

DNA Chemical Damage and Its Detected

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Abstract

The DNA chemical damage is a very important researching project in chemistry, life science and related fields. In this paper, the biological function of DNA damage, the reason causing damage and the detecting technology of DNA damage are summarized and discussed.

Keywords: Living body, DNA damage, Detect

DNA is the main genetic material in living organism. Apart from few viruses using RNA as genetic material, all other organism's genetic material are DNA, which contains all of the genetic information needed for the growth, development and reproduction of organism. As a kind of macromolecule compound, DNA is consist of deoxyribonucleoside monophosphate which are connected each other by phosphodiester bond. DNA has two pieces of polynucleotide chains, which is double helix structure with hydrogen bonds that formed between the base pairs. The DNA damage and its effect to living organism are significant research projects in life science, medicine, chemistry and some other areas, and are also the research hot spots. In this paper, the biological function of DNA damage, the reason causing damage and the detecting technology of DNA damage are summarized and discussed.

1. DNA damage and its biological significance

In living organism, DNA is very stable or conservative, and its primary structure is hardly changed in vital processes. This is the fundamental guarantee for the cells, even for the whole living body maintaining and passing down of the vital characters. But the stability of the DNA molecule structure is not absolute, many factors that from the living organism or the environment could cause some abnormal change of the DNA structure, which is known as DNA damage. Because DNA damages are the changes of molecular structure, which almost all of them accompanied with bonds' breakage or formation, it is essentially one kind of chemical damages. Most of the DNA damage could be restored by the repair system in organism, and the unrepaired DNA damage is very little. But just the very a little unrepaired DNA damage maybe has a magically significant influence on the organism (Liu Dianfeng, 2006, p. 3-12; Hoeijmakers, 2001, p.366-374). If this damage occurs in the double-strand of the normal DNA, the structure of the DNA and its function maybe changed. If the damage occurs in the replication process of the DNA, it will lead to base mismatched, absent, and some other damages. And it will generate erroneous encoded RNA and consequently affect the DNA translation, if the damage occurs in the process of transcription. Gene mutation caused by DNA damage may lead to aging of the organism, cancer or some other diseases related to gene. However, not all the gene mutation is harmful to organism. Some of the gene mutation which is beneficial to organism maybe retained, and eventually maybe lead to the biological evolution. From this significance, we can say that DNA damage is one of the important factors for biological evolution. Artificial DNA damage or artificial induced mutation is an important method for the treatment of cancer or genetic diseases, With the development of the genetic engineering, artificial induced mutation have increasingly being used to improve or even create species(Wang, 2003, p. 557-558).

2. Causes of DNA damage

Causes of DNA damage are varied. They can be generally divided into physical and chemical factors, the latter is the major cause.

2.1 DNA damage caused by physical factors

DNA damage can be caused by some physical factors, such as high temperature, ultraviolet rays and other ionizing radiation. For example, Ultraviolet rays can cause thymine dimerization to yield thymine dimmers. The formation of thymine dimmers can cause a deformation of the DNA's double helix structure, and consequently affects DNA unwinding and other processes, and even lead to the cessation of DNA replication and transcription.

In addition, the ultraviolet rays can also cause the cross-linking of DNA double strand and DNA with protein. Most of DNA damage caused by ionizing radiation is not direct damage, but firstly inducing a large number of free radicals in organism, and then the free radicals will lead to various types of DNA damage. Additional, due to the rupture of phosphodiester bond caused by ionizing radiation, DNA double-strand breaks will be induced, (Miyakoshi, 2000, p.293-302). For example, the mechanism ultraviolet sterilization is just to damage the DNA of bacteria, disturbing the replication process of DNA. The radiation therapy of cancer is also using ionizing radiation to damage the DNA of cancer cells, to stop its division or even make it to death (Charles, 1995, p.115-121).

2.2 DNA damage caused by chemical factors

There are a lot of chemical factors that can lead to DNA damage, such as the base molecular isomerization, O_2 , H_2O_2 and other chemicals yielded by biological metabolism, various types of free radical generated spontaneously or induced in organism. In addition, something, including heavy metal ions, medicine, pesticide and their metabolites, entered the body through various ways can also lead to DNA damage. Some of the heavy metal ions have no direct damage to DNA, but they play a important medium role in the oxidative damage of DNA (Xi, 2003, p.662-667; Lloyd, 1999, p.23-36).

Base molecular isomerization can lead to DNA damage. Isomerization can occur on cytosine (C), thymine (T), adenine (A) and guanine (G), namely all of the DNA bases. The isomerization will enable the position of hydrogen bonds between base pairs changed, then result in the bases-mismatched in the replication process, and bring about DNA damage, such as adenine pairing with cytosine, thymine pairing with guanine and so on (Sun, 2005, 18(3): 325-330). Among the four bases of DNA molecule, cytosine, adenine and guanine bases have exocyclic-amino ($-NH_2$). The exocyclic-amino can be removed under the influence of water, oxidants and free radicals and some other substances, turning the cytosine into uracil, adenine into hypoxanthine and guanine into xanthine. The deamination damage of DNA bases can lead to erroneous encoding in the replication process due to bases-mismatched, and then lead to gene mutation. Among the deamination of DNA bases, the deamination of cytosine changing into uracil has an important biological significance, because it can kill cells by inhabiting the synthesis of RNA and DNA. Nitrite induce the deamination of adenine to form hypoxanthine, and the latter can be paired with cytosine in the DNA replication process, resulting in $A \cdot T \rightarrow G \cdot C$ conversion. Guanine can turn into xanthine through deamination. Xanthine cannot be matched with other bases, and this will lead to base deletion, erroneous encoding or the termination of DNA replication process (Wink, 1991, p.1001-1003).

Alkylating agent can also cause serious DNA damage because of the alkylation of bases (Ito, 1994, p.273-285). There are many different kinds of Alkylating agent, such as the mustard gas which had been used as a chemical weapon, alkyl sulfate-ester and sulfonic-esters which are widely used in industry, N-nitroso-compound, imine, active halogenated hydrocarbon, epoxides and so on. All of the alkylating agents are electrophilic compounds, which can transfer active alkyl to nucleophilic position of the DNA bases. Some of the alkylating agent have two alkylate functional group, and they can react with two positions of DNA, resulting in the cross-linking of strand within DNA or with another DNA strand or with protein, such as nitrogen mustard, sulfur mustard, carcinogen diethylnitrosamine, anticancer medicine cyclophosphamide, cisplatin, mitomycin and so on. Cross-linking of DNA is a kind of serious DNA damage which can stop the unwinding, the replication and transcription process of DNA or even lead to the death of cells. In fact, for the therapeutic purposes, some anticancer drugs are just making use of their cross-link reaction with DNA to inhabit the growth, division of cancer cells and thereby kill them (Li, 2004, p.262-271).

Purine base or pyrimidine base can be removed by hydrolysis, and base alkylation can also help to the shedding of bases. Several reasons, such as the action of active free radical, fluoride, peroxide and some metal ions, can break DNA strand. DNA double-strand break means death to the monoploid cells of bacteria. Some compounds with a similar structure to DNA base, such as 5-bromouracil, 5-fluorouracil and 2-aminopurine, can bond to DNA in the replication process instead of normal DNA base. When DNA base analogues get into DNA molecular, they can lead to the base mismatch, causing the gene mutation. For example, the incorporation of 5-bromouracil in DNA will lead to the $G \cdot C \rightarrow A \cdot T$ transition. The base analogues have been used as a mutagen of gene mutation in genetic engineering (Ma, 1998, p. 48-50). Intercalated agent can cause DNA damage through inserting into DNA double-strand. Peroxide and free radical can open the ring of DNA base and some other compounds can modify directly the bases or other position of DNA strand. Dozens of studies have shown that hydroxyl radicals and superoxide free radicals produced by automobile exhaust, cooking smoke or cigarette smoke can react with guanine, generating 8-hydroxy-deoxyguanosine. Nowadays, 8-hydroxy-deoxyguanosine has become a recognized biomarker compound of the oxidative damage of DNA (Xi, 2003, p. 259-262).

3. Detection of DNA Damage

Determining the injury site and style is an important component of DNA damage study. In principle, any detecting methods of molecular structure can be used for DNA damage detection. Because the experimental condition is under controlled and the initial chemical composition, chemical structure and spatial structure are known, it is relatively easy for the detection of damage outside the living organism. However, for DNA damage within the living organism, the detection of it is much more hard and complicated due to the extremely complex biochemical environment, so there is very few methods can be used directly to detect the DNA damage within the living organism. By means of study of the cell survival, division and aberrance, we can determine whether there had been DNA damage and the extent of the damage. In recent years, with the development of detecting technology, the study of DNA damage at molecular level has attracted considerable attention, and has also made certain progress. The site and style of DNA damage caused by some physical or chemical factors have been affirmed.

3.1 Electrophoresis technologies

The changing of electrophoresis properties can be used to detect DNA damage. Single cell electrophoresis technique (SCGE) which is also named comet assay, is a rapid, sensitive and simple method for DNA damage detection, and has been widely used in the detection of DNA strand break damage, DNA crosslinking damage or other single-cell DNA damage (Merk, 1999, p. 167-172). If using pulsed electric field, it is called pulsed field gel electrophoresis (PFGE). This technology can be used for the detection of DNA double-strand breaks, and as the resolution and sensitivity having been greatly improved, the minimum amount of radiation inducing DNA strand breaks can be as low as 2Gy (Rojas, 1999, p.225-254). Denaturing gradient gel electrophoresis (DGGE) is primarily used to detect the point mutation in DNA fragments, and latter for the research of the microbial community structure. The developed temperature gradient gel electrophoresis (TGGE) is widely used in microbial molecular ecology, and nowadays, it plays an important role for the study of microbial community structure. One of the commonly used methods in detecting gene mutation is PCR-single strand conformation polymorphism analysis (PCR-SSCP). It is much more simple and sensitive, and can detect 70% -95% of the mutations. However, the technology cannot identify the site and property of the mutation (Wei, p.22-25).

3.2 Spectroscopic methods

Another kind of technology for DNA damage detection is making use of the spectrum property changes after being damaged. Fluorescent spectrophotometry (FP) is depend on the intrinsic fluorescence of some compounds, to detect the DNA adducts, such as polycyclic aromatic hydrocarbons. The developed method in present is respectively synchronous fluorimetric spectrophotometry, fluorescence emission spectrophotometry and cryogenic laser methods. Fluorescent spectrophotometry is of extremely high sensitive, and its detection limit is $10^6 \sim 10^8$. Synchronous fluorimetric determination is one of the most frequently used method, but it is not suitable for determination DNA damage induced by non-fluorescent intercalated agent (Vahakangas1985,p.1109-1115).Chromatography, such as denaturing high performance liquid chromatography (HPLC), take the changes of the DNA structure into account to detect the single nucleotide polymorphisms and heritable mutations (Hu, 2005, p.308-309; Wang, 2005, p.34-37). Under partially denaturation conditions, high performance liquid chromatography can effectively distinguish the heteroduplex DNA formed by mutated base and normal base with the normal double-strand DNA. Because the resolution of denaturing high performance liquid chromatography can be achieved 1bp /1kb, and the whole operating process can be programmed, scaled and automated, so the test time have been greatly reduced, and the experimental accuracy rate have been increased. To be a powerful tool for the screening gene mutation, it has a pretty good application prospect.

3.3 Biological methods

Making use of the biological response within the organism, such as immunoreaction, DNA damage can also be detected. The elementary principles of immunoassay is based on the response between antigen and antibody, using the antibody of DNA adducts to detect the corresponding DNA adducts in the injured tissue. The commonly used methods are competitive immunoassay, solid-phase competition or non-competitive enzyme-linked immunosorbent assay methods, ultra-sensitive enzymatic radioimmunoassay and so on (Ding, 2007, p. 4753-4760). Immunoassay sensitivity of DNA adducts detection is one adduct each 10^7 nucleotides, and it is very easy to be done in the cultured cells in vitro, such as lymphocytes. The advantage of it is simple, low cost and no need to enzymolysis DNA strand. However, it is not suitable for the detection of non-antigenic adducts or unknown antigen adducts, and need a large amount of DNA, further more there is antigen-antibody cross-reactivity. Many mutagenic agents can induce sister chromatid exchange within the cultured mammalian cells and the body of mammals, and the occurrence frequency have a quantitative relationship with DNA

breakage and repair. Therefore, the use of sister chromatid exchange analysis can determine whether DNA damage occurs (Yager, 1985, p. 135-139). Cleaving enzyme fragment length polymorphism analysis is a new type of gene mutation detection methods, which can detect the gene mutation on the DNA fragments more than 1 kb in length. Moreover, as the sites of gene mutation can be accurately determined, and the simple operation, good reproducibility, it shows a great superiority. Compared with the other enzymatic detection methods, it does not need a mismatch heteroduplex to provide enzyme cleavage site, thus eliminating the process of the formation of heteroduplexes (Jenkins 1999, p. 37-43). Allele specific amplify method, which is based on PCR technology, is a kind of single nucleotide mutation detection method and can conventionally analyze the replacement of known bases or small fragments absence mutation and insertion mutation. As the rapidity, good reproducibility, low-cost, no isotopic pollution and high efficiency, it is a promising method for large-scale mutation screening (Seki, 2000 p. 299-302).

3.4 DNA chip and molecular probe Technologies

DNA chip and molecular probes developed in recent years are new methods for detecting DNA damage. In the rapidly and efficiently detection of genome sequence variation and mutation, gene expression spectrum analysis, disease diagnosis, drug screening, pathogen detection, disease-related genome sequence, copy number changes and the detection of nucleotide sequence polymorphism, DNA chip has a broad perspectives in application (Shaon, 1996, p. 639-643). Synthetic oligonucleotide molecular probe can find out the mutation and its properties through the analysis of hybridization signal, and it is expected to open up a series study methods of DNA chemical modification and DNA damage detection (Gong, 2007, p. 4882-4883).

3.5 Other methods

³²P-postlabeling method can detect the DNA adducts, even if the structure of adducts is unknown. The highest sensitivity make it can detect out one adduct from each $10^8 \sim 10^{10}$ nucleotides, in other words, it can be detected as long as there is only one adduct in one cell. If combine the ³²P-postlabeling method with high performance liquid chromatography, its sensitivity and specificity can be greatly enhanced (Zhao, 1998, p. 102-103). The detecting method of DNA damage based on the changes of mechanical properties of DNA after being damaged has also been reported (Marszalek, 2007, p. 809-813).

4. Peroration

The study of DNA damage and repair is one of the important topics in life sciences and related fields, which is of great theoretical and practical significance. In recent years, with the adventure and development of the new DNA damage detecting technology, a remarkable progress has been made in the study of DNA damage and repair. Through direct or indirect determination of the biological marker of DNA damage and the changes of physical and chemical properties caused by DNA damage, many sites, styles and consequence of DNA damage have been identified. However, because of the specialty and complexity of molecular structure, physiological environment, the damage processes of DNA, most of the damage mechanism of DNA are, more or less, speculation. Many research works are making use of DNA fragments, simulated physiological conditions. So far, it is still very difficult to study the mechanism of DNA damage within living body, and further improvement is awaited the adventure and development of advanced technology.

References

- Charles J., Robbins J H, Bohr V. A. (1995). Gene specific DNA repair of damage induced in familial Alzheimer disease cells by ultraviolet irradiation or by nitrogen mustard. *Mutat. Res.*, 336, 115-121.
- Ding Guoxiang; Wang Zhi; Wang Li; et al. (2007). Comparative analysis of the result of hepatitis B virus detected by MEIA with preS1Ag and DNA. *Modern Preventive Medicine*, 24, 4753-4760.
- Gong J., Sturla S. J. (2007). A synthetic nucleoside probe that discerns a DNA adduct from unmodified DNA. *J. Am. Chem. Soc.*, 129(16), 4882-4883.
- Hoeijmakers J. H. (2001). Genome maintenance mechanisms for preventing cancer. *Nature*, 411, 366-374.
- Hu Jun, Shi Guixin, Li Lushi. (2005). The detection of single nucleotide polymorphisms, by denaturing high-performance liquid chromatography. *Journal of Clinical Laboratory Science*, 4, 308-309.
- Ito T., Nakamura T. (1994). Roles of transcription and repair in alkylating mutagenesis. *Mutat. Res.*, 314, 273-285.
- Jenkins G. J. S., Takahashi N., Parry J. M. (1999). Inverse restriction site mutation (iRSM) analysis. Mutation detection involving the formation of restriction enzyme sites in target genes. *Mutagenesis*, 14(1), 37-43.

- Li Rui, Lu Zhisong, Qiao an, et al. (2004). Study on the formaldehyde-induced DNA damage with comet assay. *Acta Biologiae Experimentalis Sinica*, 37(4), 262-271.
- Liu Dianfeng, Yu Sansan, et al. (2006). DNA damage checkpoint, damage repair, and genome stability. *J. Genetics and genomics*, 5, 3-12.
- Lloyd D. R., Phillips D. H. (1999). Oxidative DNA damage mediated by copper, iron and nickel fenton reactions: evidence for site-specific mechanisms in the formation of double-strand breaks, 8-hydroxydeoxyguanosine and putative intrastrand cross-links. *Mutat. Res.* 424, 23-36.
- Ma Huiping, Zhao Yongliang and Yang Guangyu. (1998). Application of Induced Mutation Technology for Crop Breeding, *Hereditas*, 4, 48-50.
- Marszalek P. E. (2007). Nanomechanical Fingerprints of UV Damage To DNA. *Small*, 3(5), 809-813.
- Merk O., Speit G. (1999). Detection of crosslinks with the Comet assay in relationship to genotoxicity and cytotoxicity. *Environ. Mol. Mutagen*, 33,167-172.
- Miyakoshi J., Yoshuda M., et al. (2000). Exposure to strong magnetic field at power frequency potentiates X-ray-induced DNA strand breaks. *J. Radiat. Res.*, 41,293-302.
- Rojas E. (1999). Single cell gel electrophoresis assay: methodology and applications. *J. Chromatogr. B*, 722, 225-254.
- Seki T., Tanaka T., Nakamura Y. (2000). Genomic structure and multiple single-nucleotide polymorphisms of the thiopurine S-methyltransferase gene. *J. Hum. Genet.*, 45,299-302
- Shaon D., Smith J. S., Brown P. O. (1996). A DNA microarray system for analyzing complex DNA samples using 2-color fluorescent-probe hybridization. *Genome Res.*, 6,639-643.
- Sun Julong, Yang Guanghui, He Guozhong, et al. (2005). DFT study on tautomerism of gaseous cytosine. *Chinese Journal of Chemical Physics*, 18(3), 325-330.
- Vahakangas K., Haugen A., Harris C. C. (1985). An applied synchronous fluorescence spectro- photometric assay to study benzo[a] pyrene-diolepoxide-DNA adducts. *Carcinogenesis*, (8), 1109-1115.
- Wang Gongyao, Huang Daixin. (2005). Denaturing high-performance liquid chromatography and its application in forensic DNA analysis, *Forensic Science and Technology*, 3, 34-37.
- Wang Y., Li J., Lin Z. (2003). Studies on the application of Cre/LoxP site-specific recombination system on plant male sterility and hybrid heterosis. *Molecular plant breeding*, 1(4), 557-558.
- Wei Taiyun, Lin Hanxin and Xie Lianhui. (2002). Optimization of the conditions affecting PCR-SSCP analysis. *Journal of Fujian Agricultural University (Natural Science)*, 01, 22-25.
- Wink D. A., Kasprzak K. S., Maragos C. M., et al. (1991). DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science*, 254, 1001-1003.
- Xi Zhuge, Cao Fuhuan and SunYongmei; et al. (2003). Study on the mechanism of oxidative damage of DNA induced by reactive oxygen species due to metal ions. *Acta Scientiae Circumstantiae*, 23(5), 662-667.
- Xi Zhuge, Li Guanxian, Sun Yongmei, Cao Fuhuan. (2003). Oxidative Damage of DNA and Formation of Its Biomarker 8-Hydroxydeoxyguanosine Induced by Heated Cooking Oil Vapors. *Journal of Environment and Health*, 20(5), 259-262.
- Yager J. W., Cohn K. L., Spear R. C., et al. (1985). Sister-chromatid exchanges in lymphocytes of anatomy students exposed to formaldehyde embalming solution. *Mutat. Res.*, 174, 135-139.
- Zhao C., Koskinen M., Hemminki K. (1998). ³²P-postlabelling of N6-adenine adducts of epoxybutanediol in vivo after 1,3-butadiene exposure. *Toxicol. Lett.*, 12, 102-103.