Calsyntenin 1 mRNA expression sensitivity to ionizing radiation in human hepatocytes and carcinoma cells and blood cells of BALB/c mice

Eon-Seok Leeab*, Won-Tae Kim*, Ga-Young Park, Manwoo Leea* and Tae Gen Son

Research Center, Dongnam Institute of Radiological and Medical Science, Busan, Republic of Korea; aDepartment of Biochemistry, Pusan National University School of Medicine, Yangsan, Republic of Korea

ABSTRACT
Whether calsyntenin 1 (CLSTN1) and serine hydroxymethyltransferase 2 (SHMT2) are sensitive to low-dose ionizing radiation (LDIR) has not yet been defined. Therefore, here, we aimed to investigate changes in gene expression in hepatic cell lines and in blood cells and serum from irradiated mice. Levels of SHMT2 and CLSTN1 were decreased in cancer (HepG2) and normal (HH) hepatocytes exposed to various radiation doses. The mRNA expression of SHMT2 and CLSTN1 was altered in response to 0.5 or 4 Gy of radiation at 24 h post irradiation in vivo. However, plasma SHMT2 protein levels did not change at either radiation dose relative to those in the non-irradiated group. Plasma CLSTN1 protein levels notably decreased in response to 4 Gy of radiation but were not altered in response to 0.5 Gy of radiation. Thus, our findings suggest that the mRNA and protein levels of CLSTN1 might be useful biomarkers for radiation exposure. Further studies are needed to characterize the molecular regulation and functional roles of SHMT2 and CLSTN1 in response to radiation.

1. Introduction
Somatic mutations, cytogenetics, and gene expression have been investigated using blood cells, because the hematopoietic system contains radiation-sensitive cells that are easily sampled (Park et al., 2017; Sun et al., 2020). However, current methods are applied only to acute conditions or blood isolated from individuals exposed to relatively high doses of radiation (International Atomic Energy Agency, 2011). The responses of biomarkers such as microRNA (miRNA) or metabolites to low-dose ionizing radiation (LDIR) have been investigated (DY Lee et al., 2012; Maes et al., 2008). The expression of several miRNAs, including miR-92b, miR-137, miR-660, and miR-656, decreases at 0.5, 6, and 24 h after irradiation (Maes et al., 2008). One study of the effect of genetics on metabolic responses of blood plasma to radiation has shown that some metabolites are involved in the responses of some functions to LDIR (DY Lee et al., 2012).

A recent study has shown that LDIR regulates the expression of mir-193b-3p, which targets Calsyntenin 1 (CLSTN1) and serine hydroxymethyltransferase 2 (SHMT2) (E-S Lee et al., 2016). The CLSTN1 gene belongs to the calsyntenin family, which is a cadherin superfamily of cell-adhesion molecules (Cheng et al., 2019). This gene mediates the axonal anterograde transport of specific vesicles that traffic amyloid precursor protein (APP) in Alzheimer disease (AD) (Konecna, Frischknecht, Kinter, Ludwig, Steuble, Meskenaite, Indermühle, Engel, Cen, Mateos, Streit, Sondergeregga et al., 2006; Ludwig et al., 2009). The biological effects of LDIR on AD have been investigated (Hwang et al., 2019; Rudobeck et al., 2017). Low-dose ionizing radiation has beneficial effects on the pathogenesis of Aβ42-associated AD (Hwang et al., 2019). Irradiation with low-dose protons increases Aβ deposition, but it had no effect on AD pathology (Rudobeck et al., 2017). Moreover, CLSTN1 is a novel candidate tumor marker for lung adenocarcinoma (Chu et al., 2017), and mitochondrial SHMT2 is overexpressed in liver, breast, and colon cancers (Bernhardt et al., 2017; Wei et al., 2018; Woo et al., 2016). Low-dose ionizing radiation induces radioreistance in human lung adenocarcinoma A549 cells via the ROS/autophagy/Nrf2 pathway (Chen et al., 2015). However, whether CLSTN1 and SHMT2 in blood samples are sensitive to LDIR has not yet been determined.

Therefore, the present study aimed to investigate the effects of various radiation doses on human hepatocytes and carcinoma cells as well as blood cells from mice to determine whether CLSTN1 or SHMT2 responds to LDIR.

2. Materials and methods
2.1. Cell culture conditions
Human hepatocellular carcinoma HepG2 cells (American Type Culture Collection, Manassas, VA,
USA) and human hepatocytes (HH) (ScienCell Research Laboratories, Carlsbad, CA, USA) were maintained at 37°C under a 5% CO₂ atmosphere in Dulbecco’s modified Eagle’s medium (DMEM; WelGENE Inc., Daegu, Korea) containing 10% fetal bovine serum (FBS; Atlas Biologicals, Inc., Fort Collins, CO, USA) and 1% antibiotic-antimycotic (Gibco, Grand Island, NY, USA), and Hepatocyte Medium (HM; ScienCell Research Laboratories).

2.2. Experimental animals

The Dongnam Institute of Radiological & Medical Sciences Institutional Animal Care and Use Committee approved all the animal experiments (Approval No: DIRAMS-AEC-2019-007). Sixty-four 5-week-old male BALB/c mice (Central Animal Laboratory, Seoul, Korea) were randomly assigned to groups that were irradiated without (0 Gy: n = 16) or with 0.15 (n = 25) or 4 (n = 23) Gy of Cesium $^{137}$Cs.

2.3. Irradiation

Cells (3 x 10⁵/well) seeded in plates were cultured under a humidified atmosphere of 5% CO₂ at 37°C overnight, then the media were refreshed and the cells were irradiated with 0, 0.25, 0.5, 1, 2, 10, 50, 100, or 200 cGy of $^{137}$Cs (LDIR system, Chiyoda Technol Corp., Tokyo, Japan) or a BIOBEAM 8000 system (Eckert & Ziegler, Berlin, Germany) at a rate of 26.5 mGy/h, 53.31 mGy/min, or 2.6 Gy/min. The non-irradiated cells remaining in the same plates, and the irradiated cells were harvested after 24 h later.

The mice were irradiated with 0.15 (rate: 6.5 mGy/h) and 4 (rate: 2.6 Gy/min) Gy of $^{137}$Cs, using the LDIR (E-S Lee et al., 2016) and BIOBEAM 8000 systems, respectively. Mice in the 0.15-Gy group were placed in individual cages to reduce the shielding effect between them; mice in the 4-Gy group were individually placed in 50-mL conical tubes with a holed cap and positioned perpendicular to the mouse phantom. All mice were sacrificed 24 h after irradiation.

2.4. Western blotting

Cells were lysed then sedimented by centrifugation at 12000 x g for 15 min at 4°C. Proteins (20 μg) were separated by SDS-PAGE, then transferred to PVDF membranes (GE Healthcare Life Sciences, Piscataway, NJ, USA). Nonspecific protein binding was blocked with 5% skim milk dissolved in TBS containing 0.02% Tween 20, then the membranes were incubated overnight at 4°C with primary antibody diluted to 1:1000. Specific binding was detected using peroxidase-conjugated secondary antibodies (diluted 1:5000). Bound proteins were visualized using a Fusion FX5 imaging system (Vilber Lourmat, Eberhardzell, Germany) and quantified using ImageJ software, version 1.47 (Schneider et al., 2012). The antibodies were anti-SHMT2 and anti-CLSTN1 (both from Abcam, Cambridge, UK), and anti-GAPDH (Advanced ImmunoChemical Inc., Long Beach, CA, USA).

2.5. Preparation of plasma and blood cells

Mice blood was collected via heart puncture, placed into heparinized tubes, and centrifuged for 20 min at 1000 x g. The supernatant was decanted and stored at −80°C. To isolate the blood cells, 1 ml of red blood cell lysing buffer (Sigma-Aldrich, St. Louis, MO, USA) was added to the pellet at 24°C for 1 to 2 min, then the mixture was centrifuged for 5 min at 500 x g. The supernatant was removed, and the separated cells were washed thrice with normal saline and stored at −80°C.

2.6. ELISA assays

Concentrations of SHMT2 and CLSTN1 in undiluted plasma samples were measured using ELISA kits (MyBioSource, Inc., San Diego, CA, USA), as described by the manufacturer. Absorbance at 450 nm was measured using a Paradigm™ microplate reader (Beckman Coulter, Fullerton, CA, USA). Levels of SHMT2 protein were quantified by comparison with a standard curve. The standard curve and equation for CLSTN1 were calculated based on data from independent experiments with recombinant CLSTN1 proteins using four-parameter logistic (4PL) regression models (MyAssays, 2020) and the ROOT modular scientific software toolkit (CERN, 2018).

2.7. Synthesis of cDNA and quantitative RT-PCR

Total RNA was isolated from mouse blood cells using miRNeasy Mini kits (Qiagen, Valencia, CA, USA) as described by the manufacturer and quantified using a NanoDrop 2000/2000 c spectrometer (Thermo Scientific, Wilmington, DE, USA). Total mRNA (1 μg) was reverse-transcribed into cDNA at 25°C for 5 min, 42°C for 30 min, and 85°C for 5 min using iScript™ cDNA Synthesis kits (Bio-Rad Laboratories, Hercules, CA, USA), then stored at −20°C. Transcripts of target genes were analyzed by qRT-PCR using a CFX96 Touch™ real-time PCR system (Bio-Rad Laboratories) at 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 40 s. Relative gene expression was quantified using the comparative $2^{-ΔΔCt}$ method (Heidary et al., 2019) and normalized to mouse GAPDH. All primers were designed using the primer3 program (Konecna, Frischknecht, Kinter, Ludwig, Steuble, Meskenaite, Indermühe, Engel, Cen, Mateos, Streit, Sonderegger et al., 2006) and the respective forward and reverse primers for qRT-PCR.
comprised SHMT2, 5′-TTA CAA GTG CTG AGG AAC GC-3′ and 3′-GCC AAT GTT GAC TCC CTC AT-5′; CLSTN1, 5′-ATG GTC GCT ACC TCA GCA AT-3′ and 3′-GCA GAG TCA TCC CAG TCC AT-5′; GAPDH, 5′-CAT CAC TGC CAC CCA GAA GAC TG-3′ and 3′-ATG CCA GTG AGC TCC CCG TTC AG-5′.

2.8. Statistical analyses

All experimental data are expressed as mean ± standard deviation. Pairs of values were compared using one-way analysis of variance (ANOVA) and Tukey post hoc multiple comparison tests. All data were statistically analyzed using Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA), and values with P < 0.01 were considered significantly different.

3. Results

3.1. Protein levels of SHMT2 and CLSTN1 in irradiated human hepatocytes

All cells were harvested at 24 h post-irradiation and further analyzed (Figure 1a). Levels of SHMT2 and CLSTN1 proteins were decreased in HepG2 and HH cells exposed to low- and high-dose radiation (Figure 1b,c, respectively).

![Figure 1](image.png)

**Figure 1.** Ionizing radiation downregulated candidate target genes. (a) Study design. Human hepatocellular carcinoma cells (HepG2) and human hepatocytes (HH) were seeded and exposed to various doses using LDIR or BIOBEAM 8000 systems. Representative western blot images of cells 24 h after irradiation. (b-c) HepG2 and HH cells irradiated at various doses were incubated for 24 h, then the SHMT2 and CLSTN1 protein levels in whole-cell lysates were determined using western blotting. Values were normalized to those of GAPDH. Image is representative of three independent experiments that yielded similar results. Relative intensity calculated using ImageJ software is shown under each blot.

3.2. Expression of SHMT2 and CLSTN1 mRNAs in blood cells from irradiated mice

Figure 2 details the experimental method and Figure 3 shows the qRT-PCR results. The expression levels of SHMT2 (Figure 3a) and CLSTN1 (Figure 3b) mRNAs at 24 h post-irradiation were equally decreased in the 0.15-Gy and 4-Gy groups compared with those in the non-irradiated control. These results showed that SHMT2 and CLSTN1 mRNA expression was downregulated in response to radiation independently of the dose of 137Cs.

3.3. Levels of SHMT2 and CLSTN1 proteins in plasma from irradiated mice

The expression of SHMT2 and CLSTN1 mRNA decreased in response to radiation but not sufficiently enough to distinguish between the effects of low- and high-doses. Therefore, we investigated whether the plasma levels of proteins expressed by both genes were decreased after exposure to radiation using ELISA. We found that SHMT2 protein levels did not change in both the irradiated groups compared with the control group (Figure 4a).

Moreover, CLSTN1 protein levels decreased in the group irradiated with 4 Gy, but not in the groups irradiated without, or with 0.5 Gy of 137Cs (Figure 4b).
4. Discussion

Blood is commonly analyzed to diagnose cancer and assess responses to irradiation (Zeegers et al., 2017), such as the composition and function of extracellular vesicles secreted from peripheral blood mononuclear cells (PBMC) (Moertl et al., 2020). Serum miRNA has potential as a biomarker of radiological damage and personalized radiotherapy (Tomasik et al., 2018). Serum miR-34a expression can distinguish irradiated from non-irradiated patients (Halimi et al., 2016).

Our previous unpublished data have shown that miR-193b-3p expression in serum from mice irradiated with 0.01 Gy of $^{137}$Cs is reduced within 3 or 6 h, but recovers 12 or 24 h post irradiation to basal levels (data not shown). The expression of miR-34a increases at 4 h, and decreases at 24-h post-irradiation in MCF-10A cells (Stankevics et al., 2013). Thus, LDIR does not appear to cause persistent changes to miRNA expression unlike cancer, other diseases, and high-dose radiation. Therefore, we predicted that CLSTN1 and SHMT2 are target genes of miR-193b-3p, and investigated whether changes in their expression could serve as biomarkers for responses to LDIR (E-S Lee et al., 2016). Here, we uncovered downregulated SHMT2 and CLSTN1 expression in irradiated blood cells. Although SHMT2 and CLSTN1 are associated with cancer (Chu et al., 2017; Chung et al., 2008; Shi et al., 2019; Yang et al., 2018), how the expression of SHMT2 and CLSTN1 changes in response to radiation at different doses has remained obscure. For example, SIRT5 is a candidate target for inhibiting serine catabolism and SHMT2 protein desuccinylation by SIRT5 inhibits serine catabolism, leading to the promotion of cancer cell proliferation.

These results suggested that CLSTN1 protein secretion into the plasma from blood cells might be regulated by high-dose, but not low-dose, gamma radiation.
The expression SHMT2 is also associated with benign lymphatic infiltration (Shi et al., 2019). The Clstn1 gene is methylated in prostate cancer (Chung et al., 2008) and serves as a biomarker of lung adenocarcinoma (Chu et al., 2017). The expression of SHMT2 and CLSTN1 is notably elevated in various cancers, and our findings showed that LDIR and high-dose radiation downregulated the expression of these genes. Because radiation causes cancer, whether the expression of these genes increases in response to radiation therapy or carcinogenesis is difficult to determine. However, the present findings indicated that SHMT2 and CLSTN1 gene expression might be considered as a single biomarker of a response to radiation rather than to carcinogenesis.

This study has some limitations. We pooled blood samples from irradiated mice because the amount of blood cells isolated from a single mouse was insufficient to measure mRNA levels of genes. Therefore, biodosimetry findings varied between the 0.15-Gy and 4-Gy groups. Plasma CLSTN1 protein levels require further investigation to determine whether responses differ between high-and low-dose exposure. The molecular regulation and functional roles of CLSTN1 in response to radiation need to be characterized in future studies.

5. Conclusions

However, the present findings indicated that SHMT2 and CLSTN1 gene expression might be considered as a single biomarker of a response to radiation rather than to carcinogenesis. Therefore, the levels of CLSTN1...
mRNA and CLSTN1 protein expression might reflect exposure to low- or high-dose radiation.

Acknowledgments

We thank Editage (www.editage.co.kr) for English language editing.

Disclosure statement

The authors have no conflicts of interest to declare, financial or otherwise.

Funding

This study was supported by the National R & D Program funded by the Ministry of Science, ICT & Future Planning through the Dongnam Institute of Radiological & Medical Sciences (DIRAMS) under grants [50491-2019 and 50491-2020].

ORCID

Manwoo Lee http://orcid.org/0000-0002-7398-8254

Data availability statement

All data generated or analyzed during this study are included in this article.

References

Bernhardt, S., Bayerlová, M., Vetter, M., Wachter, J., Amundson, S., Albert, S., Hanf, V., Lantsch, T., Uleer, C., Peschel, S., John, J., Buchmann, J., Weigert, E., Bürig, K.-F., Thomssen, C., Korf, U., Beissbarth, T., Wiemann, S., & Kantelhardt, E. J. (2017). Proteomic profiling of breast cancer metabolism identifies SHMT2 and ASC2 as prognostic factors. Breast Cancer Research, 19, 112. https://doi.org/10.1186/s13058-017-0905-7

CERN. (2018). ROOT data analysis framework. https://root.cern.ch/

Chen, N., Wu, L., Yuan, H., & Wang, J. (2015). ROS/autophagy/Nrf2 pathway mediated low-dose radiation induced radio-resistance in human lung adenocarcinoma A549 cell. International Journal of Biological Sciences, 11(7), 833–844. https://doi.org/10.7150/ijbs.10564

Cheng, K., Chen, Y. S., Yue, C. X., Zhang, S.-M., Pei, Y.-P., Cheng, G.-R., Liu, D., Xu, L., Dong, H.-X., & Zeng, Y. (2019). Calystenin-1 negatively regulates ICAM5 accumulation in postsynaptic membrane and influences dendritic spine maturation in a mouse model of fragile X syndrome. Frontiers in Neuroscience, 13, 1098. https://doi.org/10.3389/fnins.2019.01098

Chu, Y., Lai, Y.-H., Lee, M.-C., Yeh, Y.-J., Wu, Y.-K., Tsao, W., Huang, C.-Y., & Wu, S. (2017). Calystenin-1, clusterin and neutrophil gelatinase-associated lipocalin are candidate serological biomarkers for lung adenocarcinoma. Oncotarget, 8(64), 107964–107976. https://doi.org/10.18632/oncotarget.22438

Chung, W., Kwabi-Addo, B., Ittmann, M., Jelinek, J., Shen, L., Yu, Y., & Issa, J. P. J. (2008). Identification of novel tumor markers in prostate, colon and breast cancer by unbiased methylation profiling. PLoS One, 3(4), e2079. https://doi.org/10.1371/journal.pone.0002079

Halimi, M., Shahabi, A., Moslemi, D., Parsian, H., Ashgari, S. M., Sariri, R., Yogehan, F., & Zabili, E. (2016). Human serum miR-34a as an indicator of exposure to ionising radiation. Radiation and Environmental Biophysics, 55(4), 423–429. https://doi.org/10.1007/s00411-016-0661-6

Heidary, Z., Zaki-Dizaji, M., Saliminejad, K., & Khorrammkhorshid, H. R. (2019). Expression Analysis of the CRISPR2, CATSPER1, PATE1 and SEMG1 in the Sperm of Men with Idiopathic Asthenozoospermia. Journal of Reproduction and Infertility, 20(2), 70–75. https://www.jriarticle.org/article/50048

Hwang, S., Jeon, H., Hong, E.-H., Joo, H. M., Cho, K. S., & Nam, S. Y. (2019). Low-dose ionizing radiation alleviates Aβ42-induced cell death via regulating AKT and p38 pathways in Drosophila Alzheimer’s disease models. Biology Open, 8(2), bio036657. https://doi.org/10.1242/bio.036657

International Atomic Energy Agency. (2011). Cyto Genetic dosimetry: Applications in preparedness for and response to radiation emergencies. IAEA.

Konecna, A., Frischknecht, R., Kinter, J., Ludwig, A., Steuble, M., Meskenaite, V., Indermühl, M., Engel, M., Cen, C., Mateos, J.-M., Streit, P., & Sonderegger, P. (2006). Calystenin-1 docks vesicular cargo to kinesin-1. Molecular Biology of the Cell, 17(8), 3651–3663. https://doi.org/10.1091/mbc.e06-02-0112

Lee, D. Y., Bowen, B. P., Nguyen, D. H., Parsa, S., Huang, Y., Mao, J.-H., & Northen, T. R. (2012). Low-dose ionizing radiation-induced blood plasma metabolic response in a diverse genetic mouse population. Radiation Research, 178(6), 551–555. https://doi.org/10.1667/RR2990.1

Lee, E.-S., Won, Y. J., Kim, B.-C., Park, D., Bae, J.-H., Park, S.-J., Noh, S. J., Kang, Y.-R., Choi, S. H., Yoon, J.-H., Heo, K., Yang, K., & Son, T. G. (2016). Low-dose irradiation promotes Rad51 expression by down-regulating miR-193b-3p in hepatocytes. Scientific Reports, 6, 25723. https://doi.org/10.1038/srep25723

Ludwig, A., Blume, J., Diep, T.-M., Yuan, J., Mateos, J. M., Leuthäuser, K., Steuble, M., Streit, P., & Sonderegger, P. (2009). Calystenins mediate TGN exit of APP in a kinesin-1-dependent manner. Traffic, 10(5), 572–589. https://doi.org/10.1111/j.1600-0854.2009.00886.x

Maes, O. C., An, J., Sarojini, H., Wu, H., & Wang, E. (2008). Changes in microRNA expression patterns in human fibroblasts after low-LET radiation. Journal of Cellular Biochemistry, 105(3), 824–834. https://doi.org/10.1002/jcb.21878

Moertl, S., Buschmann, D., Azimzadeh, O., Schneider, M., Kell, R., Winkler, K., Tapio, S., Hornhardt, S., Merl-Pham, J., Pfaffl, M. W., & Atkinson, M. J. (2020). Radiation exposure of peripheral mononuclear blood cells alters the composition and function of secreted extracellular vesicles. International Journal of Molecular Sciences, 21(7), 2336. https://doi.org/10.3390/ijms21072336

MyAssays. (2020). Four parameter logistic regression. https://www.myassays.com/four-parameter-logistic-regression.html

Park, J. G., Paul, S., Briones, N., Zeng, J., Gillis, K., Wallstrom, G., LaBaeer, J., & Amundson, S. A. (2017). Developing human radiation biodosimetry models: Testing cross-species conversion approaches using an ex vivo model system. Radiation Research, 187(6), 708–721. https://doi.org/10.1667/RR14655.1

Rudobek, E., Bellone, J. A., Szucs, A., et al. (2017). Low-dose proton radiation effects in a transgenic mouse model of
Alzheimer’s disease - implications for space travel. *PLoS One*, 12(11), e0186168. [https://doi.org/10.1371/journal.pone.0186168](https://doi.org/10.1371/journal.pone.0186168)

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to Image: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. [https://doi.org/10.1038/nmeth.2089](https://doi.org/10.1038/nmeth.2089)

Shi, H., Fang, X., Li, Y., & Zhang, Y. (2019). High expression of serine hydroxymethyltransferase 2 indicates poor prognosis of gastric cancer patients. *Medical Science Monitor*, 25, 7430–7438. [https://doi.org/10.12659/MSM.917435](https://doi.org/10.12659/MSM.917435)

Fendler, F., https://doi.org/10.3390/ijms21030812

International biomarkers for individualization of radiotherapy. *Translational Research*, 201, 71–83. [https://doi.org/10.1016/j.trsl.2018.06.001](https://doi.org/10.1016/j.trsl.2018.06.001)

Wei, Z., Song, J., Wang, G., Cui, X., Zheng, J., Tang, Y., Chen, X., Li, J., Cui, L., Liu, C.-Y., & Yu, W. (2018). Deacetylation of serine hydroxymethyltransferase 2 by SIRT3 promotes colorectal carcinogenesis. *Nature Communications*, 9(1), 4468. [https://doi.org/10.1038/s41467-018-06812-y](https://doi.org/10.1038/s41467-018-06812-y)

Woo, C. C., Chen, W. C., Teo, X. Q., Radda, G. K., & Teck Hock Lee, P. (2016). Downregulating serine hydroxymethyltransferase 2 (SHMT2) suppresses tumorigenesis in human hepatocellular carcinoma. *Oncotarget*, 7(33), 53005–53017. [https://doi.org/10.18632/oncotarget.10415](https://doi.org/10.18632/oncotarget.10415)

Yang, X., Wang, Z., Li, X., Liu, B., Liu, M., Liu, L., Chen, S., Ren, M., Wang, Y., Yu, M., Wang, B., Zou, J., Zhu, W.-G., Yin, Y., Gu, W., & Luo, J. (2018). SHMT2 Desuccinylation by SIRT5 drives cancer cell proliferation. *Cancer Research*, 78(2), 372–386. [https://doi.org/10.1158/0008-5472.CAN-17-1912](https://doi.org/10.1158/0008-5472.CAN-17-1912)

Zeegers, D., Venkatesan, S., Koh, S. W., Low, G. M., Srivastava, P., Sundaram, N., Sethu, S., Banerjee, B., Jayapal, M., Belyakov, O., Baskar, R., Balajee, A., & Hande, M. (2017). Biomarkers of ionizing radiation exposure: A multiparametric approach. *Genome Integrity*, 8, 6. [https://doi.org/10.4103/2041-9414.198911](https://doi.org/10.4103/2041-9414.198911)