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Methods for DNA Strand Breaks Detection

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Abstract: The study of Deoxyribonucleic Acid (DNA) damage holds a wide interest within both basic and applied fields of research. Elucidating the mechanisms involved in the generation of DNA damage and the consequences of this damage, will have an enormous impact on multiple fields of scientific research and will ultimately lead to a better understanding of human disease. In this review article, a variety of experimental molecular biology techniques will be described.

Key words: DNA strand breaks, flow and laser scanning cytometry, limitations, nick translation and electron microscopy, T4 DNA ligase, TUNEL assay, ultrasound

INTRODUCTION

Advances in medicine and medical technology have resulted in a tremendous improvement in health and welfare. However, we are still faced with various diseases that are difficult to treat using contemporary medicine (Namiri *et al*., 2010). A number of exogenous and endogenous toxic agents may damage DNA, leading to genomic instability and transcriptional infidelity. Genetic or acquired defects in DNA repair mechanisms also contribute to exacerbate DNA damage (Rolig and McKinnon, 2000). The assessment of cellular DNA damage is crucial in many areas of biology including immunology, developmental biology, aging, cancer and environmental science (Freeman *et al*., 1986). At least two types of cell death are the consequence of extensive and irreversible DNA damage, i.e., apoptosis and necrosis. Apoptosis plays a central part in normal tissue homeostasis and has a role in a variety of clinical diseases characterized by either increased or decreased cell survival (Fauziah *et al*., 2010) (Fig. 1). Apoptosis is a widespread phenomenon, which plays an important role in many physiological events as well as pathological processes (Ansari *et al*., 1993). In order to establish that apoptosis is occurring, other criteria must also be used (Walker *et al*., 1995). The cell shrinkage is a ubiquitous characteristic of programmed cell death that is observed in all examples of apoptosis, independent of the death stimulus (Parichehr *et al*., 2008). Morphologic criteria for apoptosis include cell shrinkage, blebbing of the cell surface, chromatin condensation and margination, nuclear pyknosis and late fragmentation into apoptotic bodies, with remarkable preservation of the integrity of cell membranes and organelles. All of these changes are due to cleavage of various cytoplasmic and nuclear substrates (Budihardjo *et al*., 1999). Although the nuclear breakdown is widely considered to be a hallmark of apoptosis, its mechanism remains poorly understood (Montague and Cidlowski, 1996). DNA damage also occurs in necrosis. At variance with apoptosis, necrosis is not a programmed event, is characterized morphologically by early swelling, disintegration of membranes and organelles and absence of chromatin condensation (Majno and Joris, 1995). Indeed, the DNA damage that occurs in necrosis is not easily distinguishable from that of apoptosis. Thus, there has been tremendous progress in molecular biology techniques to understand the mechanism of DNA damage. In this article, different methods for DNA damage detection will be described.

LABELING DNA BREAKS USING TERMINAL TRANSFERASE (TUNEL ASSAY)

Apoptotic and programmed cell death are characterized by and indeed were first discovered from observations of, remarkable morphological changes that occur in the nucleus (Lockshin *et al*., 1998). Thus, light and electron microscopy were the first tools for the detection of apoptosis. This characteristic collapse of chromatin and ultimately the structural organization of the nucleus are triggered by the degradation of DNA, which is an active process and occurs prior to death of the cell. The degradation of DNA was subsequently found to be mediated by endonucleolytic activity that generated a specific pattern of fragments (Wyllie, 1980). DNA fragmentation is quite variable within cells and some cell types produce only High Molecular Weight (HMW) fragments. The latter observations formed the basis of a

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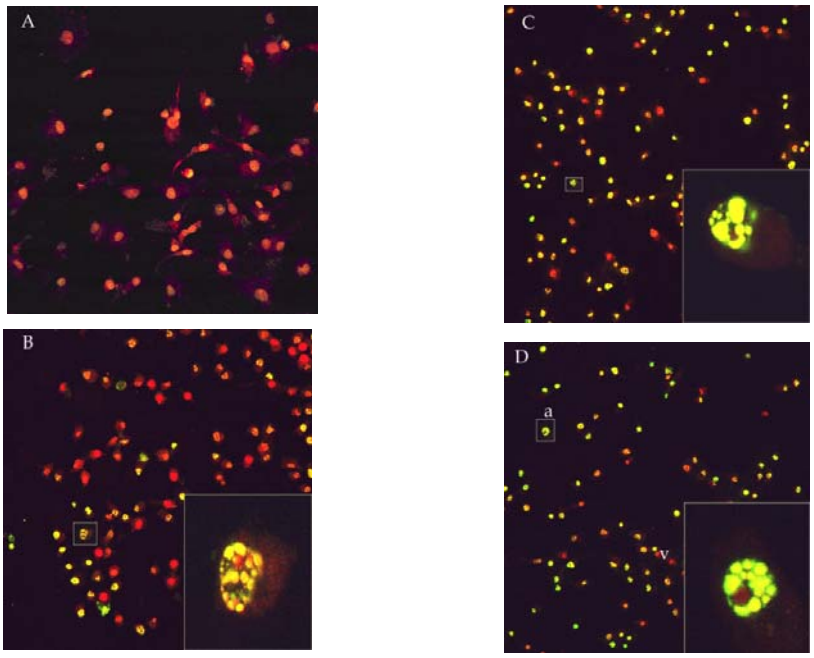

Fig. 1: Confocal micrographs of MCF-7 cells treated with AF2240 strain of NDV and stained by TUNEL technique: (A) untreated (B, C and D) treated for 24, 48 and 72 h, respectively. Cells were double stained with fluorescein-12-dUTP and propidium iodide. Viable (V) cells showed orange to red nuclei, whereas apoptotic (a) cells showed yellow to green nuclei. Note the fragmented nucleus (B, C and D). Magnification: (A-D): 40x, inserted picture (B-D): 120x (Fauziah *et al*., 2010)

convenient *in vitro* biochemical technique for the routine detection of apoptosis by resolving the fragmented DNA by conventional or pulsed field agarose gel electrophoresis. However, this technique requires relatively large amounts of material and DNA extraction. Subsequently, a variety of techniques have emerged to detect apoptotic DNA fragmentation *in situ* by exploiting the fact that the hydroxyl group at the 5' or 3' ends of the small DNA fragments becomes exposed. Nucleotide analogues can be attached to the ends by several enzymes, with Terminal deoxynucleotide Transferase (TdT) being the most popular (Gavrieli *et al*., 1992; Mundle *et al*., 1994). The assays are typically fluorescence-based, either by the direct incorporation of a nucleotide to which a fluorochrome has been conjugated, or indirectly using fluorescent dye conjugated antibodies that recognize biotin or digoxigenin tagged nucleotides. The assays have been formatted for light and confocal microscopy as well as flow cytometry, thereby greatly facilitating the detection and quantitation of apoptosis *in situ*. The first end-labeling protocol developed for the detection of DNA fragmentation in apoptosis was the Terminal Uridine Nucleotide End Labeling (TUNEL) technique of Gavrieli *et al*. (1992). This method exploited the ability of the enzyme, terminal transferase, to add biotin-conjugated nucleotides onto the 3' OH of a DNA strand. By using either a fluorescently tagged or radioactively labeled nucleotide analog, the DNA fragments become detectable.

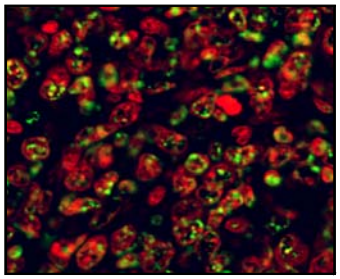

Fig. 2: TUNEL labeling of breast cancer tissue treated with 250 mg/kg of Neem. Note small dots of green fluorescent stained among the chromosomes clot in the nucleus overlapping the PI red fluorescent staining for the DNA nucleus (Magnification x 600) (Fauziah *et al*., 2011)

Formulation of the reaction buffer with cobalt ensures that the enzyme can add multiple bases to the 3'-end of each strand. As mentioned above, all types of 3'-end can be labeled, including those of single and double stranded DNA as well asrecessed, protruding and blunt ends. The enzyme appears to have a preference for single stranded and 3'-protruding ends. The method can be used on cell suspensions and monolayers as well as frozen or paraffin tissue sections. The methods use either digoxigenin-conjugated dUTP detected by stain-ing with a FITC-conjugated anti-digoxigenin antibody or biotin-conjugated dUTP detected by staining with FITC-conjugated streptavidin (Fauziah *et al*., 2011) (Fig. 2).

Limitations: DNA fragments with 3'-OH ends can be produced in a number of situations where apoptosis is not occurring. For example, some forms of DNA damage produce DNA breaks or nicks with 3'-OHs. Moreover, the DNA degradation that occurs during necrosis also produces fragments with 3'-OH that would be labelled by TUNEL or ISEL (*In situ* End Labeling). Over-reliance on these techniques has led to considerable controversy in studies in brain where, following some insults, both apoptosis and necrosis occur simultaneously making it very difficult to establish and quantitate true apoptotic cell death (Roy and Sapolsky, 1999; Gilmore *et al*., 2000; Chun, 2000; Darzynkiewicz, 1993). It is evident, therefore, that TdT-based labelling techniques should not be used as the sole criterion for establishing the nature of the cell death mechanism. In order to establish that apoptosis is occurring; other criteria must also be used. Since it is possible to use multiple fluorochromes in the same experiments, another marker such as the appearance of annexin on the cell surface can be used simultaneously. Once it has been established that the cell death is indeed apoptotic, then the TdT-based assays can be used for routine quantitation by microscopy or flow cytometry (Walker *et al*., 1995; Darzynkiewicz, 1993).

ANALYSIS OF APOPTOSIS BY FLOW AND LASER SCANNING CYTOMETRY

One advantage of strand break labeling with fluorochromes is that such cells can rapidly be analyzed by flow cytometry. When cellular DNA content also is measured in these cells, the bivariate analysis of such data provides information about DNA ploidy or the cell cycle phase specificity of apoptosis (Gorczyca *et al*., 1992; Gorczyca *et al*., 1993). Laser Scanning Cytometer (LSC) is the microscope-based cytofluorimeter which allows one to measure rapidly, with high sensitivity and accuracy, fluorescence of individual cells (Kamentsky, 2001; Darzynkiewicz *et al*., 1999). The instrument combines advantages of both flow and image cytometry. Cells staining on slides eliminates their loss that otherwise occurs during repeated centrifugations in sample preparation for flow cytometry. Another advantage of LSC stems from the possibility of localization of cells on slide for their visual inspection or morphometry after the initial measurement of large cell population and electronic selection (gating) of cells of interest. Visual examination is of particular importance because the characteristic changes in cell morphology (Kerr and Wyllie, 1972) are still considered the gold standard for positive identification of apoptotic cells. Furthermore, the measured cells can be bleached and re-stained with another set of dyes (Bedner *et al*., 2001). The cell attributes measured after re-staining can be correlated with the attributes measured before, on a cell by cell basis (Bedner *et al*., 2001; Li and Darzynkiewicz, 1999) (Fig. 3). Fixation and permeabilization of the cells are the initial essential steps to success-fully label DNA strand breaks. Cells are briefly fixed with a cross linking fixative such as formaldehyde and then permeabilized by suspending them in ethanol or using detergents in the subsequent rinses. By cross linking low MW DNA fragments to other cell constituents, formaldehyde prevents extraction of the fragmented DNA, which otherwise occurs during repeated centrifugations and rinses required by this procedure (Gorczyca *et al*., 1992; Darzynkiewicz *et al*., 1992; Darzynkiewicz *et al*., 1997). The 3'OH-termini of the DNA breaks serve as primers and become labelled in this procedure with Bromodeoxyuridine (BrdU) when incubated with 5-Bromo-2'-deoxyuridine-5'-Triphosphate (BrdUTP) in a reaction catalyzed by exogenous Terminal deoxynucleotidyl Transferase (TdT) (Li and Darzynkiewicz, 1995; Li *et al*., 1996). The incorporated BrdU is immunocytochemically detected by BrdU antibody conjugated to FITC (Li and Darzynkiewicz, 1995). The latter is a reagent widely used in studies of cell proliferation to detect BrdU incorporated during DNA replication (Dolbeare and Selden, 1994). The overall cost of reagents is significantly lower and sensitivity of DNA

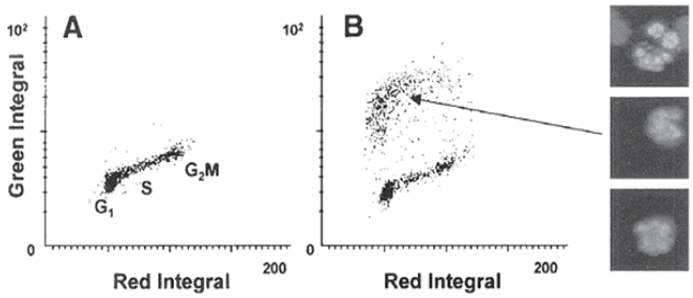

Fig. 3: Detection of apoptotic cells after labeling DNA strand breaks with BrdUTP. U-937 cells were untreated (A) or treated with tumor necrosis factor-α in the presence of cycloheximide (B) (Li and Darzynkiewicz, 2000; Bedner *et al*., 2000)

strand breaks detection is higher when BrdUTP is used as a marker, compared to the alternative labeling with biotin (or digoxygenin) or directly fluorochrome-tagged deoxynucleotides (Li and Darzynkiewicz, 1995). This method is useful for clinical material, such as obtained from in leukemias, lymphomas and solid tumors (Halicka *et al*., 1997) and can be combined with surface immunophenotyping.

The procedure of DNA strand break labelling is rather complex and involves many reagents. Negative results, therefore, may not necessarily mean the absence of DNA strand breaks but may be due to methodological problems, such as loss of TdT activity, degradation of BrdUTP, etc.

Nick translation and electron microscopy: (Rigby *et al*., 1997) Introduced nick translation procedure in 1977. This technique is used to label purified DNA. This method relies on combined 5'→3' polymerase and 5'→3' exonuclease activities of Escherichia coli DNA polymerase I. In addition to DNA damage detection (Hashimoto *et al*., 1995) the method was adapted to study the location of DNase I-sensitive regions within cellular DNA. (Levitt *et al*., 1979) selectively labeled nuclease sensitive sites in DNA in isolated nuclei by nick-translation using treatment with low concentrations of DNase I. It was recently adapted to the electron microscopic level in order to discriminate between active and inactive regions of inter-phase chromatin (Thiry, 1991). The technique is applied to the ultrathin sections of biological material and includes two successive steps, the enzymatic labeling reaction followed by an immunocytochemical detection step. The newly synthesized DNA strands are visualized by an indirect immunogold labelling technique. Nick translation using mild digestion with DNase I allows preferential labelling of actively transcribing DNA regions. When DNase I treatment is omitted the technique detects the pre-existing single-stranded DNA breaks and can be used for visualization of DNA damage *in situ* at the electron microscopic level (Sugimoto *et al*., 1999).

Using T4 DNA ligase to detect DNA breaks and apoptosis: In situ ligation technique was developed to label a particular type of DNA damage, namely, full double strand breaks, directly in fixed tissue sections (Didenko and Hornsby, 1996; Didenko *et al*., 1998; Didenko *et al*., 1999). The technique uses double-stranded oligoprobes with specific ends, which are attached by T4 DNA ligase to the ends of DNA breaks (Fig. 4).

In situ ligation can be combined with TUNEL, allowing co detection of free 3'OH groups *in situ* and with immunohistochemical staining of cellular proteins. The types of double-strand breaks which can be detected by *in situ* ligation occur in various cellular processes, the majority of which are related to cell damage and death.

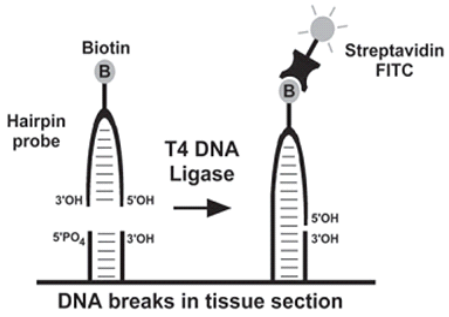

Fig. 4: Principle of *in situ* ligation assay

The basics of *in situ* ligation labeling of apoptotic cells rely on the fact that double-strand breaks produced by apoptotic endonucleases are either blunt-ended or have short 3' or 5' extensions. Applications of *in situ* ligation to the investigation of the other sources of double strand breakage in DNA are currently lacking. These other sources of double strand breaks include free radicals, ionizing radiation and various radiomimetic and chemotherapeutic agents, capable of inducing the breaks directly, or indirectly as a result of the damaged DNA repair, recombination and replication. In addition, two physiological forms of intentional double strand breakage, generating a small number of breaks, occur in lymphoid cell differentiation during V(D)J recombination and class switch recombination (Lieber *et al*., 1998). However detection of these breaks is problematic unless specialized methods of signal enhancement are used.

Combination of comet and TUNEL assays: There are many biological circumstances that require the use of small cell samples. To extract DNA damage information from these samples, techniques that rely upon the evaluation of DNA damage at the level of single cells is required. The Single Cell Gel Electrophoresis (SCGE) or "comet" assay is the most widely applied method for the detection of DNA damage in single cells. In this approach damaged DNA in individual cells is electrophoresed away from a nucleus into an agarose gel followed by staining.

The analysis of DNA damage in single cells was first reported in the studies of (Ryberg and Johanson, 1997). In this technique DNA damage was detected by:

- Lysing cells embedded in agarose on slides
- Promoting partial DNA unwinding
- Staining DNA with a dye

This method was improved by the subsequent addition of an *in situ* agarose gel electrophoresis step (Osstling and Johanson, 1984) to allow easier discrimination between damaged and undamaged DNA. This approach was further refined by (Singh *et al*., 1988)

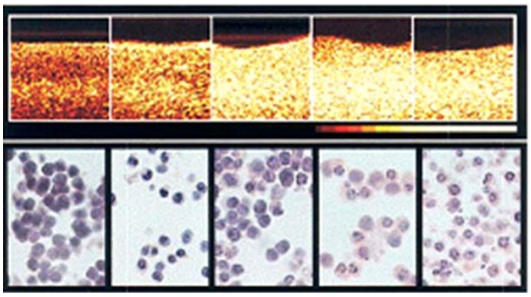

Fig. 5: Ultrasound imaging of apoptosis and correlative histology. Top panels present results of ultrasound imaging of apoptotic cells. Each panel is a representative ultrasound scan of a pellet of AML cells. From left to right, panels correspond to cells treated with cisplatinum for 0, 6, 12, 24 and 48 h. At 0 and 24 h, histological analysis indicated that 1.6 and 87% of all cells showed nuclear fragmentation, respectively. By 6 h of drug exposure 72% of the cells exhibited prominent nuclear condensation changing from a nuclear diameter 70% of the cellular diameter before addition of the drug, to a diameter 40% of the cellular diameter at 6 h. Additionally, after the 6 h time point, 95% of all cells exhibited nuclear condensation or fragmentation (Czarnota *et al*., 2000)

who included exposure to alkaline/high salt conditions, which allowed a direct correlation between electrophoretic migration distance and the extent of DNA damage. Recently, a convenient protocol to directly tag 3'-hydroxyl groups of DNA has become available. In this method, 3'-hydroxyl groups are labelled with biotinilated-dUTP or fluoresceinated-dUTP via TdT then visualized by standard fluorescence techniques.

Ultrasound: The high frequency ultrasonic detection of programmed cell death has been demonstrated *in vitro*, *in situ* and *in vivo* using a variety of different apoptosis inducing methods (Czarnota *et al*., 2000) (Fig. 5). This method provides a useful image based adjunct for the detection of programmed cell death in a laboratory setting as well as being a powerful potential clinical tool which can be used to monitor tumour responses to treatment. Data collected to date indicates that this capability of high-frequency ultrasound is based on interactions of high-frequency ultrasound waves with the chromosomal nuclear material in cells, which undergoes structural changes of condensation and subsequent fragmentation with the process of programmed cell death (Czarnota *et al*., 2000) (Fig. 5). The ultrasound-based approach to detecting apoptosis has a number of potential applications which range from embryological studies of development where apoptosis plays an important role, to assessing organ viability for the purposes of transplantation, a situation where the presence of programmed cell death is correlated to clinical outcome (Wood, 1998).

REFERENCES

Ansari, B., P.J. Coates, B.D. Greenstein and P.A. Hall, 1993. In situ end-labeling detects DNA strand breaks in apoptosis and other physiological and pathological states. J. Pathol., 170: 1-8.

Bedner, E., P. Smolewski, P. Amstad and Z. Darzynkiewicz, 2000. Activation of caspases measured in situ by binding of Fluorochrome-Labeled Inhibitors of Caspases (FLICA): Correlation with DNA fragmentation. Exp. Cell Res., 260: 308-313.

Bedner, E., L. Du, F. Traganos and Z. Darzynkiewicz, 2001. Caffeine dissociates complexes between DNA and intercalating dyes: Application for bleaching fluorochrome-stained cells for their subsequent restaining and analysis by laser scanning cytometry. Cytometry, 43: 38-45.

Budihardjo, I., H. Oliver, M. Lutter, X. Luo and X. Wang, 1999. Biochemical pathways of caspase activation during apoptosis. Annu. Rev. Cell Dev. Biol., 15: 269-290.

Chun, J., 2000. Cell death, DNA breaks and possible rearrangements: An alterna-tive view. Trends Neuro Sci., 23: 407-409.

Czarnota, G.J., M.C. Kolios, J. Abraham, M. Portnoy, F.P. Ottensmeyer, J.W. Hunt and M.D. Sherar, 2000. Ultrasound imaging of apoptosis. Brit. J. Cancer, 81: 520-527.

Darzynkiewicz, Z., 1993. Detection of DNA Strand Breaks Associated with Apoptosis. In: Paul Robinson, J., (Ed.), Handbook of Flow Cytometry Methods. Wiley-Liss, Inc., New York, pp: 206-207.

Darzynkiewicz, Z., S. Bruno, G. Del Bino, W. Gorczyca, M.A. Hotz, P. Lassota and F. Traganos, 1992. Features of apoptotic cells measured by flow cytometry. Cytometry, 13: 795-808.

Darzynkiewicz, Z., G. Juan, X. Li, W. Gorczyca, T. Murakami and F. Traganos, 1997. Cytometry in cell necrobiology: Analysis of apoptosis and accidental cell death (necrosis). Cytometry, 27: 1-20.

Darzynkiewicz, Z., E. Bedner, X. Li, W. Gorczyca and M.R. Melamed, 1999. Laser scanning cytometry. A new instrumentation with many applications. Exp. Cell Res., 249: 1-12.

Didenko, V.V. and P.J. Hornsby, 1996. Presence of double-strand breaks with single-base 3' overhangs in cells undergoing apoptosis but not necrosis. J. Cell Biol., 135: 1369-1376.

Didenko, V.V. and J.R. Tunstead and P.J. Hornsby, 1998. Biotin-labeled hairpin oligonucleotides. Probes to detect double-strand breaks in DNA in apoptotic cells. Am. J. Pathol., 152: 897-902.

Didenko, V.V., D.J. Boudreaux and D.S. Baskin, 1999. Substantial background reduction in ligase-based apoptosis detection using newly designed hairpin oligonucleotide probes. Biotechniques, 27: 1130-1132.

Dolbeare, F. and J.R. Selden, 1994. Immunochemical quantitation of bromodeoxyuridine: Application to cell cycle kinetics. Meth. Cell Biol., 41: 297-316.

Fauziah, O., I. Aini, G. Motalleb, B.E. Zulkapli and R. Asmah, 2010. Oncolytic effect of Newcastle disease virus AF2240 strain on the MCF-7 breast cancer cell line. Cell J., 12(1): 17-24.

Fauziah, O., G. Motalleb, S.L. Tsuey Peng, R. Asmah, S. Fakurazi and G. Pei, 2011. Extract of Azadirachta indica (Neem) Leaf Induces Apoptosis in 4T1 Breast Cancer BALB/c Mice. Cell, 13(2): 107-116.

Freeman, S.E., A.D. Blackett, D.C. Monteleone, R.B. Setlow, B.M. Sutherland and J.C. Sutherland, 1986. Quantitation of radiation, chemical-, or enzyme induced single-strand breaks in nonradioactive DNA by alkaline gel electrophoresis: Application to pyrimidine dimers. Anal. Biochem., 158: 1191-1129.

Gavrieli, Y., Y. Sherman and S.A. Ben-Sasson, 1992. Identification of Programmed Cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell Biol., 119: 493-501.

Gilmore, E.C., R.S. Nowakowski, V.S. Caviness and K. Herrup, 2000. Cell birth, cell death, cell diversity and DNA breaks: How do they all fit together? Trends Neuro Sci., 23: 100-105.

Gorczyca, W., S. Bruno, R.J. Darzynkiewicz, J. Gong and Z. Darzynkiewicz, 1992. DNA strand breaks occurring during apoptosis: Their early in situ detection by the terminal deoxynucleotidyl transferase and nick translation assays and prevention by serine protease inhibitors. Int. J. Oncol., 1: 639-648.

Gorczyca, W., J. Gong, B. Ardelt, F. Traganos and Z. Darzynkiewicz, 1993. The cell cycle related differences in susceptibility of HL-60 cells to apoptosis induced by various antitumor drugs. Cancer Res., 53: 3186-3192.

Halicka, H.D., K. Seiter, E.J. Feldman, F. Traganos, A. Mittelman, T. Ahmed and Z. Darzynkiewicz, 1997. Cell cycle specificity during treatment of leukemias. Apoptosis, 2: 25-39.

Hashimoto, S., T. Koji, J. Niu, T. Kanematsu and P.K. Nakane, 1995. Differential staining of DNA strand breaks in dying cells by non-radioactive in situ nick translation. Arch. Histol. Cytol., 58: 161-170.

Kamentsky, L.A., 2001. Laser scanning cytometry. Meth Cell Biol., 63: 51-87.

Kerr, J.F.R., A.H. and A.R. Wyllie, 1972. Curie Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer, 26: 239-257.

Levitt, A., R. Axel and H. Cedar, 1979. Nick translation of active genes in intact nuclei. Dev. Biol., 69: 496-505.

Li, X. and Z. Darzynkiewicz, 1995. Labeling DNA strand breaks with BrdUTP. Detection of apoptosis and cell proliferation. Cell Prolif., 28: 571-579.

Li, X. and Z. Darzynkiewicz, 1999. The Schrödinger's cat quandary in cell biology: Integration of live cell functional assays with measurements of fixed cells in analysis of apoptosis. Exp. Cell Res., 249: 404-412.

Li, X. and Z. Darzynkiewicz, 2000. Cleavage of poly (ADP-ribose) polymerase measured in situ in individual cells: Relationship to DNA fragmentation and cell cycle position during apoptosis. Exp. Cell Res., 255: 125-132.

Li, X., M.R. Melamed and Z. Darzynkiewicz, 1996. Detection of apoptosis and DNA replication by differential labeling of DNA strand breaks with fluorochromes of different color. Exp. Cell Res., 222: 28-37.

Lieber, M.R., 1998. Warner-Lambert/Parke-Davis Award Lecture. Pathological and physiological double-strand breaks: Roles in cancer, aging and the immune system. Am. J. Pathol., 153: 1323-1332.

Lockshin, R.A., Z. Zakeri and J.A. Tilly, 1998. When Cells Die: A Comprehensive Evaluation of Apoptosis and Programmed Cell Death. Wiley-Liss, New York.

Majno, G. and I. Joris, 1995. Apoptosis, oncosis and necrosis: An overview of cell death. Am. J. Pathol., 146: 3-15.

Montague, J.W. and J.A. Cidlowski, 1996. Cellular catabolism in apoptosis: DNA degradation and endonuclease activation. Experientia, 52: 957-962.

Mundle, S., A. Iftikhar, V. Shetty, S. Dameron, V. Wright-Quinones, B. Marcus, J. Loew, S. Gregory and A. Raza, 1994. Novel in situ double labeling for simultaneous detection of proliferation and apoptosis. J. Histochem. Cytochem., 42: 1533-1537.

Namiri, M., H. Baharvand and N. Aghdami, 2010. Methods for isolation of bone marrow stem cells: Comparative analysis. Cell J., 12(4): 439-446.

Osstling, O. and K.J. Johanson, 1984. Microelectrophoretic study of radiationinduced DNA damage in individual mammalian cells. Biochem. Biophys. Res. Comm., 123: 291-298.

Parichehr, H., O. Fauziah and G. Motalleb, 2008. Effect of *Berberis vulgaris* aqueous extract on the apoptosis, sodium and potassium in hepatocarcinogenic rats. Iranian J. Basic Med. Sci., 11(2): 62-69.

Rigby, P., M. Dieckmann, C. Rhodes and P. Berg, 1997. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol., 113: 237-251.

Rolig, R.L. and P.J. McKinnon, 2000. Linking DNA damage and neurodegeneration. Trends Neurosci., 23: 417-424.

Roy, M. and R. Sapolsky, 1999. Neuronal apoptosis in acute necrotic insults: Why is this subject such a mess? Trends Neuro Sci., 22: 419-422.

Ryberg, B. and K.J. Johanson, 1997. Estimation of DNA Strand Breaks in Single Mammalian Cells. In: Hanawalt, P.C., E.C. Friedberg and C.F. Fox, (Eds.), DNA Repair Mechanisms. Academic Press, NY, pp: 465-468.

Singh, N.P., M.T. McCoy, R.R. Tice and E.L. Schneider, 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res., 175: 184-191.

Sugimoto, T., A. Takeyama, C. Xiao, T. Takano and H. Yamamoto, 1999. Ichikawa Electron microscopic demonstration of nick end-labeled DNA fragments during capsaicin-induced apoptosis of trigeminal primary neurons in neonatal rats. Brain Res., 818: 147-152.

Thiry, M., 1991. *In situ* nick-translation at the electron microscope level: a tool for studying the location of DNase I-sensitive regions within the cell. J. Cytochem. Histochem., 39(6): 871-874.

Thiry, M. and D. Nase, 1991. I-sensitive sites within the nuclear architecture visualized by immunoelectron microscopy. DNA Cell Biol., 10: 169-180.

Walker, P.R., S. Pandey and M. Sikorska, 1995. Degradation of chromatin in apoptotic cells. Cell Death Differ., 2: 93-100.

Wood, K., 1998. Transplantation biology: Recent advances 5th basic sciences sym-posium of the transplantation society. Mol. Med. Today, 4: 56-57.

Wyllie, A.H., 1980. Glucocorticoid induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature, 284: 555-556.