Cell transformation in tumor-development: a result of accumulation of Misrepairs of DNA through many generations of cells

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Abstract

Development of a tumor is known to be a result of accumulation of DNA mutations in somatic cells. However, the processes of how DNA mutations are produced and how DNA mutations accumulate in somatic cells are not yet clear. There are several types of DNA mutations; however point mutations are the main type of mutations that can remain and accumulate in cells. Severe DNA injuries are the causes for DNA mutations; however Misrepair of DNA is an essential process for transforming a DNA injury into a “survivable and inheritable” DNA mutation. In somatic cells, Misrepair of DNA is the main source of DNA mutations. Since the surviving chance of a cell by Misrepair of DNA is low, accumulation of DNA mutations can take place only possibly in the cells that can proliferate. Tumors can only develop in the tissues that are regenerable. The accumulation of Misrepairs of DNA needs to proceed in many generations of cells, and cell transformation from a normal cell into a tumor cell is a slow and long process. However, once a cell is transformed especially when it is malignantly transformed, the deficiency of DNA repair and the rapid cell proliferation will accelerate the accumulation of DNA mutations. The process of accumulation of DNA mutations is actually a process of aging of a genome DNA. Repeated injuries and repeated cell regenerations are the two preconditions for tumor-development. For cancer prevention, a moderate and flexible living style is advised.

Keywords

Tumors, tumor-development, DNA mutations, accumulation of DNA mutations, cell transformation, properties of tumor cells, Misrepair, accumulation of Misrepairs, aging, Misrepair-accumulation theory, Misrepair of DNA, regenerable cells, deficiency of DNA repair, malignant tumor, aging of a genome DNA, self-accelerating, in-homogenous, and cancer-prevention

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Tumors, called also neoplasms, have two groups: benign tumors and malignant tumors. Those tumors that develop from epithelium are called carcinoma, which include pre-malignant carcinoma and malignant carcinoma. A malignant carcinoma is also called cancer, and a cancer can develop from a pre-malignant carcinoma. There are four types of malignant tumors in respecting to their cell-origins: carcinoma (e.g. skin cancer), sarcoma (e.g. osteosacoma), lymphoma (e.g. leukemia), and blastoma (e.g. neuroblastoma). It is known that development of a tumor is a result of accumulation of DNA mutations in somatic cells. A tumor cell is a transformed cell from a normal cell by DNA mutations, which make the cell able to proliferate independently. Although we have had made enormous progress on diagnosis and therapy of some tumors, the common mechanism of tumor development is not fully understood. The key processes on cell transformation, including how DNA mutations are produced and how DNA mutations accumulate in cells, are not yet clear. Most types of tumors are aging-associated; however none of traditional aging theories can interpret the phenomenon of tumor-development. In the present paper, we will demonstrate that our novel aging theory, the Misrepair-accumulation theory (Wang et al, 2009), is distinct in this aspect. The Misrepair-mechanism that is proposed in this theory can explain the processes of production and accumulation of DNA mutations in cell transformation. Our discussion tackles the following issues:

I. Characteristics of tumor cells and tumor-development

1.1 Independence of tumor cells on dividing and surviving
1.2 Tissue-selectivity in tumor-development

II. A generalized concept of Misrepair

III. Production and accumulation of DNA mutations in somatic cells

3.1 Misrepair of DNA: the main source of DNA mutations in somatic cells
3.2 Accumulation of DNA mutations: through many generations of somatic cells
3.3 Accelerated accumulation of DNA mutations in malignant tumor cells
3.4 Aging of a genome DNA

IV. Successive acquisitions of new properties of tumor cells

V. Preconditions for cancer-development: repeated injuries + repeated cell-regenerations

VI. Conclusions

I. Characteristics of tumor cells and tumor-development

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Tumor-development is a result of uncontrollable cell proliferation of an immortal cell. An immortal cell is a transformed cell from a normal somatic cell after acquiring new properties through DNA mutations. Rapid expansion of a tumor tissue, invasion of tumor cells into neighbor tissues, and metastasis of tumor cells to other locations through blood circulation ..., all these behaviors of a tumor may lead to a rapid failure of one or multiple organs. To understand the common mechanism of cell transformation, analyzing the behaviors of tumor cells and the manners of tumor-development is essential. Apart from the properties of tumor cells, tumor-development has two characteristics: age-related and tissue-selected. In our view, tissue-selectivity of tumors gives us an important clue for exploring the mechanism of cell transformation.

1.1 Independence of tumor cells on dividing and surviving

Except blood cells, all of the cells in an organism need to survive in a tissue environment where they can communicate with each other directly or via extracellular matrixes (ECMs). The cells in a tissue are interdependent on each other for functioning and for surviving; and loss of contact with neighbor cells/ECMs will lead to cell death, which is called “loss of anchor apoptosis” or “anoikis”. A normal stem cell needs to be stimulated by external signals for proliferation, and cell division will be terminated by withdrawing of the stimulating signals and/or by a stopping signal. Cell-contact is a universal stopping signal for terminating cell proliferation, and this phenomenon is called “cell-contact inhibition”. The behaviors of normal cells in a tissue are strictly and precisely controlled by their tissue environment, and none of them is independent.

Differently, a tumor cell has lost its dependence on other cells for proliferation and even for survival. It can undergo cell division without being stimulated, and the cell division cannot be stopped by cell-contact. With these two properties, namely, the property of stimulator-independent mitotic division and the property of loss of cell-contact inhibition, a tumor cell can proliferate unlimitedly into a clone, developing into a tumor. Apart from these two properties, the tumor cells in a malignant tumor have additional properties, including production of matrix metalloproteinase (MMP), anoikis-resistance, and acquired mobility. With the ability of producing MMPs to digest ECMs, the tumor can invade into neighbor tissues. With the property of anoikis-resistance, a tumor cell can survive without anchoring to neighbor cells/ECMs, and it can immigrate passively to other organs via blood flow. A tumor cell can immigrate actively if it has additionally obtained mobility. Therefore, these five properties are the most important properties for tumor cells:

1. Stimulator-independent mitotic division
2. Loss of cell-contact inhibition
3. MMP-production
4. Anoikis-resistance
5. Acquired mobility

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1.2 Tissue-selectivity in tumor-development

Tumors are classified into two groups in respecting to the ages of onset of tumors: one is aging-associated tumors, and the other is genetic tumors. Aging-associated tumors are the tumors that develop mainly in the people age over 50 years old, with increasing incidence with age. Most of human tumors are aging-associated, including lung cancer, gastric cancer and colorectal cancer. Genetic tumors are the tumors that develop mainly in the children less than 6 years old, but rarely in adults. Genetic tumors are often rare tumors, such as neuroblastoma, neurofibromatosis, and muscle tissue sarcoma. Genetic tumors develop often in the tissues, where aging-associated tumors do not develop. In contrast, aging-associated tumors mainly develop from epithelial cells (including glandular cells), hepatocytes, and glial cells, of which genetic tumors hardly develop. The phenomenon of tissue-selectivity in tumor-development reminds us that aging-associated tumors develop mainly in the tissues that are regenerable. Normally, a DNA mutation can occur to any one of somatic cells, but why does cell transformation take place only in regenerable cells?

Tumor-development is a part of aging of an organism, and a reasonable aging theory should be able to interpret this phenomenon. However, most of traditional biological theories, including gene-control theory (Fabrizio et al, 2010; McCormick et al, 2012) and damage (fault)-accumulation theory (Kirkhood, 2005), fail to explain tumor-development. Tumors have a great diversity on cell-origin, and different individuals may develop the same form of tumor at different ages. Therefore, development of a tumor cannot be possibly a result of controlling of certain genes, which should work in the same way in different individuals. Damage (fault)-accumulation theory emphasizes that it is the “intrinsic faults”, due to the limitation of repair/maintenance, that accumulate and lead to aging (Kirkhood, 2005). However, it is found that the DNA mutations in somatic cells are mainly produced by Mismatch repair (Misrepair) of DNA rather than by “faults” (Natarajan et al, 1993; Bishay et al, 2001). For a cell that suffers from a DNA injury, lack of DNA repair would lead to cell death rather than DNA mutation. On interpreting the phenomenon of tumor-development, these two theories are not tenable. Differently, with a concept of Misrepair mechanism, the Misrepair-accumulation theory is able to explain tumor-development on the mechanisms how DNA mutations are produced and how they accumulate during cell transformation.

II. A generalized concept of Misrepair

The term of Misrepair is traditionally used on DNA, referred to as the Mis-match repair of DNA. Misrepair of DNA is a strategy of DNA repair for increasing the surviving chance of a cell when its DNA is severely injured. Actually, incorrect repairs take place also on cells and tissues, and scar formation is an example. On this basis, we proposed in our Misrepair-accumulation theory a generalized concept of Misrepair for describing all types of incorrect repairs. The new concept of Misrepair is defined as incorrect reconstruction of an injured living structure, and it is applicable to all living structures including molecules (DNA), cells, tissues (Wang et al, 2009). DNA Misrepair is a special case of Misrepair. In situation of a severe injury, when complete repair is impossible to achieve, Misrepair, a repair with altered

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materials and in altered remodeling of a structure, is a way to maintain the structural integrity and increase the surviving chance of an organism. Without Misrepairs, an individual could not possibly survive to the age of reproduction; thus Misrepair mechanism is a surviving mechanism for an organism and for a species.

Misrepairs have three characteristics: unavoidable, with alternation of structure, and irreversible. Therefore Misrepairs will accumulate in an organism. Accumulation of Misrepairs will gradually disorganize the structure of a molecule, a cell, or a tissue, appearing as aging of it. Misrepairs have a tendency to accumulate focally in a tissue, because an old Misrepair makes the local part of tissue have reduced repair-efficiency and increased damage-sensitivity. Accumulation of Misrepairs is thus self-accelerating, focalized, and inhomogeneous. Aging may take place on the levels of molecules, cells, and tissues respectively; however aging of a multi-cellular organism takes place essentially on tissue level. Although remaining of aged cells can be a part of aging of a tissue, an irreversible change of the spatial relationship between cells/ECMs in a tissue is essential and sufficient for leading to a decline of tissue functionality and body functionality. Aging of an organism does not always require aging of cells. In summary, aging of an organism is a result of accumulation of Misrepairs on tissue level. Misrepair mechanism is beneficial for the survival of a species, and this is the evolutionary advantage of aging mechanism. Aging of an individual is a sacrifice for that.

III. Production and accumulation of DNA mutations in somatic cells

DNA mutations are the genetic basis for tumor-development and they are responsible for the alteration of cell phenotypes of tumor cells. The type and the amount of DNA mutations are different in different forms of tumors and even in the same form of tumors but from different individuals. It would be too time-consuming to study all the DNA mutations in each tumor. More important is to study the common mechanism in all tumors: A. how DNA mutations are produced in somatic cells and B. how DNA mutations accumulate in cells.

3.1 Misrepair of DNA: the main source of DNA mutations in somatic cells

Genetic changing can take place by different mechanisms. An entire chromosome can be lost or gained during cell mitosis. Large-scale mutations with loss or gain of part of a chromosome can be a result of abnormal chromosomal division during cell mitosis, genomic amplification, virus-integration, or chromosome translocation. Small-scale mutation such as point mutation (alteration, deletion or insertion of one or two base-pairs) may occur to any part of a DNA. For a normal somatic cell, a change on chromosomes, such as loss or gain of entire or big part of a chromosome, may lead to cell death, since such a big change on DNA may largely alter cell phenotype and lead to failure of cell functionality. Differently, a point DNA mutation is often mild, not essentially altering cell phenotype; therefore, it can “survive” in a cell and accumulate in the offspring cells. In another word, those DNA changes that may accumulate and contribute finally to cell transformation are mainly point mutations.

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In normal somatic cells, point DNA mutations can be introduced before and during DNA duplication; however a promoting factor is an injury of DNA. The DNA duplication and DNA repair in a normal cell has great accuracy. However, in situations of severe injuries such as double-strand breaks of DNA (DSBs), a perfect DNA repair may not possibly be achieved in a permitted time. Non-homologous end joining of broken DNAs or other pathways for re-linking DNAs have to be promoted to prevent cell death from failure of DNA (Moore et al, 1996). Such a repair will result in alteration of DNA sequence, such as alternation, deletion, or insertion of base-pairs; therefore the repair in this way is a “Misrepair”. Misrepair of DNA is not a result of failure of DNA repair, but a result of active DNA repair. Misrepair of DNA is an essential process for transforming a DNA injury (lesion) into a “survivable and inheritable” DNA mutation. Like the Misrepairs in other aging changes, DNA Misrepairs are made for increasing the surviving chance of a cell; however they will manifest their side-effects later in tumor-development. Misrepair of DNA can only take place when a cell has normal functionality on DNA repair. In contrast, for a cell with defect on DNA repair, a DNA injury will lead to cell death rather than a DNA Misrepair. In the following discussion, the term of “Misrepair of DNA” is used for describing the process of Misrepair of DNA, and the term of “DNA Misrepair” is used for describing the result of that, namely, a DNA mutation.

Many studies have shown that Misrepair of DNA is the main source of DNA mutations. The misrepaired DSBs are found to be the main lesions as the origin of both chromosomal abnormalities and gene mutations (Natarajan et al. 1993; Bishay et al, 2001). Misrepair of DNA is one of the surviving mechanisms of a cell under radiation, but is also the origin of tumor-development (Rothkamm et al, 2002). The “misrepaired DNA damage” is a causal factor for gene mutation and development of cancer and aging (Suh et al, 2006). Although Misrepair of DNA is a strategy for cell survival, the chance is low. There are three reasons for the low surviving chance of a cell from Misrepair of DNA: A. a cell that has DNA injuries may have also severe injuries on other parts of the cell, e.g. cell membrane and cytoskeleton; B. some DNA Misrepairs may lead to cell death by introducing toxic gene mutations; and C. those cells that have been altered on cell phenotype by a DNA Misrepair may be soon removed by immune-defending system in the tissue. A recessive DNA Misrepair, which does not alter cell functionality and cell phenotype, however can possibly make a cell survive. Thus recessive DNA mutations are the main type of DNA mutations that can remain and accumulate in cells.

### 3.2 Accumulation of DNA mutations: through many generations of somatic cells

Since the chance of a cell to survive through Misrepair of DNA is low, the opportunity of the same cell to survive two times of Misrepair is almost zero. Accumulation of DNA Misrepairs in one cell is hardly possible. However, for the cells that are reproducible, namely the stem cells, DNA mutations can be inherited and accumulate in their offspring cells. Therefore, accumulation of DNA mutations can only proceed possibly in reproducible cells, and this is one reason why tumors develop selectively in regenerable tissues. For example, for part of a tissue that is frequently exposed to certain damage, if the surviving chance of the cells from a kind of DNA injury through Misrepair of DNA is 1‰, the surviving chance of the same cells

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from a second time of DNA injury is 1‰ x 1‰ = 1/100000. Namely, few cells can survive from two times of such kind of DNA injuries. However, if a stem cell, which has survived by a DNA Misrepair (the first Misrepair), has reproduced 2 x 10^7 x 10^3 (= 2 x 1000000) offspring cells, all of these cells will inherit this DNA Misrepair (mutation). When these cells suffer again from the same kind of DNA injury, some of them, possibly one or two cells, may survive by another Misrepair of DNA (the second Misrepair). Thus, DNA Misrepairs accumulate in these survived cells. To have this number of offspring cells, namely 2 x 10^6, a stem cell needs to proliferate for at least 17 generations (2 x 10^6 = 2 x 2^{16} = 2^{17}). By cell proliferation, more offspring cells will inherit these two DNA mutations, and some of them may possibly survive by a third DNA Misrepair. In this way, by repeated DNA injuries and repeated cell proliferations, more and more DNA mutations are produced and accumulate in the offspring cells from one mother cell. Finally one of the cells may be transformed after obtaining a new property (Figure 1). Accumulation of DNA mutations need to proceed in many generations of cells. For example, for accumulating ten times of DNA mutations by Misrepair of DNA, a cell needs to proliferate at least 86 generations (2 x 10^{3x10} = 2 x 10^{6x5} = 2 x 2^{16x5} = 2 x 2^{85} = 2^{86}).

Cell proliferation in a normal tissue is accurately controlled; and it needs to be promoted by stimulators for adapting to different situations. Thus a stem cell and its offspring cells, which we call “a group of cells”, need many years, possibly 20-40 years, for proliferating sufficient generations. If there are numbers of stem cells in a local tissue, the load of DNA damage will be shared by “more groups of cells” and the opportunity of accumulation of DNA Misrepairs in “one group of cells” is reduced. Therefore, in somatic cells, accumulation of DNA mutations is a slow process, and cell transformation needs a long time. As human being, we seem to have higher rate than animals on tumor-development, and one reason is that we live longer than them, from which we have longer time for accumulation of DNA mutations! Simpler organisms such as insects and worms do not develop cancers, because they live too short (Campisi et al, 2000)! Low-dose of aspirin is found to be effective in reducing the risk of tumor-development (Brotons et al, 2014). One mechanism may be: cell repair including repair and Misrepair of DNA is inhibited by aspirin, and the process of accumulation of DNA Misrepairs is slowed down in cells.

A risking element for accelerating the accumulation of DNA mutations is repeated exposure for a long term to radiation or to DNA-toxic substances. Rapid accumulation of DNA mutations and high rate of cancer-development may take place to the people, who have been exposed to intensive nuclear radiation like that in Chernobyl Disaster. Slow accumulation of DNA mutations is a result of daily exposure for many years to a similar aggressive substance in food or in air. This is one reason why gastric cancer, colorectal cancer and lung cancer have high incidence in old people. Differently, genetic tumors develop mainly in the children who have had acquired gene mutations before birth. A genetic DNA mutation exists in every somatic cell, and the defect on cell functionality caused by genetic mutation may make the accumulation of DNA mutations start earlier and proceed more rapidly. Cell transformation may therefore take place earlier in these individuals.

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Figure 1. Accumulation of DNA Misrepairs (mutations): proceeding in many generations of cells

Most of the cells that suffer from severe DNA injuries will die, and only a few of them may survive through Misrepair of DNA (A). If a cell can proliferate, the DNA Misrepair (the 1st Misrepair) in the cell can be inherited by its offspring cells. If some of the offspring cells can survive from a DNA injury by another DNA Misrepair (the 2nd Misrepair), DNA Misrepairs can accumulate in these cells (B). By cell proliferation, more offspring cells will

damage to DNA

Normal cells

One of the cells survived by 1st DNA Misrepair

Cell proliferation

One of the cells with 1st Misrepair survived by 2nd DNA Misrepair

Cell proliferation

One of the cells with 1st and 2nd Misrepairs survived by 3rd DNA Misrepair

Cell proliferation and accumulation of DNA Misrepairs (1st + 2nd + 3rd +...)

One of the cells transformed

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inherit these two DNA mutations, and some of them may possibly survive by the 3rd DNA Misrepair. In this way, by repeated cell proliferation and repeated DNA injuries, DNA mutations are produced and accumulate in the offspring of one mother cell (D). Finally one of the cells can be transformed after obtaining a new property (E). Accumulation of DNA mutations need to proceed in many generations of cells; and cell transformation of a normal cell to a tumor cell is a long and slow process.

3.3 Accumulation of DNA mutations in malignant tumor cells: self-accelerating and in-homogenous

Malignant tumor cells are different from normal cells on several aspects. I. Normal cells have full differentiation with full efficiency on DNA repair and other functions; whereas tumor cells have low degree of cell differentiation with functional deficiency on cell repair and on DNA repair. Because of the deficiency of DNA repair, SOS-like Misrepair pathways have to be often promoted in malignant tumor cells. To some DNA injuries, full repair can be achieved in a normal cell, but cannot probably in a malignant tumor cell. The frequency of Misrepairs in malignant tumor cells is higher than that in normal cells. II. Cell proliferation of a normal stem cell is strictly controlled, whereas that of tumor cells is out of control and unlimited. III. The tolerance of a normal cell to a dominant DNA mutation is low, since an abnormal phenotype will lead to cell death or apoptosis. Differently, a malignant tumor cell can be tolerant to some DNA mutations because of immaturity on functionality. The surviving chance of a tumor cell from a DNA injury though Misrepair of DNA is higher than that of a normal cell. For example, a malignant tumor cell being anoikis-resistant is able to survive even if it cannot produce some proteins that are essential for cell-anchoring (Table 1). Taken together, accumulation of DNA mutations is accelerated in malignant tumor cells by rapider cell proliferation, reduced efficiency of DNA repair, and higher tolerance to mutations. The malignancy of a tumor cell is a result of DNA mutations; and in return, the malignancy will increase the frequency of DNA mutations in this group of cells. Accumulation of DNA Misrepairs (mutations) is therefore self-accelerating in the same group of tumor cells. Most of the mutations that are detected in malignant tumor cells could be produced during the progression of the tumor.

Table 1. Accumulation of DNA mutations: rapider in malignant tumor cells than in normal cells

|                          | Normal cells | Malignant tumor cells |
|--------------------------|--------------|-----------------------|
| Cell differentiation     | High         | Low                   |
| DNA repair               | Full         | Deficient             |
| Cell proliferation       | Well-controlled | Unlimited            |
| Tolerance to DNA mutations | Low         | High                  |

In a malignant tumor, accumulation of DNA mutations is more and more rapid, but the rates of accumulation of DNA mutations can be different in different tumor cells. During the growing of a tumor, tumor cells differentiate when some of them obtain new mutations, and
sub-groups or sub-sub-groups of tumor cells with different mutations appear. As shown in Figure 2, although all of the tumor cells in a tumor have some common DNA mutations, such as mutation A (MA), they can be different on other mutations. For example, some cells with MA have additional mutations respectively: MB1, MB2 or MB3, and some cells with (MA+MB1) have additional mutations respectively: MC1, MC2 or MC3. The difference on the type and on the number of DNA mutations makes the cells into different sub-groups, such as sub-groups of (MA+MB1), of (MA+MB2), and of (MA+MB3), and even sub-sub-groups, such as sub-sub-groups of (MA+MB1+MC1), of (MA+MB1+MC2), and of (MA+MB1+MC3). Therefore, the accumulation of DNA mutations is in-homogenous in different sub-groups of tumor cells.

![Diagram of tumor cells with different DNA mutations](image)

**Figure 2. In-homogenous accumulation of DNA mutations in different sub-groups of tumor cells**

In a malignant tumor, the rates of accumulation of DNA mutations can be different in different tumor cells. With the growing of tumor, tumor cells will differentiate when some of them obtain new mutations, and sub-groups and sub-sub-groups of tumor cells with different mutations appear. For example, some cells with MA have additional mutations respectively: MB1, MB2 and MB3, and some cells with (MA+MB1) have additional mutations respectively: MC1, MC2 and MC3. The difference on type and on number of DNA mutations makes the cells into different sub-groups, such as sub-groups of (MA+MB1), of (MA+MB2), and of (MA+MB3), and
even sub-sub-groups, such as sub-sub-groups of \( (MA+MB1+MC1) \), of \( (MA+MB1+MC2) \), and of \( (MA+MB1+MC3) \). Therefore, the accumulation of DNA mutations is in-homogenous in different sub-groups of tumor cells.

3.4 Aging of a genome DNA

Accumulation of DNA mutations can be understood as a process of aging of a genome DNA, since this process alters gradually the structure as well as the functionality of the genome DNA. Similarly to a scar on the skin, a DNA Misrepair (mutation) is a kind of aging change on DNA. Aging of a genome DNA, the accumulation of DNA Misrepairs, needs to proceed in daughter DNAs through many generations of cells. In somatic cells, aging of a genome DNA is slow, but in malignant tumor cells, that is rapid. Aging of a DNA may lead to tumor-development, and death of the organism from a cancer will make all genome DNAs including the “aged ones” disappear.

IV. Successive acquisitions of new properties for cell transformation

The new properties for transforming a cell into a tumor cell are essentially acquired by changes of DNA in the cell. The most important properties for cell transformation are stimulator-independent mitotic division, called here as property A (PA), loss of cell-contact inhibition (PB), ability of MMP-production (PC), anoikis-resistance (PD), and acquired mobility (PE). Each of these properties can be a final effect of alterations of multiple genes through DNA mutations. Acquiring of a new property of a cell through DNA mutations is a complex process, and it may be different in different tumors and in different individuals. However, important is to know whether these properties are acquired successively in a certain sequence or simply in a random way. Theoretically, occurrence of a DNA mutation and obtaining of a new property of a cell are random. However, for tumor-development, new mutations and new properties should be able to remain and accumulate in cells. A mutation in a single cell will disappear, if this cell dies before cell division. Thus a mutation will have increased chance to remain if it takes place in the cells that can proliferate. An ideal sequence of acquiring of new properties for tumor-development should be the sequence that is beneficial for accumulation of new mutations. Based on this logic, we find out that one of the best sequences is: PA to \((PA+PB)\), then to \((PA+PB+PC)\), then to \((PA+PB+PC+PD)\), and finally to \((PA+PB+PC+PD+PE)\) (Figure 3).

![Diagram of new properties sequence]

**PA:** stimulator-independent mitotic division  
**PB:** loss of contact inhibition  
**PC:** ability of MMP-production  
**PD:** anoikis-resistance  
**PE:** acquired mobility

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The most important properties of tumor cells are stimulator-independent mitotic division, the property A (PA), loss of cell-contact inhibition (PB), ability of MMP-production (PC), anoikis-resistance (PD), and acquired mobility (PE). These properties may be acquired by a normal cell successively in a certain sequence. In our view, one of the best sequences of acquiring of these properties for cell transformation is: PA to (PA+PB), then to (PA+PB+PC), then to (PA+PB+PC+PD), and finally to (PA+PB+PC+PD+PE).

The first property to be acquired should be PA, since this property helps make copies of a DNA mutation in daughter cells. In the cells with this property, the DNA mutations for PA and other mutations that take place later will have increased chance to remain. Although cell proliferation is still restricted by cell-contact inhibition in this stage, cells can proliferate in certain environments where there is not this inhibition. In contrast, if a cell had at first acquired one of other properties, the corresponding DNA mutations cannot remain when the single carrying cell dies. Without PA, accumulation of new properties is difficult in cells. The second property should be PB, since this additional property makes the cells with PA overcome the limit of cell-contact inhibition for proliferation. The properties of PA+PB will increase cell number and copies of DNA mutations, and can transform a normal cell into a tumor cell. Other three properties, including PC, PD, and PE, are not essential for all tumors, but essential for malignant tumors. PC and PD should be acquired earlier than PE, because PC and PD are sufficient for the aggressive behaviors of a tumor. PC, by digesting ECMs in tissues, enables the local invasion of a tumor. PD enables a cell survive even if it has lost its contact with neighbor cells; therefore the two properties, PC and PD, are both needed for a faraway metastasis of a tumor cell. Taken together, PA and PB are the two essential properties for all types of tumor cells, and they should be obtained before clonal evolution. PC, PD, and PE are possibly acquired successively during the procession of a tumor from pre-malignant to malignant.

V. Preconditions for cancer-development: repeated cell injuries + repeated cell regenerations

Repeated cell injuries are the promoters of DNA mutations, and cell proliferation enables the accumulation of DNA mutations in cells. Thus repeated cell injuries and repeated cell regenerations are the two preconditions for tumor-development. For example, gastric cancer and colorectal cancer are results of repeated injuries of mucosa and repeated regenerations of epithelial cells for repair. Development of breast cancer may be a consequence of repeated proliferation-regression of mammary glands. Proliferation of mammary cells is promoted by increase of hormones; and death of lobule cells is promoted by decrease of these hormones. DNA mutations may take place when some “strong” cells survive from “the edge of death”. The DNA mutations in stem cells of terminal ducts may accumulate after many circles of proliferation-regression. Some cancers such as colorectal cancer and breast cancer have in some cases genetic predisposition. One of the genetic impacts on oncogenesis is by enhancing cell proliferation. High level of hormones, which can promote cell proliferation, is found to be a cause for the high sensitivity of some people to cancers. For example, the high risk of breast

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cancers in the daughters of the women who have had breast cancers is due to the high levels of estrogen and progesterone in these individuals (Henderson et al, 2000).

For cancer prevention, it is therefore important to reduce the opportunity of repeated injuries to the same part of an organ. We need to make efforts from several aspects. Firstly, it is better live in a moderate style for avoiding unnecessary damage-exposure such as smoking, too much eating and drinking, prolonged sun-bath, and violent sports. Secondly, we should therapy actively acute inflammations for preventing them from becoming chronic. Thirdly, we need to reduce the opportunity of a long-term exposure to the same type of damage. Some substances in food or in air are probably not damaging directly, but the half-degraded products of them can be aggressive. It is reported that gastric cancer has high incidence in Asia whereas colorectal cancer has high incidence in Europe. The difference on the habits on food-consummation between Asians and Europeans can be a reason for this difference on tumor-development (Brenner et al, 2009). Therefore, increasing the diversity of foods can be a way to reduce the risk of these two forms of cancers. When we have a large diversity of foods, the opportunity of damage of certain food to the same part of gastric-intestinal mucosa will be reduced. That is to say, the load of damage will be shared by more groups of cells in different parts of digestive mucosa if we have more kinds of foods. Thus, when we have a flexible life on eating, on moving, and on working, we will have reduced risk to be exposed to the same type of damage, physical or chemical. Taken together, a moderate and flexible living style can be helpful in reducing the risk of cancer-development.

VI. Conclusions

In somatic cells, DNA mutations are mainly produced by Misrepair of DNA. Accumulation of DNA mutations can only proceed in the cells that are reproducible. This is one reason why tumor-development takes place mainly in the tissues that are regenerable. Accumulation of DNA mutations needs to proceed in many generations of cells; therefore cell transformation from a normal cell into a tumor cell is a slow and long process. However, once a cell is transformed, especially when it is malignantly transformed, rapid cell proliferation and deficiency of DNA repair will accelerate the accumulation of DNA mutations. The process of accumulation of DNA mutations is in fact a process of aging of a genome DNA. Repeated cell injuries and repeated regenerations of cells are the two preconditions for tumor-development. Therefore, for cancer prevention, a moderate and flexible living style is advised.

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