Pollution of ionising radiation diagnostic dose and its targeted and non-targeted effects on human primary fibroblast cells

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التلوث بالجرع القليلة للإشعاع وتأثيراته المباشرة وغير المباشرة على خلايا الانسان الاولية المولدة للألياف

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المستخلص

تعتبر التأثيرات البيولوجية للجرع القليلة من الاشعة السينية احدى المشاكل في بحوث الاشعاع والتي قد تؤدي الى التحطم الكروموسومي في الخلايا المشععه بشكل مباشر او في الخلايا المجاورة للخلايا المشععة . اظهرت كلا الخلايا المشععة بشكل مباشر او الخلايا المجاورة استجابات تحطميه متأخرة في الخلايا الابناء تحت مفهوم عدم الاستقرار الجيني المستحث بالإشعاع . في هذه الدراسة حاولنا لتقليد تأثيرات جرع الاشعة السينية المستخدمة في الكشف (0.5rad) باستخدام خلايا الانسان الاولية المنتجة للألياف وهذه الاشعة تعتبر كملوث للبيئة .

شععت الخلايا بمقدار(0.5rad) من الاشعة السينية واعتبرت هذه الخلايا كمجموعة الخلايا المشععة بشكل مباشر , بينما استحثت الخلايا المجاورة بواسطة نقل الوسط الزرعي من الخلايا المشععة وإضافتها لخلايا غير مشععة , كلا المجموعتين المباشرة والمجاورة نميت حتى الجيل 24 لغرض قياس التأثيرات المتأخرة ثم قياس التحطم الكروموسومي والتحطم الكلي للـ DNA في كلا المجموعتين والخلايا الابناء باستخدام التحليل الكروموسومي وتحليل المذنب . لوحظت الاستجابة الاولية للتحطم الكروموسومي في الخلايا المشععة بشكل مباشر مع ذلك كانت تلك الاستجابة معنوية غير ان الخلايا المجاورة اظهرت تحطم كروموسومي غير معنوي من الناحية الاحصائية مقارنة مع مجموعة السيطرة . اظهرت نتائج تحليل المذنب الخاصة بقياس التحطم الكلي للـ DNA استجابة اولية عالية ومعنوية لتحطم الـ DNA في كلا المجموعتين المباشرة والمجاورة. كما اظهرت الخلايا الابناء لكلا المجموعتين المباشرة والمجاورة ارتفاع ملحوظ ومعنوي في تحطم الكروموسومات و الـ DNA بعد 24 جيل .اقترحت النتائج ان الجرعة القليلة المستخدمة في التشخيص ممكن ان تؤدي الى عدم استقرار كروموسومي في خلايا الانسان الاولية المنتجة للألياف وعدم الاستقرار هذا يعد العلامة الحقيقية لنشوء السرطان

Abstract

One of the key issues of current radiation research is the biological effect of low doses of X-ray, which can instigate to chromosomal damage in direct irradiated and bystander cells. Additionally, the direct irradiated and bystander cells demonstrate delayed damage responses within their progeny, underlying radiation-induced genomic instability (GI). In this study we tried to mimic the effects of diagnostic procedure doses of X-ray (0.5 rad) on human fibroblast cells, which are considered as a one of the environmental polluter.

Human fibroblast cells were irradiated with 0.5 rad X-ray, which were considered the direct irradiated cells; whilst radiation-induced bystander effects (BE) were induced by media transfer. Both cell populations were propagated until 24 generations for delayed responses. The direct, bystander and their progeny cells were subjected to chromosomal analysis and comet assay to measure chromosomal and total DNA damage in these cells.

Early response of chromosome damage was observed in the direct irradiated cells; however it was statistically insignificant. Bystander cells showed higher chromosomal damage than the irradiated cells; but it was insignificant compare to the corresponding control groups. Conversely, comet data demonstrated a significant DNA damage in both direct irradiated and bystander cells as initial responses. Interestingly, progeny of direct irradiated and bystander cells showed a significant chromosomal instability and DNA damage after 24 population doublings following irradiation. The findings suggested that the diagnostic procedure doses of X-ray could cause a profound chromosomal instability within human primary fibroblast cells; which are considered as a hallmark of carcinogenesis.

Introduction

In recent years, many observations following radiation exposure, both in vivo and in vitro, indicate that radiation induces complex biological damage described as non-targeted effects of ionising radiation; these include radiation-induced genomic instability (GI) and radiation- induced bystander effects (BE). Radiation-induced GI produces effects that are observed in the progeny of irradiated cells, which are thought to be a critical step in the onset and progression of cancer. Radiation-induced BE are a damaging response exhibited in unirradiated cells that have received molecular signals produced by irradiated cells through molecules in the irradiated cell conditioned media (ICCM) or via intercellular communication through cell gap junctions (1-4).

Bystander cells exhibit a wide range of biological responses, with many phenotypic similarities to GI. The nature of the soluble transmitting factor(s) is yet to be fully understood, but cytokines including IL-8 (5), TGF-b (6), and TNF-a (7, 8), as well as calcium fluxes, nitric oxide (NO) (9) and reactive oxygen species (ROS) (10) have been suggested as mediators of bystander responses. A role for plasma membrane-bound lipid rafts has also been indicated (11). Recently, miRNA has been shown to be a potential mediator of the bystander effect (12). Interestingly, miRNA molecules have been found in exosome multi-protein complexes, which are known to be one of the cell-cell communication signals (13), secreted by healthy and nonhealthy cells. In addition, exosomes have been found to be associated with the process of non-targeted effects of ionising radiation (14).

Non-targeted effects have been identified in a wide range of cell types (15, 16), following exposure to different types and doses of ionising radiation and studied using many different experimental designs such as grid technique, medium transfer, microbeam and co-culture (7, 17-21), and using both in vivo and in vitro models (22, 23). Furthermore, there are some discrepancies between research laboratories as to whether bystander responses in particular, can be induced using certain radiation quality or dose and in certain cell types (24). The cause is unclear; however, it is important to investigate some of these factors and attempt to elucidate potential

sources of such discrepancies. Interestingly, in addition to the above- mentioned factors that influence non-targeted effects of ionizing radiation, recent studies by two groups have demonstrated the possible involvement of serotonin in the induction of radiation-induced bystander signals. These groups have suggested a correlation between serum serotonin levels and toxicity of harvested irradiated cell medium that may contribute to the variability of bystander response in some studies (25).

The aim of this study was to investigate the effects of low dose of ionising radiation (procedure diagnostic dose) on human fibroblast, which can be considered as a profound pollution in our modern life.

Materials and methods

Cell Culture

Human primary fibroblast cells were cultured in EMEM media supplemented with 15% fetal bovine serum, (FBS, Sigma St. Louis, MO); 2 mM L-Glutamine (Gibco) and 1% (v/ v) penicillin/streptomycin solution (Sigma) in a humidified 5% CO2 incubator at 378C. For cell propagation 1.5 X 10^6 cells were seeded per 75 cm2 tissue culture flask (T75).

Irradiation

Cells were grown in T75 flasks to 80% confluence. Cells were irradiated with 0.5 Rad of X rays. The irradiation was carried out at the Gray Institute for Radiation, Oncology and Biology (University of Oxford), using a Siemens Stabiliplan X-ray machine (Siemens, Munich, Germany). A media transfer method [irradiated cell conditioned media (ICCM) transfer] was used for the investigation of radiation-induced bystander effect. Several experiments were performed and bystander populations were established according to the treatment regime, as detailed below.
A flask of cells that received direct irradiation was incubated at 37C for a further 24 h and cells were harvested for analysis. For bystander experiments the ICCM was collected 4 h after irradiation of cells and filtered through a 0.2 lm filter [which was pre-treated with 1% BSA to reduce protein binding to the filter (24)]. Then the filtered ICCM media was transferred to fresh cells at 80% confluence and incubated for 24 h to induce bystander effects.

Chromosomal analysis

Cells were harvested for metaphase preparation at 60–70% confluence using our established method (26). Briefly, cells were treated with 20 ng/ml demecolcine (Sigma) for 1.5 h in a humidified 5% CO2 incubator at 378C. Cells were centrifuged at 259g for 10 min. The supernatant was discarded, while the cell pellet was re-suspended with a hypotonic solution [75 mM potassium chloride solution (KCl, Sigma) for 20 min at 378C]. The hypotonic cell suspensions were centrifuged at 180g for 10 min. The supernatant was aspirated, and the cell pellet was fixed twice with 25% acetic acid in methanol. Fixed cells were then dropped onto clean slides, and stained using the Giemsa solid staining technique. Slides were coded and at least 100 metaphases were analyzed per group.

Comet Assay

Single-cell gel electrophoresis or comet assay is a simple and sensitive method to quantify total DNA damage (double-strand breaks, single-strand breaks and base damage) in individual cells (27, 28). The comet assay was performed as described (29, 30). Briefly, microscope slides were coated with 1% normal melting point agarose (NMPA) and allowed to dry over night. The coated slides were then placed on a metal tray on ice. Twenty thousand cells were re- suspended with 200 ll of 0.6% low melting point agarose (LMPA) and placed immediately onto chilled pre-coated slides. The cell- LMPA suspensions were flattened with cover slips, which were removed after 5–10 min. The slides were then transferred to a Coplin jar, which was filled with cold lysis buffer (2.5 M NaCl, 100 mM EDTA pH 8.0, 10 mM Tris-HCl pH 7.6, and 1% Triton X-100, pH . 10). The jar was kept at 48C overnight. The slides were then moved to a horizontal electrophoresis tank filled with electrophoresis buffer (0.3MNaOHand1mMEDTA,pH13)at48Cfor40min.The electrophoresis was run for 30 min, at 19 V, 300 A. Slides were neutralized with neutralizing buffer (0.4 M Tris-HCl, pH 7.5), washed with distilled water, and immediately stained with a 1:10,000 dilution of SYBR Gold (Molecular Probes/Invitrogen, Carlsbad, CA). The slides were analyzed using Komet 5.5 Image Analysis Software (Kinetic Imaging Technology /Andor, Germany).

Statistical Analyses

For analysis of comet assays, at least 200 cells per group were analyzed; statistical analysis was performed using the Mann-Whitney Test, utilising the median of raw data. The Mann-Whitney test deals with the median of nonparametric data and it is an acceptable test for comet data (31, 32). Each experiment was carried out 3 times. Analysis showed no significant inter-experimental variation; therefore, data from these experiments was pooled. Chromosomal data, represented as mean aberrations per cell, was subjected to the Mann-Whitney test. For each group, 100 metaphases were analyzed.

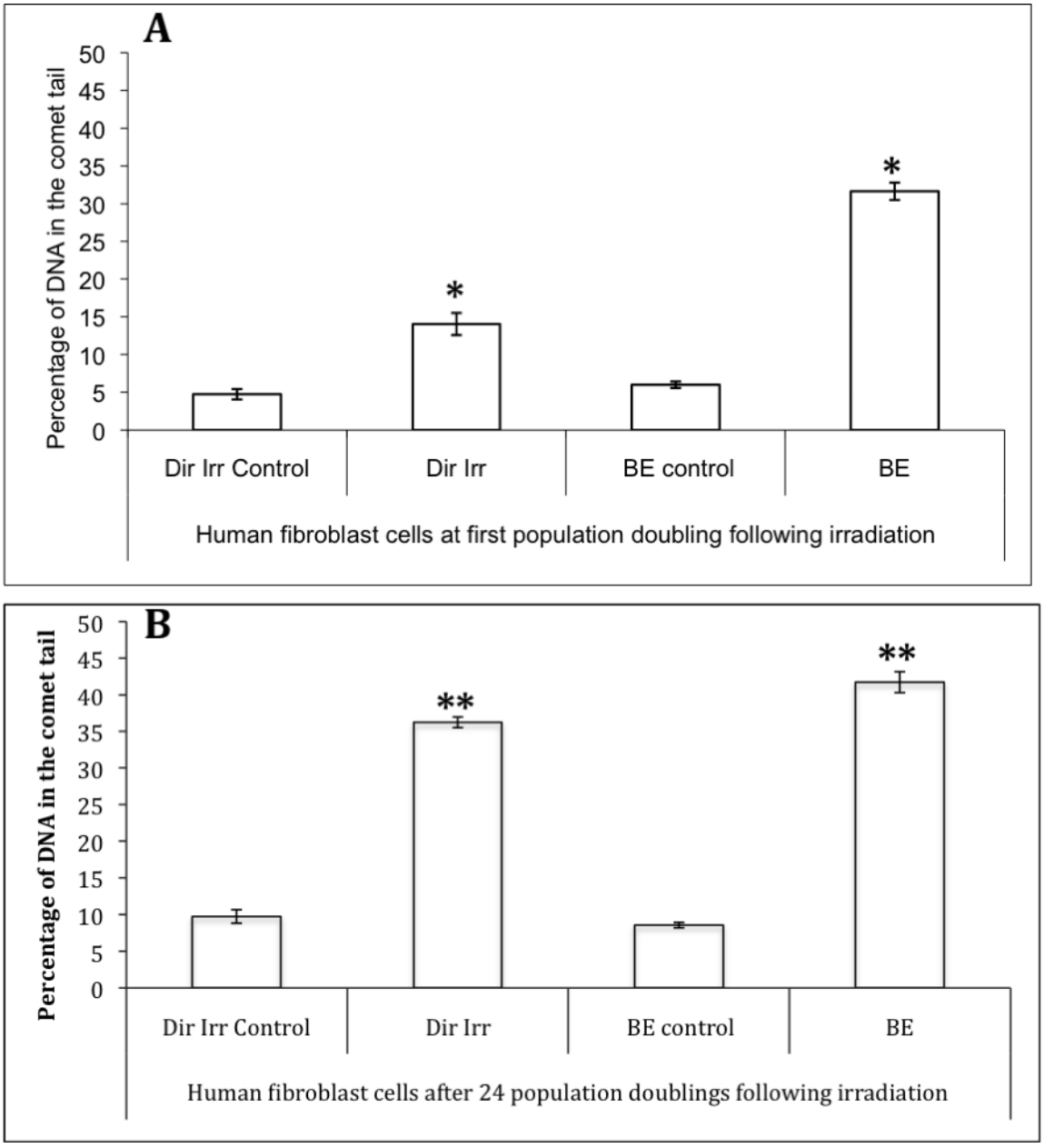
Results and discussion

It was first necessary to prove that IR has a biological effect on cells. Therefore, human primary fibroblast cells were hit with 0.5 rad X-ray and subjected to comet assay, measuring the total DNA damage. The results showed that IR significantly ($p \leq 0.01$) induced DNA damage in the directly irradiated human fibroblast cells compared to the unirradiated human fibroblast (control cells) (Fig 1A). Data suggested that IR could induce inflammation instigating to DNA damage. Much evidence has reported that IR can mediate inflammation in human lung cells *in vivo* (26) and *in vitro* (27). As very well known that inflammation can lead to DNA damage (28, 29). In addition, IR can active endotoxic proteins (30), which can cause damage to the cells components including DNA (31).

We also tested whether IR was able to induce BE in the human fibroblast by transferring ICCM to non-treated/fresh human fibroblast cells to induce BE within these cells as described in the materials and methods section. Our finding demonstrated that the ICCM significantly ($p \leq 0.01$) induced DNA damage in the bystander cells (Fig 1A). The BE could be mediated by cytokines that secreted by IR

treated cells. This suggestion was supported by Ishida, et al, who reported that IR can increase in pro-inflammatory cytokines, such as tumour necrosis factor- α (TNF-α), interleukin (IL)-1β and IL-6 (32). These cytokines can play a crucial role in the induction of BE. Rastogi and co-authors demonstrated that cytokine inflammatory signals such as TNF-α can converge on a COX-2 dependent pathway leading to induce BE in the mice bone marrow (33). Moreover, IL-6 and IL-8 are likely to be involved in the induction of BE (34).

The Irradiated and bystander fibroblast cells were propagated up to 24 population doublings to investigate GI within the progeny of these cells, and whether IR can be involved in the late DNA damage. The progeny of both direct treated and bystander fibroblast cells showed a significant delayed DNA damage ($p \leq 0.0001$) compared to the control (Fig 1B). Data suggested that the damaged cells with in the progeny of direct irradiated and bystander cells could be observed because of the telomeric instability, that are induced by IR (35)Al-Mayah has reported that normal cells can display GI following low doses of X-ray. He suggested that exosomes' cargo has an important role in the induction of non-targeted effects of IR (36).



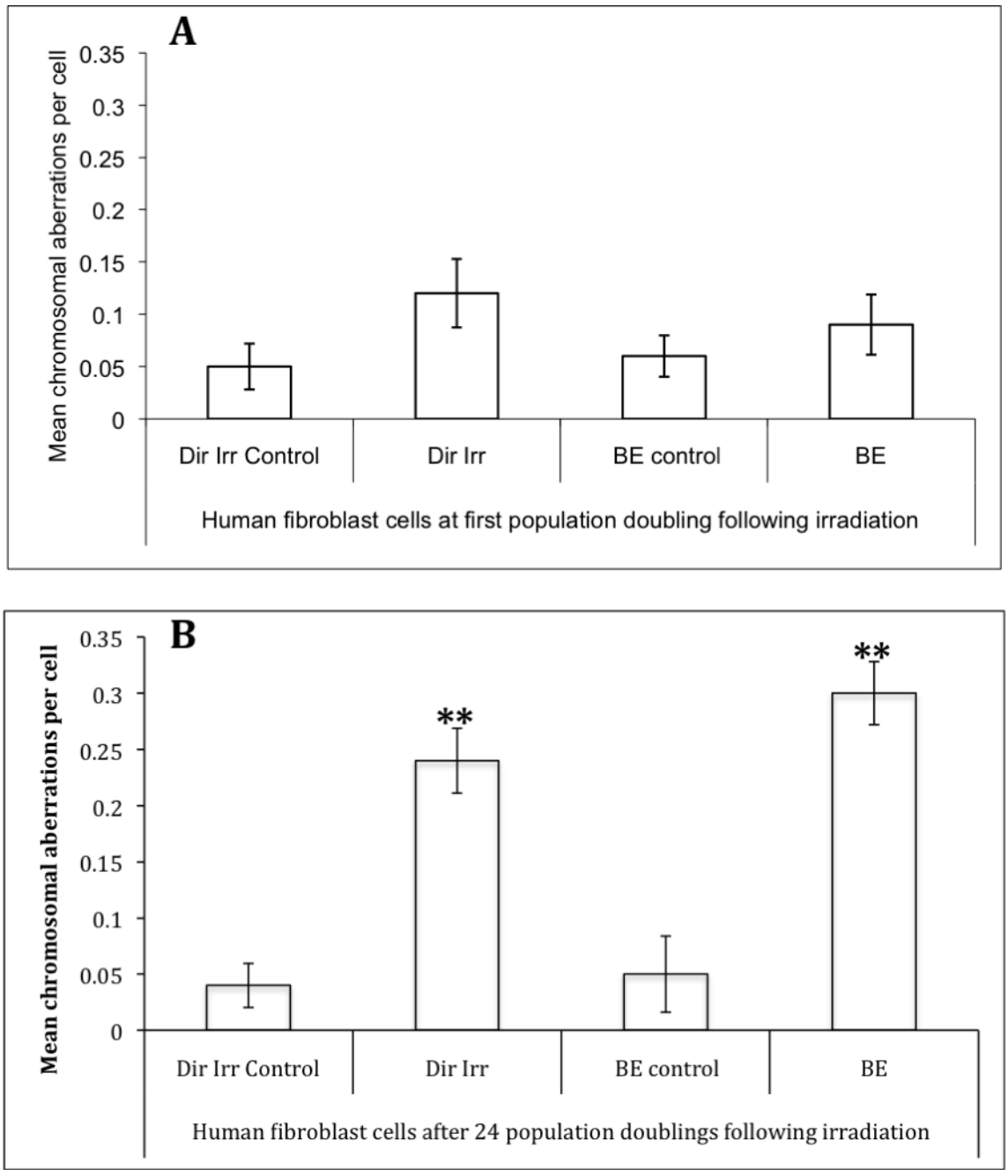
Figure(1): Early and late total DNA damage in human primary fibroblast cells following 0.5 rad X-ray irradiation.

Panel A represents the initial direct and bystander total DNA damage including single-stranded break, double-stranded break and DNA base damage in the human fibroblast following 0.5 rad X-ray. Both direct irradiated and bystander cells showed a significant DNA damage (*$p \leq 0.01$) compared to the corresponding controls. Comet data in panel B demonstrated that the progeny of direct irradiated and bystander cells exhibited a high significant delayed DNA damage (**$p \leq 0.0001$) after 24 population doublings post irradiation. The finding suggested that diagnostic dose of X-ray is able to induce a significant genomic instability in human fibroblast after 24 generation following irradiation.

We repeated the experiment using chromosome analysis in order to measure the initial and delayed effect of low dose of X-ray in the direct irradiated, bystander and the progeny of human fibroblast cells.

The direct and bystander fibroblast cells demonstrated an early chromosomal aberrations following irradiation; however, they were statistically insignificant as shown in figure 2 (A). Our finding suggested that initial chromosomal damage could be repaired by DNA repair mechanism. As mentioned above in figure 1 A that comet data showed single-stranded break, double stranded break and DNA base damage, in which more than 90% of single stranded break can be repaired by one of DNA repair mechanisms. Therefore, chromosome aberrations were insignificantly observed at in fibroblast cells at first generation post low dose irradiation.

Interestingly, human fibroblast cells demonstrated profound chromosomal instability ($p \leq 0.0001$) within survival progeny of both direct irradiated and bystander cells after 24 generations post irradiation (See figure 2, B). Our chromosomal data confirm our comet data suggestion, that IR could be involved in the induction of GI, but in a tissue specificity manner. Kim and co-worker have documented that media from irradiated prostate epithelial cells has IL-1β, IL-6 and TNF-α. They suggested that IR can stimulate pro-inflammatory cytokines secretion (37). These long lived signals (cytokines) can induce stress and injury, which can instigate GI and BE (38). Al-Mayah and co-authors have documented that exosomes protein and RNAs molecules play a crucial role in the chromosomal instability induction by X-ray. Our data suggested that low doses of X-ray can induce chromosomal instability in the human primary fibroblast, leading to cancer underlying radiation-induced genomic instability.



Figure(2): Initial and delayed mean chromosomal aberrations in human primary fibroblast cells following 0.5 rad X-ray irradiation.

Panel A represents early chromosome aberrations in the human fibroblast following 0.5 rad X-ray. Both direct irradiated and bystander cells showed an insignificant chromosomal damage compared to the corresponding controls. Nevertheless, panel B demonstrated that the progeny of direct irradiated and bystander cells exhibited high significant delayed chromosome aberrations (**$p \leq 0.0001$) after 24 population doublings post irradiation. The data suggested that low dose of X-ray is able to induce a significant chromosomal instability in human fibroblast after 24 generation following irradiation underlying radiation induced genomic instability.

Conclusions

Low doses of ionising radiation, X-ray, can cause initial chromosomal damage in the direct irradiated human fibroblast cells. Moreover, the ICCM of both human fibroblast cells has an ability to induce BE in the unirradiated/fresh human fibroblast. In addition, GI was significantly observed in the progeny of both irradiated and bystander cells after 24 population doublings following irradiation.

Acknowledgement

The Authors would thank Professor Munira Kadhim, Mrs Deborah Bowler, Miss, Kim Chapman, Mr Scott Bright, Dr Mark Hill and James Thompson for valuable technique.

References

1.**Azzam EI, Little JB.(2004)** .The radiation-induced bystander effect: evidence and significance. Human & experimental toxicology. Feb;23(2):61-5.

2.**Boyd M, Ross SC, Dorrens J, Fullerton NE, Tan KW, Zalutsky MR, et al.(2006).**Radiation-induced biologic bystander effect elicited in vitro by targeted radiopharmaceuticals labeled with alpha-, beta-, and auger electron-emitting radionuclides. J Nucl Med. 2006 Jun;47(6):1007-15.

3.**Kadhim MA, Moore SR, Goodwin EH.(2004).** Interrelationships amongst radiation-induced genomic instability, bystander effects, and the adaptive response. Mutation research. Dec 2;568(1):21-32.

4.**Morgan WF, Sowa MB.(2007).**Non-targeted bystander effects induced by ionizing radiation. Mutation research. Mar 1;616(1-2):159-64.

5.**Facoetti A, Ballarini F, Cherubini R, Gerardi S, Nano R, Ottolenghi A, et al. (2006).**Gamma ray-induced bystander effect in tumour glioblastoma cells: a specific study on cell survival, cytokine release and cytokine receptors. Radiation protection dosimetry.;122(1-4):271-4.

6.**Burr KL, Robinson JI, Rastogi S, Boylan MT, Coates PJ, Lorimore SA, et al. (2011).**Radiation-induced delayed bystander-type effects mediated by hemopoietic cells. Radiation research. Jun;173(6):760-8.

7.**Moore SR, Marsden S, Macdonald D, Mitchell S, Folkard M, Michael B, et al. (2005).**Genomic instability in human lymphocytes irradiated with individual charged particles: involvement of tumor necrosis factor alpha in irradiated cells but not bystander cells. Radiation research. Feb;163(2):183-90.

8.**Kadhim MA, Hill MA, Moore SR.(2006).** Genomic instability and the role of radiation quality. Radiation protection dosimetry.122(1-4):221-7.

9.**Shao C, Prise KM, Folkard M.(2008).**Signaling factors for irradiated glioma cells induced bystander responses in fibroblasts. Mutation research. Feb 1;638(1-2):139-45.

10.**Matsumoto H, Hamada N, Takahashi A, Kobayashi Y, Ohnishi T. (2007).**Vanguards of paradigm shift in radiation biology: radiation-induced adaptive and bystander responses. Journal of radiation research. Mar;48(2):97-106.

11.**Hamada N, Matsumoto H, Hara T, Kobayashi Y. (2007).**Intercellular and intracellular signaling pathways mediating ionizing radiation-induced bystander effects. Journal of radiation research. Mar;48(2):87-95.

12.**Aypar U, Morgan WF, Baulch JE. (2011).**Radiation-induced epigenetic alterations after low and high LET irradiations. Mutation research. Feb 10;707(1-2):24-33.

13.**Mathivanan S, Ji H, Simpson RJ. (2011).**Exosomes: extracellular organelles important in intercellular communication. Journal of proteomics. Sep 10;73(10):1907-20.

14.**Al-Mayah AH, Irons SL, Pink RC, Carter DR, Kadhim MA.(2012).** Possible role of exosomes containing RNA in mediating nontargeted effect of ionizing radiation. Radiation research. May;177(5):539-45.

15.**Azzam EI, de Toledo SM, Little JB.(2001).** Direct evidence for the participation of gap junction-mediated intercellular communication in the transmission of damage signals from alpha -particle irradiated to nonirradiated cells. Proceedings of the National Academy of Sciences of the United States of America. Jan 16;98(2):473-8.

16**.Lorimore SA, McIlrath JM, Coates PJ, Wright EG. (2005).**Chromosomal instability in unirradiated hemopoietic cells resulting from a delayed in vivo bystander effect of gamma radiation. Cancer research. Jul 1;65(13):5668-73.

17.**Lorimore SA, Kadhim MA, Pocock DA, Papworth D, Stevens DL, Goodhead DT, et al. (1998).**Chromosomal instability in the descendants of unirradiated surviving cells after alpha-particle irradiation. Proceedings of the National Academy of Sciences of the United States of America. May 12;95(10):5730-3.

18.**Mothersill C, Seymour CB. (1998).**Cell-cell contact during gamma irradiation is not required to induce a bystander effect in normal human keratinocytes: evidence for release during irradiation of a signal controlling survival into the medium. Radiation research. Mar;149(3):256-62.

19.**Kadhim MA, Marsden SJ, Goodhead DT, Malcolmson AM, Folkard M, Prise KM, et al. (2001).**Long-term genomic instability in human lymphocytes induced by single-particle irradiation. Radiation research. Jan;155(1 Pt 1):122-6.

20**.Shao C, Stewart V, Folkard M, Michael BD, Prise KM.(2003).**Nitric oxide-mediated signaling in the bystander response of individually targeted glioma cells. Cancer research. Dec 1;63(23):8437-42.

21.**Bowler DA, Moore SR, Macdonald DA, Smyth SH, Clapham P, Kadhim MA. (2006).**Bystander-mediated genomic instability after high LET radiation in murine primary haemopoietic stem cells. Mutation research. May 11;597(1-2):50-61.

22.**Morgan WF. (2003).**Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo,

clastogenic factors and transgenerational effects. Radiation research. May;159(5):581-96.

23.**Morgan WF. (2003).**Non-targeted and delayed effects of exposure to ionizing radiation: I. Radiation-induced genomic instability and bystander effects in vitro. Radiation research. May;159(5):567-80.

24.**Sowa MB, Goetz W, Baulch JE, Pyles DN, Dziegielewski J, Yovino S, et al.(2010).**Lack of evidence for low-LET radiation induced bystander response in normal human fibroblasts and colon carcinoma cells. International journal of radiation biology. Feb;86(2):102-13.

25.**Poon RC, Agnihotri N, Seymour C, Mothersill C. (2007).**Bystander effects of ionizing radiation can be modulated by signaling amines. Environmental research. Oct;105(2):200-11.

26.**Huo M, Cui X, Xue J, Chi G, Gao R, Deng X, et al. (2012).**Anti-inflammatory effects of linalool in RAW 264.7 macrophages and lipopolysaccharide-induced lung injury model. The Journal of surgical research. Dec 4.

27.**Shimizu K, Taniyama Y, Sanada F, Azuma J, Iwabayashi M, Iekushi K, et al. (2012).**Hepatocyte Growth Factor Inhibits Lipopolysaccharide-Induced Oxidative Stress via Epithelial Growth Factor Receptor Degradation. Arteriosclerosis, thrombosis, and vascular biology. Nov;32(11):2687-93.

28.**Budworth H, Snijders AM, Marchetti F, Mannion B, Bhatnagar S, Kwoh E, et al. (2012).**DNA repair and cell cycle biomarkers of radiation exposure and inflammation stress in human blood. PLoS ONE.;7(11):e48619.

29.**Martin OA, Redon CE, Dickey JS, Nakamura AJ, Bonner WM. (2011).**Para-inflammation mediates systemic DNA damage in response to tumor growth. Communicative & integrative biology. Jan;4(1):78-81.

30**.Konig H, Claus H, Varma A, editors.(2010).** Prokaryotic cell wal compounds. Springer-Verlag, Berlin, Heidelberg, New York.

31.**Jaiswal YK, Jaiswal MK, Agrawal V, Chaturvedi MM. (2009).**Bacterial endotoxin (LPS)-induced DNA damage in preimplanting embryonic and uterine cells inhibits implantation. Fertility and sterility. May;91(5 Suppl):2095-103.

32.**Ishida T, Nishiumi S, Tanahashi T, Yamasaki A, Yamazaki A, Akashi T, et al. (2012).**Linoleoyl ethanolamide reduces lipopolysaccharide-induced inflammation in macrophages and ameliorates 2,4-dinitrofluorobenzene-induced contact dermatitis in mice. European journal of pharmacology. Nov 28.

33.**Rastogi S, Coates PJ, Lorimore SA, Wright EG. (2012).**Bystander-type effects mediated by long-lived inflammatory signaling in irradiated bone marrow. Radiation research. Mar;177(3):244-50.

34.**Pasi F, Facoetti A, Nano R. (2010).I**L-8 and IL-6 bystander signalling in human glioblastoma cells exposed to gamma radiation. Anticancer research. Jul;30(7):2769-72.

35.**Berardinelli F, Antoccia A, Cherubini R, De Nadal V, Gerardi S, Tanzarella C, et al. (2011).**Telomere alterations and genomic instability in long-term cultures of normal human fibroblasts irradiated with X rays and protons. Radiation protection dosimetry. Feb;143(2-4):274-8.

36.**Al-Mayah ahj.(2012).**radiation-induced genomic instability and cellular communication: mechanistic investiga tions. oxford: oxford;

37.**Kim HJ, Park JW, Cho YS, Cho CH, Kim JS, Shin HW, et al.(2013).** Pathogenic role of HIF-1alpha in prostate hyperplasia in the presence of chronic inflammation. Biochimica et biophysica acta. Jan;1832(1):183-94.

38.**Lorimore SA, Coates PJ, Wright EG.(2003).**Radiation-induced genomic instability and bystander effects: inter-related nontargeted effects of exposure to ionizing radiation. Oncogene. Oct 13;22(45):7058-69.