Sam68 is cleaved by caspases under apoptotic cell death induced by ionizing radiation

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The RNA-binding protein Sam68, a mitotic substrate of tyrosine kinases, has been reported to participate in the cell cycle, apoptosis, and signaling. In particular, overexpression of Sam68 protein is known to suppress cell growth and cell cycle progression in NIH3T3 cells. Although Sam68 is involved in many cellular activities, the function of Sam68, especially in response to apoptotic stimulation, is not well understood. In this study, we found that Sam68 protein is cleaved in immune cells undergoing apoptosis induced by γ-radiation. Moreover, we found that Sam68 cleavage was induced by apoptotic stimuli containing γ-radiation in a caspase-dependent manner. In particular, we showed that activated caspase-3, 7, 8 and 9 can directly cleave Sam68 protein through in vitro protease cleavage assay. Finally, we found that the knockdown of Sam68 attenuated γ-radiation–induced cell death and growth suppression. Conclusively, the cleavage of Sam68 is a new indicator for the cell damaging effects of ionizing radiation.

Keywords: Sam68; ionizing radiation; caspase; cleavage; apoptosis

INTRODUCTION

Src-associated substrate in mitosis of 68 kDa (Sam68), otherwise known as KHDRBS1, was first characterized as a mitotic target of Src tyrosine kinase in mouse NIH3T3 fibroblasts [1, 2], and as a tyrosine phosphorylated protein associated with the T cell–specific Src kinase p56lck in leukemic T-cell lines and mitogen-activated T lymphocytes [3]. Sam68 belongs to the signal transducer and activator of the RNA (STAR) family of RNA-binding proteins that link signaling pathways to RNA processing and contain a heteronuclear ribonucleoprotein particle K homology (KH) domain [4]. The KH domain of Sam68 is flanked by conserved N- and C-terminal sequences required for RNA binding activity [5, 6]. The RNA-binding ability of Sam68 is harbored within the GRP33, Sam68, GLD-1 (GSG) domain, which is required for homodimerization and sequence-specific binding to RNA targets [7, 8]. The GSG domain is flanked by a proline-rich WW domain (a protein–protein interaction domain containing two conserved tryptophan residues), Src homology domain (SH3) binding regions, and SH2-interacting tyrosine-rich motifs, which mediate interplay with numerous cell signaling components in response to different stimuli and critically regulate Sam68 function [4, 7, 8].

Sam68 is implicated in a number of cellular processes, including signal transduction, transcription, RNA metabolism, cell cycle regulation, carcinogenesis and apoptosis [4, 9]. In mouse fibroblasts, Sam68 overexpression inhibits G1 to S phase progression and induces apoptosis in an RNA-binding–dependent manner [10]. These findings may, in part, explain the many roles in cellular and viral function previously attributed to Sam68. However, its apoptotic function remains unclear.

Apoptosis is a process of cell death used by organisms to eliminate superfluous, cancerous or virus- or bacteria-infected cells [11–13]. It is initiated by the activation of caspases, a family of cysteine proteases that cleave after Asp residues [14–16]. Caspases are present in most healthy cells...
as inactive precursors known as procaspases, which undergo proteolytic processing to generate the active enzyme when an apoptotic signal is received [15]. While caspase-8 and -9 participate in the initiation phase of apoptosis, caspase-3, -6 and -7 are involved in the execution phase of apoptosis [14–16]. Caspase-2 can function both as an initiator and an effector caspase [17–19]. Proteolytic cleavage of critical cellular proteins, such as poly(ADP-ribose) polymerase (PARP), DNA-dependent protein kinase, lamin B, and protein kinase C-δ by executioner caspases is associated with cell death [14, 20, 21].

Although Sam68 is involved in many cellular activities via regulation of its RNA-binding ability and its substrate proteins, the function of Sam68, especially in response to apoptotic stimulation, is not well understood. In this study, we showed that Sam68 is cleaved by activated caspases in response to ionizing radiation and treatment with apoptotic stimuli. Our results indicate that cleavage of Sam68 might be a phenomenon associated with loss of cell viability and a new indicator for the cell damage effects induced by ionizing radiation and pro-apoptotic agents.

MATERIALS AND METHODS

Reagents
Anti-Sam68 (C-20), anti-caspase-3, 6, 10 and anti-PARP were purchased from Santa Cruz Biotechnology Inc. (Delaware, CA). Anti-caspase-3, 6, 8, 10, anti-Actin, and broad caspase inhibitor (z-VAD-fmk) were purchased from Cell Signaling Technology Inc. (Denver, MA). Camptothecin, propidium iodide (PI), and the MTT assay reagents were purchased from the Sigma Chemical Co. (St Louis, MO).

Cells and cell culture
IM-9, human B lymphoblast, Jurkat (A3), human T lymphoma and its subclone I9.2 cell lines were cultured in RPMI 1640 medium containing 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 U/ml) at 37°C under an atmosphere of 5% CO₂. NIH3T3 mouse fibroblasts and human lymphoblastoid cell lines (IM-9, human B lymphoblast, Jurkat (A3), human T lymphoma, and its subclone I9.2 cell lines) were cultured in DMEM containing 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 U/ml) at 37°C under an atmosphere of 5% CO₂. All cells were purchased from the American Type Culture Collection (Manassas, VA).

Exposure of cells to γ-radiation
Cells were uniformly irradiated at room temperature with various doses of a 137Cs-γ-source (dose rate, 5.41 Gy/min; IBL 437 C type H, CIS Biointernational, France). Control cells for each dose were simultaneously exposed to sham radiation.

Western blot analysis
Cells were harvested, rinsed with ice-cold PBS and lysed in homogenization buffer (50 mM Tris-Cl, pH 6.8) containing protease inhibitor (1 mM phenylmethylsulphonyl fluoride), 10% sodium dodecyl sulphate (SDS) and 10% glycerol. Protein concentrations of whole cell lysates were determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Hertfordshire, UK). Western blotting was performed using standard protocols, and the membrane was visualized by enhanced chemiluminescence (ECL-solution, Amersham, Buckinghamshire, UK).

MTT assay
Cells were seeded in 96-well microtiter plates at 1 × 10⁴ cells per well and incubated for 24 h in 100 μl of culture media prior to treatment with γ-radiation, as indicated. MTT (100 μl; 5 mg/ml) was added to the cells and they were then incubated for another 2–4 h. After the supernatant was removed, 100 μl of DMSO per well was added to the cells and mixed on a shaker for 15 min. The absorbance at 570 nm was measured by a microplate reader.

Annexin V–FITC analysis
Each sample was prepared according to the instructions of the annexin V–FITC apoptosis detection kit (Sigma). A total of 20 000 events were collected per sample using flow cytometry, and debris was excluded by scatter gating. The quadrants for data analysis were determined for single Annexin V–FITC and PI-stained samples, as well as for no-dye negative control samples. Data analysis was by Cell Quest software (Becton Dickinson, Canada), and the percentage of cells in early apoptosis, late apoptosis, necrosis and viable (unaffected) quadrants was calculated and compared by ANOVA Two Factor with Replication. The assay was performed in triplicate. P<0.05 was considered a significant result.

In vitro translation and protease cleavage assay
The entire sam68 gene has been cloned, and in vitro transcription and translation of cDNAs encoding the human Sam68 protein was performed with the TNT Coupled Reticulocyte Lysate System (Promega, Madison, WI) according to the manufacturer’s protocols. Biotin-labeled Sam68 protein was incubated with each active recombinant caspase in reaction buffer (50 mM HEPES, pH 7.4, 50 mM NaCl, 0.1% Chaps, 10 mM sodium EDTA, 5% glycerol and 10 mM DTT) for 1 h at 37°C and then subjected to SDS-PAGE. Cleaved fragments were detected by anti-streptavidin-HRP (Sigma).

siRNA transfection with Amaxa-Nucleofector
Scrambled siRNA and siRNA for Sam68 were designed using Dharmacon’s siRNA database (Lafayette, CO). siRNAs were transfected into cells using Amaxa Nucleofector system according to the manufacturer’s recommendations. IM-9 cells were transfected with program U-9 and immediately transferred to a 60-mm dish containing prewarmed medium, followed by incubation at 37°C in a humidified atmosphere containing 5% CO₂.
**Propidium iodide staining analysis**
Cells were harvested and then fixed in 75% ethanol for at least 1 h. Cells were then washed with PBS and resuspended in PBS buffer containing 100 μg/ml of RNase A (sigma) and 50 μg/ml of propodium iodide (PI) (Invitrogen), followed by incubation at 37°C for 30 min. The percentage of cells in sub-G1 was analyzed by a FACS-Caliber along with Cell Quest Pro software (BD biosciences).

**RESULTS**

**Sam68 is cleaved during γ-radiation–induced apoptosis**
Although Sam68 is known to play a crucial role in the apoptotic pathway, the way in which Sam68 is regulated by pro-apoptotic stimuli has not been understood to date. Thus, to investigate the regulation of Sam68 under apoptotic conditions, we analyzed the level of Sam63 proteins in various cell types after γ-radiation. First, IM-9, NIH3T3 and Jurkat cells were exposed to various doses of γ-radiation, after which apoptosis analysis was performed. As we expected, γ-radiation induced apoptotic cell death in IM-9 and NIH3T3 cells, and to a lesser extent in Jurkat cells (Fig. 1A). We would like to note that a Jurkat cell is known as a p53-null cell. Thus, a Jurkat cell might be less sensitive to γ-radiation due to a lack of p53. Whereas, HeLa cells, which are known as radioresistant cells, demonstrated little, if any, of apoptotic cells after γ-radiation (Fig. S1A). Interestingly, we found that cleavage of Sam68 by γ-radiation was observed in both Jurkat and IM-9 cells (Fig. 1B). Moreover, IM-9 cells were more sensitive to γ-radiation than Jurkat T cells (Fig. 1B). As we have illustrated in Fig. 1B, full-length Sam68 proteins have been measured as ~68 kDa, and the size of the cleaved Sam68 protein was estimated at ~60 kDa. Considering the anti-Sam68 antibody used in this experiment recognizes the c-terminal region of the Sam68 protein, where the epitope is located, we postulate that the cleavage site of Sam68 protein by γ-radiation is located in the N-terminal region. We were unable to detect cleavage of Sam68 in NIH3T3 (Fig. 1B) or HeLa cells (Fig. S1B). Thus, our data suggest that the cleavage of Sam68 is cell-type specific and, in particular, more sensitive in immune cell types.

**The cleavage of Sam68 is induced by apoptotic stimuli as well as by γ-radiation in a caspase-dependent manner**
To determine whether other apoptotic stimuli as well as γ-radiation is be able to cleave Sam68 proteins, we measured the level of cleaved Sam63 protein after TRAIL or camptothecin

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**Fig. 1.** Sam68 is cleaved during γ-radiation–induced apoptosis. (A) Cell viability of IM-9, NIH3T3 and Jurkat cells after exposure to γ-radiation. Cells were cultured for 24 h after exposure to γ-radiation, and the percentage of cells undergoing apoptosis was estimated using the Annexin V–FITC method. (B) Induction of Sam68 cleavage in IM-9, Jurkat and NIH3T3 cells by γ-radiation. Cells were exposed to γ-radiation and then further incubated for 24 h. Cells were harvested and whole cell lysates were prepared. The levels of Sam68 and Actin proteins were determined by western blot analysis.
treatment. First, we performed apoptosis analysis using annexin V–FITC staining in Jurkat cells. We showed that the populations of annexin V–positive cells were increased by TRAIL or camptothecin (CPT) treatment at similar level to that produced with γ-radiation at 30 Gy (Fig. 2A).

The activation of caspases is a common feature of apoptotic pathways, because activated caspases are capable of cleaving a large number of apoptotic mediator proteins. Thus, we investigated whether caspase activation is required for cleavage of Sam68 during apoptotic cell death. To test this, Jurkat cells were cultured in the presence of a broad inhibitor of caspases (z-VAD-fmk) for 1 h prior to induction of apoptosis by γ-radiation, TRAIL, or camptothecin. The levels of cleaved Sam68 were determined by western blot analysis. We found that cleavage of Sam68 after treatment with TRAIL, camptothecin or γ-radiation (30 Gy) was completely inhibited in the presence of z-VAD-fmk (Fig. 2B). These results suggest that a caspase-mediated signal is involved in the apoptotic cleavage of Sam68.

**Involvement of a range of caspases in Sam68 cleavage in vitro**

As illustrated in Fig. 2, Sam38 might have been cleaved by caspases or caspase-activated proteases. Thus, to determine whether Sam68 is a direct substrate for caspases, Sam68 was translated in vitro and incubated with recombinant active caspase-1, 2, 3, 6, 7, 8, 9 and 10. Surprisingly, we found that several caspases were able to cleave Sam68, resulting in cleavage products similar to the fragment generated in vivo (Fig. 3). Caspase-6, 7, 8 and 9 cleaved Sam68 very efficiently, and this cleavage was totally inhibited by each of the caspase inhibitors (Fig. 3). However, caspase-1, 2, 3 and 10 didn’t cleave Sam68 proteins (Fig. 3). These results suggest that Sam68 is a direct substrate of caspase-6, 7, 8 and 9.

**Caspase-8 is mediated via radiation-induced Sam68 cleavage**

Since caspase-8 can promote effector caspase activation [22], and radiation-activated caspase-8 is mediated in p53-independent apoptosis in glioma cells [23], we tested whether caspase-8 is required for the cleavage of Sam68 upon apoptotic condition. To test this, we compared the levels of cleaved Sam68 induced by ionizing radiation in wild type Jurkat, caspase-8-deficient Jurkat cells (I9.2 cells) and Fas-associated protein with death domain (FADD)-deficient Jurkat cells (I2.1 cells). We would like to note that FADD is an adaptor molecule that links death receptors to caspase-8 through its death domain.
during γ-radiation–induced apoptosis. We found that wild-type Jurkat cells and FADD-deficient I2.1 cells showed a similar pattern of Sam68 cleavage from 5 to 30 Gy, whereas caspase-8–deficient I9.2 cells exhibited Sam68 cleavage to a lesser extent (Fig. 4). However, the Sam68 cleavage at 30 Gy of γ-radiation was still detectable in I9.2 cells (Fig. 4). These results indicate that a deficiency of caspase-8 results in a reduction in Sam68 cleavage and that other caspases contribute to the cleavage of Sam68 in apoptotic cells induced by γ-radiation.

**Sam68 knockdown attenuates γ-radiation–induced apoptosis**

To assess whether Sam68 cleavage is functionally linked to growth suppression or apoptosis induced by γ-radiation, the Sam68 was knocked down by its specific siRNA, and cell viability was measured. We demonstrated that the levels of pro-Sam68 and cleaved Sam68 proteins were decreased in cells transfected with Sam68 siRNA (Fig. 5A). We found that γ-radiation–induced cell growth suppression was inhibited by Sam68 knockdown (Fig. 5B). Moreover, we also found that γ-radiation–induced cell death was inhibited by Sam68 knockdown (Fig. 5C). Our findings indicate that Sam68 is a key regulator of γ-radiation–induced cell death.

**DISCUSSION**

The RNA-binding protein Sam68 has been proposed as a key regulator of apoptosis and cell cycle progression. For instance, overexpressed Sam68 induces cell cycle arrest and apoptosis, and this is dependent on its ability to bind RNA [10]. In addition, Sam68 has been known to regulate the ratio of anti-apoptotic Bcl-x(L) or pro-apoptotic Bcl-x(s) mRNAs through an alternative splicing mechanism in order to exert its pro-apoptotic function [24]. As an upstream regulatory mechanism of Sam68, SUMO modification of Sam68 was known to enhance its ability to repress cyclin D1 expression and to inhibit its ability to induce apoptosis [25]. However, the study of upstream regulatory signaling mechanisms for Sam68 modulation during apoptosis is poorly investigated. In this study, we demonstrated that Sam68 protein is cleaved by γ-radiation and that this phenomenon is restricted in immune cells. Moreover, we also demonstrated that Sam68 cleavage can be induced by various apoptotic stimuli as well as by γ-radiation in a caspase-dependent manner. In addition, we found that Sam68 is a substrate of various caspases such as caspase-6, 7, 8, and 9. Finally, we showed that Sam68 is required for γ-radiation–induced cell death.

Although previous research revealed the possibility of control of cell cycle progression via the Sam68 protein [10], the study of Sam68 regulation of apoptosis is poorly investigated. In this study, we found that the Sam68 protein can be cleaved upon apoptotic cell death induced by various apoptotic inducers such as γ-radiation and DNA-damaging agents (Fig. 1B). Moreover, the γ-radiation-induced cleavage of
Sam68 was demonstrated in immune cells such as Jurkat and IM-9 cells, but not in other cell types. These data let us postulate that \( \gamma \)-radiation can cleave the Sam68 protein in an immune cell–specific manner.

Caspase activation, as a key component of the apoptotic pathway, can be initiated by a DNA-damaging stimulus such as ionizing radiation, and occurs through a cascade in which the activation of initiator caspases leads to the activation of downstream executive caspases [26, 27]. Many previous studies have identified target substrates that are cleaved by caspases during apoptosis [28]. Here, we demonstrated that cleavage of Sam68 was induced by a range of apoptotic stimuli and was completely inhibited by a broad caspase inhibitor (Fig. 2), suggesting that Sam68 is a target of caspases. Moreover, we demonstrated that the Sam68 protein was cleaved by caspases 6, 7, 8 and 9 (Fig. 3) and that deficiency of caspase-8 reduced the cleavage of Sam68 induced by \( \gamma \)-radiation (Fig. 4). These data suggest that the Sam68 protein is a substrate of various caspases and can be cleaved by the active caspase pathway during DNA damage-induced cell death. We then asked, what is the functional role of the cleaved Sam68 protein in the apoptotic pathway? To determine this, we tested whether the caspase-induced cleavage of Sam68 is required for ionizing radiation–induced apoptosis. We found that the knockdown of Sam68 attenuated \( \gamma \)-radiation–induced cell death (Fig. 5), suggesting that Sam68 contributes to the pro-apoptotic pathway during \( \gamma \)-radiation–induced cell death.

Here, we demonstrated that the Sam68 protein was cleaved by high-dose irradiation from 5 to 30 Gy in a dose-dependent manner, but not by radiation doses less than 5 Gy. Similarly, apoptotic cells were dose-dependently increased as those high-dose radiations in those cell lines. This phenomenon lets us postulate that the cleaved Sam68 protein plays a pro-apoptotic function in high-dose irradiated cells.

Further, we demonstrated that high-dose irradiation induced the cleavage of Sam68 in a caspase-dependent manner, whereas low-dose radiation (lower than 0.5 Gy) didn’t induce the cleavage of Sam68. At this point, we consider how low-dose irradiation might affect the function of Sam68 in the immune system. In fact, many previous reports have shown that low-dose irradiation improves the immune function, based on radiation hormesis theory [29–31]. For instance, low-dose irradiation has been known to facilitate anti-tumor cytotoxicity of natural killer cells without influencing cellular proliferation [30] or apoptosis and to induce...
immune-stimulatory responses in human primary monocytes [29]. However, the mechanism by which low-dose irradiation regulates immune responses has not been elucidated yet. Thus, investigation of the effect of low-dose irradiation on Sam68 function might prove to be interesting and very worthwhile.

CONCLUSION

Overall, this study provides the first demonstration that Sam68 protein is cleaved in immune cells under apoptotic conditions and that Sam68, as a direct substrate, can be cleaved by various caspases. Thus, we propose that the formation of cleaved Sam68 protein can be used as a significant indicator of radiation-induced apoptosis.

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REFERENCES

1. Taylor SJ, Shalloway D. An RNA-binding protein associated with Src through its SH2 and SH3 domains in mitosis. Nature 1994;368:867–71.
2. Fumagalli S, Totty NF, Hsuan JJ et al. A target for Src in mitosis. Nature 1994;368:871–4.
3. Vogel LB, Arthur R, Fujita DJ. An aberrant lck mRNA in two human T-cell lines. Biochim Biophys Acta 1995;1264:168–72.
4. Lukong KE, Richard S. Sam68, the KH domain-containing superSTAR. Biochim Biophys Acta 2003;1653:73–86.
5. Chen T, Boisvert FM, Bazett-Jones DP et al. A role for the GSG domain in localizing Sam68 to novel nuclear structures in cancer cell lines. Mol Biol Cell 1999;10:3015–33.
6. Lin Q, Taylor SJ, Shalloway D. Specificity and determinants of Sam68 RNA binding. Implications for the biological function of K homology domains. J Biol Chem 1997;272:27274–80.
7. Di Fruscio M, Chen T, Bonyadi S et al. The identification of two Drosophila K homology domain proteins. Kep1 and SAM are members of the Sam68 family of GSG domain proteins. J Biol Chem 1998;273:30122–30.
8. Jones AR, Schedl T. Mutations in gld-1, a female germ cell-specific tumor suppressor gene in Caenorhabditis elegans, affect a conserved domain also found in Src-associated protein Sam68. Genes Dev 1995;9:1491–504.
9. Rajan P, Gaughan L, Dalgliesh C et al. Regulation of gene expression by the RNA-binding protein Sam68 in cancer. Biochem Soc Trans 2008;36:505–7.
10. Taylor SJ, Resnick RJ, Shalloway D. Sam68 exerts separable effects on cell cycle progression and apoptosis. BMC Cell Biol 2004;5:5.
11. Arends MJ, Wyllie AH. Apoptosis: mechanisms and roles in pathology. Int Rev Exp Pathol 1991;32:223–54.
12. Kaufmann SH, Hengartner MO. Programmed cell death: alive and well in the new millennium. Trends Cell Biol 2001;11:526–34.
13. Zimmermann KC, Bonzon C, Green DR. The machinery of programmed cell death. Pharmacol Ther 2001;92:57–70.
14. Cohen GM. Caspases: the executioners of apoptosis. Biochem J 1997;326 (Pt 1):1–16.
15. Nicholson DW. Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 1999;6:1028–42.
16. Salvesen GS, Dixit VM. Caspase activation: the induced-proximity model. Proc Natl Acad Sci U S A 1999;96:10964–7.
17. Baliga BC, Read SH, Kumar S. The biochemical mechanism of caspase-2 activation. Cell Death Differ 2004;11:1234–41.
18. Duan H, Dixit VM. RAIDD is a new ‘death’ adaptor molecule. Nature 1997;385:86–9.
19. Tinel A, Tschopp J. The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. Science 2004;304:843–6.
20. Fuentes-Prior P, Salvesen GS. The protein structures that shape caspase activity, specificity, activation and inhibition. Biochem J 2004;384:201–32.
21. Salvesen GS, Dixit VM. Caspases: intracellular signaling by proteolysis. Cell 1997;91:443–6.
22. Muzio M, Salvesen GS, Dixit VM. FLICE induced apoptosis in a cell-free system. Cleavage of caspase zymogens. J Biol Chem 1997;272:2952–6.
23. Afshar G, Jelluma N, Yang X et al. Radiation-induced caspase-8 mediates p53-independent apoptosis in glioma cells. Cancer Res 2006;66:4223–32.
24. Boise LH, Gonzalez-Garcia M, Postema CE et al. Radiation-induced bcl-2, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 1993;74:597–608.
25. Babic I, Cherry E, Fujita DJ. SUMO modification of Sam68 enhances its ability to induce apoptosis. Oncogene 2006;25:4955–64.
26. Budihardjo I, Oliver H, Lutter M et al. Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol 1999;15:269–90.
27. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. Nat Rev Mol Cell Biol 2004;5:897–907.
28. Luthi AU, Martin SJ. The CASBAH: a searchable database of caspase substrates. Cell Death Differ 2007;14:641–50.
29. El-Saghire H, Michaux A, Thierens H et al. Low doses of ionizing radiation induce immune-stimulatory responses in isolated human primary monocytes. Int J Mol Med 2013;32:1407–14.
30. Sonn CH, Choi JR, Kim TJ et al. Augmentation of natural cytotoxicity by chronic low-dose ionizing radiation in murine natural killer cells primed by IL-2. J Radiat Res 2012;53:823–9.
31. Chun SH, Park GY, Han YK et al. Effect of low dose radiation on differentiation of bone marrow cells into dendritic cells. Dose Response 2012;11:374–84.