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Review article:

DNA DAMAGE: DETECTION STRATEGIES

Sunita Kumari, Rajesh P. Rastogi, Kanchan L. Singh, Shailendra P. Singh and Rajeshwar P. Sinha*

Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India

***Corresponding author:** Dr. R. P. Sinha, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India, Ph: +91 542 2307146, Fax: +91 542 2368174, e-mail: r.p.sinha@gmx.net

ABSTRACT

Even under the best of circumstances, DNA is constantly subjected to chemical modifications. Several types of DNA damage such as SSB (single strand break), DSB (double strand break), CPDs (cyclobutane pyrimidine dimers), 6-4PPs (6-4 photoproducts) and their Dewar valence isomers have been identified that result from alkylating agents, hydrolytic deamination, free radicals and reactive oxygen species formed by various photochemical processes including UV radiation. There are a number of strategies such as PCR (polymerase chain reaction), comet, halo, TUNEL (Terminal deoxyribonucleotidyltransferase-mediated deoxyuridine triphosphate nick end labeling) assay, HPLC-Electrospray tandem mass spectrometry, FISH (Fluorescence in situ hybridization), FCM (Flow cytometry), annexin V labeling, immunological assays including immunofluorescent and chemiluminescence thymine dimer detection, immunohistochemical assay, Enzyme-linked immunosorbent assay (ELISA), Radio immunoassay (RIA), Gas chromatography-mass spectrometry and electrochemical methods, that are commonly used to detect DNA damage in various organisms. The main aim of this review is to present a brief account of the above mentioned DNA damage detection strategies for the convenience of interested readers.

Keywords: ageing; cancer; detection strategies; DNA damage; UV radiation

Abbreviations: 6-4PPs (6-4 Photoproducts); CPDs (Cyclobutane pyrimidine dimers); DSB (Double strand break); FCM (Flow cytometry); FISH (Fluorescence in situ hybridization); HPLC (High-performance liquid chromatography); PCR (Polymerase chain reaction); SSB (Single strand break); TUNEL (Terminal deoxyribonucleotidyltransferase-mediated deoxyuridine triphosphate nick end labeling); UV (Ultraviolet)

INTRODUCTION

DNA is one of the key targets for UV-induced damage in a variety of organisms ranging from bacteria to human (Sinha and Häder, 2002). Among ultraviolet radiation, UV-B (280–315 nm) is the most deleterious that induces two of the most abundant mutagenic and cytotoxic DNA lesions, such as cyclobutane-pyrimidine dimers (CPDs), 6-4 photoproducts (6-4PPs) and their Dewar valence isomers, that ultimately block the movement of DNA polymerases on DNA template (Sinha and Häder, 2002; Häder and Sinha 2005). Progression of mammalian RNA polymerase II is also interrupted by CPDs and 6-4PPs (Mitchell et al., 1989; Protić-Sabljić and Kraemer, 1986). In contrast, UV-A (315–400 nm)

radiation is less efficient in inducing DNA damage because it is not absorbed by native DNA. However, it can still produce secondary photoreactions of existing DNA photoproducts or damage DNA *via* indirect photosensitizing reactions (Hargreaves et al., 2007). Nature of bases and the flexibility of DNA play a major role to the extent of DNA damage that can occur. Sequences that facilitate bending and unwinding are favorable sites for damage formation e.g. CPDs are produced at higher rates in single-stranded DNA and at flexible ends of poly d(A)-d(T) tracts, but not in their rigid centre (Becker and Wang, 1989; Lyamichev, 1991).

Other than UV radiation, there are also a number of factors such as ionizing radiations (X-rays, γ-rays, alpha particles), acridine dye, mustard gas and bleomycin that are known to cause DNA damage. Acridine dye and acriflavin are mutagenic for bacteria and higher plants (Sugino, 1966) whereas proflavin is mutagenic for phages (Crick et al., 1961). They induce deletion and insertion of single base pair in DNA helix which result damage in the native structure of DNA. Breaks in DNA may also result from damaged DNA replication forks or from oxidative destruction of deoxyribose residues. Double strand breaks are lethal as they affect both strands of DNA and lead to the loss of genetic information (Altaf et al. 2007). Low pH causes depurination and backbone breakage of DNA (Shchepinov et al., 2001). Oxidative cytosine derivatives are the most abundant and mutagenic DNA damage induced by oxidative stresses (Daviet et al. 2007).

There are a number of repair mechanisms such as photoreactivation, nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), recombinational repair and apoptosis which are operative in organisms to enable them to withstand the damage. In humans, failure of these mechanisms leads to serious hereditary diseases such as xeroderma pigmentosum and non-polyposis colon cancer as well as non-hereditary disease such as breast cancer. Xeroderma pigmentosum is a rare sun-sensitive hereditary disease (Friedberg, 1997; 2003; Masutani et al., 2000) in which organism has 10,000 fold increased risk of skin cancer on sunlight exposure. Defects in nucleotide excision repair (NER) and mismatch repair (MMR) mechanisms cause skin cancer and colorectal cancer, respectively, due to the accumulation of mutations (Friedberg, 1995). The process of cell cycle may be checked in the presence of DNA lesion so that repair process can be operated (Zhou and Eledge, 2000). At last, when DNA damage is accumulated in the absence of above repair mechanisms, the cell undergoes apoptosis (Cory and Adams, 2002) (Fig. 1).

Over a period of few decades a number of methods have been invented to detect DNA damage in various organisms (Sinha et al., 2001; Buma et al., 1995, 2001; Quaite et al., 1992; O'Brien and Houghton, 1982; Freeman et al., 1986; Mitchell et al., 1991; Pang and Hays, 1991; Li and Waters, 1996; Ravanat et al., 2000). This review deals with several methods applied by various workers to detect DNA damage in organisms.

DETECTION STRATEGIES FOR DNA DAMAGE

In the following we present a brief account on various strategies that has been commonly used for the detection and quantification of DNA damage.

Polymerase Chain Reaction (PCR)

PCR is one of the most reliably used techniques for detecting DNA damage as the amplification stops at the site of the damage. A schematic representation of detection of DNA damage by PCR is shown in Fig. 2.

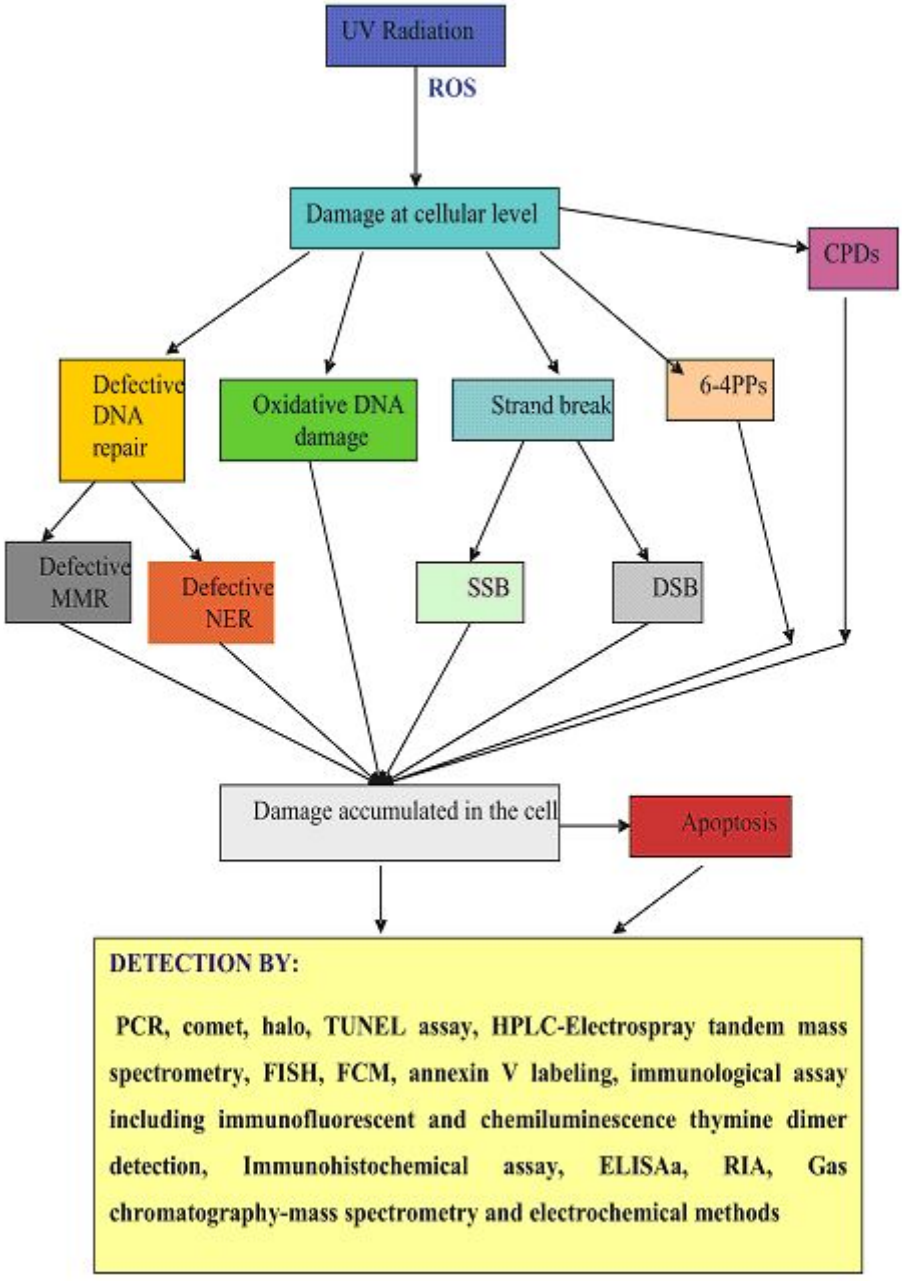

Figure 1: Schematic representation of DNA damage and commonly used detection strategies

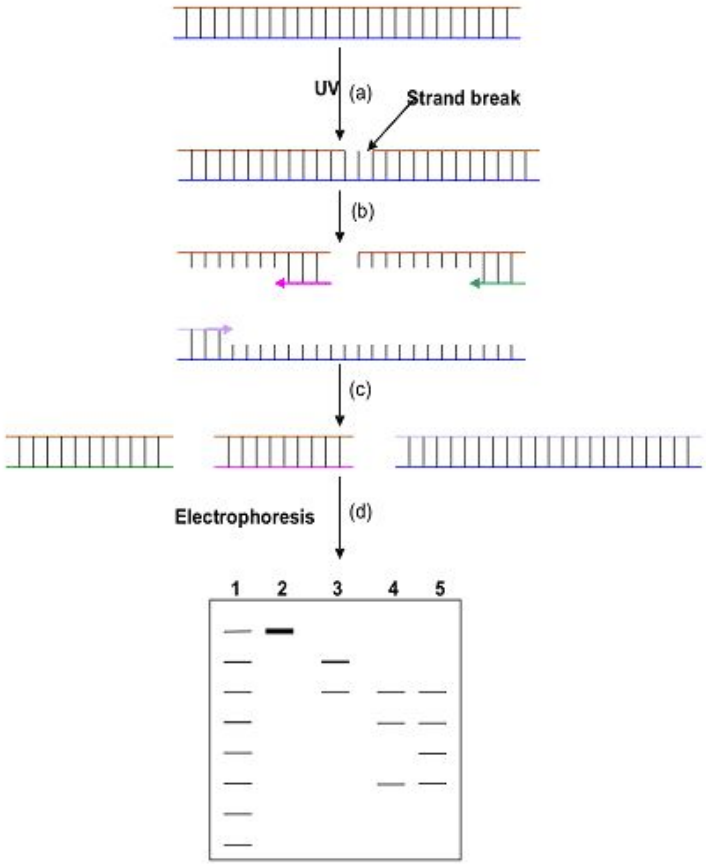

Figure 2: Schematic representation of the PCR application in DNA damage detection. Due to UV radiation or other agents, strand break occurs which result in separate amplification of the single strand that is identified after electrophoresis: (a) Strand break by UV irradiation, (b) Denaturation and annealing, (c) Amplification and (d) Gel electrophoresis showing different bands- Lane 1- Marker, Lane 2- Control, Lane 3- 1 h, Lane 4- 2 h and Lane 5- 3 h of UV treatment.

Recently, for the mapping of pyrimidine (6-4) pyrimidone photoproducts (6-4PP), TDPCR (Terminal transferase-dependent PCR) has been used (Rochette et al., 2006) which has the advantage from LMPCR (Ligation-mediated PCR) in using low doses of UV-C for mapping 6-4PP. By using PCR-based assays such as RAPD (random amplified polymorphic DNA) and rDNA amplification, Kumar et al. *(*2004) demonstrated decrease in template activity of genomic DNA of cyanobacterium *Anabaena* strain BT2 by UV-B radiation both *in vivo* and *in vitro*. An immuno-coupled PCR (ICPCR) assay has been used by Karakoula et al. (2003) to estimate T<>T (thymine dimer) formation at gene level and to compare gene and global levels of T<>T within human genomic DNA. PCR-based SINE (short interspersed DNA element)-mediated detection method was developed by Wang et al. (1999) for UV-B induced DNA damage and repair detection in mammalian genome which utilize the abundance, dispersion and conservation of SINEs. This assay is also based on the template activity of DNA region between SINEs which is amplified by using primers bind to the SINE.

Comet assay

The term Comet assay (single-cell gel electrophoresis) was first given by Olive et al. (1990). As the name indicates, it is the detection of DNA damage in individual cell and estimation of its distribution in cell population. This technique is used to detect mainly single-strand break, double-strand break, oxidative DNA damage and single-

strand break associated with incomplete excision repair sites caused by UV radiation, ultrasound, electromagnetic frequency radiation etc. Using this technique it was assessed that the two populations in the same area with different incidence of lung cancer when exposed to toxic substances shows significant difference in DNA damage, detected only by tail length (Heepchantree et al., 2006). The head of the comet appears as a spherical mass and the damaged DNA streaming from the head as a tail (Kent et al., 1995) (Fig. 3). To further improve the assay, the time and the temperature of lysis were increased to 4-5 h and 40-50 ºC, respectively (Banath et al., 1998; Olive, 1998; Olive and Banath, 1993, 1995; Olive et al., 1991; Singh et al., 1994; Vijayalaxmi et al., 1992), and alkaline lysis step was included (Singh et al., 1988). 1M NaCl was found to be sufficient to unwind DNA duplex but it should be rinsed before electrophoresis step because salt can retard migration (Olive et al., 1992). Furthermore, by incubating the lysed cells in RNase and proteinase K, the sensitivity of the assay could be increased (Singh and Stephens, 1997, 1998). DNA damage in the patients with Down syndrome has been assessed by using new optimized comet assay (Tiano et al., 2005). Recently, a modified version of comet assay i.e. apo/necro-comet-assay has been devised that differentiate viable, apoptotic and necrotic cells and determines the viability status of individual cells. It also correlate the DNA fragmentation pattern i.e. comet formed by the same cells identified by staining pattern (Morley et al., 2006).

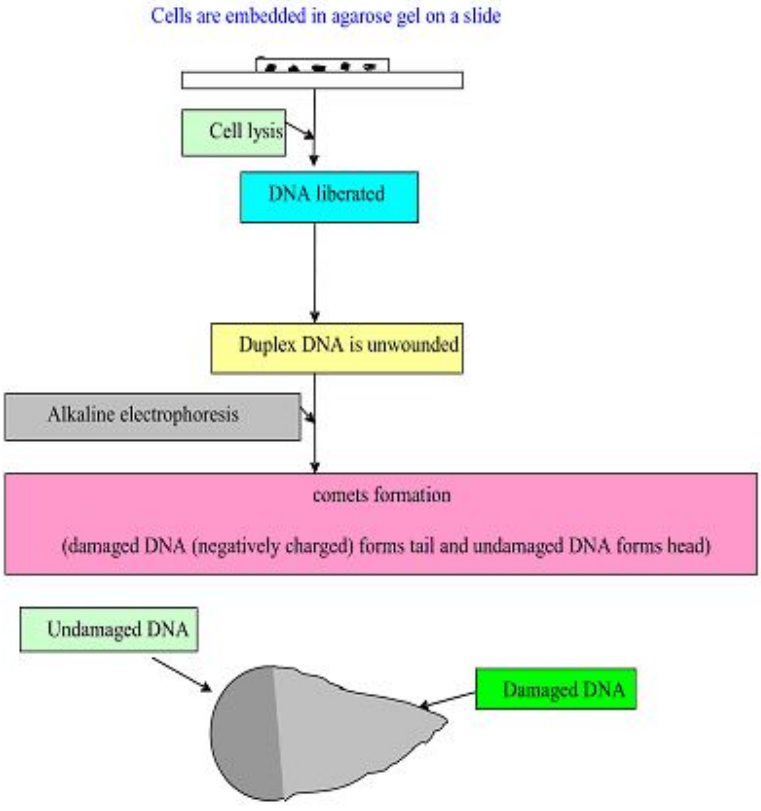

Figure 3: Schematic representation of the comet assay

Halo assay

This technique was first described by Vinograd et al. (1965) and refined by Roti Roti and Wright (1987). In this assay propidium iodide (PI), a fluorescent dye; intercalates into the DNA helix and causes the change in supercoiling status of the DNA. Thus, DNA can be seen as a fluorescent halo that changes diameter with PI concentration. At low PI concentrations (0-7.5 µg/ml) the supercoils are relaxed, while at higher PI concentrations (7.5-50 µg/ml) supercoils in the opposite winding sense are rewound. This assay can measure single cells and does not require radioactive labeling of DNA (Roti Roti and Wright, 1987) but has limitation in its sensitivity. With this technique, cells are lysed and individual nucleoids are visualized as 'halos' and thereafter, halo area can be measured by an image analysis system which determines the chromatin fragility (Woudstra et al., 1998). It was used to detect the alterations in DNA organization in individual cell (Malyapa et al., 1995). This assay can detect changes in DNA organization at radiation doses of 2 Gy, when and only the damage is not repaired (Roti Roti and Wright, 1987). After the repair of damage, the assay becomes insensitive below 10 Gy (Roti Roti and Wright, 1987; Jaberaboansari et al., 1988). For the assessment of single-strand breaks at the single cell level, this assay was improved as alkaline-halo assay. In this modified assay, the cells are first embedded in melted agarose and spread on the microscope slides, thereafter, incubated in a high-salt alkaline lysis solution followed by another incubation in a hypotonic alkaline solution and finally, stained with ethidium bromide (EB) (Sestili et al., 2006). Under these conditions, single-stranded DNA fragments diffuse radically from the nuclear cage. Fast halo assay (FHA) is a technique similar to alkaline-halo assay (AHA) but there is some modification such as simplification of the lysis, denaturation and staining procedures (Sestili et al., 2006).

Terminal deoxyribonucleotidyltransferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL assay)

As the name indicates, TUNEL assay detects DNA fragmentation by fluoresceinating the free ends of the DNA, therefore, with the help of fluorescence microscope one can detect apoptosis (Bruggeman et al., 1997). It can also detect single and double-strand breaks (Gavrieli et al., 1992; McGahon et al., 1994; Migheli et al., 1995). Due to DNA fragmentation and strand breakage, the 3'-OH termini becomes free which are enzymatically labeled with a modified nucleotide dUTP and digoxigenin, and then anti-digoxigenin antibody is used for signal detection (Pulkkanen et al., 2000). But it has limitation in sensitivity and specificity. In the apoptotic cell, DNA condensation and protein environment of DNA may interfere in the progression of this assay (Gold et al., 1994). It is very sensitive to fixation (Lucassen et al., 1995). In situ, distribution of DNA strand breaks in the rat brain following transient focal ischemia and reperfusion has been detected by Chen et al. (1997) using TUNEL assay. Pietruszewska et al. (2005) observed significant correlation between apoptotic index, degree of neoplastic cell polymorphism and tumor size by TUNEL assay. Apoptosis in the brainstems (hypoxic-ischemic injury) was also studied by TUNEL assay and heterogeneity in apoptotic index among the nuclei leads to the hypothesis of the differing vulnerability of the nuclei of the brainstem (Stecco et al., 2005).

HPLC-electrospray tandem mass spectrometry

Oxidative stresses such as inflammation, oxidative metabolism and exposure to UV radiation are known to cause oxidative DNA damages. Oxidative DNA damage leads to most common diseases such as cancer and ageing (Chance et al., 1979; Halliwell and Gutteridge, 1989; Halliwell, 1999; Beckman and Ames, 1999). HPLC-electrospray tandem mass spectrometry is a sensitive and specific assay but due to early elu-

tion property of liquid chromatography, it is not so helpful in the detection of oxidative base damages, mainly of guanine (purine base) which is most prone to oxidation. The soft ionization property of electrospray allows to assess the DNA adducts with bulky chemicals (Wolf and Vouros, 1994; Rindgen et al., 1995) and UV-induced dimeric pyrimidine photoproducts. It also allows the detection of cis-syn and trans-syn I cyclobutane thymine dimers (c-s T<>T and t-s T<>T, respectively) (Douki et al., 2000a). A urinary-8-hydroxylated species of guanine has been determined by using this assay. The analysis includes 8-hydroxylated base, ribonucleoside and deoxynucleoside and the corresponding non-oxidised species. It is used to quantify 5,6-dihydroxy-5,6-dihydrothymidine, 5-hydroxy-2-deoxyuridine, 8-oxo-7,8-dihydro-2-deoxyadenosine in isolated and cellular DNA after exposure to γ-rays (Frelon et al., 2000).

Fluorescence in situ hybridization (FISH)

It is a non-isotopic labeling and detection method which determines the copy number or relative location of disturbed cellular DNA content in nuclei or chromosome (Murthy and Demetrick, 2006). FISH is more sensitive than flow cytometry in detection of aberrant urinary cells, and required very little material (Sauter et al., 1997), but 100 % specificity was never obtained due to rare chromosomal aberrations in normal urothelium (Sauter et al., 1995). With this technique, visualization and estimation of DNA damage is carried out on a cell by cell basis (Hopman et al., 1991; Kallioniemi et al., 1992). Chromosomes with numerical aberrations are detected efficiently by this method (Sauter et al., 1997). This technique has been used efficiently in detecting HER-2/neu (c-erbB-2) alterations in the patients with breast cancer (Sidoni et al., 2006). FISH on touch preparations is an efficient method for the study of loss of heterozygosity on 1p/19q (reported in oligodendroglial tumors) testing and does not require normal DNA as control (Scheie et al., 2006). A modification of FISH is interphase-dual-color and dual-fusion fluorescence in situ hybridization (DD-FISH) which detect minimal residual disease (MRD) with chronic myelogenous leukemia (CML) after allogeneic hematopoietic stem cell transplantation (allo-HSCT) (Qian et al., 2006).

Flow cytometry (FCM)

DNA fragmentation also occurs in necrosis and autolysis other than apoptosis (Grasl Kraupp et al., 1995) and TUNEL assay may fail to differentiate various types of cell death. Regarding this, a new method known as flow cytometry has been developed for the detection of apoptosis (Koopman et al., 1994) which also has the advantage of analyzing large number of cells (Deaven, 1982; Bickham, 1990). This assay is useful in detecting chromosomal aberrations, sister-chromatid exchange, chemical adducts to DNA and DNA strand breakage (Lower and Kandall, 1990; Shugart, 1990; Deaven, 1982; Bickham, 1990; Bickham et al., 1994). Recently, nucleotide excision repair has been also detected by alkaline unwinding FCM assay (Thyagarajan et al., 2007).

Annexin V labeling

Annexin V was first reported by Inaba et al. (1984). Annexin V does not bind to the vital cells because of its inability to penetrate the lipid bilayer. However, in dead cells, the inner leaflet of the membrane is free for binding of extrinsically applied annexin V (van Engeland et al., 1998). During apoptosis, phosphatidylserine is translocated to the outer surface of the membrane, with which annexin V binds with high affinity in the presence of Ca^{+2} (Andree et al., 1990; Tait et al., 1989). B-lymphocytes undergoing chromatin condensation were strongly stained with annexin V because chromatin condensation coincides with phosphatidylserine exposure (Koopman et al., 1994). DNA fragmentation is only detected in annexin V-positive cells (Levy et al., 1998). Vital cell are nega-

tive for both propidium iodide and annexin V, apoptotic cells are negative for propidium iodide and positive for annexin V whereas dead cells are positive for both propidium iodide and annexin V (van Engeland et al., 1998). Apoptosis in tumors cells caused by X-rays has been recently detected by using (125)I-radiolabeled annexin V (Watanabe et al., 2006).

Immunological assay

It is also one of the methods commonly used for the detection of oxidative DNA damage but it has limitations because of cross-reactivity of the antibodies with normal DNA bases. Thymine glycols were detected by this method (Leadon and Hanowalt, 1983). Radiochemical, fluorescent or enzyme-conjugated secondary antibodies technique is useful in the quantification of UV photoproducts (CPDs). In this assay DNA damage can be detected and quantified very efficiently by immunoslot-blot system utilizing chemiluminescent detection (Kriste et al., 1996), secondary antibodies conjugated to alkaline phosphatase enzymes (Wani et al., 1987) and secondary antibodies conjugated to radioactive iodine (Plaza et al., 1991). Antibodies to modified nucleosides are also possible (Wallace et al., 1971).

Immuno-slot-blot assay is used to detect very low levels of adduct in very small amount of DNA. It is a very sensitive and specific assay. Malonaldehyde (MDA) is mutagenic in bacterial and mammalian system (Basu and Marnett, 1983; Marnett, 1994) and carcinogenic in rats (Spalding, 1988). Product of the metabolic processes such as lipid peroxidation and prostaglandin biosynthesis, forms endogenous adducts with DNA. The major adduct, a fluorescent pyrimidopurinone, malondialdehyde-deoxyguanosine (M_1-dG) has been measured in healthy human liver, leukocyte and breast tissue. The availability of a new monoclonal antibody (D1OA1) produced and chartacterised by Sevilla et al. (1997) made it possible to develop an immunoslot-blot assay for the detection of M_1-dG, in intact DNA using only 1µg of DNA per slot (Leuratti et al., 1998) and in leukocytes of uremic patients undergoing hemodialysis (Müller et al., 2004).

An immuno-dot-blot assay was used to detect CPDs, 6-4PPs and their Dewar valence isomers in UV-irradiated mammalian cells (Perdiz et al. 2000). To determine the frequency of thymine dimers in a variety of aquatic organisms such as cyanobacteria, phytoplankton and macroalgae, a simple and efficient quantitative method was developed by Sinha et al. (2001) which is based on use of thymine dimer-specific antibodies followed by blotting and chemiluminescence methods (Fig. 4).

In this assay a plasmid pBSK with known DNA sequence, length and number of adjacent thymine pairs was used for calibration. By using the same method it was found that there was an increase from 3.2 to 50.9 thymine dimers per mega base pair during the light period in phytoplankton assemblage exposed at the surface under natural solar radiation from a freshwater lake in Trelew, Argentina (Klisch et al. 2005). Otero et al. (2006) have also used dot-blot assay given by Sinha et al. (2001) to detect DNA damage and its intensification by cadmium in liverwort *Jungermannia exsertifolia* subsp. *cordifolia*.

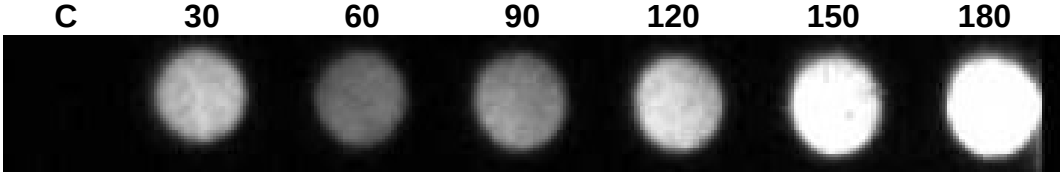

Figure 4: Detection of thymine dimer by dot-blot after increasing UV exposure time (min)

Immunohistochemical assay

This technique is carried out on fixed cells (lymphocytes, bladder cells etc.) treated with proteases and RNase in order to remove proteins and RNA, respectively, which can cross-react with DNA. Cells are then counterstained with propidium iodide for immunofluorescence to allow visualization of nuclei in adduct-negative cells. It is applicable to small amount of samples and detect adducts in specific cell types within the tissue (Santella, 1999). In this technique epitopes can be localized and specific areas can be selected without DNA extraction and hydrolysis. It is useful for *in vitro* and *in vivo* studies on cancer, oxidative stress-associated pathologic conditions such as ageing, neurodegenerative diseases, ischemia-reperfusion injury etc. (Toyokuni et al., 1997). This technique has been recently used in combination with FISH to detect HER-2/neu (c-erbB-2) alterations in the patients with breast cancer (Sidoni et al., 2006).

Radio immunoassay (RIA)

Anti-carcinogen adducts and carcinogen-modified DNA antibodies were firstly used in RIA. Antigen is synthesized in both radiolabeled (tracer) and non-labeled (inhibitor) form (Poirier et al., 1977; Umbenhauer et al., 1985) and standard curves are obtained by mixing equal amount of antibody and tracer with increasing concentration of inhibitor in a constant volume (Santella, 1999). It has the capacity to estimate 6-4 photoproducts and cyclobutane dimers in DNA (Mitchell et al., 1985). In the detection of very low quantity of cyclobutane pyrimidine dimer (CPD) in bacterioplankton and marine viruses caused by UV-B radiation, radioimmunoassay was found to be very effective (Jeffrey et al., 1996; Miller et al., 1999; Mitchell, 1996). Specific RIAs were used to monitor antibody binding sites associated with cyclobutane dimers and 6-4 photoproducts. Using this technique, biological role of 6-4 photoproducts can be envisaged (Mitchell and Rosenstein, 1987). Cyclobutane pyrimidine dimers were detected in active *Mycobacterium parafortuitum* and *Serratia marcescens* cells, using fluorescent Alexa Fluor 488 and radiolabeled (125)I secondary antibodies as reporters (Peccia and Hernandez, 2002).

Enzyme-linked immunosorbent assay (ELISA)

In this technique antigens (modified DNA) bound to the plate which is blocked by the incubation of wells with a dilute protein solution. Thereafter, unknown samples are similarly mixed with antibody before addition to the plate. Bound primary antibody is quantified with enzyme-conjugated secondary antisera by addition of appropriate substrate after incubation and washing off non-bound material (Santella, 1999).

Gas chromatography-mass spectrometry (GC-MS)

By the interaction of γ-rays with DNA, hydrolysis of water occurs (Ward, 1988) which leads to the formation of reactive oxygen species that can further react with DNA to cause various types of DNA damages (strand break, modified bases, abasic sites, DNA-protein crosslinks etc.) (von Sonntag, 1987; Cadet and Téoule, 1978; Dizdaroglu, 1985). For the detection of oxidative DNA damage, GC-MS is commonly used because of its ability to detect wide range of DNA base product. In this method polar bases are converted into thermally stable derivatives which possess mass spectra in a process called derivatization. But this method sometimes overestimate the oxidative damage due to the derivatization of hydrolyzed DNA at higher temperature in the presence of air that result in increase level of 8-OH-guanine, 8-OH-adenine and 5-OH-cytosine (Jenner et al., 1998). 8-oxo-7,8-dihydroguanine is the most common type of base damage (Floyd, 1990). Fapy (formamido-pyrimidines) derivatives are also measured by the use of GC-MS (Douki et al., 1999, 2000b).

Electrochemical methods

Electrochemical methods offer a sensitive, selective, low cost and miniaturized device for the detection of DNA damage (Paleček et al., 1998; Lee and Shim, 2001; Paleček and Jelen, 2002; Darain et al., 2004; Rahman et al., 2005; Cahová-Kuchaříková et al., 2005). DNA is an electroactive and surface-active substance yielding analytically valuable electrochemical signals. Adenine, cytosine, and guanine undergo redox processes at the mercury electrodes while guanine and adenine are oxidizable at carbon and some other solid electrodes. Some of these signals respond to the changes in DNA structure (Paleček et al., 2002; Fojta, 2002; 2004). 8-oxoguanine has been detected *via* its oxidation signal at carbon electrodes (Langmaier et al., 2003; Brett et al., 2000). The lesions such as thymine dimers could not be detected electrochemically until they are connected with distortions of DNA double helix (Fojta, 2002; 2004). Electrochemical responses of native (double-stranded) and denatured (single-stranded) DNAs differ greatly at the mercury electrodes, allowing for determination of small amounts of ssDNA in dsDNA samples (Cahová-Kuchaříková et al., 2005). Kara et al. (2007) studied the electrochemical detection of DNA damage by direct and indirect irradiation with radioactive technetium (Tc-99m) and iodine (I-131). The decrease in the guanine oxidation signal after irradiation with Tc-99m and I-131 can be attributed to conformational changes in the DNA double helix. The increase in the guanine oxidation signal after irradiation with varying Tc-99m radioactivity levels can be attributed to the hydrogen bonding break down, which results from increasing radioactivity levels of technetium.

CONCLUSION

DNA damage has been studied in a variety of organisms such as bacteria, cyanobacteria, phytoplankton, macroalgae, plants, animals and humans. It may be spontaneous or environmental that affects all living cells in a number of ways (Horio et al., 2007). There are several kinds of DNA damages and for developing some artificial repair strategies (e.g. artificial repair enzymes) against these damages in humans and other important flora and fauna, detection of DNA damage is important.

At present there are several methods available for detecting different kinds of DNA damage but with some or other limitations. PCR based assays are although very sensitive and easy to measure gene-specific DNA damage but can not quantify and recognize the kind of damage. It is completely based on template activity of damaged DNA during amplification and analysis depends on the intensity of amplified band. Other factors like pippetting of different component of PCR mixture and amount of starting material (template) for amplification may also affect the band intensity and therefore much accuracy is required to get the precise results. Comet assay can detect DNA damage in individual cell and is useful for the estimation of damage distribution in a population of cells. Halo assay has the advantage of not using radioactive substances but it is not very sensitive. TUNEL assay detects DNA fragmentation (SSB and DSB) by fluoresceinating the free ends of the DNA but it can not differentiate apoptosis from necrosis and autolysis in which DNA fragmentation is also common. A new method, flow cytometry, was developed for detection of DNA damage exclusively in apoptotic cells (Koopman et al., 1994). HPLC-electrospray tandem mass spectrometry has the problem of early elution property of liquid chromatography but it is a sensitive and specific assay for the quantification of thymine dimer and detection of oxidative damage. FISH is a non-isotopic labeling and detection method and is more sensitive than flow cytometry. Annexin V labeling has advantage in analyzing apoptosis because it does not bind to vital cells. Immunological assays are useful in estimation of CPDs and 6-4PPs and required less amount of DNA. GC-MS is mainly useful for detecting oxidative DNA damage but it has the limitation of over estimation of damage.

Thus it is clear that there is a need to combine the features of different detection methods and to develop a unique strategy that can localize damage in genome, point out the nature of damage and quantify damage and repair processes. This will be helpful in developing repair strategies and will also provide better insight into the process of carcinogenesis and ageing.

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