Carcinogenesis Models Using Small Fish

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Received April 7, 2021

Experimental animals are indispensable in life science-related research, including cancer studies. After rats and mice, small fishes, such as zebrafish and medaka, are the second most frequently used model species. Fish models have some advantageous physical characteristics that make them suitable for research, including their small size, some transparency, genetic manipulability, ease of handling, and highly ortholog correspondence with humans. This review introduces technological advances in carcinogenesis model production using small fish. Carcinogenesis model production begins with chemical carcinogenesis, followed by mutagenesis. Gene transfer technology has made it possible to incorporate various mechanisms that act on cancer-related genes in individuals. For example, scientists may now spatiotemporally control gene expression in a single fish through methods including the localization of an expression site via a tissue-specific promoter and expression control using light, heat, or a chemical substance. In addition, genome editing technology is realizing more specific and more efficient gene disruption than conventional mutagenesis, in which the disruption of the gene of interest depends on chance. These technological advances have improved animal models and will soon create carcinogenesis models that better mimic human pathology. We conclude by discussing future expectations for cancer research using small fish.

Key words carcinogenesis; transgenesis; mutagenesis; medaka; zebrafish

1. Introduction

Research is underway to understand the pathological mechanisms of human diseases along with their treatment methods. Rapid advances are being achieved in cancer research, to which experimental animals are making a significant contribution. Unlike cultured cells, experimental animals maintain the hierarchical structure of cells, tissues, organs, and individuals, while continuing to engage in vital activities. Therefore, they are extremely useful for understanding biological phenomena. Experimental animals used in this field range from invertebrates (e.g., nematodes and fruit flies) to vertebrates (e.g., rats and mice). Small fishes, such as zebrafish and medaka, are also common vertebrate models, with many reports of their use in the medical field. This review provides an overview of research into carcinogenesis using small fish as model organisms.

2. Advantages of Small Fish

The small fish used as experimental animals are mainly teleosts belonging to the orders Cyprinodontiformes, Beloniformes (including medaka), and Cypriniformes (including zebrafish). Although they are not evolutionarily close, each group is used for similar purposes. Zebrafish and medaka are particularly prominent. Native to India, zebrafish were initially used as model organisms in the United States before spreading worldwide. In contrast, the Japanese species medaka is mainly used in Japan, with mutants being actively analyzed in genetic research. Medaka have also been successfully used in developmental biology and are now linked to the medical field.

The cells, tissues, and organs in fish are similar to those of higher animals in terms of composition and function. The full genome has been sequenced in some fishes, including zebrafish and medaka, with the development of a genetic research infrastructure making more genetic data available. Genome analyses revealed that fish have a similar genetic composition to higher vertebrates, including humans. For example, medaka have approximately 20000 genes, almost the same number as humans, with 80% ortholog correspondence with humans. However, the genome size of medaka is only approximately 25% that of humans and less than 50% that of zebrafish; therefore, its small size makes it easier to be subjected to whole-genome analyses.

Another advantage of small fish is their high transparency, allowing for microscopic observations of the internal cavity while the animal is alive. Through the expression of a fluorescent protein under a cell-/tissue-specific promoter, it is possible to observe the dynamics of living cells. In addition, in vivo cell function is observable through the expression of a functional probe (such as a fluorescent protein mutant with Ca responsiveness). In recent studies using see-through medaka and Casper zebrafish, which are all chromatophore-deficient transparent species, in vivo imaging has been possible at a more advanced developmental stage.

Mammals, such as mice and rats, are expensive to maintain and difficult to analyze in a group. In contrast, the maintenance and management of multiple small fish are easier and more cost-effective, making a multi-individual analysis a...
feasible option. The very low maintenance cost is a prominent advantage of using fish over mammals as experimental animals.

In this review, we describe the application of small fish as a carcinogenesis model in the medical field.

3. Carcinogenesis Models Using Small Fish

3.1. Carcinogenesis Induced by Chemical Compounds

Zebrafish and medaka both spontaneously develop cancer, but at low frequencies. Carcinogenesis is almost absent in the first or second year of life. After 3 years of age, spontaneous carcinogenesis occurs, although rarely. Older female medaka are particularly susceptible to carcinogenesis in the liver, but not in other tissues.

Since the publication of the first study on hepatic tumorigenesis in zebrafish exposed to carcinogens, chemicals that are carcinogenic in mammals have also been shown to induce carcinogenesis in small fish, particularly zebrafish. N-Nitrosodimethylamine and N-nitrosodiethylamine (NDEA) exhibit tissue-specific carcinogenicity; the former only induces liver tumors while the latter causes liver and pancreatic cancers. In contrast, 7,12-dimethylbenz[a]anthracene and the N-methyl-alkylating agent ENU cause epidermal papilloma in the epitheli-um; however, this does not progress to invasive skin cancer. Trosourea (ENU) causes epidermal papilloma in the epithelium; however, this does not progress to invasive skin cancer. In medaka, NDEA also induces cancer of and around the liver, which is similar to the findings obtained from mammals and zebrafish. Additionally, NDEA-induced liver cancer in medaka is more prominent in adult males.

The induction of carcinogenesis using chemical substances is simple experimentally, and provides useful information on the mechanisms of onset when performed in combination with a molecular (gene) analysis. However, difficulties have been associated with the spatiotemporal control of cancer onset, with onset generally taking a long time and onset sites widely varying. Onset probability was also low. Since there are no developed lines of model animals, they must be created for each experiment. Experimental efficiency may be increased via strains that are genetically engineered to have a greater probability of developing cancer.

3.2. Development of Carcinogenesis Models Using Mutagenesis

The correlation between gene function and phenotype needs to be investigated in animal models of carcinogenesis. However, chemical carcinogenesis models are not efficient tools for identifying a specific responsible gene, for reasons already described above (section 3.1).

To overcome this issue, scientists have established forward and reverse genetics using mutagens to induce mutagenesis. The alkylating agent ENU is a powerful mutagen that efficiently induces base substitutions (A to T, AT to CG, and CG to AT) to cause point mutations in a gene; this mechanism involves transferring the ethyl group of ENU to the base of a nucleic acid. The relationship between gene mutations and phenotypes was previously examined via experiments that exposed small fish to mutagens and introduced mutations into their genomes. The forward genetic method of identifying the gene responsible for a given phenotype may contribute to the discovery of novel cancer-related genes, such as tumor suppressor genes. The large-scale screening of ENU-treated zebrafish has yielded a number of new genes that are involved in carcinogenesis. The usefulness of small fish for forward genetics has also been demonstrated through retrovirus insertional mutagenesis studies using zebrafish, which have identified several carcinogenesis-related genes.

In contrast, systematic reverse genetics using mutagenesis have been developed to investigate the correlation between mutations in known genes and phenotypes. Targeting Induced Local Lesions IN Genomes (TILLING) is a very effective technology for selecting individuals with known mutations from the gene library of ENU-derived mutants. TILLING has been implemented in various organisms. In this method, the genomic DNA and sperm of F1 individuals obtained by mating male fish exposed to ENU with wild-type females are made into a library as a set. Mutations in the target gene are screened from the genomic DNA library by PCR, and the sperm of the relevant individuals are returned to the individuals by artificial insemination in order to obtain mutants. This method is particularly valuable for establishing cancer models using small fish, which are not suitable for other common techniques that disrupt a known gene, such as knockouts or genome editing.

The TILLING method has yielded multiple zebrafish mutant lines with various cancer-related genes. Analyses of these lines revealed that the mutants exhibit various symptoms that are similar to those in humans, including carcinogenesis, and they also possess gene mutations that frequently occur in human cancer pathology. For example, tp53 is the most mutated gene in human cancers and is similar to the missense mutant tp53<sup>E241X</sup> in zebrafish treated with gamma irradiation. Although homozygotes for this mutation grow normally, 28% of adult fish eventually develop peripheral nerve sheath tumors (MPNST) (Table 1). These findings are consistent with the role of tp53 as a tumor suppressor gene that controls the cell cycle through a number of processes, such as DNA repair, cell growth arrest, and apoptosis, which play important roles when cells become cancerous.

In medaka, TILLING has generated tp53 nonsense mutants, including tp53<sup>Y186X</sup> and tp53<sup>E241X</sup>. Each homozygous mutant showed reduced apoptosis after gamma irradiation and a shorter lifespan than the wild type (median lifespans were 311 d for tp53<sup>Y186X</sup> and 228 d for tp53<sup>E241X</sup>, whereas that of the wild type is typically more than one year). tp53<sup>Y186X</sup> developed a number of tumors across different tissues, whereas tp53<sup>E241X</sup> did not exhibit obvious tumorigenesis. In comparisons of phenotypes among each mutant after backcrossing six times with the wild type, the survival curve of tp53<sup>E241X</sup> shifted closer to that of tp53<sup>Y186X</sup>, even though carcinogenesis remained as pronounced as before. These findings indicate that phenotypic variations between the two mutants were not due to differences in the tp53 mutation type, and were more likely attributed to ENU-induced mutations other than tp53. None of the MPNST-type tumors found in zebrafish mutants have been developed in medaka. Since the zebrafish mutant has a missense rather than nonsense mutation, different tp53 mutation types may have distinct effects on tumorigenesis. Alternatively, the same gene may affect the tissue specificity of carcinogenesis differently depending on the species.

The Pten gene has the second highest mutation frequency in human cancer pathology. Zebrafish have two copies of the Pten gene (piena and pienb), which is not a rare phenomenon because the teleost genome is doubled. Analyses of each
defective mutant revealed that if one Pten copy was homozygous, the mutant grew normally without any phenotypic issues. However, when both genes were homozygous, the fish died shortly after hatching.43) When one copy was heterozygous and the other was homozygous (3 pten allele deficient), the fish grew normally, but spontaneously developed hemangiosarcomas.44) These findings suggest that the haploinsufficiency of Pten genes predisposes zebrafish to hemangiosarcoma.

### 3.3. Transgenic Models for Carcinogenesis

#### 3.3.1. Melanoma Model

Transgenic technology is essential for obtaining lineages that express functional genes, such as cancer-related genes or fluorescent markers for the visualization of cells. Transgenic forms of various experimental species have been created. The application of this technology is very important for maximizing the advantages of small fish in cancer research. In the technology, the expression unit for the gene of interest is integrated into the genome after injecting artificial DNA that combines a coding sequence downstream of the promoter into fertilized egg cells. Promoters spatially control gene expression. A protein of interest may be expressed throughout the body using a ubiquitous promoter or in limited cell/tissue types using a tissue-specific promoter. The expression of cancer-related genes in the desired spatial distribution within individuals allows us to confirm their involvement in carcinogenesis and use them to develop novel therapies. Carcinogenesis may be visually monitored by expressing a fluorescent protein that shares a promoter with cancer-related genes.

The earliest transgenic fish expresses an oncogene under the control of a tissue-specific promoter that successfully induces carcinogenesis in the target tissue (Fig. 1a). For example, in a study on melanoma, the function of an oncogene found in one small fish was examined in another small fish. Hybrids of different species within the genus Xiphophorus, pigmented platyfish (Xiphophorus maculatus) and non-pigmented swordtails (X. helleri), spontaneously develop melanoma.45) The gene responsible was identified by analyzing the phenotype of this hybrid strain through genetic backcrossing, which resulted in the sequence of Xmrk (Xiphophorus melanoma receptor kinase), a novel receptor tyrosine kinase gene related to the epidermal growth factor receptor (EGFR), eventually being isolated.46–48) To verify that a gene functions as an oncogene, it must be expressed in an organism. However, Xiphophorus is generally live-bearing and unsuited for transgenic technology. Therefore, Xmrk was expressed in the more suitable medaka under the control of the pigment cell-specific promoter mtf; and transgenic fish developed melanoma with 100% penetrance, demonstrating that Xmrk is an oncogene that induces carcinogenesis by itself.49)

Cdkn2ab, the fish ortholog of human CDKN2A, was recently identified as a new tumor suppressor gene. Xiphophorus cdkn2ab exerted strong inhibitory effects on cancer when overexpressed in the Xiphophorus melanoma cell line. Additionally, Xiphophorus cdkn2ab expression strongly suppressed carcinogenesis in the mitf-Xmrk medaka melanoma model, while the knockdown of cdkn2ab stimulated carcinogenesis.50)

Cancer-causing mutations have been reported in the zebrafish model. In human melanoma, mutations frequently occur in genes of the mitogen-activated protein kinase (MAPK) signaling pathway, such as the serine/threonine kinase RAF and the GTPase RAS. The constitutively active mutants BrafV600E, NrasQ61K, and HrasG12V (or HrasG12C) are strongly associated with carcinogenesis. When expressed in zebrafish, BrafV600E does not cause melanoma by itself. However, melanoma was induced when BrafV600E was expressed in tp53-deficient fish, showing that the mutation is carcinogenic only when combined with other defects.51,52) Similarly, NrasQ61K did not induce melanoma when expressed alone, but strongly induced the onset of melanoma when crossed with the tp53-deficient line.53)

In contrast, the expression of HrasG12V-induced melanoma by itself.54,55) Since RAS acts on multiple effector pathways, the combined activation of MAPK and AKT signaling pathways may explain HrasG12V oncogenic activity. A previous study demonstrated that the expression of the dominant interfering AKT suppressed HrasG12V-induced carcinogenesis.55)

#### 3.3.2. Temporary Control of Oncogenic Gene Expression

Carcinogenesis may be confirmed through the forced expression of oncogenic genes. However, early carcinogenesis increases the difficulties associated with keeping organisms alive until reproductive maturity and hampers the development of model animal lines. Therefore, technologies that temporar-
ily control gene expression are valuable for establishing lines of carcinogenesis models, and various similar pioneering techniques have been applied in nematodes and *Drosophila*. The application of this technology to small fish enabled the generation of a fish carcinogenesis model, in which the expression time of oncogenic genes is controlled. The application of heat shock promoters (e.g., the widely used heat shock protein 70, hsp70) and optogenetics allows for heat- and light-induced gene expression. Promoters, such as hsp70, contain a heat shock element (HSE) that functions as a binding site for the heat shock transcription factor Hsf. Under stress-free conditions, the effects of Hsf are suppressed by binding to the Hsp90 protein complex. When the temperature increases, Hsf is released from the complex and binds to HSE, stimulating transcription. Previous studies used Hsps in small fish, inducing gene expression locally through infrared laser irradiation or in the entire body through hot water baths (Fig. 1b). Many studies have used the latter method to generate carcinogenesis models. For example, a heat shock stimulation activated Hsp70-controlled AML1-ETO (a fusion protein synthesized via translocation, observed in leukemia) or BCR-ABL1 (an oncogene in zebrafish), and resulted in a myeloid leukemia-like pathology. Optogenetics involves the control of cell function by applying light-sensitive structural changes in the domain of light-responsive proteins, this technique is also used to uncage chemicals with light (Fig. 1e). An example in zebrafish combines optogenetics with another technique described at the end of this section.

In addition to technology that uses heat and light, the chemical induction of gene expression is also available and includes the well-known TET-OFF and TET-ON systems, in which gene expression is controlled via the binding of doxycycline (Dox), a tetracycline analog with greater stability, to a transactivator (tTA), generated with a tetracycline repressor from *Escherichia coli* and the transactivation domain of the transcription factor VP16. When binding with Dox, tTA cannot activate gene expression under the control of the tetracycline operator-dependent minimum promoter (TRE) (TET-OFF). In contrast, Dox binding activates rtTA to induce gene expression (TET-ON). The TET-ON system has frequently been applied to zebrafish, with the expression unit being controlled by TRE (Fig. 1d). One zebrafish experiment expressed rtTA under the tissue-specific *fabp10a* promoter, and activated rtTA binding to TRE induced oncogene expression. These findings demonstrated that exposure to Dox induced liver cancer, while its removal suppressed it.

Hormone-dependent activity may be conferred on a functional protein, such as a transcription factor or kinase, by...
fusing the ligand-binding domain of a hormone receptor to the functional protein (Figs. 1d, 1e). For example, the oncogene-estrogen receptor ligand binding site Myc-ER2 may be expressed under the control of a T cell and B cell-specific rag2 promoter. In this system, the ER2-specific hormone analog 4-hydroxy-tamoxifen induced leukemia, whereas its removal suppressed it.77) This technique has also been combined with optogenesis (Fig. 1e). In zebrafish that ubiquitously express ER2 and express KRASG12V in a manner that is dependent on ER2 nuclear translocation, photoactivation in the presence of caged cyclofen induced the transient expression of KRASG12V (cyclofen is a synthetically modified estrogen receptor inducer). In this experiment, carcinogenesis was not observed with a single photoactivation treatment; however, tumors formed in some individuals after repeated photoactivation cycles.69)

3.4. Application of Genome Editing In TILLING, mutants of the target gene are selected via screening, and there is no guarantee that the desired mutant may be obtained without extensive efforts, making the process inefficient. However, genome editing technology has the potential to revolutionize reverse genetics. Genome editing is designed to localize endonucleases at the sequence of interest and cleave DNA at a specified target site. Three methods are now commonly used: zinc-finger nucleases, transcription activator-like effector nucleases, and the clustered regularly interspaced short palindromic repeats RNA-guided Cas9 nucleases.78–80) The cleavage of DNA activates the cellular gene repair system, including error-prone non-homologous end joining (NHEJ) and homology-directed repair (HDR). The former is an inaccurate repair process that frequently causes mutations, such as nucleic acid insertion or deletion, at the repair site; therefore, NHEJ is used to disrupt target-gene functions. These gene repair processes are often used to introduce gene mutations into zebrafish.76) Furthermore, cleavage at two distant sites in the genome may induce the loss of long regions. If a DNA fragment with a homologous sequence is present during DNA cleavage, highly accurate HDR may be used to insert a new gene. These techniques will allow for the creation of animal models that faithfully mimic human mutations.

4. Perspectives Cancer research has continued to develop increasingly efficient methods for the creation of effective model organisms by focusing on specific genes and mutations. To achieve this efficiency, many efforts have been made to apply various technologies, starting with chemical carcinogenesis. Extensive research using experimental animals, including small fish, has led to numerous discoveries about genes and mutations and the elucidation of signaling pathways involved in carcinogenesis. Experimental animals that mimic the mutations of cancer-related genes found in human tumors showed an increased probability of carcinogenesis. Therefore, these mutations are likely involved in carcinogenesis and cancer growth. We expect similar research to continue in the future. Model fish will contribute to multiple research areas, such as experiments using treatments generated from a novel therapy paradigm, as recently reported.82) However, it is important to note that many human cancers do not show mutations in cancer-related genes that are recognized as oncogenic in animal models, and, thus, these genes only explain a small part of carcinogenesis.

Genomic DNA is always at risk of damage from endogenous factors (e.g., inflammation and reactive oxygen species (ROS)) and environmental factors (e.g., exogenous chemi-

**Fig. 2. Example of a Genome Mutation Analysis**

Chromosomal distribution of mutations and LOH. (a) Cultured cells, established from wild-type and dCMP transferase activity-deficient mutant (H489N) embryos, were used in a NGS genome analysis of chromosome 19. The proportion of mutant alleles lined up along the left arm of chromosome 19. Regarding each mutation identified by NGS, the value obtained by dividing the number of mutant allele reads by that of the total reads was mapped on the left arm of chromosome 19. Values obtained from each individual colony of cells are distinguished by a different color. The vertical axis represents the value of mutant allele reads/total reads, and the horizontal axis represents the number of bases from the top of chromosome 19. Pale yellow bars indicate the regions including the locations at which the peak calling value of mutant alleles was lower than that of normal alleles. (b) Distribution of LOH on the left arm of chromosome 19. A colony with the most prominent "mutant allele proportion" fluctuation pattern was selected from 6 clones in each genotype (wild type: Clone 2 and H489N: Clone 1), and 21 or 24 independent subcolonies were recovered by reseding cryo-preserved cells. Mutations present in each subclone were identified for 33 or 34 chromosomal positions by capillary sequencing. Several positions in each subclone were homozygous for mutant or normal alleles, and were defined as LOH. Each subclone may be divided into several LOH patterns, and these LOH patterns are shown. These patterns included homozygous for a mutant allele (black square) and normal alleles (white circle). The rate of clones having the distribution pattern of the corresponding LOH is indicated on the left of each bar. LOH caused by NDEA was markedly higher in the H486N mutant than in the wild type. This figure was cited from ref. 94. (Color figure can be accessed in the online version.)
cals, UV, radiation, and viral infections). Mutations occur upon gene damage, and the accumulation of these mutations causes cancer (multi-stage carcinogenesis). Investigations on the relationship between mutation accumulation and carcinogenesis are as important as those that focus on individual cancer-related genes. If DNA damage is not repaired, replication forks stall, leading to insertions, deletions, and other abnormal conditions that impair chromosomal function.82–84) This genomic instability leads to the loss of heterozygosity (LOH), a condition that is closely related to carcinogenesis. In a diploid organism, such as humans, one allele typically continues to function even when the other allele is impaired or deleted because of a gene mutation or inactivating epigenetic event. Therefore, cells function normally and carcinogenesis does not occur. However, when the remaining functional allele is also impaired, the probability of carcinogenesis increases. The mechanisms regulating the occurrence of LOH currently remain unclear. 

A recent study focused on the mechanisms underlying the generation of LOH in medaka models. Rev1 is a translesion synthesis (TLS) DNA polymerase that replaces stalled replicative DNA polymerases and resolves replication blocks by inserting a nucleotide opposite the modified base on the template.85) The dCMP transferase activity of Rev1 allows it to bypass apurinic/apyrimidinic (AP) sites or G-adducts,86,87) and the C-terminal domain functions as a scaffold protein that facilitates interactions between other TLS enzymes.88–90) A carcinogenic mechanism analysis was then performed using TILLING-selected Rev1 mutant fish.92) Researchers have also investigated the effects of NDEA on medaka mutants defective in each of these functions. The scaffold function-deficient mutant showed strong sensitivity to the cytotoxic effects of NDEA, while the mutant with reduced dCMP transferase activity exhibited markedly weaker toxicity, but a higher tumorigenic rate than wild-type fish. The frequency of NDEA-induced mutations was similar between the mutant with reduced dCMP transferase activity and the wild type; the frequency of LOH was higher in the former (Fig. 2). This experiment demonstrated the relationship between LOH and carcinogenesis, indicating for the first time that Rev1 also functions in carcinogenesis, indicating for the first time that Rev1 also functions in carcinogenesis. The distribution of mutations in individual cancer cells differs even within the same tissue.93) Additionally, cancer cells in metastasized tumor tissues have more mutations than before metastasis.93,94) These findings indicate that individual cancer cells have a history of mutations that constantly develop, leading to continuously changing traits. Therefore, it is important to monitor temporal changes in genomic mutations using animal models that mimic mutations in human cancer cells. The spatiotemporal control of cancer onset in model animals allows us to investigate the relationship between mutations at each cancer cell stage and the state of cancer cells or tissues during various stages, such as carcinogenesis, cancer tissue growth, and post-metastasis. This analysis will provide insights into the processes of these stages processes through anti-cancer therapy. With advances in technologies to generate model animals, future studies will take advantage of the unique characteristics offered by each experimental organism, including small fish.

**Conflict of Interest** The authors declare no conflict of interest.

**References**

1. Walter R. B., Obara T., _Comp. Biochem. Physiol. C Toxicol. Pharmacol._, 178, 156–162 (2015).
2. Kasahara M., Naruse K., Sasaki S., _et al._, _Nature_ (London), 447, 714–719 (2007).
3. Howe K., Clark M. D., Torroja C. F., _et al._, _Nature_ (London), 496, 498–503 (2013).
4. Lander E. S., Linton L. M., Birren B., _et al._, _Nature_ (London), 409, 860–921 (2001).
5. Venter J. C., Adams M. D., Myers E. W., _et al._, _Science_, 291, 1304–1351 (2001).
6. Wakiyama Y., Pristayzhnyuk S., Kinoshita M., Tanaka M., Ozato K., _Proc. Natl. Acad. Sci. U.S.A._, 98, 10046–10050 (2001).
7. White R. M., Sessa A., Burke C., Bowman T., LeBlanc J., Ceol C., Bourque C., Dovey M., Gessling W., Burns C. E., Zon L. I., Rev1ell C, 2, 183–189 (2000).
8. Kent M. L., Spitsbergen J. M., Matthews J. M., Fournie J. W., Westerfield M., “Diseases of Zebrafish in Research Facilities,” Zebrafish International Resource Center, 2016.
9. Matthews J. L., _Methods Cell Biol._, 77, 617–643 (2004).
10. Smolowitz R., Hanley J., Richmond H., _Biol. Bull._, 203, 265–266 (2002).
11. Masahito P., Aoki K., Egami N., Ishikawa T., Sugano H., _Jpn. J. Cancer Res._, 80, 1058–1065 (1989).
12. Stanton M., _J. Natl. Cancer Inst._, 34, 117–130 (1965).
13. Mizgirev I. V., Majorova I. G., Gorodinskaya V. M., Khudoley V., Revskoy S. Y., _Toxicol. Pathol._, 32, 514–518 (2004).
14. Mizgirev I. V., Revskoy S. Y., _Cancer Res._, 66, 3120–3125 (2006).
15. Spitsbergen J. M., Tsai H-W., Reddy A., Miller T., Arbogast D., Hendricks J. D., Bailey G. S., _Toxicol. Pathol._, 28, 716–725 (2000).
16. Spitsbergen J. M., Tsai H-W., Reddy A., Miller T., Arbogast D., Hendricks J. D., Bailey G. S., _Toxicol. Pathol._, 28, 705–715 (2000).
17. Beckwith L. G., Moore J. L., Tsao-Wu G. S., Harshbarger J. C., Cheng K. C., Lab. Invest., 80, 379–385 (2000).
18. Brown-Petserson N. J., Krol R. M., Zhu Y. L., Hawkins W. E., _Toxicol. Sci._, 50, 186–194 (1999).
19. Ishikawa T., Shimamine T., Takayama S., Hinton D. E., _Jpn. J. Cancer Res._, 53, 909–916 (1985).
20. Lauren D. J., Teh S. J., Hinton D. E., _Cancer Res._, 50, 5504–5514 (1990).
21. Liu Z., Kullman S. W., Bencic D. C., Torten M., Hinton D. E., _Mutat. Res._, 539, 43–53 (2003).
22. Nekazawa T., Hamaguchi S., Kyono-Hamaguchi Y., _J. Natl. Cancer Inst._, 75, 567–573 (1985).
23. Teh S. J., Hinton D. E., _Aquat. Toxicol._, 41, 141–159 (1998).
24. Nolan P. M., Hugill A., Cox R. D., _Brief. Funct. Genomics Proteomics_, 1, 278–289 (2002).
25. Coghill E. L., Hugill A., Parkinson N., Davison C., Glenister P., Clements S., Hunter J., Cox R. D., Brown S. D., _Nat. Genet._, 30, 255–256 (2002).
26. Raby L., Voelkel P., Le Bourhis X., Angrand P. O., _Cancers_ (Basel), 12, 2168 (2020).
27. Neumann J. C., Chandler G. L., Damoulis V. A., Fustino N. J., Lillard K., Looijenga L., Margraf L., Rakheja D., Amatruda J. F., _Comp. Biochem. Physiol. C Toxicol. Pharmacol._.
[86] Haracska L., Prakash S., Prakash L., J. Biol. Chem., 277, 15546–15551 (2002).

[87] Nelson J. R., Lawrence C. W., Hinkle D. C., Nature (London), 382, 729–731 (1996).

[88] Guo C., Fischhaber P. L., Luk-Paszyc M. J., Masuda Y., Zhou J., Kamiya K., Kisker C., Friedberg E. C., EMBO J., 22, 6621–6630 (2003).

[89] Nelson J. R., Gibbs P. E. M., Nowicka A. M., Hinkle D. C., Lawrence C. W., Mol. Microbiol., 37, 549–554 (2000).

[90] Ohashi E., Murakumo Y., Kanjo N., Akagi J., Masutani C., Hanoka F., Ohmori H., Genes Cells, 9, 523–531 (2004).

[91] Tissier A., Kannouche P., Reck M. P., Lehmann A. R., Fuchs R. P., Cordonnier A., DNA Repair, 3, 1503–1514 (2004).

[92] Fujikawa Y., Ishikawa-Fujiwara T., Kuo T., Shinkai N., Shoji T., Kawasaki T., Kamei Y., Sakuraba Y., Sato A., Kinoshita M., Gondo Y., Yuba S., Tsujimura T., Sese J., Todo T., Genes Cells, 25, 124–138 (2020).

[93] Gerlinger M., Rowan A. J., Horswell S., et al., N. Engl. J. Med., 366, 883–892 (2012).

[94] Vogelstein B., Papadopoulos N., Velculescu V. E., Zhou S., Diaz L. A. Jr., Kinzler K. W., Science, 339, 1546–1558 (2013).