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NEUTRAL COMET PROFILES: RELIABLE SYSTEM FOR ANALYSES OF DNA STRAND BREAKS DISTRIBUTION

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Summary: This paper reports the conversion of 2-dimensional to 3-dimensional comet profiles produced by different mutagens able to induce DNA double-strand breaks – radiomimetic agent (bleomycin), restriction endonuclease (Eco RI), ionizing (gamma rays and ^{7}Li-ion beam) and non-ionizing (UVC) radiation. The 2-dimensional comet profiles obtained after application of the neutral comet assay were analyzed using ImageJ software. The obtained data reveal that the 3-dimensional comet profiles can be utilized as a reliable hallmark of the predominant type of DNA breaks induced by agents with different mode of action.

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Abbreviations: 2-D – 2-dimensional; 3-D – 3-dimensional; DSB – double-strand breaks; dsDNA – double-stranded DNA; LET – linear energy transfer; RGB – red, green, blue; SCGE – single cell gel electrophoresis; ssDNA – single-stranded DNA.

INTRODUCTION

Single cell gel electrophoresis (SCGE) or the comet assay is a sensitive method used to evaluate the genotoxicity of different environmental agents. The neutral comet assay is performed under non-denaturing conditions and is capable of measuring the rate of DNA double-strand breaks (DSB) on a single cell level. The cells are embedded in a layer of low melting point agarose on a surface of a microscope slide. The lysis with detergents and high salt leads to depletion of histones and release of DNA thus forming a structure termed nucleoid. Nucleoids represent supercoiled loop DNA attached to a network called nuclear matrix (Afanasieva et al., 2013). The theory explains that the relaxed loops with breaks migrate to the anode thus forming structures resembling comets.

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It is known that different agents produce various levels of DNA fragmentation, but no studies have been performed in order to identify the predominant type of strand breaks in comet DNA under neutral conditions. The genotoxic effects are determined by assessment of the type and frequency of the induced primary DNA breaks which can have an impact on cell fate. It is commonly accepted that the neutral protocol is capable to distinguish exclusively DSB. The structural organization of DNA into neutral comets is not yet completely clarified. It is established that comet tails are formed by loops of relaxed DNA sized between 12 and 400kb (Jackson et al., 1990; Shaposhnikov et al., 2009; Afanasieva et al., 2013) and linear single-stranded (ssDNA) or double-stranded (dsDNA) fragments, associated with the nuclear matrix or left free (Afanasieva et al., 2010; Mladinic et al., 2012). In order to elucidate the distribution of the DNA fragments in comet tails, selected neutral comet profiles (2-dimensional, 2-D), obtained after exposure to different DSB-inducing agents, were analysed further by commercially available 3-dimensional (3-D) image analysis software.

We suppose that the high intensity peaks obtained by this conversion represent the distribution of distinct DNA fragments resulting from DSB induction.

MATERIAL AND METHODS

Image analysis

For image analysis of the pixel intensities freeware ImageJ v 1.43 (National Institute of Health, Bethseda, MD) as well as the surface plot 3-D plug-in, both downloaded from the NIH website (http://rsb.info.nih.gov/ij) were used. The representative comet images of pea and barley nuclei were selected from previous studies obtained after application of the neutral comet assay described in details in Georgieva and Stoilov (2008), Nikolova et al. (2013) and Stoilov et al. (2013). Nuclei were treated with various DSB-inducing agents: radiomimetic antibiotic (200μg/ml bleomycin), ionizing radiation (low linear energy transfer, LET radiation-100Gy gamma rays; high LET radiation-50Gy ^{7}Li-ion beams), non-ionizing radiation (9kJ/m^2 UVC) and restiction endonuclease (0.5u/μl Eco RI).

RESULTS

The comet images were compared utilizing the color histogram module (RBG-red, green, blue) of the ImageJ software and visual observation of the color distribution. Fig. 1 (A-E) shows the distribution of the dsDNA fragments viewed as high intensity peaks in the comet tails. Our results indicated good correlation between the observed distribution patterns and the DNA breaks induction capacity of the agent applied. Comet tails specific for agents having a predominantly direct mode of action for DSB induction (as high LET radiation) displayed a more representative DNA damage response and higher intensity peaks in the blue area and beyond.

As shown in Fig. 1, 3-D image analyses of the neutral comets offered higher resolution of the images produced by the DSB-inducing agents applied. Our approach seemed to visualize the predominant type of DNA breaks induced

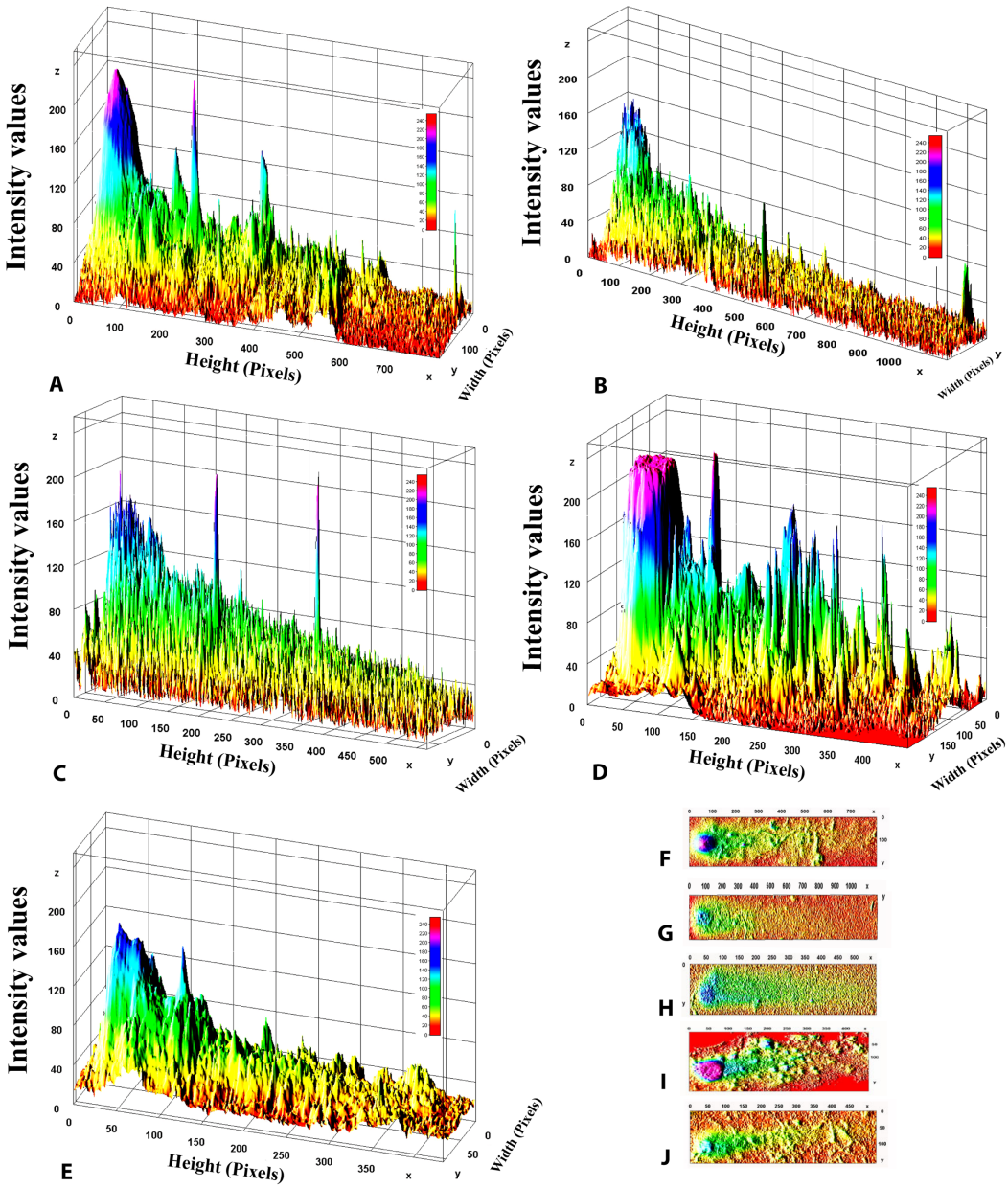

Figure 1. The surface rendition of the primary DNA damage after 3-D reconstructions of neutral comet images - side views (A-E) and their corresponding 2-D top views (F-J). A, F - 200µg/ml bleomycin; B, G - 0.5u/µl Eco RI; C, H - 100Gy gamma rays; D, I - 50Gy ^{7}Li-ion; E. J - 9kJ/m^2 UVC.

by agents with different mode of action, i.e. low LET and high LET radiation (Fig. 1 C, D). It is important to note that the length of the comet tails did not correlate with the genotoxic properties of the DSB –inducing agents (Fig. 1 F-J).

DISCUSSION

In this paper, we propose the conversion of 2-D comet images to their 3-D forms as a suitable tool for analyzing the nature of comet tails. The standard

software packages for the comet assay measure the basic comet parameters as Tail DNA %, tail moment and Olive moment, etc. Along with other shape and intensity parameters, they contribute to DNA damage assessment as a whole (Gyori et al., 2014). The interpretation of parameter values serves for agent's characterization by assessment of the ability to produce primary DNA lesions.

The present study proposes the conversion of standard 2-D comet images to 3-D images for better characterization of the nature and efficiency of DSB induction. The suggested approach is simple, sensitive, easy to handle and inexpensive. Its major advantage is the potential to perform comparative studies on the effects of different genotoxic agents. This approach can serve as a tool for the visualization of linear dsDNA fragments in comet tails belonging to heavily damaged nuclei (Afanasieva et al., 2010; 2013). Our data are in agreement with the loop theory and offer quick screening of DNA damaging effects. The results obtained contribute towards refinement of the visualization of DNA breaks induction and can be applied for agents with unknown mode of action. Moreover, the approach presented here could be very useful for analysis of comets obtained by the Comet-FISH methodology and specific DNA damage distribution in comet tails.

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REFERENCES

Afanasieva K, M Chopei, M Zazhytska, M Vikhreva, A Sivolob, 2013. DNA loop domain organization as revealed by single-cell gel electrophoresis. *BBA Molecular Cell Research*, 1833: 3237–3244.

Afanasieva K, M Zazhytska, A Sivolob, 2010. Kinetics of comet formation in single-cell gel electrophoresis: Loops and fragments. *Electrophoresis*, 31: 512–519.

Georgieva M, L Stoilov, 2008. Assessment of DNA strand breaks induced by bleomycin in barley by the comet assay. *Environ Mol Mutagen*, 49: 381–387.

Gyori BM, G Venkatachalam, PS Thiagarajan, D Hsu, M-V Clement, 2014. OpenComet: An automated tool for comet assay image analysis. *Redox Biology*, 2: 457–465.

Jackson DA, P Dickinson, PR Cook, 1990. The size of chromatin loops in HeLa cells. *EMBO J*, 9: 567–571.

Mladinic M, D Zeljezic, SA Shaposhnikov, AR Collins, 2012. The use of FISH-comet to detect c-Myc and TP 53 damage in extended-term lymphocyte cultures treated with terbuthylazine and carbofuran. *Toxicol Lett*, 211: 62–69.

Nikolova I, M Georgieva, L Stoilov, Z Katerova, D Todorova, 2013. Optimization of Neutral Comet Assay for studying DNA double-strand breaks in pea and wheat. *J BioSci Biotechnol*, 2: 151–157.

Shaposhnikov S, E Frengen, AR Collins, 2009. Increasing the resolution of the comet assay using fluorescent in situ hybridization-a review. *Mutagenesis*,

24: 383–389.

Stoilov L, M Georgieva, V Manova, L Liu, K Gecheff, 2013. Karyotype reconstruction modulates the sensitivity of barley genome to radiation-induced DNA and chromosomal damage. *Mutagenesis*, 28:153–160.