REVIEWS

The role of disparity-mutagenesis model on tumor development with special reference to increased mutation rate

Mitsuru Furusawa

Neo-Morgan Laboratory Inc., Biotechnology Research Center, Kawasaki, Japan

Correspondence: Mitsuru Furusawa. Address: Neo-Morgan Laboratory Inc., Biotechnology Research Center, 907 Nogawa, Miyamae-ku, Kawasaki 216-0001, Japan. Email: furusawa@neo-morgan.com

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Abstract

Human solid tumors are believed to have very high mutation rates at least in the early stage of extension. Irrespective of whether the increased mutation rate is a necessary condition for the tumor development or not, extremely high mutation rates such as in excess of the so-called "threshold" would before long result in the natural death of tumor cells. In reality, however, we are dying by cancer. Thus, it has been hypothesized that tumor cells should make a quick transition from the higher mutation state to the lower one. According to our "disparity-mutagenesis model", however, carcinogenesis could continue without any incident even under a prolonged period of high mutation rates. Namely, if lagging-strand-biased mutations far beyond the threshold of mutation rate are introduced in tumor cells, the tumor could progress to malignant extension without extinction. The results of evolution experiments using mutator microorganisms are discussed in terms of carcinogenesis.

Key words

Disparity-mutagenesis, Disparity-mutator, Error threshold, Solid tumor, Evolution

1 Introduction

The progress of studies on human solid tumors tends to be slow mainly because of the technical problems. The problem of in vivo mutation rates of the tumors is no exception. Till this day, enthusiastic arguments have been developed concerning the relationship between the mutation rates and cancer progression. Judging from the arguments that appeared in previous studies, it is reasonable to consider that in the precancer stage of human solid tumors the mutation rate might be considerably increased [1-4]. Irrespective of the high mutation rate being an essential condition for carcinogenesis, when the mutation rate exceed the threshold value [5], the tumor cells are dying from accumulated deleterious mutations. To avoid these seen from the tumor cell's perspective, the tumor cells must decrease their own mutation rates somehow as soon as possible [2]. Is there any different manner to attain the same goal, avoiding catastrophe? In the present article, it will be shown that if the biased-mutagenesis in the lagging strand is hypothesized, cells will be able to continue to replicate even at mutation rates exceeding the threshold value. This idea originally comes from the principle of the "disparity theory of evolution [6, 7]. Lastly, evolution experiments using mutator microorganisms with biased-mutagenesis will be discussed from the viewpoint of carcinogenesis.

2 Previous arguments about the mutation rates and cancer: human solid tumors have a stage showing abnormally high mutation rates

Human solid tumor and evolution: The process of human solid tumor extension is regarded as an evolutionary process [1]. Underlying causes of tumor disease are heritable somatic mutations. Mutations can be introduced at any stage of ontogeny. Especially, stem cells dwelling in adult organs bear a high risk of becoming cancerous, because they have to divide for a thousand times over a lifetime [2]. Mutations that have been firstly introduced in oncogenes or tumor-suppressor genes in stem cells would induce cellular replication, collaterally resulting in raising the probability of the next mutation in the stem cell group. At this state, however, mutation rate does not rise. Continuous cell divisions thus induced accumulate many mutations. A small number of the mutations may injure the DNA-repairing system and result in the increase of mutation rate. Then, the DNA-repairing function may be too late for the quick replication, and mutation rates are increased more and more.

Increased mutation rate may work to the tumor's advantage. This is because the tumor has to necessarily adapt to changing new environments that have been produced by the tumor itself. Thus, at the early stage of carcinogenesis, increased mutation rate is believed to be an indispensable event. For a limited period of time, at least, it can be presumed that mutation rates exceed the so-called "threshold" of mutation [5]. A prolonged period of time of such a high mutation rates will cause self-destruction of tumor cells by means of the accumulation of an excess of deleterious mutations. To avoid the self-destruction, it is predicted that at the later stages of cancer extension, mutation rates might be decreased and go into a stabilized phase [2]. As a secondary event, the increased mutation rate induces chromosomal mutations by the destruction of telomeres. In addition, phenotypic changes in tumor cells would be triggered by the disturbance of the DNA methylation system [2].

Nowell proposed the "clonal evolution theory", in which the process of carcinogenesis assimilates to the evolution of unicellular organisms [8]. All of the solid tumors are originated from a single cell. From a clonal population, the best adapted mutant cell is positively selected. By repeating this process of clonal expansion and selection, the malignancy is enlarged in a step-by-step manner. Thus, tumor cells have accumulated many mutations and are genetically much more unstable comparing normal cells.

Cancer stem cell theory: Pierce proposed the concept of "cancer stem cells (CSCs)" [9]. CSCs fundamentally have the same properties as normal organ-specific stem cells and are in a dormant state. In a precise sense, it should be said that CSCs in a patient have not yet been proved, According to the CSCs theory, a single CSC produces a single solid tumor, but the genetic constitution of CSCs existing in a single tumor is not homogeneous. Repeated replication accumulates mutations and finally CSCs become malignant cells that can metastasize. In the case of the development of human colon cancer, a number of mutations are believed to be introduced into cancer-related genes in a step-wise manner [10]. There is a speculation that after the final hit of mutations, the colon tumor consists of a homogeneous genetic constitution. There has been, however, no certain evidence whether CSCs are indispensable to metastasis or relapse [1].

Early tumor cells have mutator phenotype: In a solid tumor cell, thousands or maybe hundreds of thousands of random mutations are accumulated all over the genomic DNA [3]. For instance, a hereditary non-polyposis colon cancer harbors mutated mismatch-repair enzymes that increase the mutation rate up to one hundred times higher than that of the normal enzyme, indicating that the colon cancer cell is probably a mutator [3, 11]. Many mutations due to the mutator are not the rate limiting factor for the growth or the extension of cancer but merely represent a parallel relationship. In inherited retinoblastoma, it is reported that two mutations occurring in the RBI tumor suppressor gene increase the mutation rate by 10 to 10,000 fold [12]. Microsatellite instability can be seen usually in sporadic colon cancers and the cause of the instability seems mainly to be the defect of mismatch-repairing [3].

At the beginning of carcinogenesis the accumulation of mutations starts. Among the newly introduced mutations, there will be mutations that trigger further increase of mutation rate, and the tumor cell will become a stronger mutator. When this cycle has piled up, mutations are accumulated exponentially in a cell. Positive selection against mutator may tend to increase the number of mutators in a solid tumor. Furthermore, a clonal selection against mutator may increase the ratio of mutators in the cell population of the tumor. In a bacterial system, it was shown that a similar selection process resulted in the increase of the appearance frequency of mutator by more than 500 hold [13].

Most probably, mutator phenotype is necessary for tumor cells to adapt to variable environments. The fate of a tumor cell population would depend on the probability of producing mutations that will be able to adapt well to upcoming unknown environments. These situations would be inevitable in the process of carcinogenesis. The ability of tumor cells to overcome such a big hurdle would depend on their mutation rate, so that their mutation rates should be increased, at least in early stages of carcinogenesis [3, 11, 14].

A crucial factor for carcinogenesis is not mutation rate but selection: Bodmer proposed another idea. Although carcinogenesis can be regarded as the process of somatic evolution, its key factor is selection and a mutator phenotype is not necessarily for carcinogenesis. Thus, an abundance of mutations observed in the genome of tumor cells should be assumed as a merely parallel phenomenon [4].

It has been postulated that six mutations are needed for carrying through a process of carcinogenesis and that the upper limit of mutation rate is 10,000 fold that of the normal rate. Simulations showed that a successive advantageous mutation was the leading player for the promotion of carcinogenesis [4].

3 The disparity-mutagenesis model and solid tumor progression

3.1 Mutation rates of human solid tumors are believed to exceed the threshold

In this article, the developmental process of human solid tumor is regarded as a kind of evolution. Differences among the implications of the clonal selection theory, the CSCs theory and the mutator theory are not consciously considered, because in a broad sense, these three theories belong to the category of somatic evolution. At the precancer stage of human solid tumors, mutation rate is believed to be exceptionally high and this situation might be prolonged for a significant period of time. Bodmer's "selection theory" also accepts high mutation rates in carcinogenesis.

Because of technical reasons, it is difficult to know the precise mutation rate of in situ human solid tumors. The condition is predicted that, at least in precancer state, mutation rates might increase beyond the threshold value for a significant period of time. Based on the current concept of evolutionary biology, rapid cell replications and a prolonged period of high mutation rates bring about a quick shrinkage of the tumor size, and the tumor cells sooner or later become extinct [2].

3.2 Disparity-mutagenesis model operates by increasing the threshold of mutation rate

The disparity-mutagenesis model deduced from the principle of "disparity theory of evolution" [6, 7, 15] nicely explains the reason why tumor cells do not become extinct in a situation where mutation rates exceed the so-called "threshold". It is predicted that mutations accompanying DNA replication occur disproportionately in the lagging strand. The main argument for this prediction is as follows. The error frequency might be significantly higher in the lagging-strand, since a more complex system is used in the synthesis of the lagging strand compared to that of the leading strand [6]. Indeed, biased-mutagenesis was observable in the lagging strand in *Escherichia coli* (E. coli) [16].

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The basic operation principle of the disparity-mutagenesis model of evolution is shown in Figure 1. The replicore has one origin of replication (*ori*) at the upper end of a linear DNA. When it replicates semi-conservatively, random point mutations are deterministically introduced to the lagging strand and each mutation once introduced is certainly inherited.

Conclusions obtained from the pedigree shown in Figure 1b are as follows. 1) Each replication produces two daughter DNAs. One has the same genotype as its parent has, while the other inevitably has a different genotype by added mutations. 2) The ancestral genotype is guaranteed forever and any genotype that appeared in the past is guaranteed at any generation.

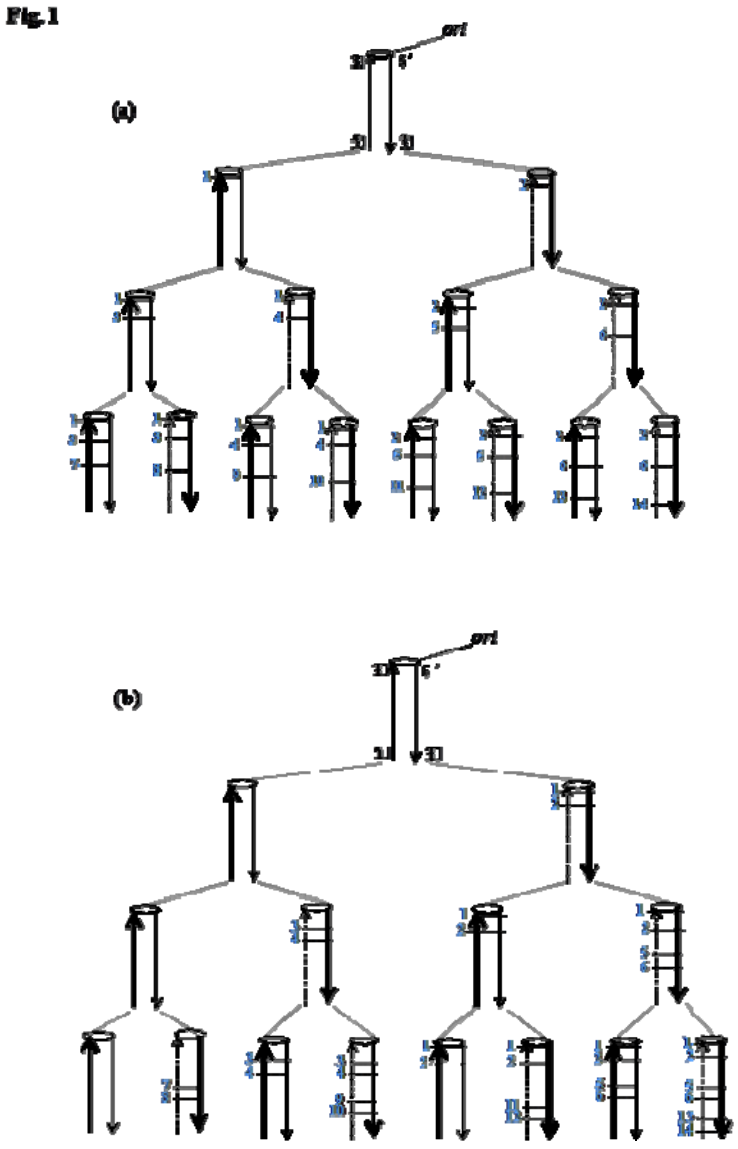

Figure 1. The parity and disparity mutagenesis models

(a)The parity model: the number of point mutations introduced in the leading and lagging strand in each DNA replication are 1 and 1, (b) The disparity model: the mutation number in the reading and lagging strand are 0 and 2, respectively. Mutations once introduced are passed down to successive generations. *ori*, the origin of replication; a broad long arrow, a parental template DNA strand; a continuous fine arrow, a newly synthesized leading strand; a dashed fine arrow, a newly synthesized lagging strand; dotted bars crossing the double-stranded DNA, point mutations; each number (blue) with the bar, a base substitution occurring at a different site.

That is to say, the creation of diversity with "guarantee of principal" is realized. This model system serves as an unbeaten strategy. When unchanged environment continues for a long period, the ancestral genotype can be used. When the environment is changed, an adequate mutant is selected from previously provided mutants. The newly selected mutant can continue to produce their offspring in the same manner. It is noticeable that this deterministic model has basically no mutation threshold when environments are stable, because the ancestral genotype is always guaranteed. However, when environments change dramatically, the ancestral mutant is no longer possible to adapt to the new environment. The ability to increase mutation rates would be beneficial to adapt to new environments. On the other hand, when mutation rates are too high, there would be no chance to find out appropriate mutants from preexisting individuals. This is because they must have been accumulated an excess of deleterious mutations. However, it can be easy to predict that the threshold must be significantly increased.

In fact, our previous experiment clearly show that Eigen's error-threshold in his quasispecies moved up or disappeared when a mixture of error-less and error-prone polymerase was used [15, 17]. Our experimental condition corresponds to realizing the fidelity difference between the leading and lagging strands in DNA replication.

DNA-type genetic algorithm (neo-Darwinian algorithm) with disparity–mutagenesis clearly showed that at appropriate mutation rates the algorithm well resolved an optimization problem, namely the so-called "knapsack problem", and the threshold was considerably increased compared with the conventional "parity-mutagenesis" model where mutations occurred evenly in the lagging and leading strands. As expected, when mutation rates exceeded a given threshold value, the population size and fitness scores were gradually decreased and finally populations became extinct [18].

3.3 The biased-mutagenesis makes it easier to procure a set of mutant genes responsible for carcinogenesis

Let us consider the case where an organ-specific stem cell becomes cancerous. As a necessary condition for this it is postulated that six mutations (a, b, c, d, e, f) must be introduced in this order in particular cancer-related genes [4]. It is easy to speculate that more the mutation rates increase, the more the chance of meeting this condition increases. There is, however, a problem. When the mutation rate is so high, the essential effect of the first a-mutation for carcinogenesis will be cancelled by the next mutations. This probability seems to be rather high. This is because there is a possibility in that the additional mutations introduced in the coding or non-coding regions of the gene with a-mutation would cancel the effect of first-hit a-mutation. Under high mutation rates, even if positive selection works for the mutations responsible for carcinogenesis, it would be difficult to keep a set of six mutations (a, b, c, d, e, f) staying intact in a cell for prolonged period of time. Even more so, if the required number of mutations responsible for carcinogenesis is higher.

Our disparity-mutagenesis model dramatically ameliorates these circumstances. This situation will be explained below by using a metaphor.

There is a slot machine with six frames. Each frame has ten numbers, from 0 to 9. Each frame is given a name A, B, C, D, E and F from the left, respectively. If you get a full set of A=1, B=2, C=3, D=4, E=5, F=6 you win. When you handle the steering wheel, the six numerical sequences turn simultaneously, the probability to get the right answer is 1/1,000,000, i.e. 1,000,000 times of trials, on average, are necessary for win.

But at this point, in order to introduce the concept of the guarantee of principal, you use illegal tricks. When a correct number appears in a correct frame, you immediately lock in the number using a magnet. The next trial must be done using the remaining five frames in the same manner. If you are repeating this handling, how many trials are needed to win? The answer is 10 times on average. In this metaphor, to lock in a correct number is comparable to the guarantee of principal. Even if the machine has a much higher number of frames, the answer is always 10 times. As predicted in the case of human solid tumors, when a stepwise introduction of mutations (such as 1→2→3→4→5→6) is an essential requirement, 60 trials on average are necessary.

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3.4 How does the disparity-mutagenesis model work in living tumor cells?

Under rapid cell-cycles with high mutation rates at a precancer state, the biased-mutagenesis model could serve to protect tumor cells from the harmful effect of deleterious and lethal mutations. Because, as deleterious mutations are introduced exclusively in the lagging strands, the counterpart daughter DNA synthesized by the leading strand tends to stay intact. At the same time, a beneficial mutation for a tumor cell once trapped in the lagging strand will be fixed on the leading strand in the next replication step, and the mutation can be transferred and protected in the descendant cells through the leading strand. This protection mechanism might principally work in tumor cells with mutator phenotype (Figure 1b). Accordingly, the tumor cells not only can avoid the risks of extinction, but also can evolve step-by-step and finally acquire the terminal phenotype as malignant tumor cells.

Concerning this, a landmark experiment with *Paramecium tetraurelia* would be noteworthy. Depurinations/ depyrimidinations and DNA-strand breaks (mainly, single-strand breaks) had been accumulated with ageing when the cells were asexually reproduced by binary fission [19].

We pointed out the risks of continuous mutator phenotypes in asexual reproduction [7]. Asexually-reproduced paramecia are destined to die before long. Most probably, these DNA lesions observed might not be due to replication errors but might be due to the loss of ribosomal activity, the decreased activity of repair system [19], and the inhibition of telomere elongation. Mutations caused by disparity-mutagenesis may act rather as the factor which delays the progression of ageing and the timing of dying when reproduced asexually. Needless to say, autogamy (self-fertilization) rescues them from aging and the death. By the way, tumor cells never fertilize. Thus, immortality of mutator tumor cells might be guaranteed mainly by autonomous telomere elongation and the disparity-mutagenesis.

From the viewpoint of the stepwise introductions of mutations, common mutations observed in human tumors would be noticeable. Especially, mutations in TP53 tumor-suppressor gene are the most plausible candidate for the master mutation which triggers or maintains tumor development [20]. This is because different human tumors show overexpression or mutations of P53 protein which enhance the transition from G0 to G1 stage in a cell cycle. From tumor's perspective, the precious mutations introduced in P53 on chromosome 17 should be protected from an upcoming storm of mutations. Otherwise, the function of the mutations might be cancelled soon. This protection might be realized by disparity-mutagenesis.

Xenoplastic transplantation into immunodeficient mice is the only way to identify human CSCs. The general scenario for normal stem cells, in which a stem cell unequally divides into a stem cell and a differentiated cell, seems to be not simply applicable to CSCs. In a single tumor, a differentiated cell may be able to turn back to CSC state and *vice versa* [1, 21, 22]. If this may happen, the disparity-mutagenesis model might be highlighted as a key mechanism, by which the tumor cell can maintain its immortality and malignancy even under such a complicated situation.

Aflotoxin B1 (AFB1) exposure triggers human hepatocellular carcinoma. Various animal models have been used to clarify the molecular mechanism of pathogenesis. Chemically-specific DNA adducts of this carcinogen is a key event for the early stage of this tumor development, and hotspot mutations in *p53* in hepatocellular carcinoma is confirmed [23]. This mutation must occur without relation to the disparity-mutagenesis. However, in the later stages of cancerous expansion, the disparity-mutagenesis model might work as a main mechanism.

3.5 Tumor cells are specialists in evolution

A synergistic effect between the system developing the guarantee of principal and positive selection would give solid tumors an ability to overcome severe and unstable environmental pressures. Sometimes solid tumor becomes advance so quickly that they deserve to be called a specialist in the acceleration of evolution.

The constitution of a tumor cell is very complex. In many cases, they are polyploidy or heteroploidy, and chromosomal mutations occur. Probably hundreds or more of *oris* exist in a single chromosome. If you focus simply on a replicore unit, it can be predicted that the performance of the guarantee of principal might be developing as shown in Fig. 1b. Notwithstanding, quite a number of tumor cells might be dying by severe negative selection pressures in vivo. Some of them, however, can survive; at the time, the biased-mutagenesis might play a starring role.

As mentioned above, stepwise introductions of mutations are necessary for carcinogenesis [4, 8]. A set of mutant genes that are necessary for carcinogenesis may not exist together in a single replicore, but they are mostly scattered around different replicores or even different chromosomes. Therefore, in order to keep the set of the mutated genes in descendant cells for extended periods of time, recombinations such as sister-chromatid exchange, gene conversion and translocation may play an important role. Polypoidy would also act as a promoting factor for tumor development, since it may increase the chance to make a set of combined mutations favorable for carcinogenesis. In addition, although successive positive selection is an important factor [4], but here the role of the lagging-strand-biased-mutagenesis would likely to be emphasized for the quick development of human solid tumors.

According to these contexts, it can be said that a human solid tumor is a kind of "disparity-mutator" [7]. Hereafter, results of evolutionary experiments using disparity-mutators of microorganisms will be presented as a mimic of the somatic evolution of human solid tumors.

4 Evolution of disparity-mutators of microorganisms mimics somatic evolution of human solid tumors

4.1 *Escherichia coli* mutator

E. coli has a circular DNA genome consisting of 4.6×10^6bp and about 4,300 genes. There is one *ori*. One termination site for DNA synthesis is located at the symmetric point of the *ori*. During replication, as the genome is synthesized simultaneously in two directions from the *ori*, a half of the genomic DNA is synthesized using the leading strand and the remaining half using the lagging strand. Both strands are synthesized by polα and proofreading is operated by dnaQ. Mutant *dnaQ49* is a temperature-sensitive mutator. At 37℃, the proofreading activity is deleted. Therefore, *dnaQ49* appears to demonstrate the net errors in the base-paring process in which polα is committed without the help of dnaQ. Our previous experiment with *dnaQ49* showed that the error frequency in the lagging strand synthesis was estimated about 100 times higher than that of the leading strand [16]. When *dnaQ49* mutators were cultured at 37℃ with gradually increased concentration of different antibiotics, they were able to make colonies at the presence of saturated concentrations of all antibiotics tested [24].

The results with Ofloxacin (gyrase inhibitor; one of Quinolones) are most noteworthy. In order to obtain a *dnaQ49* strain with moderate tolerance to Ofloxacin (MIC=256μg/ml), one mutation had to be firstly introduced in gyrA, followed by a secondary mutation in topoIV. A similar stepwise introduction of two mutations in these two genes is observable in E coli samples which were collected from Quinolone-treated patients. It should be emphasized that two positions of nucleotide replacement observed in gyrA and topoVI from the in vitro experiment were identical with those from the clinic-based samples. In the in vitro experiment, no other base replacement, including synonymous one, was found as far as sequenced, except the above-mentioned two replaced bases. The super-Ampicillin-tolerant *dnaQ49* strain (resistant to 30mg/ml) thus obtained was highly sensitive to other antibiotics similar to intact E. coli or intact *dnaQ49*. Thus, it can be concluded that the *dnaQ49* adapted exclusively to Ampicillin given as a selection pressure.

When *dnaQ49* was treated with a mutagenic compound at 24℃ where no mutator phenotype was expressed, the *dnaQ49* was unable to produce colonies at higher concentrations than 100μg/ml of Ampicillin [24].

There are several other experiments in which bacterial disparity-mutators displayed high adaptabilities to different conditions and showed genome-wide improvements [25-27].

4.2 Mutator of budding yeast

Haploid *Saccharomyces cerevisiae* has sixteen chromosomes, 1.2 × 107 bp genomic DNA and about 6,000 genes. It is estimated that hundreds of oris exist in the total genome. As a eukaryote, *S. cerevisiae* is more closely related to human compared to E. coli. *S. cerevisiae* pol3 (polδ) is specializing in synthesizing the lagging strand. As *pol3-01* is a mutator with deleted proofreading activity, biased mutations may occur in the lagging strand. Accordingly, it is one of the disparity-mutators.

The mutator yeast *pol3-01* was cultured gradually raising the temperature to isolate temperature-resistant strains. Two temperature-resistant lines that make colonies at 40℃ have been isolated. Genetic analysis showed that at least two stepwise mutations were necessary for acquiring this phenotype. Namely, the first mutation at hot1 locus provided 38.5℃-resistant properties, and by the addition of the second mutation (not identified) the mutant was able to make colonies at 40℃ [28].

There are several other experiments in which yeast disparity-mutators displayed high adaptabilities to different conditions [29-31].

Summary: The following conclusions can be deduced from the above-mentioned experiments using disparity-mutators of living microorganisms. 1) Growth rates of disparity-mutators and of the intact cells are nearly equal, when cultured in normal conditions. 2) The principle of disparity-mutagenesis model may be applicable to all living prokaryotic and eukaryotic cells. 3) The prolonged period of high mutation rates, in that most probably average mutation rates exceed the threshold value, does not necessarily lead to the death of organisms [32, 33]. 4) To attain a final intended phenotype, a number of appropriate mutations should be introduced in a correct order.

These performances shown by the disparity-mutators of microorganism in adaptive evolution appear to strongly suggest that human solid tumors would also have a disparity-mutator phenotype as well. Judging from the performance shown by yeast *pol3-01* mutant, the exquisite evolutionary abilities of biased-mutagenesis model could be applicable for understanding the development of human solid tumors.

Recently, it was reported that mutation rates in the lagging and leading strands were balanced by mismatch repair enzymes in yeasts [34]. If this is the case in human, the balancing effect on tumor cells should be reconsidered in terms of mutation rates and evolutionary adaptabilities. For instance, when the mismatch repair system is injured by a mutation, it may not only affect the total mutation rate but also affect the fidelity difference between the lagging and leading strands, indicating that evolution speed of the tumor cells might be changed.

It also remains to be examined how precisely the biased-mutagenesis model works in eukaryotic cells. For example, simulations of evolution experiments with cells that harbor plural chromosomes and multiple *oris* in a chromosome should be carried out.

5 A paradoxical concept for tumor suppression

It has been proposed that drugs decreasing the mutation rate of tumor cells may cause tumor suppression [2]. This idea might come from an assumption that random mutations accompanying DNA replication occur independently in the leading and lagging strand syntheses.

According to our disparity-mutagenesis model, any treatment by which excess mutations are simultaneously introduced in both leading and lagging strands of tumor cells may act as an effective inhibitor of tumor development. This is because, the "parity-mutator" of budding yeasts, in which the proofreading domains of polδ and polε were simultaneously deleted, could not be isolated [35]. This destructive effect of "parity-mutagenesis" on a living cell was also deduced from the experiments with the neo-Darwinian algorithm, in that the parity-mutagenesis with high mutation rates definitely resulted in the decrease of fitness scores and/or the extinction of the population [18]. Thus, chemicals that decrease concurrently the fidelity of both polε and polδ of tumor cells could be possible drug candidates.

Though the true reason why the above-described double-mutations-mutant yeast is not viable is obscure, it was suggested that the total mutation rate sufficiently exceeded the threshold [35]. Based on the disparity-mutagenesis model, another explanation would be possible, in that as excess mutations are evenly introduced in both strands in each replication, a once established "good" genotype for surviving would be easily canceled by additional mutations.

6 Closing remarks

Based on the principle of the disparity theory of evolution, implications of mutator phenotype for human solid tumor progression and experimental evolution were discussed. It is concluded that the disparity-mutator phenotype might play a central role for these systems, especially when mutation rates are sufficiently high. All these observations and statements presented here are probably true for human tumors other than solid tumors, and also for tumors in all kinds of animals.

In our previous simulation studies [6, 7, 15] and adaptive evolution experiments with living organisms [24, 28], we have never used another disparity-mutator which performed in biased-mutagenesis in the leading strand. There is a decent reason. Namely, it has been shown that the speed of molecular clocks of mammals was faster than those of other vertebrates. Then, it was presumed that the cause of their faster molecular clock might be due to the frequent amino acid replacements in the proofreading domain of polδ, indicating that their proofreading activities of polδ repeatedly might go up-and-down in the past. Accordingly, in these species, the speed of evolution might be frequently controlled during evolutionary process. It is also confirmed that in all vertebrates, amino acid substitution rates of the polymerase domain of polδ and polε, and those of the proofreading domain of polε are low, compared to those of the proofreading domain of mammalian polδ [15, 36].

In vertebrates, although the precise role of polδ and polε in replicating DNA has not yet been clarified, it is presumed that ancestors of mammals might have many occurrences of the disparity-mutator phenotype, in which excess mutations might be introduced exclusively in the lagging strand [15]. Consequently, it can be predicted that the fidelity of the leading strand of existing human being might stay high and so for developing human solid tumor cells as well.

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