Impact of Ionizing Radiation on the Expression of CDC25A Phosphatase in vivo

Seyed Mostafa Mir (MSc)
Department of Clinical Biochemistry, School of Medicine, Babol University of Medical Sciences, Babol, Iran

Esmaeil Samadian(MSc)
Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Sahar Alijanpour(MSc)
Department of Medical Genetics, Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran

Alireza Khoshbin Khoshnazar (PhD)
Department of Clinical Biochemistry, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

Hamid Haghighatfard (MSc)
Department of Medical Biotechnology, Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran

Seyed Hossein Sadeghi (MSc)
Department of Medical Genetics, School of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran

Medical Cellular & Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Corresponding author:
SeyedHosseinSadeghi

Tel: +989190334976

E-mail: Hsadeqi86@gmail.com

Address: ShahidBeheshti University of Medical Sciences, Tehran, Iran

Received : 06 Dec 2014
Revised: 09 Feb 2015
Accepted: 12 Feb 2015

ABSTRACT

Background and Objective: The cell division cycle 25 (CDC25)is a familyof highly conserved dual-specificity phosphatases that activate cyclin-dependent kinase complexes. These complexes are the main cell cycle regulators. Mammalian cells ,exposure to DNA damaging radiations such as ionizing radiation and ultraviolet light, prevent cell cycle progression by activation of checkpoint pathways and lead to cell death.

Methods: In this study, mice were exposed to different doses of ionizing radiation. Their total cellular protein was extracted from the bone marrow. After determining and matching the protein concentrations, CDC25A phosphatase levels were measured by western blotting.

Results: The results showed that exposure to different doses of ionizing radiation in vivo significantly increased the expression of CDC25A compared to control group ($P < 0.05$).

Conclusion: Exposure to ionizing radiation increases the expression of CDC25A phosphatase, which increases the possibility of tumorigenesis in that area by increasing bone marrow cell proliferation.

Keywords: Cell Cycle, CDC25A, Ionizing Radiation, Cyclin-Dependent Kinase.

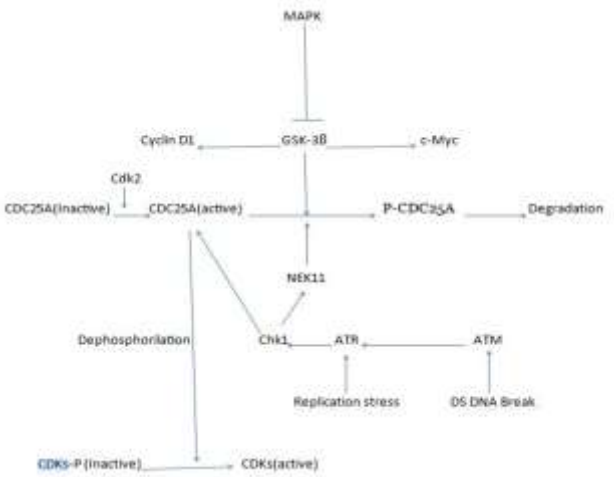
This paper should be cited as: Mir SM, Samadian E, Alijanpour S, Khoshbin Khoshnazar A, Haghighatfard H, Sadeghi SH[Impact of Ionizing Radiation on the Expression of CDC25A Phosphatase in vivo] . mljgoums. 2016; 10(5):22-26

INTRODUCTION

Double-strand break (DSB) in the DNA is resulted from exposure to ionizing radiation that induces genomic instability, dysregulation of cell cycle and tumorigenesis (1, 2). Cyclin-dependent kinases (CDKs) are the key regulator of cell cycle progression. CDKs become inactivated via phosphorylation of their two residues on the loop binding to ATP (threonine-14 and tyrosine-15) by Myt1 and Wee1 kinases.When the cell cycle progression is dependent on the activity of CDKs, the dual-specificity phosphatase (CDC25) activates the CDK-cyclin complex that leads to cell cycle progression by removing the inhibitory phosphate from the CDKs (3).The cell division cycle 25 (CDC25) is a family of highly conserved dual-specificity phosphatases that activate the CDKC and regulate cell cycle. The family has three isoforms; CDC25A, CDC25B and CDC25C (4), with 40-50% identity (5, 6). The CDC25A interacts with many genes in the cell (Figure 1), and mostly causes activation of CDK2-cyclinA and CDK2-cyclinE complexes, which leads to G1 to S phase transition. However, it is also responsible for the G2 to M transition by activating CDK1-cyclinB (7, 8).CDC25A is predominantly expressed in the G1 phase and stabilized in mitosis by phosphorylation of CDK1-cyclinB. At the end of mitosis, CDC25A level reduces due to destruction caused by anaphase 10). promoting complex/cyclosome ubiquitin (9, CDC25A is destroyed in embryonic stem cells after induction of DSBs by ionizing radiation (11). Today, the use of radiation and its application in human life are undeniable necessities. On the other hand, the biological side effects of radiation have always been of interest to researchers (12). When eukaryotic cells are exposed to DNA damaging agents such as ionizing radiation and ultraviolet (UV), they inhibit cell cycle progression by activating DNAdamage checkpoints, leading to cell death (13).

CDC25A is a suitable target for damage response during mitosis because it accumulates during mitosis (14), and plays an important role in response to damage caused by DNA exposure to ionizing radiation (15, 16). Eliminating CDC25A suppresses cell proliferation and gives the cell a chance to repair the DNA. The exposure of eukaryotic cells to ionizing radiation activates checkpoints and stops the cell cycle (17). Although overexpression of CDC25A has been observed in a large number of cancers, the molecular mechanism of this overexpression is not clear (4). This study aimed to evaluate cell cycle suppression or progressionand CDC25A expression level after exposure to different doses of ionizing radiationin vivo.

Figure 1-*CDC25A* gene interacts with certain genes involved in the cell cycle



MATERIAL AND METHODS

The healthy female mice (without any infection) were used in this study. The animals used for experiments were kept in controlled temperature and humidity with access to food and water. All testing phases were approved by the Ethics Committee for Laboratory Animals at GolestanUniversity of Medical Sciences, Iran. Test animals were divided into four groups of three and a control group. Each group was exposed to different doses of gamma-radiation including (1.25, 1.5, 1.75, 2). Gamma rays from cobalt-60 obtained from the radiotherapy center of the Golestan Province had dose rate of 67.55 cGy per minute. The exposure time was at the 2.13-3.55 min range, irradiated field size of 30 × 30 cm^2, and source-to-surface distances of 80cm. The femoral bone marrow of the mice was removed and placed in test tubes. About 150 µl of lysis buffer [containing 5% SDS, 100 mMTris-Hcl (pH=7.4), 150 mMNacl, 5 mM EDTA, 5% sodium deoxycholate, 10 % glycerol] were added to the tubes containing bone marrow cells. Then, 10 µl of protease inhibitor cocktail was added to the samples. Pierce BCA protein assay kits (Thermo Fisher Scientific) and bovine serum albumin (BSA) were used to determine the concentration of cellular proteins and plotting the standard curve, respectively. The proteins were separated by SDS-PAGE electrophoresis on 12% gel based on the molecular weight. Then, the 5x sample loading buffer containing 10% SDS, 0.5% bromophenol blue, 60 mM of Tris-HCI (pH 6.8), 50% glycerol, and 14.4 Mm of 2-mercaptoethanol was added to each tube. The samples were placed at boiling temperature for 5 minutes. Then, 35µl of protein was loaded into each well for blotting. The protein samples were separated on 12% polyacrylamide gel with current of 110 volts for 45 minutes, and transferred to nitrocellulose membrane (Thermo) via the buffer (15.6 mMTris-base, 120mM glycine, 10% methanol with pH 8.4). Blots in the 3% skim milk along with phosphate buffer saline [(PBS) with final concentration of 0.1% Tween] were placed on a rotator for 2-3 hours at room temperature. The antibodies were diluted in 3% skim milk (1:1000 ratio) and used against CDC25A phosphatase at 4 ^oC, and then incubated in PBS-Tween (PBS-T).The blots were washed three times with PBS-T for 5, 10 and 15 minutes at room temperature. Then, the blots were incubated with HRP conjugated secondary antibody, goat anti-mouse IgG, diluted with 3% skim milk (1:2,000) in PBS-T for 1-2 hours at room temperature, on a rotator. The blots were washed and exposed to autoradiography film at various times, 5 minutes after incubation with enhanced chemiluminescence (ECL). Finally, the films were printed and observed. The data was analyzed by SPSS (version 19), using analysis of variance (ANOVA), Tukey's honest significant difference test and Dunken test with significance level set at 0.05.

RESULTS

Exposure to different doses was done at four points to identify the relationship between the dose and CDC25A protein expression changes. The presence of the CDC25A phosphatase in bone marrow cell lysates was evaluated. After Western blotting, the bands related to the CDC25A phosphatase were observed in irradiated mice (Figure 2). In addition, ß-actin was used as internal control.
At this stage, Image J software was used to convert the Western blot images into quantitative data by giving the color intensity and area of each band a numerical score. It should be noted that the numbers calculated by the Image J software are only figures that represent specific coordinates of the selected curves.In other words, these numbers do not represent the amount of proteins in each band based on milligrams or any other unit.

DISCUSSION

In recent years, significant improvements have been made in better identification of cell cycle and factors affecting it. While several factors affect the cell cycle, UV and ionizing radiation are among the factors that cause DNA damage. Based on severity of the damage, these factors may either cause cell cycle arrest to repair the damaged DNA or accelerate the cell cycle and cause cancer (1, 2). Meanwhile, the changes in the expression of some genes such as overexpression of proto-oncogenes interrupt the cell cycle. The proto-oncogene *CDC25A* is highly regulated during the cell cycle because of its importance in determining the cell's fate. Misregulation of the CDC25A leads to accumulation of DNA damage and loss of genomic integrity (19). The CDC25A phosphatase is destroyed in case of DNA damage replication arrest (13). The DNA damages are ignored and macromolecular damage is accelerated by misregulation of CDC25A phosphatase function in checkpoints (8, 18). Bone marrow is more sensitive to ionizing radiationsince it contains cells with active cell cycle that are constantly dividing (19). In the present study, in vivo irradiation with different doses led to significant overexpression of CDC25A phosphatase compared to the control, which could indicate cell cycle acceleration. *CDC25A* is a cellular proto-oncogene gene and its activation in the early stages of tumor progression causes DNA replication stress. This can lead to malignancy because of induced DNA damage response (DDR) and selection of defective cells in the DDR (20). Consistent with the results of the present study, Ming-Yii Huang et al. demonstrated the overexpression of a large number of genes in colorectal cancer after radiotherapy. In the mentioned study, bioinformatics database analysis showed that 30 of these genes were involved in the DDR, immune response and complement pathways as well as the coagulation cascade. The *CDC25A*was among the overexpressed genes in the colorectal cancer after radiation (21). However, the results of many studies differ from our results, which could be due to several reasons such as different target tissues selected (bone marrow in the present study), dosage, study conditions (in vivo or in vitro), mutations, etc. For example, study of Jodi Yanagida et al. on expression of skin cells showed that exposure of mice to UV radiation degrades CDC25A phosphatase and leads to cell cycle arrest (22). Cell cycle arrest at the checkpoints enables the cell to repair DNA damage and reduce tumorigenesis. Moreover, some mutations affect the stability of the CDC25A protein. Goloudina AR et al. reported that S75A mutation destroys CDC25A in normal and stressed cells (23). It is suggested to investigate the tumorigenic and cell arrest capability of proliferating cells exposed to ionizing radiation in vitro, compared to control cells.

CONCLUSION

CDC25A is an important proto-oncogene and its overexpression accelerates cell cycle and tumorigenesis. The results of this study show that the presence of ionizing radiation increases CDC25A phosphatase expression. Moreover, there may be the possibility of cell cycle acceleration and tumorigenesis in people exposed to radiations.

ACKNOWLEDGMENT

The authors wish to acknowledge the staff of Laboratory of Clinical Biochemistry, Laboratory Sciences Research Center and Radiotherapy Center of Panje Azar Hospital. Cooperation and assistance of Ms.Shamsabadi is also gratefully acknowledged.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this manuscript.

REFERENCES

1. Helleday T, Lo J, van Gent DC, Engelward BP. *DNA double-strand break repair: from mechanistic understanding to cancer treatment.* DNA repair. 2007; 6(7): 923-35.

2. Jazayeri A, Falck J, Lukas C, Bartek J, Smith GC, Lukas J, et al. *ATM-and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks.* Nature cell biology. 2005;8(1):37-45.

3. Malumbres M, Barbacid M. *Mammalian cyclin-dependent kinases.* Trends in biochemical sciences. 2005; 30(11): 630-41.

4. Boutros R, Lobjois V, Ducommun B. *CDC25 phosphatases in cancer cells: key players? Good targets?* Nature Reviews Cancer. 2007; 7(7): 495-507.

5. Sadhu K, Reed SI, Richardson H, Russell P. *Human homolog of fission yeast cdc25 mitotic inducer is predominantly expressed in G2.* Proceedings of the National Academy of Sciences. 1990;87(13):5139-43.

6. Sexl V, Diehl JA, Sherr CJ, Ashmun R, Beach D, Roussel MF. *A rate limiting function of cdc25A for S phase entry inversely correlates with tyrosine dephosphorylation of Cdk2.* Oncogene. 1999; 18(3):573-82.

7. Blomberg I, Hoffmann I. *Ectopic expression of Cdc25A accelerates the G1/S transition and leads to premature activation of cyclin E-and cyclin A-dependent kinases.* Molecular and cellular biology. 1999; 19(9): 6183-94.

8. Molinari M, Mercurio C, Dominguez J, Goubin F, Draetta GF. *Human Cdc25 A inactivation in response to S phase inhibition and its role in preventing premature mitosis.* EMBO reports. 2000;1(1):71-9.

9. Jinno S, Suto K, Nagata A, Igarashi M, Kanaoka Y, Nojima H, et al. *Cdc25A is a novel phosphatase functioning early in the cell cycle.* The EMBO journal. 1994; 13(7):1549-56.

10. Mailand N, Podtelejnikov AV, Groth A, Mann M, Bartek J, Lukas J. *Regulation of G2/M events by Cdc25A through phosphorylation-dependent modulation of its stability.* The EMBO journal. 2002; 21(21): 5911-20.

11. Koledova Z, Kafkova LR, Krämer A, Divoky V. *DNA Damage-Induced Degradation of Cdc25A Does Not Lead to Inhibition of Cdk2 Activity in Mouse Embryonic Stem Cells.* Stem Cells. 2010; 28(3):450-61.

12. ShabestanimonfaredA,aMHoA. Compare of dosimetery biologic with dosimetery Physical at radiation worker city Babol. Magazine science Medical Mazandaran. 2006;16(51):43-50.

13. Falck J, Mailand N, Syljuåsen RG, Bartek J, Lukas J. *The ATM–Chk2–Cdc25A checkpoint pathway guards against radioresistant DNA synthesis*. Nature. 2001;410(6830):842-7.

14. Mailand N, Podtelejnikov AV, Groth A, Mann M, Bartek J, Lukas J. *Regulation of G2/M events by Cdc25A through phosphorylation-dependent modulation of its stability*. The EMBO journal. 2002; 21(21):5911-20.doi: 10.1093/emboj/cdf567.

15. Mailand N, Falck J, Lukas C, Syljuåsen RG, Welcker M, Bartek J, et al. *Rapid destruction of human Cdc25A in response to DNA damage.* Science. 2000; 288(5470):1425-9.

16. Zhao H, Watkins JL, Piwnica-Worms H. *Disruption of the checkpoint kinase 1/cell division cycle 25A pathway abrogates ionizing radiation-induced S and G2 checkpoints.* Proceedings of the National Academy of Sciences. 2002; 99(23):14795-800.

17. Kang T, Wei Y, Honaker Y, Yamaguchi H, Appella E, Hung M-C, et al. *GSK-3β targets Cdc25A for ubiquitin-mediated proteolysis, and GSK-3β inactivation correlates with Cdc25A overproduction in human cancers.* Cancer cell. 2008; 13(1):36-47.

18. Bartek J, Lukas J. *Pathways governing G1/S transition and their response to DNA damage.* FEBS letters. 2001; 490(3):117-22.

19. Cangi MG, Piccinin S, Pecciarini L, Talarico A, Dal Cin E, Grassi S, et al. *Constitutive overexpression of CDC25A in primary human mammary epithelial cells results in both defective DNA damage response and chromosomal breaks at fragile sites.* International Journal of Cancer. 2008; 123(6): 1466-71.

20. Löbrich M, Shibata A, Beucher A, Fisher A, Ensminger M, Goodarzi AA, et al. *γH2AX foci analysis for monitoring DNA double-strand break repair.* Cell Cycle. 2010; 9(4): 662-9.

21. Huang M-Y, Wang J-Y, Chang H-J, Kuo C-W, Tok T-S, Lin S-R. *CDC25A, VAV1, TP73, BRCA1 and ZAP70 gene overexpression correlates with radiation response in colorectal cancer.* Oncology reports. 2011;25(5):1297-309.doi: 10.3892/or.2011.1193.

22. Yanagida J, Hammiller B, Al-Matouq J, Behrens M, Trempus CS, Repertinger SK, et al. *Accelerated elimination of ultraviolet-induced DNA damage through apoptosis in CDC25A-deficient skin.* Carcinogenesis. 2012; 33(9): 1754-61.

23. Goloudina A, Aksenov N, Pospelov V. *Degradation of Cdc25A phosphatase in HeLa cells under normal and stress conditions*. Tsitologiia. 2003; 46(5): 423-30.