A secretome analysis reveals that PPARα is upregulated by fractionated-dose γ-irradiation in three-dimensional keratinocyte cultures

Jeeyong Lee[1], Hyun-Ji Kim[1] and Jae Youn Yi[1*]

[1] Division of Radiation Effects, Korea Institute of Radiation and Medical Sciences, Seoul, Korea

1. Abstract

Studies have shown that γ-irradiation induces various biological responses, including oxidative stress and apoptosis, as well as cellular repair and immune system responses. However, most such studies have been performed using traditional two-dimensional cell culture systems, which are limited in their ability to faithfully represent *in vivo* conditions. A three-dimensional (3D) environment composed of properly interconnected and differentiated cells that allows communication and cooperation among cells via secreted molecules would be expected to more accurately reflect cellular responses. Here, we investigated γ-irradiation–induced changes in the secretome of 3D-cultured keratinocytes. An analysis of keratinocyte secretome profiles following fractionated-dose γ-irradiation revealed changes in genes involved in cell adhesion, angiogenesis, and the immune system. Notably, peroxisome proliferator-activated receptor-α (PPARα) was upregulated in response to fractionated-dose γ-irradiation. This upregulation was associated with an increase in the transcription of known PPARα target genes, including angiopoietin-like protein 4, dermokine and kallikrein-related peptide 12, which were differentially regulated by fractionated-dose γ-irradiation. Collectively, our data imply a mechanism linking γ-irradiation and secretome changes, and suggest that these changes could play a significant role in the coordinated cellular responses to harmful ionizing radiation, such as those associated with radiation therapy. This extension of our understanding of γ-irradiation-induced secretome changes has the potential to improve radiation therapy strategies.

2. Results

2.1 *Fractionated-dose ionizing radiation induces differential expression of various genes in 3D-cultured keratinocytes*

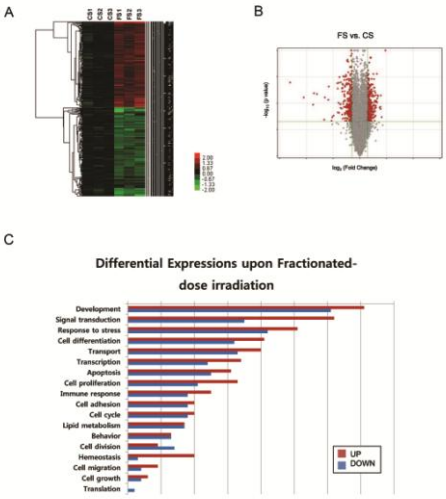

Fig. 1. Microarray analysis of irradiated samples.
(A) Total RNA from the indicated samples was analyzed using Affimetrix microarrays. Upregulated (red) and downregulated (blue) genes are shown. (B) Genes that were differentially expressed by more than 50% relative to control samples with a p-value < 0.05 were selected for analysis. Spots corresponding the selected genes are denoted in red. (C) Differentially expressed genes were categorized according to their functions using GO:functional annotations. Upregulated genes are shown in red, and downregulated genes are shown in blue.

2.2 *Fractionated-dose ionizing radiation induces differential expression of the secretome in 3D-cultured keratinocytes*

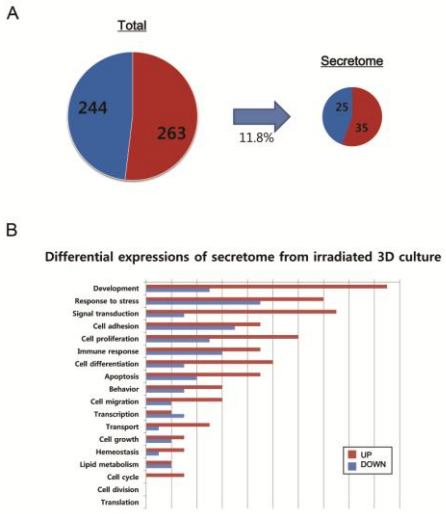

Fig. 2. Irradiation differentially regulates the secretome of keratinocytes.
(A) Differentially expressed genes were trimmed to 11.8% of the total by selecting genes categorized as being associated with the extracellular region. A total of 60 differentially expressed genes, 35 upregulated and 25 downregulated, were selected for further analysis. (B) The selected genes were categorized according to their functions using GO:functional annotations. Upregulated genes are shown in red, and downregulated genes are shown in blue.

2.3 *PPARα might play a role in secretome changes*

A

Gene	GeneBank Accession ID	Fold change (FS/CS)	p-value
PPARα	NM_001001928	1.67	0.02
PPARβ/δ	NM_001171818	0.92	0.02
PPARγ	NM_005037	1.16	0.04

B

Gene	GeneBank Accession ID	Fold Change (FS/CS)	p-value
Angiopoietin like protein 4	NM_001039667	1.66	0.04
Dermokine	NM_001035516	0.50	0.02
FXYD ion transporter 5	NM_001164605	1.67	0.02
IGF-like protein 2	NM_000612	1.60	0.03
Kallikrein-related peptide 12	NM_019598	2.05	0.02
PPARα	NM_001001928	1.67	0.02

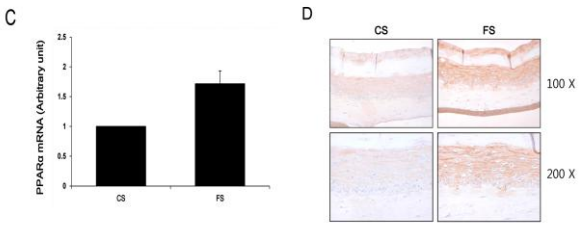

Fig. 4. PPARα is upregulated by exposure to γ-irradiation.
(A) Fold-changes in the expression of PPARs are shown. PPARα expression was significantly increased by γ-irradiation compared to controls. (B) Known PPARα target genes and fold changes in their expression upon γ-irradiation. (C) Real-time RT-PCR confirmed microarray data, showing that PPARα is upregulated by γ-irradiation in 3D-cultured keratinocytes. (D) Immunohistochemistry, performed on 3D-cultured keratinocytes using an anti-PPARα antibody, confirmed microarray data showing that PPARα is upregulated in FS.

3. Conclusions

PPARα in keratinocytes could act as a homeostatic regulator of inflammation and tissue repair. Radiation-induced skin injury occurs in about 95% of patients receiving radiation treatment for cancer (McQuestion, 2011; Salvo et al., 2010). Control of inflammatory waves, improved wound healing, and stabilization of the skin barrier are imperative for minimizing such injuries. Therefore, PPARα agonists and antagonists have the potential to become important therapeutic agents for the treatment of γ-irradiation–induced skin damage. Specifically, our analysis suggests that the undesirable consequences of long-term exposure to ionizing radiation could be alleviated by PPARα agonists. Although our understanding of PPARα functional responses to γ-irradiation is obviously incomplete, our data could provide new experimental support for future applications of PPARα modulators.

REFERENCES

1. Cox JD. Radiation oncology. Jama. 1991;265(23):3165-7.
2. Lataillade JJ, Doucet C, Bey E, Carsin H, Huet C, Clairand I, et al. New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy. Regenerative Medicine. 2007;2(5):785-94.
3. Zschenker O, Streichert T, Hehlgans S, Cordes N. Genome-wide gene expression analysis in cancer cells reveals 3D growth to affect ECM and processes associated with cell adhesion but not DNA repair. PloS one. 2012;7(4):e34279.
4. Blanquart C, Barbier O, Fruchart JC, Staels B, Glineur C. Peroxisome proliferator-activated receptors: regulation of transcriptional activities and roles in inflammation. The Journal of steroid biochemistry and molecular biology. 2003;85(2-5):267-73
5. Heinaniemi M, Uski JO, Degenhardt T, Carlberg C. Meta-analysis of primary target genes of peroxisome proliferator-activated receptors. Genome biology. 2007;8(7):R147.
6. Warters RL, Packard AT, Kramer GF, Gaffney DK, Moos PJ. Differential gene expression in primary human skin keratinocytes and fibroblasts in response to ionizing radiation. Radiation research. 2009;172(1):82-95.
7. Williams JP, McBride WH. After the bomb drops: a new look at radiation-induced multiple organ dysfunction syndrome (MODS). International journal of radiation biology. 2011;87(8):851-68.
8. Mothersill C, Seymour CB. Bystander and delayed effects after fractionated radiation exposure. Radiation research. 2002;158(5):626-33.
9. Kippenberger S, Loitsch SM, Grundmann-Kollmann M, Simon S, Dang TA, Hardt-Weinelt K, et al. Activators of peroxisome proliferator-activated receptors protect human skin from ultraviolet-B-light-induced inflammation. The Journal of investigative dermatology. 2001;117(6):1430-6.
10. Bey E, Prat M, Duhamel P, Benderitter M, Brachet M, Trompier F, et al. Emerging therapy for improving wound repair of severe radiation burns using local bone marrow-derived stem cell administrations. Wound Repair and Regeneration. 2010;18(1):50-8.
11. Nunn AV, Bell J, Barter P. The integration of lipid-sensing and anti-inflammatory effects: how the PPARs play a role in metabolic balance. Nuclear receptor. 2007;5(1):1.
12. Friedmann PS, Cooper HL, Healy E. Peroxisome proliferator-activated receptors and their relevance to dermatology. Acta dermato-venereologica. 2005;85(3):194-202.
13. Romics L, Jr., Kodys K, Dolganiuc A, Graham L, Velayudham A, Mandrekar P, et al. Diverse regulation of NF-kappaB and peroxisome proliferator-activated receptors in murine nonalcoholic fatty liver. Hepatology. 2004;40(2):376-85.
14. McQuestion M. Evidence-based skin care management in radiation therapy: clinical update. Seminars in oncology nursing. 2011;27(2):e1-17.
15. Salvo N, Barnes E, van Draanen J, Stacey E, Mitera G, Breen D, et al. Prophylaxis and management of acute radiation-induced skin reactions: a systematic review of the literature. Current oncology (Toronto, Ont). 2010;17(4):94-112.