# Adverse Outcome Pathway application to radiation risk assessment on leukemia

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## 1. Introduction

An adverse outcome pathway (AOP) is a new concept that identifies the sequence of molecular and cellular events required to produce a toxic effect when an organism is exposed to a substance [1]. Recently, this concept has been applied in the field of radioprotection research for the safety regulation in chronic and low dose radiation exposure [2]. Promoted and coordinated by the OECD, the AOP concept has been suggested to increase our understanding of the biological processes that contribute to the risk of radiation. It is a conceptual framework that portrays existing knowledge concerning the linkage between two anchor points - the Molecular Initiating Event (MIE), and an Adverse Outcome (AO), connected by a chain of Key Events (KE) and the relationships between them (KER) [3]. We used the AOP framework to outline and evaluate the evidence linking ionizing radiation with leukemia from molecular initiating events to the adverse outcome through intermediate key events.

## 2. Methods and Results

### *2.1 Leukemia mouse model and irradiation*

The AKR/j mouse strain was originally developed as an inbred line with a high incidence of spontaneously arising leukemia [4, 5]. In order to evaluate the risk of developing leukemia according to radiation exposure, AKR/j mice were follow-up for a total of 32 weeks after irradiation (0.5, 1 and 3 Gy, n=15/group, Cs-137, 3.25 Gy/min). Depending on the occurrence of leukemia, spinal deformation, shortness of breath, and decreased movement were observed. The survival rate and body weight of mice decreased over time after irradiation, especially in the 3 Gy group. Organ hypertrophy (spleen, thymus, lymph nodes, and liver), which are the main symptoms of leukemia caused by leukocyte cells infiltrating each tissue, is also caused by radiation exposure of 0.5 Gy or more.

### *2.2 Histopathological assessment and complete blood count (CBC) analysis in leukemia mice model*

Leukemia is a type of blood cancer that generally arises in the bone marrow and results in an overabundance of immature leukocytes, that is, undeveloped blasts [6, 7]. In the normal case, less than 2% of blasts are observed in the blood and bone marrow, but it was confirmed that the proportion of blasts increased to more than 30% in the bone marrow smear sensitive to radiation (Diff-Quick, Thermo Fisher). In addition, CBC analysis showed an increase in white blood cells (WBC), lymphocytes (Lymphocytosis), and neutrophils performing an inflammatory response (NEU), and a decrease in platelets (PLT) and red blood cells (RBC) in radiation-exposed group.

### *2.3 Immunologic classification of lymphoblastic leukemia by fluorescence-activated cell sorter*

To analyze the immunologic classification of lymphocytes (immune cell ratio: T cell/B cell) from which leukemia cells are derived and to determine the degree of activation, cells in the lymph node tissue as the main lesion site were isolated and flow cytometric analysis was performed [8]. It was confirmed that the number of white blood cells in the lymphoid tissue increased according to the radiation dose. There was no significant difference in the change of T immune cells, but it was confirmed that the distribution of B immune cells (CD220; CD45R, CD19) was significantly increased by radiation exposure.

### *2.4 Changes in organelles observed by Image Flow Cytometry*

Mouse solenocytes were obtains and stained with gamma-H2AX antibody as a highly specific and sensitive molecular marker for monitoring DNA damage initiation and resolution [9], GM130 for Golgi membranes protein [10] and DRAQ5 for nuclear staining [11], 32 weeks after irradiation. At least 1,000 cell images were analyzed. Gamma-H2AX fluorescence intensity at 32 weeks after exposure, increased compared to controls group. As morphologic changes, the dispersion of the Golgi apparatus increased linearly with increasing radiation dose

### *2.5 Correlation analysis*

The relationship between the risk of leukemia as adverse outcomes with key events (body weight, survival rate, organ hypertrophy, blood histology, immune cell types, and organelles) were analyzed by Spearman correlations according to radiation dose. Several experimental parameters were correlated with increased radiation doses, including body weight

(− 0.303, P < 0.05), white blood cell count (0.298, P < 0.05), the weight of spleen (0.326, P < 0.05) and thymus (0.344, P < 0.05) in leukemia mice.

### *2.6 Statistical Analysis*

All data are presented as the mean ± SD from three independent experiments with three replicates each. Statistical analyses were performed using GraphPad Prism 7 software. Spearman's rank correlation coefficient was used to evaluate the correlation between dose and key event using SPSS software (version 23; SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered to be statistically significant.

## 3. Conclusions

These experimental data on leukemia model mice provide the important information for the future development of leukemia risk estimation system. Among examined key events in our AOP model on leukemia, some measured parameters were correlated with the exposed level of radiation. These suggest that our findings as key events in AOP framework could be used for the risk estimation on the radiation [12].

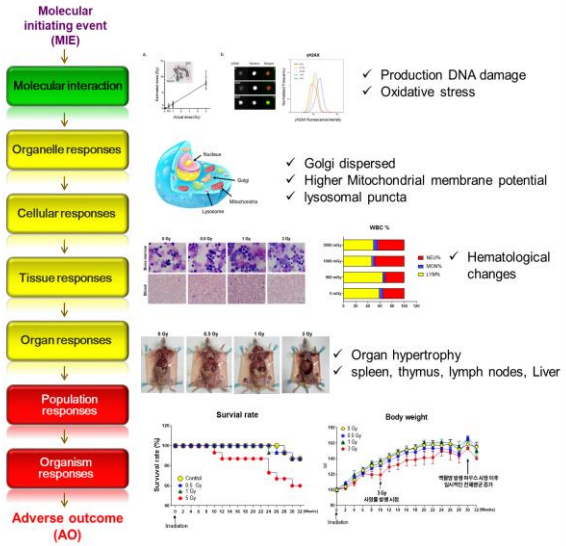


Fig.1. Radiation-induced leukemia key events applying adverse outcome pathways frame.

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