Ikaros can enhance immune activity though the interaction with Autotaxin in LDIR exposed immune cells

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Introduction

Ikaros, one of transcription factors, plays major roles in the differentiation and biology of leukocytes, including all classes of lymphocytes (NK, T, and B cells), monocytes/macrophages, and dendritic cells. Ikaros was also shown to regulate early neutrophils differentiation. Therefore, Ikaros appears to be a major determinant in the development and function of immune system. Autotaxin (ATX), which is also called nucleotide pyrophosphatase/phosphodiesterase 2 (NPP2), is an exo-enzyme originally identified as a tumor cell autocrine motility factor. ATX functions as a lysophospholipase D, converting lysophosphatidylcholine (LPC) into the lipid mediator lysophosphatidic acid (LPA). LPA bind together with specific G protein-coupled receptors, which elicit a wide range of cellular responses including the cell proliferation, migration and neurite remodeling. In the Recent report, ATX stimulate human endothelial cells (HUVECs) growth and cytokine production. In our previous study, we showed that low-dose ionizing radiation (LDIR) enhanced the cell proliferation cell coupled with Ikaros phosphorylation. In addition, we found that LDIR increased the expression level of cyclin E and cdk2 protein in IM-9 B lymphoblast cells. In this report, therefore, we try to find Ikaros binding proteins after LDIR in IM-9 lymphoblastic cell lines to examine whether the effects of LDIR induced cell proliferation are one of immune activation responses or not.

Materials and Methods

γ-irradiation on cells

IM-9 B lymphoblast cells were then uniformly irradiated at room temperature with 0.05 Gy dose of a 137 Cs γ-source (dose rate of 5.41 Gy/min) (IBL 437 C type H, CIS Biointernational, France).

Determination of cell cycle stage by FACS

We analyzed cell cycle proportion of the irradiated cells using FACS (Beckman Coulter, Inc.) and activity of cell cycle regulatory proteins (Cyclin E and Cdk2) using western blotting.

Immunoprecipitation analysis and western blotting

The following primary antibodies were used for immunoblotting and immunoprecipitation: Lysate was immunoprecipitated with anti-Ikaros (Santa Cruz Biotechnology). The expression of the Ikaros and ATX was evaluated by the western blot and immunohistochemical methods.

Result

Low-dose of Ionizing Radiation Enhances Cell cycle progression

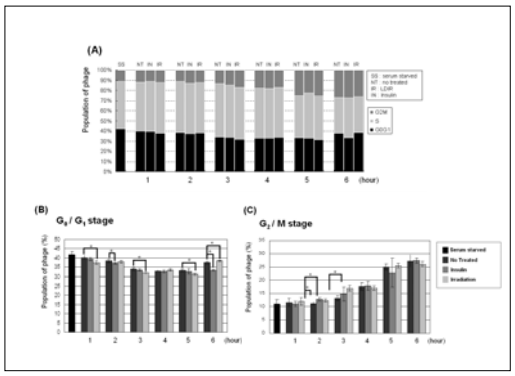

Figure 1. The effects of cell cycle transition induced by LDIR (*P<0.05).

In order to identify the LDIR-induced cell proliferation effects of γ-rays on immune cell, IM-9 B lymphoblast cells were irradiated with 0.05 Gy γ-rays (dose rate 5.43 Gy/min) and then we analyzed the levels of cell cycle stages in

various times using FACS (Fig. 1). The cells exposed to LDIR are faster in cell cycle progression through the G1, S, and G2 phases than non-LDIR cells. And the expression level of CDK2 and cyclin E proteins, one of the G1/S transition regulators, are increased in the cells exposed to LDIR (Fig. 2).

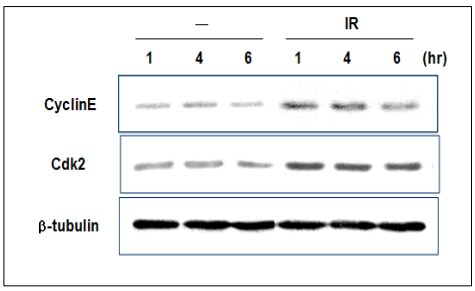

Figure 2. Up-regulation of cell cycle regulatory molecules induced by LDIR.

Ikaros and ATX interaction is increased after LDIR

In our previous study, we found that the decline of its DNA binding activity with Ikaros phosphorylation induced by LDIR promoted the cell proliferation. Ikaros had known to have lots of important role in many different cellular processes of immune cells. To characterize the specific role of it in each different biological process, we tried to find proteins associated with Ikaros through the in vitro screening system. As a result, we found that Autotaxin bound with Ikaros using immunoprecipitation assay. The their binding affinity was increased in LDIR exposed to IM-9 cells (Fig. 3).

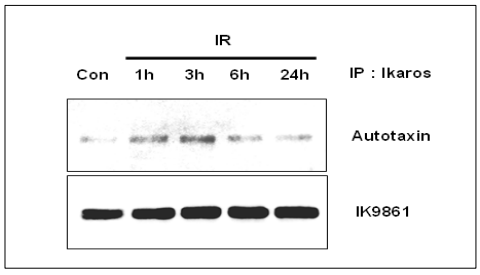

Figure 3. Binding activity between Ikaros and ATX increased by LDIR.

Discussion

The representative biological benefits of LDIR are the enhance DNA repair activity, stimulation of immune response and the cellular proliferation. Ikaros protein in the cells exposed to LDIR was phosphorylated in its serine/threonine residues and then stimulates the proliferation and cell cycle progression. However, It is hard to say that this phenomenon is the effects of LDIR-induced immune activation response. Therefore we try to find Ikaros associated with a protein or Ikaros target genes. In this study, we provide evidence for an interaction between Ikaros and ATX. ATX has lysophospholipase D activity that converts LPC into LPA. LPA has many hall marks of a wound healing factor: it stimulates the proliferation and migration, it enhances the production of various pro-inflammatory cytokines (such as IL-1b, IL-3, IL-6 and IL-8), which all are key events during immune response. Ikaros transcription factors are essential regulators of lymphopoiesis and the development of the immune cells. Here we propose two hypothesis that, (1) Phosphoylated Ikaros in the cells exposed to LDIR translocate to cytosol and binds ATX so that Ikaros assist its inserting to cell membrane. (2) Ikaros assist to transform into cleaved ATX in cell membrane (Fig. 4). Theses results indicate that Ikaros could activate immune response through the interaction with ATX in immune cells exposed to LDIR.

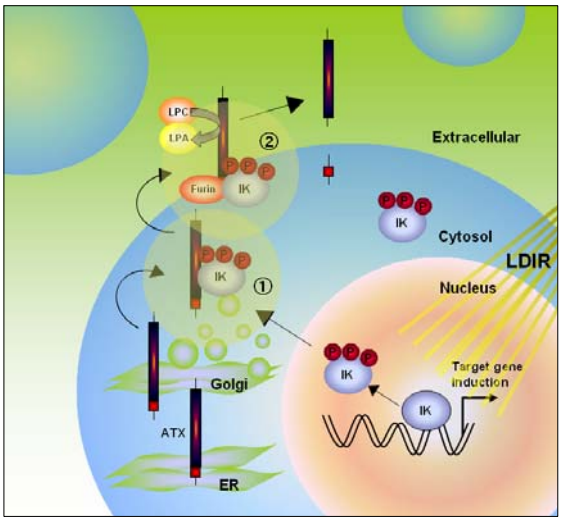

Figure 4. The hypothesis of Ikaros regulation in low dose irradiated IM-9 cells

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