Effects of ionizing radiation on the immune system

Ph.D. thesis

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Introduction

The biological effects of ionizing radiation are deterministic or stochastic. Deterministic effects have clinical symptoms, always cause damage above a certain threshold dose, which depends on the radiosensivity of the individual organ and tissues. In the case of stochastic effects, the probability of the manifestation of the effect (mainly the risk of developing cancer) is increased with the dose. Stochastic effects, therefore, have no threshold dose, theoretically even a single radiation hit can lead to cancer, so the fundamental goal of radiation protection is to limit the radiation exposure to the reasonably acceptable lowest level.

Studying the effects of low doses (range below 100 mGy) is presently in the focus of radiological research. Recently it has been shown that low dose effects are mostly not targeted, meaning that alterations can be observed not only in the irradiated cell, but in cells, which were not directly hit by radiation. These effects are called non-targeted effects of radiation and they can manifest as genome instability and bystander effects. Genome instability means increased frequency of spontaneous mutations within the progeny of directly irradiated cells several generations later. Bystander effect is the phenomenon, when effects of irradiation are observed not only in the directly irradiated cells, but also in cells in the immediate or wider neighbourhood of directly irradiated cells, most often manifested in DNA damage or gene expression changes. The discovery of non-targeted effects of radiation highlighted that radiation affects the functionality and survival of much more cells than it would be expected according to the principle of targeted effects.

The number of studies on the role of extracellular vesicles (EVs) in radiation-induced bystander effects is limited, although EVs are ideal subjects for transmitting signals from one cell to the others. EVs are membrane-bound vesicles that are actively emitted by the cells into the extracellular space from where other cells take them up and thus have a prominent role in transferring nucleid acids (miRNA, mRNA), proteins and lipids. The content of EVs depends on the type, metabolic state and stimuli of the producing cell. Their role is described in physiological conditions (immunological processes such as activation of T cells, induction of immune tolerance, activation of

Tregs), stress responses (pro-inflammatory or anti-inflammatory signals, stress response of ionizing radiation), and pathological conditions (autoimmune diseases or cancer). EVs are rich sources of miRNAs. These evolutionarily conserved, small (about 22 nucleotide long) non-coding RNAs are responsible for transcriptional and post-transcriptional regulation of biological processes. MiRNAs are specifically packaged in the EVs, therefore the miRNA content of the EVs does not necessarily reflect the miRNA content of the emitting cell. MiRNAs were associated with radiation-induced tissue response and as a possible mediator of radiation-induced bystander effect. The miRNAs are transmitted through the EVs from the direct cells to the bystander cells and are able to transmit the radiation-induced bystander effect.

The immune system is responsible for recognizing foreign and altered self structures and destroying them by actively immune responses. In the first phase of the immune response, foreign cells are contacted with professional antigen-presenting dendritic cells (DCs). On the cell surface of the DCs, the adhesion and costimulatory molecules (e.g. CD40, CD80, CD86), the antigen-pattern recognising receptor (e.g. TLR4), receptor for mediating antigen uptake and processing and presentation (e.g. DEC205) and the protein involved in T-cell inhibition (e.g., B7H1) allow the DC cells to modulate the functioning of the effector cells of the adaptive immune system. Regulatory T cells (Tregs) are a unique group of CD4+ lymphocytes and play an important role in maintaining immune homeostasis. Tregs are formed in the thymus, however, in various environmental conditions (typically in the presence of TGFβ) Treg cells may also be formed from CD4+Foxp3- T cells on the periphery. Treg cells play a role in inhibition of T cells, which can take place in several ways: they produce cytokines that inhibit activation of T cells (IL-10, TGFβ), they can cause cytolysis of T cells, they can cause T cell metabolic disturbances (e.g. IL-2 withdrawal, cAMP transfer) or inhibition of DC maturation.

Aim of the study

We know much less about the biological effects of low doses and their (short and long-term) health effects than about the effects of high dose radiation. Studying effects

of low doses is a key topic today because of the widespread use of medical diagnostic procedures with radiation exposure. Since these procedures target increasing number of people, including children, the long-term consequences of low dose medical exposures becomes a crucial public health issue. This is the reason why, understanding the mechanisms responsible for the developing of low dose effects has become a hot spot in radiation biology in recent decades. In our research, we are investigating the effects of low and high dose ionizing radiation on the numberal and functional changes of the cells of immune system.

The role of EVs and the active involvement of EV-mediated miRNAs in the development of radiation-induced bystander effect *in vivo* has not been studied yet. Thus, we developed an *in vivo* model in which EVs isolated from the irradiated mice were injected intravenously into naïve, so-called bystander animals, and the effects of EV transfer were studied in the spleen cells of the bystander mice. The miRNA content of EVs isolated from bone marrow and blood of irradiated mice was also analyzed. The changes caused by irradiation were concluded from miRNA-regulated signal transduction pathways.

Materials and methods

Experimental animals and the irradiation schedule

6 to 12 week-old of C57B1 / 6 mice were used for the experiments. The animals were injected intraperitoneally (i.p.) with ketamine-xylazine solution for the time of irradiation. The animals were placed in a plastic restrainer, and whole body irradiation was performed by 60Co-γ or X-ray irradiation source. Animals were sacrified by intraperitoneal admission of lethal dose of penthobarbital.

Isolation of immune cells from spleen and their characterization

From the spleen of animals single cell suspensions were made at different time points after irradiation. After removing of red blood cells, the single-cell splenic suspension was used for further phenotyping, apoptosis testing, for isolation of cell populations (DCs and Tregs), and for functional testing. CD4+CD25+ Tregs and CD4+CD25- T

cells were separated by magnetic separation. For isolating DCs, the spleen was disrupted not only mechanically and enzymatically, as well. From this spleen cell suspension, CD11c+ DC cells were selected on the basis of a positive selection by magnetic separation.

The phenotypic characterization of the cell populations and the comparison among the subpopulatiopns were performed by identifying the surface and intracellular proteins of the cells. Cell surface proteins were labeled with the appropriate fluorescently labeled monoclonal antibodies: B7H1, CD3, CD4, CD8, CD11c, CD19, CD25, CD40, CD80, CD86, CTLA4, DEC205, Foxp3, GARP, GITR, IA-b (MHCII), NK1.1. To detect nuclear and cytoplasmic proteins, cells were permeabilized before dyeing. Immuno-phenotyping was performed by a FACSCalibur flow cytometer and evaluated by CellQuest software.

Measurement of DNA damage, apoptosis and cell division

DNA damage measurements were performed by detecting H2AX histone protein phosphorylation. The γH2AX assay was performed on the spleen cells of the irradiated and the bystander animals, both by immunocytochemistry and by flow cytometry. The cells were fixed in both methods and then permeabilized. Aspecific staining was blocked on cells, and then cells were incubated with primary antibody against γH2AX, then with fluorescently labeled secondary antibody. During immunocytometric staining, the cover plates containing the labeled cells were plated with DAPI-labeled adhesive on a slide, and the evaluation was performed with a phase contrast microscope with a magnification of 100x. For flow cytometric analysis, the ratio of γH2AX + cells was determined by a flow cytometer.

TUNEL assay was used for estimation of apoptosis. The single cell suspension was labeled with markers for characterization of spleen cell subpopulations, then fixed and buffered into dUTP-FITC and TdT enzyme solution. Samples were measured and evaluated by flow cytometry.

To detect cell division kinetics, expression of the Ki67 intracellular proliferation marker was measured by a flow cytometer. The spleen cell suspension was labeled with the specific extracellular marker, and then an intracellular staining followed by

Ki67-PE antibody was used. During the cytometric analysis, Ki67 + (dividing) and Ki67- (dormant) fractions were identified.

Measuring cytokine gene expression

For measuring cytokine production of splenocytes, RNA was isolated from the spleen cell suspension and from isolated Treg cells, and cDNA was prepared. Quantitative RT-PCR reactions were performed from the cDNA. The expression pattern was normalized to β-actin using a comparative Ct method. The cytokines tested were GDF-15, GM-CSF, IFNγ, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, LTβ, TGFβ, TNFα.

CFSE labeling and proliferation inhibition of T cells

To investigate whether effector T cells transform into Treg cells, CD4+CD25- cells were labeled with CFSE fluorescent cell dye, and then injected intravenously into control animals. Two groups of T cells were used for injection: one group of cells isolated from non-irradiated (0 Gy, control) mice, and the other group of cells isolated from animals irradiated with 2 Gy. Six days after injection animals were sacrificed and their spleen cells was analysed by flow cytometry to measure the ratio of CFSE+Foxp3+ cells to those Foxp3+ cells measured previously (before injection).

To test the functional integrity of the Treg cells, T cell suppression assay was performed. CD4+CD25- T cells were co-cultured with CD4+CD25+ Tregs derived from mice irradiated with 0 Gy or 2 Gy dose. T cell proliferation was determined by the proportion of incorporation of 1 μC methyl-^{3}H-thymidine added to the culture and measured by a liquid scintillation counter. The relative proliferation of CD4 + CD25-T cells was expressed as percent (%), where 100% was the degree of proliferation of T-cells cultured without Treg cells.

Examination of antigen uptake and presentation of DCs

Antigen capture was assayed on isolated DCs, which were separated by magnetic separation. The cells were incubated with FITC-labeled ovalbumin peptide (SIINFEKL), washed and the ratio of the labeled cells was measured by flow cytometry.

For the antigen presentation assay, the cells were incubated in the presence of SIINFEKL peptide. Once washed with buffer, the cells were labeled with H2b-SIINFEKL-FITC (MHCI-bound protein) antibody. From the ratio of stained / total cell we estimated the efficiency of antigen uptake.

Isolation of EVs and i.v. transfer

The bone marrow cells were isolated from the femoral bone and tibia 24 hours after irradiation. The bones were cut off from epiphyseal and the bone marrow was flushed out of the diaphysis. The single-cell suspension supernatant was collected and incubated overnight with ExoQuick solution. The next day the pellet, i.e. the EV obtained, was further purified with a G-25 column to remove the ExoQuick polymer from the EV samples. Plasma EVs were obtained from the blood plasma of the treated and control animals by the Exiqon Exosome Isolation kit.

Treatment of bystander animals was performed as follows: EVs from bone marrow of control and irradiated animals were injected via tail vein into naïve mice. The animals were sacrificed 24 hours later and peripheral blood and splenocytes were collected for further examinations.

Determination of the miRNA profile of bone marrow and plasma EVs

To determine the miRNS profile, bone marrow EVs were sent to ExiqonServices for analysis. The company carried out RNA isolation, captured the miRNA profile by PCR reaction and made the first phase of data evaluation. To analyze the miRNA array data, the Ct values calculated by Exiqon were taken as the basis. The expression change was compared to the 0 Gy samples in pairs and data of 3 independent samples were averaged.

The DIANA-miR-Path software was used to analyze the possible biological role of differently expressed miRNAs found in the 0.1 Gy and 2 Gy samples. The program defines the potential signaling pathways controlled by the miRNAs in comparison with the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) signaling path databases.

Statistical analysis

The statistical analysis was done with Student t-test using GraphPad Prism 5. The effects were consisted significant if $p < 0.05$.

Results

Radiation-induced cell number changes and apoptosis in splenocytes

We first investigated how acute low and high dose irradiation affects the proportion of different subpopulations of spleen cells at different time points after irradiation. The following spleen cell subpopulations were studied: CD3+CD4+ helper T cells, CD3+CD8+ cytotoxic T cells, CD4+ CD25+ Treg cells, NK cells, CD11c+ DC cells, and CD19+ B cells. One day after 0.01 Gy irradiation, increased cell number was observed in total splenocytes and the tendency was similar in most of the examined spleen cell subpopulations. Due to irradiation with higher doses (0.05 Gy - 0.5 Gy), a dose-dependent decrease was measured in the number of total splenocytes and this change was similar in NK, B and Treg cells. The 2 Gy irradiation resulted in a pronounced reduction of cell number in all spleen cell subpopulations, but we found significant differences between the individual subpopulations. 25% of total splenocytes survived the first day after irradiation. CD8+ T cells showed a similar tendency, but CD4+ T cells, NK cells, DCs, and especially Treg cells had significantly higher surviving fractions. B cells were significantly more sensitive to 2 Gy irradiation than any other cell type. At the third day following irradiation the spleen cell numbers reached the minimum. Irradiation with 0.05 Gy decreased the total number of total splenocytes approx. by 25% and this ratio did not change following 0.1 Gy and 0.5 Gy irradiation. The relative numbers of Tregs, NK cells, and DCs were lower after 0.1 Gy irradiation than after 0.5 Gy irradiation, suggesting a possible hypersensitivity of these cells to low doses. The number of CD4+, CD8+ T cells and B cells showed a similar tendency, a dose-dependent decrease in the range of low doses. The differences observed on the first day after 2 Gy irradiation were still existing on the third day. The most resistant populations were Tregs, NK cells and DCs. Seven days after irradiation, the number of total splenocytes changed slightly from that seen on the third day after irradiation although the specificity of the changes differed in the range of low and high

doses. Seven days after the high dose (0.5 Gy - 2 Gy) irradiation, all examined cell populations showed a slight increase in cell numbers towards the pre-irradiation cell numbers.

Spleen cell subpopulations had different rate of cell number decrease following irradiation, therefore we further the apoptosis of the spleen cell subpopulations. The splenocytes were isolated 4 hours after irradiation and used directly for apoptosis assay. Spontaneous apoptosis of the individual cell populations showed significant differences. The spontaneous apoptosis of CD4+ and CD8+ T cells was similar to the apoptosis of total splenocytes, but in the B cells it was much lower, while in NK cells, number of Tregs and DCs was much higher. Low dose irradiation reduced the rate of apoptosis in most of the examined populations. The most pronounced changes were between 0.01 Gy and 0.1 Gy in case of NK and DC cells. It is interesting to note that the proportion of apoptotic cells already increased slightly in the population of CD8+ T and B cells due to 0.05 Gy dose irradiation. High dose (0.5 Gy - 2 Gy) irradiation resulted in increased apoptosis in most of the examined populations. In response to 2 Gy irradiation, nearly a quarter of the total splenocytes died. B cells were the most sensitive, CD4+, CD8+ T cells and Tregs were more resistant compared to total splenocytes, while NK and DC cells were the most resistant.

The results showed that CD4+ T cells are much more resistant to radiation than the total splenocytes, and the Treg cells are even more resistant. This difference resulted in an increased proportion of Treg cells within CD4+ T cells following high dose (2 Gy) irradiation. To find an explanation of this changed ratio of Tregs and CD4+ T cells, the kinetics of apoptosis of CD4+Foxp3+ (Treg) cells was determined 4 hours, 1 day, and 3 days after 2 Gy irradiation. The kinetics of apoptosis among these cell populations were different: number of apoptotic total splenocytes reached the maximum 4 hours after irradiation, while number of apoptotic T cells (both effector T cells and Tregs) reached the maximum one day after irradiation. Three days after irradiation, apoptosis of Tregs reached again the control level, whereas in the case of CD4+ T cells this has not yet occurred. These results suggest that Treg cells are less sensitive to radiation-induced apoptosis than the effector T cells, and cell regeneration is also happening earlier. In lymphopenic conditions, the cells' homeostatic division promotes cellular

recovery in the body. Inequalities of cell division may also play role in the observed changes in ratio of lymphocyte subpopulations. The difference between the proliferation rate of effector T cells and Treg cells changed from the initial 74/26 to 59/41 on the 11th days of irradiation. According to these data we can conclude that in whole-body irradiated mice, Treg cells of irradiated mouse proliferated more dynamic than effector T cells which, together with the decreased apototic rate, resulted in the above-described Treg enrichment in the spleen.

Changes in cytokine gene expression after whole-body irradiation

We determined the cytokine gene expression profile of spleen cells of the irradiated mice. Among the investigated cytokines, there were Th1 (IL-2, IL-12, IFN-γ, LT-β and TNF-α) and Th2 (IL-4, IL-6, IL-10, TGF-β) cytokines, and hematopoietic growth factors (GM-CSF, IL-5). We also investigated the change in GDF15 expression after irradiation. High dose (2 Gy) irradiation increased the expression of most studied cytokines. Changes were detected following low dose irradiation, as well, but they were much smaller and less pronounced compared to changes after high dose irradiation. IFN-γ and IL-2 expression decreased after low dose irradiation and increased after 2 Gy irradiation at each examined time point. IL-12 expression decreased at low dose and increased at high dose 4 hours after irradiation and returned to the control level after 3 days. The expression of LT-β cytokine was reduced by almost 50% at the third day after 2 Gy irradiation, but low dose irradiation did not affect it. IL-4 gene expression was reduced at both low and high doses 4 hours after treatment. The change in gene expression level due to low dose irradiation was still measurable three days later, but the change measured after 2 Gy irradiation disappeared at the same time point. IL-6 expression was bi-phasic: 4 hours after each dose a significant increase was detected. Later, 1 day after irradiation, its level fell below the control value and returned to the normal value at the third day after treatment. The level of IL-10 gene expression did not change at initial time points, the most significant dose-dependent increase was measured 3 days after irradiation. The expression of GDF-15 was reduced on the first day after low dose irradiation, in turn, its level increased for long term after 2 Gy irradiation, and this enhanced level could be detected even until the third day. GM-CSF gene expression was not affected by

low-dose irradiation. However, its level showed a two-phase curve following 2 Gy: the most significant overexpression was observed 4 hours and 3 days after irradiation, however 1 day after irradiation, the expression of the gene decreased. Both low and high dose irradiation resulted in an increase in expression of IL-5 at each studied timepoint.

Functional changes of Tregs after 2 Gy irradiation

Radiation may alter the functional integrity of Treg cells in several ways: by reducing or increasing the amount of cell surface receptors or by modifying cytokine production. Expression of three specific Treg cell surface proteins (CTLA-4, GARP and GITR) was measured 3 days after irradiation. The ratio of Tregs expressing the CTLA-4 molecule increased almost twice in response to 2 Gy irradiation. The levels of GARP and GITR also increased on the Treg cells following irradiation. These findings show that the expression of activation markers on Tregs has been moderately increased after 2 Gy irradiation.

Treg cells express several cytokines which are important in maintenaning a suppressive phenotype. Of these, the change in expression of IL-10 and TGFβ cytokines was investigated in Treg cells isolated from 2 Gy irradiated mice. IL-10 mRNA expression showed a mild increase and TGFβ expression remained at baseline level. According to these results, only in level of IL-10 cytokine can be detected any changes due to irradiation, although this difference is relatively small and does not necessarily reflect the actual change in the amount of secreted cytokine.

The ability of Treg cells to inhibit the proliferation of effector T cells was tested *in vitro*. We found that Treg cells from irradiated animals were less able to reduce the ratio of proliferating T cells than Treg cells from control animals. This significant alteration of suppressive capacity suggests that 2 Gy irradiation impairs the functional integrity of Treg cells.

Functional change of DCs due to 2 Gy irradiation

The mature mouse spleen DCs are characterized by the expression of CD80, CD86 and CD40 molecules, which is enhanced by activation. After low dose irradiation, the

expression of these receptors remained unchanged, with the exception of a mild increase in the CD80 after 0.25 Gy irradiation and a slight decrease in the number of DCs expressing DEC205. All of these costimulatory proteins involved in the induction of T cell response were expressed on DCs at increased level following 2 Gy irradiation. The expression of the B7-H1 marker, involved in the inhibition of the T cell response, was elevated on the cells, as well. We have also examined whether irradiation has an effect on the antigen uptake and presentation of DCs. We found that after 0.1 Gy irradiation, DCs took up less labeled OVA peptide than control cells. High dose irradiation did not alter the ability of the cells to uptake antigens. The 0.25 Gy irradiation reduced the antigen presentation of DCs compared to the control one day after irradiation. These results are in line with what we observed in changes in expression of the cell surface DEC205 after 0.25 Gy dose irradiation: DEC205 decreased while antigen presenting ability (correspondingly) dropped compared to control. After the high dose (2 Gy) irradiation we did not see any significant difference.

EV transfer changed spleen cells ratio

We measured the changes in the number of lymphocytes and the proliferating ability of lymphocyte subpopulations (CD4+ and CD8+ T cells, CD19+ B cells, NK cells and DCs) in EV recipient animals. Significant bystander effect was measured in CD4+ and CD8+ T cell populations, but not in B cells and NK cells. After 2 Gy irradiation, the rate of proliferation decreased in all the examined subpopulations. The proliferation rate of the cells was not changed by the EV transfer itself (i.e., the injection of EVs from non-irradiated animals). In 2 Gy bystander animals, the rate of proferating cells decreased similarly to direct 2 Gy irradiated animals but at much less extent. In contrast, the number of DCs did not decrease in bystander animals after none of the doses. TLR4 protein expression on the surface of the DCs increased after 2 Gy direct irradiation, but surprisingly the TLR4+ DCs in the bystander animals TLR4 significantly decreased and this decrease was not related to the dose.

The ratio of γH2AX foci increased in spleen cells due to in vivo EV transfer

The number of γH2AX foci increased in directly irradiated mice dose-dependently. In the bystander animals, the number of foci and the ratio of γH2AX-positive cells also increased, albeit to a lesser extent than in the spleen cells of directly irradiated animals, and did not show any correlation with the dose. These results show that EVs are able to activate DNA damage repair signaling pathways in spleen cells of bystander animals and this bystander effect can be observed even at low doses.

EV transfer changed the proportion of blood proteins

Of the 105 proteins (cytokines, chemokines and growth factors) tested, only 7 and 9 proteins changed after direct 0.1 Gy and 2 Gy irradiation, and 5 and 8 proteins changed in blood plasma of 0.1 Gy and 2 Gy bystander animals, respectively. Functionally, most of the altered proteins were chemokines or chemokine ligands. The changes was were small, it exceeded a 2-fold decrease or increase only in case of some a few proteins.

Irradiation affects the miRNA content of EVs – both in bone marrow and peripheral blood

On average, 500 miRNAs pers sample could be identified in EVs derived from bone marrow of control mice. We did not find any miRNA that typically appears or disappears due to irradiation but miR-124, miR-346, miR-449c and miR-381 were present while miR-695 and miR-761 were absent from the EVs of 2 Gy irradiated animals. Twenty differently expressed miRNAs were found in EVs of 0.1 Gy irradiated mice compared to control mice, and 90 differently expressed miRNAs in 2 Gy samples. In addition, we found 8 miRNAs, which changed both after 0.1 Gy and 2 Gy irradiation: five miRNA expression levels decreased and three miRNA expression levels increased. The change in the levels of these miRNAs was dose-dependent.

In the EVs isolated from the plasma, 174 miRNAs were identified, which were present in all samples. From these miRNAs 7 and 11 were changed at least 1.3-fold in the 0.1 Gy and in the 2 Gy sample, respectively.

In order to explain the role of differentially expressed miRNAs in phenotypic and functional changes of cells in bystander animals, DIANA miRPath software was used.

With DIANA we analyzed the signaling pathways affected by differentally expressed miRNAs from 0.1 Gy and 2 Gy animals and compared 2 different pathway databases (KEGG and GO). Based on GO database, the pathways affected by our 8 miRNAs were in association with cell differentiation, metabolic pathways, cell growth, motility and cell death. Using KEGG database, signaling pathways related to radiation induced cell response, DNA repair mechanisms, and hematopoietic system were identified.

When examining the miRNA content of the EVs isolated from the plasma of 0.1 Gy and 2 Gy animals, 35 and 60 pathways were found to be affected on the KEGG database, respectively. Although we did not find shared miRNAs differentially expressed in plasma EVs of both 0.1 Gy and 2 Gy irradiated mice, we could identify 26 common pathways, including acute myeloid leukemia (AML), TCR signaling, mitogen-activated protein kinase (MAPK), TGFβ, FoxO, Hippo signaling pathways which affected by changed miRNAs.

Conclusions

- we described the effects of low dose *in vivo* irradiation on quantitative and functional (cytokine gene expression level) changes of different splenocyte subpopulations
    - *in vivo* low dose irradiation had a significant effect on all subpopulations
    - different mechanisms were activated by low and high dose irradiation *via* different signaling pathways
        - high doses: rapid cell number decrease, seven days after irradiation regeneration still lasted
        - low doses: the effect was more persistent, seven days after irradiation no or weak signs of regeneration were detected
- Radiation response of Treg cells is complex:
    - on the one hand, high dose irradiation increased the Tregs ratio within CD4+ splenocytes, which is probably due to its low apoptotic rate and its pronounced proliferative ability

    - on the other hand, the T cell inhibitory capacity of irradiated Tregs decreased significantly, indicating the changed functional integrity of the cells
- ionizing radiation enhanced the activation of DCs
    - expression of activation markers on cell surface increased
    - antigen presentation capacity of the DCs decreased
- an *in vivo* model was constructed to evaluate the role of EVs in the development of radiation-induced bystander effect
    - EVs from the bone marrow of the irradiated mice can activate the repair of DNA damage in spleen cells of the recipient animals and can cause quantitative and phenotypic changes in the various splenocyte subpopulations
    - in the background of the changes in direct irradiated and bystander animals are different mechanisms
    - EVs can transmit signals of radiation-induced immune response and inflammation in peripheral blood
    - miRNAs isolated from EVs are potential participants in the signaling pathways in DNA damage repair, hematopoiesis and various immunological processes in both the bone marrow and the blood
    - we demonstrated the important role of EVs in radiation-induced systemic bystander effect, which is most likely mediated by miRNAs

List of publications

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