA replications in the S-phase of the mitotic cycle.

Cell conditions under which the DNA recombination errors would occur are extremely diverse. Almost all experiments hitherto carried out to elucidate the cause of carcinogenesis have failed to achieve their purpose but have nevertheless revealed unexpected results which have clarified the diversification of cell conditions under which gene mutations would be induced at the time of the DNA replication in tissue cell mitosis.

Tissue cells suffering from both endogenous diseases, or senile decaying, and

also from exogenous diseases, or from chemical, and physical treatments or from any of many other carcinogenic agents, both partly die and partly become necrotic. The nuclei of these wounded cells generally disintegrate or break down into various grades of DNA fractions. In addition, tissue cells attacked with viruses contain not only heterogenous RNA or DNA different from the nuclei of host cells but also disintegrated DNA fractions. Under these cell conditions, it is assumed that various disintegrated nucleic acid fractions can diffuse from nuclei into the cytoplasm and further infiltrate into cell nuclei surrounding the wounded cells.

On the other hand, wound-healing tissue cells appear around wounded cells and actively repeat mitosis for repairing the wounded tissues. The most important cell biological phenomenon occurring around the wounded tissue cells is that the wound-healing tissue cells which actively repeat mitosis contain various grades of DNA fractions or heterogenous nucleic acid fractions transferred from cells attacked by viruses. Under such cell circumstances, it is highly possible to assume that DNA recombination errors would occur in the S-phase of mitotic cycle more frequently than in renewals of normal tissue cells. Accordingly, it is not too emphatic to say that all experiments hitherto engaged in for inquiring into the cause of carcinogenesis did nothing but to reveal diverse cell conditions or to provoke conditions under which the occurrence of somatic cell mutations by DNA recombination errors were more greatly accelerated.

In the situation of a coexistence of dead or necrotic cells together with mitotic cells of wound-healing tissue, these cell groups together likely function as a crucible in which the replication of DNA, copy-choice errors or recombination errors of genes would take place in the S-phase of mitotic cycles, and various kinds of somatic cell mutations would be produced in high frequency (Fig. 2).

Among various kinds of somatic cell mutations produced in the cell crucible, a cell which by chance suffers from a deficiency of SFR genes is considered to become an incipient cancer cell or stem cell. And this mutant cell alone, without fail, can survive by repeating mitosis, although the fate of many other mutant cells is unknown. In the field of medicine, the above mentioned cell biological phenomena are a short cut to the causes of carcinogenesis and as stated by Harris (1962): "the tumors appear to arise from areas of cellular repair and regeneration following the destruction of tissue by carcinogen".

As the result of cell biology investigation, it becomes clear that an incipient cancer cell is a somatic cell mutant and diverse carcinogens and viruses, which hitherto for a long time have been believed to be the causes of carcinogenesis are nothing but ways and means for giving rise to cell conditions under which the frequent occurrence of recombination errors would be induced at the time of chromosome DNA replication.

Therefore, it is concluded that the chemical applications or physical treatment which bring about those cell conditions for facilitating carcinogenesis have common properties for disintegrating the nucleic acids of treated cells into various grades of DNA fractions; and those chemicals which kill or coagulate the nuclei of treated cells instantly may as a whole lack this aptitude for carcinogenesis.

5. Denial of a plurality of cause for carcinogenesis

As stated in the foregoing chapter, the cause of cancers is but a single and common one for all kinds, i.e. the deficiency of SFR genes. However, the cell conditions, under which somatic cell mutations would be brought about, are extremely diversified as stated before. Now this mistaking of the diversification of cell conditions as being the cause of cancers has induced a firm belief in a plurality

Fig. 2. Schematic drawing of the development of tumor tissue in *Stizolobium* induced artificially. Arrows indicate growth directions of the tumor and the tumor-bearing tissue cells. 'A' is the initial wounded zone. 'B' the necrotic zone: disintegration of nuclei and diffusion of chromatin granules or DNA fractions conspicuous even in the cytoplasm. 'C' the zone of repair: many cells are in mitosis and in them disintegrated DNA fractions are also visible. 'D' the mutable zone: inner border layer of tumor tissue, where cancer incipient cells induced by DNA recombination errors repeat the mitosis. 'E' the tumor zone: out-growth of incipient cancer cells without their entering into cell differentiation (Wada 1966).

of cause for carcinogenesis in the field of medicine. It is this belief which is the second reason for the cause of carcinogenesis being left as an unsolved problem to-day.

The above mentioned mistake is mainly attributed to an insufficient analysis of the gene activities among the stem cells, the tumor cells and the tissue cells of cancer-bearing organs. Intrinsically, the genomes of these three cell groups are same. However, their phenotypes show conspicuous differences with each other.

Tumors are composed of a massive aggregation of a great number of stem cells, which have become enlarged. Corresponding to their cell aggregation, tumor cells alter their characteristics from those in the original stem cells as well as in the tissue cells of tumor-bearing organs with reference to morphology, physiology, and immunology.

The different phenotypes among these three cell groups are, of course, caused by alterations of gene activities, in accordance with the alterations in enzyme activities, the latter being a result of those alterations in the genes in the active or in the dormant states. It is obvious that different metabolites which may respond immunologically, are produced among the three cell groups. In a special way, the suppression of gene activities participating in cell differentiation may alter greatly the kinds of active or of dormant genes, and produce special metabolites different from those in tumor-bearing organ tissue cells.

The series of alterations in gene activities among stem cells, tumor cells, and tissue cells of tumor-bearing organs takes place genetically in all kinds of cancers, such as in the stomach, the liver, the lung, the skin, and so on respectively. It is, therefore, clear that different characteristics or immunological responses in different cancers are manifested by a shift in the activities of intrinsic genes of the cancer-bearing organs but not by the presence of cancer-specific genes or enzymes. Based on this cell biology consideration, the author denies a plurality of cause for carcinogenesis.

The causal, analytic studies on cancers have been promoted widely in medicine, biology, biochemistry, virology, pharmacology, immunology, and other fields. Treatment with chemicals, physical agents, radiations, infection of viruses, analysis of karyotypes of cancer cell chromosomes, induction of unusual cells in cell cultures by treatment with carcinogens, detection of special nucleic acids in various cancers, and control of DNA duplication with chemicals, etc. . . . all these have no logical relation to elucidate the cause of carcinogenesis but are only serviceable to help an understanding of tumor cell characteristics by secondary or tertiary alterations caused by a massive aggregation of cancer cells.

At present, projects for cancer research are highly specialized and push into the minutest details in each specialized field and each investigator is inquiring into the cause of carcinogenesis, each in his own specialized field with very little attention to other fields. So long as any aspect in cancers is a manifestation of cell life, all the findings at molecular levels in each specialized field of research should be re-investigated by synthesizing them *morphologically*, to constitute the molecular organization of the cancer cell backed by information from the genes. In conclusion, all the organization of cancer cells is nothing but biological phenomena which take place in the frame of autonomous morphogenesis according to the principle of 'chance and necessity' (Monod 1972) in the evolution of organisms.

6. *The meaning from the aspect of cell biology of dedifferentiation in cancer cell with special reference to their genotype and phenotype*

In the field of medicine, cancer cells are dealt with as dedifferentiate or non-differentiate cells, and they are also considered to be in an embryonal state. Very

few genetic considerations are given to the dedifferentiation phenomena of cancer cells and cancer-bearing organ tissue cells. As already stated, the spindle formation is an autonomous morphogenesis controlled by genes (SFI genes). And the development of normal tissues in each organ is due to the balanced activities of SFI and SFR genes. In the case of cancer cells, the dedifferentiation starts from a deficiency of SFR genes in the cancer-bearing organ tissue cells, but all other genes in the cells do not suffer from any aberrations. Moreover, the dedifferentiation of organ tissue cells is by no means a specific event confined to cancer cells alone.

In the case of normal organ tissue cells, the tissue renewal of each organ starts from the dedifferentiation of tissue cells which form a karyokinetic spindle as a result of SFI gene activity. The SFI gene activation is caused by stimulation or wound-hormones correspondingly to internal or external demands placed on the organs. The renewal of organs is then accomplished by cell proliferation and by organ-specific cell differentiation. The difference of dedifferentiation between cancer cells and normal tissue cells is genotypically confined to the presence or absence of SFR gene activity. From the cell biological viewpoint, it is concluded that the dedifferentiation of organ tissue cells is itself by no means malignant or benign, so long as the SFR genes act in harmony with the SFI genes.

Most of the cancer specific phenomena investigated by doctors, biochemists, enzymologists, or immunologists are introduced by special environments brought about as the result of a massive aggregation of cancer cells in a grown tumor but not showing the sum of the individual cancer cell characters. An aggregation of a great number of cancer cells in a grown tumor brings about, as a whole, a new morphology and also show various kinds of new physiological activities to assist in keeping cell life in the new environment.

The metamorphosis from cancer cell aggregation to a tumor appears mainly as a synthesized result of the continuous cell proliferation, the effects of surrounding organ tissues, and the suppression of cell differentiation. Corresponding to these environmental changes, some of the genes in the tumor cells are newly activated and some of the others become dormant. Accordingly, the various characteristics investigated in tumor cells are different from those in tumor-bearing organ tissue cells in their physiology, their enzymology, or their immunology. However, all these special characteristics of tumor cells are caused by renewed activities of the intrinsic genes in organ tissue cells, but are not caused by cancer-specific genes or enzymes.

Problems on dedifferentiation in tumor cells have hitherto been discussed without considering the genotypic relations among tumor cells, cancer cells, and organ tissue cells. This is a third reason which adds confusion to the attempts to clarify the entity of cancer cells and the cause of carcinogenesis at the present stage. Most recently, immunological research on cancer cells was greatly expected to offer a solution and the medical treatment for cancer problems. This research has revealed the presence of high sensitive proteins for immunity in tumor cells. Many of the findings recognized in tumor cells have been discussed as cancer-specific immunity phenomena. However, a fresh cancer cell group composed of a small number of cells shows no immunological responses. This contradiction in im-

munity may prove the truth that the immunity believed to be cancer-specific is nothing but that produced by newly activated intrinsic genes of the cancer-bearing organ tissue cells as mentioned above. In other words, no new substances responding to immunity have been produced in young, fresh tumor cells which have not yet suffered from alterations in morphology and in physiology caused by a massive aggregation of cancer cells.

The only genotypically different substance between incipient cancer cells and cancer-bearing organ tissue cells is confined to the substance produced as a result of the deficiency of SFR genes. In future, if such a substance would be determined immunologically and isolated biochemically, mankind would be rid of the fear of cancers.

7. *The roles of nucleoli in spindle formation*

Current cytology books depending on fixed figures refer to no relationship between the spindle formation and the nucleoli, the latter which disappear before the appearance of the spindle. Complicated electron microscopic figures of nucleoli in resting nuclei, and the distribution of RNA and DNA shown with autoradiographic treatments are important descriptions of nucleoli, and many inferences are discussed about the role of the nucleolus.

The so-called mitotic apparatus proposed by some animal cytologists and its biochemical interpretations have brought about a strained judgement on the spindle formation which completely disregards the unitary composition of karyokinetic spindles both in the higher and the lower organisms (Wada 1972a). It is a well known fact that spindle bodies at metaphase in all the eukaryotes fundamentally appear as fusi-form, independent of the presence or the absence of centrioles (Fig. 1). The trigger for the transformation of the nuclear sap colloidal sol into a fusi-form or a tactoid is considered to be due to the disintegration of nucleoli, and especially to the release of RNA at the end of prophase (Wada 1966). The tactoid formation of nuclear sap colloidal sol is strongly supported by the birefringent figures of plant and animal metaphase spindles demonstrated under the polarization microscope in living cells (Inoué 1953).

The cause and effect relation between nucleoli and spindles is clearly demonstrated by the disintegration of nucleoli and the appearance of spindles at metaphase and vice versa at telophase. This may be strong enough evidence to verify the fact that the RNA in nucleoli plays a role as a catalizer-like reaction to transform the dispersed protein globules in the nuclear sap colloidal sol into fibrils.

In other words, the nucleoli in each resting nucleus of meristematic cells and also in the cancer cells may be reservoirs of substances which mainly participate in the transformation of nuclear sap colloidal sol into a tactoid. Accordingly, the nucleoli in meristematic cells or in cancer cells are generally conspicuous in their size or number, while the nucleoli in senile cells or differentiated grown tissue cells generally diminish in size and number or degenerate. The biological meanings of these facts are disregarded in current cytology books. With regard to the role of the nucleoli, the author emphasizes the reinvestigation of nucleoli as a reservoir of spindle formation inducer substance or the precursor of such substance.

On the other hand, it is a very difficult problem to determine when the spindle formation repressor substances would be produced and where they would be stored, whether in the nuclei or in the cytoplasm. And also concretely unknown is the mechanism which prevents mitotic cells from entering into cell differentiation through the deficiency of SFR genes. In immunological studies on cancers, the findings about the α -fetoprotein or the carcinoembryonic antigen discussed in some kinds of cancer-bearing organs are considered to have some relation to a materialistic explanation for the effect due to the deficiency of SFR genes.

All the problems concerning biochemical, biophysical and immunological properties of the spindle formation inducer substances, the repressor substances as well as the substances produced as a result of the deficiency of SFR genes remains as fields to be explored in future studies.

In the beginning of this paper, the author has compared the relationship of spindles between normal cells and cancer cells to that between atoms and their isotopes. The substantial difference in the spindles between normal cells and cancer cells is inferred to be concealed in the constituents of the nucleoli, just as the difference of atoms and their isotopes is confined to the constitution of their nucleus.

8. *Problems brought up by the somatic cell mutation theory of carcinogenesis*

The interpretation according to which the cause of cancer is attributed to a mutation of genes which control spindle formation does lay a new base line from which to reinvestigate cancer problems cell biologically. The true state of the cancer problem is hitherto being groped for in darkness. In spite of diversified research projects in modern scientific fields, no cancer investigators have ever considered the entity of spindles which are independent, absolutely necessary organs for making one cell into its two independent daughter cells by mitosis, e.g. for cell proliferation.

According to the atractoplasm theory, the role of the spindles in mitosis, their developmental process, the colloidal properties, and also role of nucleoli with regard to the spindle formation are explained in the foregoing chapters. However, quantitative studies on various properties of the spindle at the colloid chemical, biochemical, or biophysical levels still offer no solutions.

As a precondition for the analysis at the molecular level, it is strongly required that new experimental techniques be developed which isolate the spindle tactoid from the surrounding cytoplasm as a complete entity. Until this technical development is achieved, no biochemical studies on spindle bodies will be significant for cell biology. When this new technical success is achieved, the studies on karyokinetic spindles will bring new light to the knowledge of the spindle formation inducer substance and the repressor substance. And in addition epoch-making progress on cancer studies will be expected in the field of biochemistry, pharmacology or immunology. The so-called isolated mitotic apparatus offers no contributions to the exact analysis of the spindle body (Wada 1969a, b, 1972a, b).

The development of new techniques to isolate nucleoli completely free from the nuclear elements may also be an important factor for distinguishing the nature

of cancer cells from normal organ tissue cells. In addition, too, nucleolar substances may offer the possibility of a suggestion about the nature and distribution of both the spindle formation inducer substance and the repressor substance.

Therefore, in exploiting new fields on the subject of cancer, it is most important both to develop new, original experimental techniques and also to be released from various authorized concepts and circumstances perduring from the past into present.

Concluding remarks

The extensive contributions to cancer problems carried out by a great number of investigators at enormous research expense during this century have not yet brought any fundamental solutions to the cause of carcinogenesis. The coexistent intrinsic nature of all cancers is the continuous repetition of mitosis without any entering into cell differentiation.

From the cell biology viewpoint, it is a well known fact that cell differentiation takes place antagonistically toward cell proliferation. The latter starts from cells activated under the information of spindle formation inducer genes. Consequently, to elucidate the cause of carcinogenesis, all research efforts should be centered, first of all, on clarifying the nature of the mitotic spindles. This suggestion may be like 'the bolt from the blue', because mitotic spindles have hitherto been scarcely taken into account by cancer investigators and only very sparingly in the cytology books of to-day.

With regard to cell proliferation in normal tissue cells or in cancer cells, the karyokinetic spindle is a pivot of the mitotic activity and *an absolutely necessary organ to divide a cell into two independent daughter cells*. Nevertheless, studies on cancers have been pushed forward without making any searching inquiry into the entity of the mitotic spindles. It may be a fruitless project to continue in various research fields investigations on cancers while simultaneously looking upon the role of the spindles as something extraneous and unrelated. This passing over of the spindles is fundamentally attributed to those incorrect explanation of mitotic spindles described in current cytology books.

Reinvestigating the studies previously conducted on carcinogenesis, the author is not inclined at all to reproach cancer investigators for their out of focus contributions but thinks rather that the editors of cytology publications and books should assume the responsibility for stoutly holding to the assumption of the breakdown of the nuclear membrane in mitosis without publicizing adequately during these more than forty years the atractoplasm theory.

Summary

1. According to the atractoplasm theory, the cause of carcinogenesis is ultimately attributed to a deficiency of SFR genes which give information to repress the spindle formation for the cell differentiation.

2. This deficiency of SFR genes takes place as a result of somatic cell mutation induced by DNA recombination errors.

3. All the agents and the treatments, and also the cells attacked by viruses,

all of which heretofore have been considered to be the causes of carcinogenesis are nothing but the causes inducing cell conditions under which various grades of DNA fractions would appear in the disintegrated nuclei of wounded cells. These DNA fractions permeating into neighbouring mitotic cell nuclei of wound-healing tissue participate in giving rise to DNA recombination errors.

4. Among various kinds of mutant cells induced by recombination errors, those cells encountered having a deficiency of the SFR genes by chance become incipient cancer cells or stem cells.

5. The genomes of the stem cells, the tumor cells and the tissue cells of tumor-bearing organs are the same, but the first two cell groups have a deficiency of SFR genes.

6. Corresponding to the increase of a vast number of cells in growing tumors, the morphology and the metabolism of the tumor cell groups alter conspicuously, and the kinds of genes in active or dormant states alter as well. Accordingly, many newly produced substances appear differing from those in tumor-bearing organ tissue cells, as well as from those in incipient cancer cells. Therefore, various immunological reactions found in tumors imply neither the evidence for the presence of cancer-specific substances nor that for a plurality of cause for carcinogenesis.

7. The only cancer-specific substance which should be determined immunologically and isolated biochemically is logically the substance which may be produced as a result of a deficiency of SFR genes.

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