Frontiers in Bioscience Scholar

Front. Biosci. (Schol Ed). **2022**; 14(1): 002
http://doi.org/10.31083/j.fbs1401002

Original Research

Influence of photon, proton and carbon ion irradiation on differentiation, maturation and functionality of dendritic cells

Laila König[1,2,3,4,*,†], Adriane Hommertgen[2,3,†], Lena Orschiedt[1,2], Juliane Hörner-Rieber[1,2,3,4], Stephan Brons[2,4], Peter E. Huber[1,2,3], Jürgen Debus[1,2,3,4], Stefan Rieken[1,2,3,4,5]

[1]Department of Radiation Oncology, University of Heidelberg, 69120 Heidelberg, Germany
[2]National Center for Radiation Research in Oncology (NCRO), Heidelberg Institute of Radiation Oncology (HIRO), 69120 Heidelberg, Germany
[3]Molecular and Radiation Oncology, DKFZ 69120 Heidelberg, Germany
[4]Heidelberg Ion Beam Therapy Center, 69120 Heidelberg, Germany
[5]Department of Radiation Oncology, Göttingen University, 37075 Göttingen Germany
*Correspondence: Laila.Koenig@med.uni-heidelberg.de (Laila König)
[†]These authors contributed equally.

Academic Editors: Gustavo Caetano-Anollés
Submitted: 17 November 2021 Revised: 20 December 2021 Accepted: 27 December 2021 Published: 20 January 2022

Abstract

While the primary purpose of radiotherapy (RT) is the elimination of cancer cells by inducing DNA-damage, considerable evidence emerges that anti-neoplastic effects extend beyond mere tumor cell killing. These secondary effects are based on activation of dendritic cells (DCs) via induction of antitumoral immune reactions. However, there is an ongoing debate whether or not irradiation of the DCs themselves may negatively affect their maturation and functionality. Human monocytes were irradiated with different absorbed doses (1 × 15 Gy relative biological effectiveness (RBE), 5 × 2 Gy (RBE), 1 × 0.5 Gy (RBE)) with photons, protons and carbon ions. Differentiation and maturation of DCs were assessed by staining of corresponding cell surface molecules and functional analysis of irradiated DCs was based on *in vitro* analysis of phagocytosis, migration and IL-12 secretion. Irradiation of CD14-positive DCs did not alter surface phenotypes of immature DCs and mature DCs. Not only differentiation, but also functionality of immature DCs regarding phagocytosis, migration and IL-12 secretion capacity was not negatively influenced through RT with photons, protons or carbon ions as well as with different dose levels. After proton irradiation migratory capacity of immature DCs was increased. Our experiments reveal that phenotypic maturation of DCs remains unchanged after RT with different fractionations and after irradiation with particle therapy. Unaffected functionality (phagocytosis, migration and cytokine secretion) after RT of DCs indicated possible persistent potential for inducing adaptive immune response. Additional effects on the immunogenic potential of DCs will be investigated by further functional assays.

Keywords: Dendritic cells; Monocytes; Maturation; Phagocytosis; Radioimmunology; Radiotherapy; Particle therapy; Carbon ion; Protons; Monocyte derived

1. Introduction

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) which play a crucial role in initiating tumor immunity. Immature DCs (iDCs) reside in peripheral tissue, where they are activated and matured upon encounter of pathogens. Phagocytosed antigens such as bacteria, viruses or damaged (tumor) tissue are processed by the immunoproteasome in the iDC and ultimately presented as epitopes on major histocompatibility complex (MHC) molecules expressed by the DC. The so-called monocyte-derived dendritic cells (MO-DC) are generated from human $CD14^+$ monocytes which eventually differentiate over immature monocyte-derived DCs (iMO-DCs) [1–3]. Proinflammatory cytokines further induce differentiation into mature monocyte derived DCs mMO-DCs, which cross-present the antigen derived epitopes to T-cells on MHC I molecules in associated tumor-draining lymph nodes [2,4,5]. Through increased release of tumor-associated antigens (TAA) and immune-activating danger-signals (DAMPs) after RT, DCs are recruited. Cross-presentation of TAA leads to activation of cytotoxic T-lymphocytes, therefore playing a pivotal role in adaptive immune response and immunogenic cell death [6,7]. During this maturation process of DCs, Interleukin-12 (IL-12) is produced and plays an important role in the activation of natural killer cells and T lymphocytes [8,9]. The costimulatory molecules CD80 and CD86 are increasingly expressed on the cell surface and subsequent binding of CD80/CD86 molecule to the CD28 molecule expressed on T cells delivers, in addition to T-cell receptor (TCR) engagement, the co-stimulatory signal for successful T cell activation followed by clonal expansion of the antigen-specific T cell population.

Radiotherapy (RT) is one of the main pillars of oncological therapy. Primary purpose of RT is elimination of cancer cells by inducing DNA-damage that either causes induction of tumor cell death or inhibition of the proliferating capacity of these cells in the high dose region. However, considerable evidence emerges, that antineoplastic effects extend beyond these mechanisms. Furthermore, controversial data on the effects of radiation dose (low dose RT with doses $<$1 Gy vs. high dose RT with doses $>$1 Gy) as well as fractionation can be found in the literature [10–12] and may be of clinical interest, since the high dose region reflects the irradiated tumor and/or lymphatic region, whereas the low dose region reflects the surrounding tissue.

On the molecular level, high dose irradiation has been shown to upregulate stress proteins, which can function as neoantigens, activating APC on the one hand [13], but has also been shown to massively kill blood cells, such as lymphocytes after whole-body irradiation *in vivo* [14,15] and to reduce DC function *in vitro* [10]. Regarding low dose irradiation, results are also controversial showing stimulated expression of APC and increased IL-12 levels [16,17] but also a decrease of T-cell proliferation due to reduced APC and T-cell interaction [18]. Additionally, most of the DC-mediated processes depend on their state of differentiation, maturation and migration capacity, which might all be influenced by irradiation. These above-mentioned secondary effects may have the potential to contribute to anti-tumor responses in a local, but also systemic manner via activation of the immune system outside of the irradiated volume (abscopal effects) [19,20].

Despite of the well understood physical aspects of particle therapy, radiobiology and its clinical relevance regarding activation of the immune system are still scarcely understood and we did not find any data describing direct effects on immune cells like DCs. Particle therapy consists not only of low-linear energy transfer (LET) RT with protons but also heavier high-LET ions like carbon ions (C12). Although often assumed to be a low-LET treatment, the LET of protons is heterogeneous, with values up to 10 times that of photons over the last 2 mm of the beam range (5 to 20 keV/um) at the edge of the spread-out Bragg peak (SOBP) [21]. High LET has the potential to intensely damage cells due to DNA damage and may therefore induce higher proportions of cell death. These effects are well-known in tumor cells and therefore we hypothesized that particle therapy of dendritic cells might also decrease their function.

In this study we investigated for the first time the effects of different irradiation types (photon, proton and carbon ion RT) and dose concepts (low-dose, normofractionated and hypofractionated/ablative RT) on the phenotype and functionality of MO-DCs.

2. Methods

2.1 Isolation of PBMC and generation of MO-DCs

$CD14^+$ monocytes were isolated from peripheral blood mononuclear cells (PBMCs) from healthy donors obtained from the Blutbank Mannheim by MACS® technology using C14 MicroBeads (Miltenyi Biotec, Germany). Isolated monocytes (5×10^6 cells) were cultivated in 5 mL MO-DC differentiation medium (RPMI 1640) supplemented with 250 IU/mL IL-4 and 800 IU/mL GM-CSF in T25 flasks, and incubated at 37 °C and 5% CO_2. On day 3, 5 mL of MO-DC differentiation medium was added to the MO-DC cultures. On day 7, MO-DCs differentiation medium (Miltenyi Biotec, Germany) was replaced by 5 mL of MO-DC maturation medium (Miltenyi Biotec, Germany) (RPMI 1640 containing L-Glutamin and tumor necrosis factor alpha (TNFα)). MO-DCs were harvested either on day 7 or day 11 using ice-cold PBS 5 mM EDTA to detach cells from the cell culture flask.

2.2 Irradiation

Photon irradiation was performed with a biological cabinet x-ray irradiator (XRAD 320 Precision X-ray Inc., N. Bradford, CT, MO, USA) with 320 kV and 12.50 mA and a 110 cGy/min dose rate at absorbed doses of 1×0.5 Gy (low dose RT) and 1×15 Gy (hypofractionated/ablative RT) on day 0 and 5×2.0 Gy (normofractioned RT) on day 0–4. Proton and carbon ion (C12) irradiation were applied using an extended Bragg Peak for cell culture flasks (30 mm) at Heidelberg Ion Beam Therapy Centre (HIT). Irradiation was performed using an active raster scanning technique with a horizontal beamline [22]. For protons an energy of E = 69.1–92.3 MeV/u with a LET = 3.9 keV/μm (range: 3.0–8.5 keV/μm) was used. For carbon ions, an energy of E = 1.56–2.12 GeV (specific energy of 129.8–176.6 MeV/u) with a LET = 60 keV/μm (range: 45–175 keV/μm) was used. Both resulted in a 30 mm spread out Bragg Peak (SOBP).

Since relative biological effectiveness (RBE)-values are dose-dependent, biological dose estimations were made according to the local effect model (LEM) IV in pre-experimental calculations and a mean RBE of 1.2 for protons [23] and 2.5 for C12 [24] was used. Therefore, physical absorbed doses of 1×0.42 Gy, 5×1.67 Gy and 1×12.5 Gy (RBE of 1.2) for protons and 1×0.2 Gy, 5×0.8 Gy and 1×6 Gy for C12 (RBE of 2.5) were applied on day 0–4. Radiotherapy was performed at room temperature. Cell viability was measured on day 7 and 11 using LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit (Thermo Fisher Scientific, MA, USA), **Supplementary Fig. 1**.

2.3 Phenotypic analysis

To investigate whether irradiated monocytes are able to differentiate into DCs, we irradiated CD14-positive PBMCs on day 0 (-4) with different radiation doses. To analyze the differentiation to iMO-DCs and mMO-

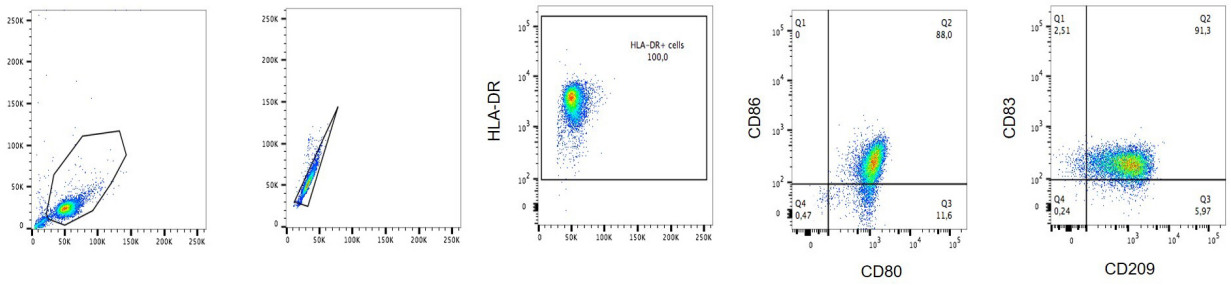

Fig. 1. Gating strategy. Cells are gated for alive cells (FSC-A vs. SSC-A) and single cells (FSC-A vs. FSC-H). The population of HLA-DR$^+$ cells was then used to determine the population with a high CD80 and CD86 co-expression. CD80$^+$CD86$^+$ cell population is gated on CD83$^+$ and CD209$^+$ cells. Final gating parameters are: HLA-DR$^+$CD80$^+$CD86$^+$CD83$^+$CD209$^+$ for mMO-DCs.

DCs, cells were labeled with specific monoclonal antibodies for characteristic surface markers. Non-specific binding was assessed using appropriate isotype controls. Mean fluorescence intensity (MFI) was measured via flow cytometry. Antibodies used were: CD14 PE, #clone HB15e (eBiosciences); CD80 PerCP-eFlour710, #clone 2D10.4 (eBiosciences); CD83 PE, #clone HB15e (eBiosciences); CD86 Pacific blue, #clone GL-1 (Bioledgend); CD209 PE-Cy7, clone# eB-h209 (eBiosciences); HLA-DR APC, clone# L243 (Bioledgend). iMO-DCs were defined as CD14$^-$, C80$^+$, CD83$^-$, CD86$^-$, HLA-DR$^+$ CD209$^+$, whereas mMO-DCs were defined as CD14$^-$, C80$^+$, CD83$^+$, CD86$^+$, HLA-DR$^+$, CD209$^+$ following a defined gating strategy (Fig. 1).

2.4 Functional assays

Different functional assays have been performed to distinct timepoints. Fig. 2 gives an overview of the experimental setup.

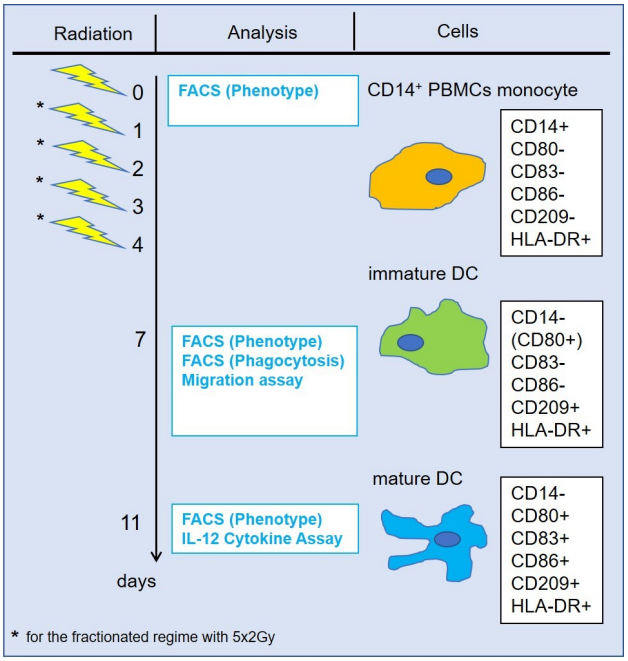

Fig. 2. Overview of the experimental setup. Radiation was performed on the day of the CD14$^+$ monocytes isolation, which was defined as day 0. For the fractionated regimen with 5 $\times$ 2 Gy, irradiation was done once daily on days 0–4, whereas for the single fraction regimes, irradiation was performed only on day 0.

2.4.1 Phagocytosis assay

To analyze phagocytotic capacity of iMO-DCs and mMO-DCs cells were incubated with Fluorescein isothiocyanate-labled (FITC-labeled) Dextran (Sigma) (1 mg/mL) for 60 minutes at 37 °C. A control sample was kept on ice (4 °C) to check for non-specific binding. Uptake was measured analyzing the MFI (mean flourescence intensity) of Fluorescein isothiocyanate (FITC) signal via flow cytometry.

2.4.2 Migrational analysis

In order to analyze the migrational ability of iDCs, modified Boyden chamber assays were performed: Polycarbonate membranes with 8-μm pores were coated with 0.5 μg/cm^2 Collagen I (Corning, Bodenheim, Germany) and stored overnight at 4 °C before the experiments. Next, 5 $\times$ 10^5 cells/mL were loaded into the upper chamber of a 48-well modified microchemotaxis chamber (Multiwell Chemotaxis Chamber, Neuro Probe, MD, USA). The lower well contained cell culture medium containing SDF-1α (0.1 μg/mL), as indicated. An 8-μm pore size polycarbonate membrane separated the lower and upper chambers. Methods have been described in detail by our work group before [25]. After 5 hours of incubation at 37 °C, transmigrated iDCs on the lower chamber side were stained with methylene blue and counted with a Leica DC300F microscope. The number of invading cells was counted using a phase-contrast microscope. Two fields were randomly selected per well, and the number of the cells was recorded by an investigator blinded to experimental set-up.

2.4.3 Cytokine measurements

Cell supernatants were harvested on day 11 from *in vitro* culture experiment on generating CD14 derived MO-DC. Cells supernatants were analyzed for IL-12 Cytokine

levels using an IL-12 ELISA kit (Coud-Clone Corp, TX, USA).

2.5 Statistical analysis

Data is displayed as means +/– standard deviations (SD). Comparisons between two groups were performed using Student's *t*-test or Wilcoxon rank test (software: SPSS 24, IBM Corporation, NY, USA). Asterisk in figures indicates statistical significance ($p < 0.05$) between the respective samples and control (ctr) in black and for samples of same fractionation schemes between different RT-techniques in grey.

3. Results

3.1 Irradiation of CD14-positive PBMCs does not alter surface phenotypes of iMO-DCs and mMO-DCs

Irradiation of monocytes on day 0 with photons, protons and carbon ions mostly showed no significant change in the expression profile of characteristic surface markers of iMO-DCs ($CD14^-$, $CD209^+$, $HLA\text{-}DR^+$) compared to the untreated control on day 7. Only after 5 × 2 Gy (RBE) proton irradiation, level of iMO-DCs was significantly increased compared to untreated control and photon irradiation (Fig. 2). Furthermore, the surface marker profile of mMO-DCs ($CD14^-$, $CD80^+$, $CD83^+$, $CD\ 86^+$, $CD209^+$, $HLA\text{-}DR^+$) on day 11 was not significantly different to the untreated control (Fig. 3A,B).

3.2 Irradiated iMO-DCs maintain their phagocytic capacity

Phagocytosis activity of MO-DCs was analyzed by measuring the uptake of FITC-labeled Dextran by MO-DCs. Uptake of FITC-labled Dextran by iMO-DCs was analyzed at 37 °C culture condition. Incubation of iMO-DCs with FITC-labled Dextran at 4 °C culture condition represents the condition of passive internalization of FITC-labled Dextran by iMO-DCs following the biochemical process of diffusion.

Uptake of FITC-labeled Dextran did not show significant differences between irradiated MO-DCs compared to untreated MO-DCs. Moreover, no significant differences between FITC-labeled Dextran uptake could be detected within the three treatment groups (Photons/C12/Protons) of irradiated MO-DCs (Fig. 4).

3.3 Migrational activity of iMO-DCs is not altered after photon and C12 irradiation but increased after proton irradiation

Migration of iMO-DCs was analyzed on day 7. Compared to untreated control, migratory capacity was significantly decreased after 1 × 15 Gy photon and 1 × 0.5 Gy (RBE) C12 irradiation, although differences after C12 irradiation were small. However, treatment of $CD14^+$ cells on day 0 with protons induced a significant increase in the migratory capacity of iMO-DCs compared to the untreated control, irrespective of dose and fractionation scheme (Fig. 5).

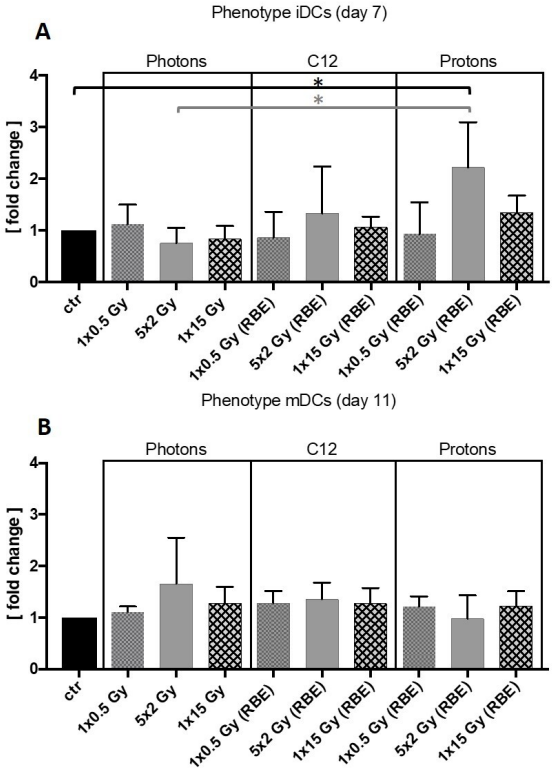

Fig. 3. Effect of irradiation on the amount of iMO-DCs and mMO-DCs. (A) Effect of irradiation on the amount of iMO-DC generated from $CD14^+$ cells. $CD14^+$ cells were irradiated with Photons: 1 × 0.5 Gy, 5 × 2 Gy, 1 × 15 Gy; Carbon Ions (C12) 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE); Protons 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE). iMO-DCs were identified on day 7 of the maturation process using flow cytometry. Frequencies of iMO-DCs are depicted as fold change with respect to non-irradiated iMO-DCs (ctr). Analysis performed using Student's *t*-test (asterisk indicates statistical significance $p < 0.05$) between the respective samples and control (crt) in black and for samples of same fractionation schemes between different RT-techniques in grey. All phenotypic assays were performed in triplicates. (B) Effect of irradiation on the amount of mMO-DC generated from $CD14^+$ cells. $CD14^+$ cells were irradiated with Photons: 1 × 0.5 Gy, 5 × 2 Gy, 1 × 15 Gy; Carbon Ions (C12) 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE); Protons 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE). mMO-DCs were identified on day 11 of the maturation process using flow cytometry. Frequencies of mMO-DCs are depicted as fold change with respect to non-irradiated mMO-DCs (ctr). All phenotypic assays were performed in triplicates.

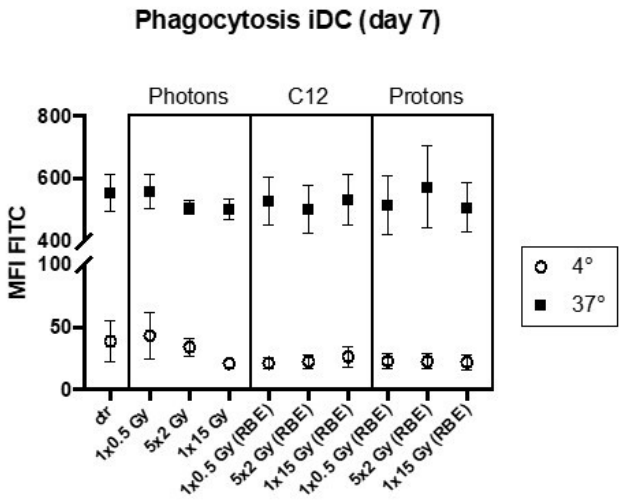

Fig. 4. Measurement of phaygocytic capacity of iMO-DCs measured on day 7 using FITC-labeled Dextran. $CD14^+$ Monocytes were irradiated on day 0 with Photons: 1 × 0.5 Gy, 5 × 2 Gy, 1 × 15 Gy; Carbon Ions (C12) 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE); Protons 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE). Non-irradiated iDCs were used as control (ctr). Uptake of FITC-labeled Dextran by iMO-DCs was analyzed at 37 °C culture condition and 4 °C culture condition. All phagocytosis assays were performed in triplicates.

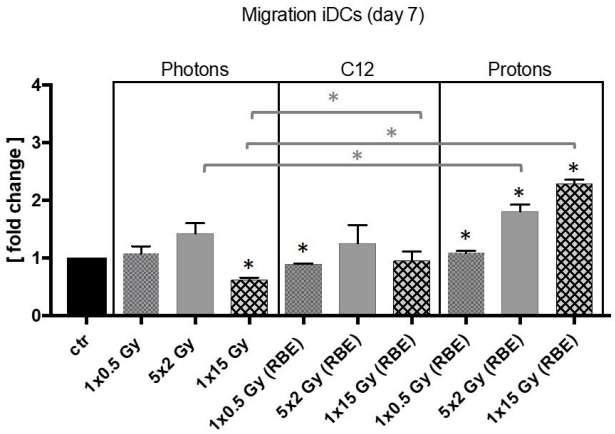

Fig. 5. Effect of irradiation on migratory capacity of iMO-DC. $CD14^+$ cells were irradiated with Photons: 1 × 0.5 Gy, 5 × 2 Gy, 1 × 15 Gy; Carbon Ions (C12) 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE); Protons 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE). Migration assay was performed with iMO-DCs on day 7 of the maturation process. Migrated iMO-DCs are depicted as fold change with respect to migrated non-irradiated iMO-DCs (ctr). Statistical analysis performed using Student's *t*-test. Asterisk indicates statistical significance $p < 0.05$ between the respective samples and control (crt) in black and for samples of same fractionation schemes between different RT-techniques in grey. All migration assays were performed in triplicates.

3.4 Irradiation does not impair cytokine production of IL-12 in mMO-DCs

IL-12 secretion was measured using supernatants from MO-DC differentiation set up. IL-12 cytokine levels were non-significantly different comparing supernatants harvested from untreated MO-DCs to supernatants harvested from treated MO-DCs. Furthermore, no significant differences of IL-12 levels were detected within supernatants harvested form the different treatment groups (MO-DCs irradiated with Photons, C12, Protons) compared to untreated control (Fig. 6).

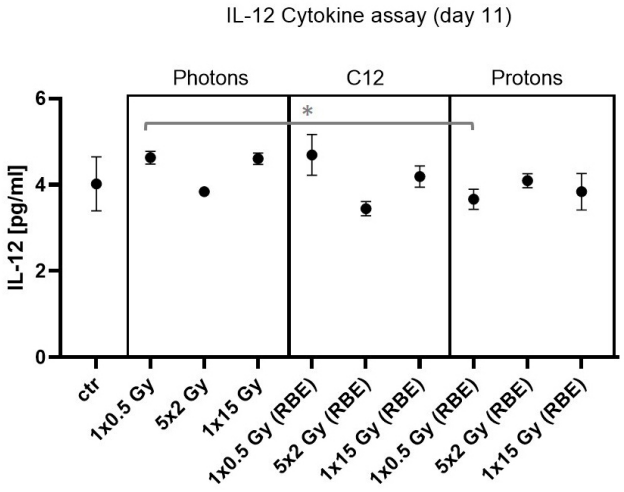

Fig. 6. IL-12 Cytokine levels in cell supernatants of mDC measured on day 11. $CD14^+$ Monocytes were irradiated on day 0 with Photons: 1 × 0.5 Gy, 5 × 2 Gy, 1 × 15 Gy; Carbon Ions (C12) 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE); Protons 1 × 0.5 Gy (RBE), 5 × 2 Gy (RBE), 1 × 15 Gy (RBE). Non-irradiated iDCs were used as control (ctr). Statistical analysis performed using Student's *t*-test. Asterisk indicates statistical significance $p < 0.05$ of samples of same fractionation schemes between different RT-techniques in grey. All cytokine assays were performed in triplicates.

4. Discussion

Although extensive research is carried out in the field of radiation oncology regarding the local tumor tissue or the surrounding normal tissue effects, the influence of irradiation on immune activation due to DCs is not satisfactorily investigated so far. Especially the influence of different doses and fractionation as well as different radiation types is unclear.

Most of preclinical experiments are performed using single RT doses only, thus comparability with the clinically used concepts is disputable. Strength of our experiments is that we used different doses: low dose, high dose and a normofractionated dose regimen. Since antigen contact with DC might occur in different places, radiation doses on

DCs might differ considerable. For example, in the tumor itself the RT dose can be very high like in hypofractionated concepts like stereotactic body radiotherapy (SBRT) and is then applied in a single dose or in a small number of fractions (e.g., 1 × 15 Gy). On the other hand, it is possible that DCs in the tumor itself might be exposed to normofractionated doses over several days before they start migrating to the lymph nodes (e.g., 5 × 2 Gy). Another possible place where DC might be activated during a course of RT is the surrounding tissue, where only low doses (e.g., 1 × 0.5 Gy) of the prescribed total dose arrive.

Our results demonstrate that irradiation with different dose regimens and RT techniques did not negatively influence the phenotype and differentiation of iMO-DCs to mDCs, which is supported by the data of Merrick *et al*. [26], where no significant alteration of surface markers was seen after RT with 2, 8 and 30 Gy.

Since specific immune responses against TAA depend on the ability of DCs to internalize antigen and migrate to lymph nodes for further T-cell activation, we subsequently tested phagocytic and migrational capacities. Firstly, we could demonstrate that phagocytic and migrational capacities iMO-DCs are not negatively altered after irradiation and secondly that high-LET RT with protons and C12 did also not negatively influence functionality of iMo-DCs, despite our initial hypothesis. Furthermore, after proton irradiation with 5 × 2 Gy and 1 × 15 Gy, migrational activity showed a slight increase. Our findings are similar to the results of Merrick *et al*. [26] which suggest that functionality of irradiated DC is not significantly changed by different RT doses.

Only one other published paper regarding photon irradiation of human DCs was found which reported partially contradictory results regarding differentiation and functionality: Cao *et al*. [10] showed that CD86 expression, which is a marker for mMO-DCs, and their functional capacity, measured by T-cell activation is downregulated by irradiation. However, they did not investigate detailed phagocytic or migrational capacity as well as secreting of the immunostimulatory cytokine IL-12 of DCs. An additional factor that decreases comparability of these results, is the fact that they used DCs generated from PBMCs obtained from patients. with multiple sclerosis. It is not unlikely that in this setting, *in vitro* generated DCs are negatively impaired in their functional capacity due to the underlying autoimmune disease. A comparison with DCs derived from healthy donors is lacking and results should therefore be taken with precaution.

We also investigated wether irradiation alters IL-12 cytokine production of mMO-DCs and found no decrease in IL-12 production, supporting the hypothesis of preserved functional ability of DCs after RT. Thus, our overall results regarding phenotype and functional capacities of DCs after irradiation are supported by the data given from Merrick *et al*. [26]. However, the experimental set up lacks a positive control, e.g., experiments on irradiated DCs stimulated with an IL-12 inducing agent. Within the given experimental set up, incapability of the maturation medium used in this experimental set up to induce IL-12 secretion of mature DCs cannot be excluded.

One possible limitation of our analysis is certainly the artificial *in vitro* situation which cannot fully represent the situation *in vivo* including the interaction with the microenvironment. Furthermore, while we did show a possible preserved IL-12 secretion of irradiated mMO-DCs, we did not investigate the direct interaction between DCs and T-cells which represents the final activation step in immune response and anti-cancer cell activity. Overall, the model used for DCs generation represents an artificial model thus *in vitro* generated Mo-DCs might differ in their functionality from in vivo Mo-DCs. Moreover, this work cannot exclude an impact of irradiation on other subsets of DC (cDCs, pDCs). Our data however support the notion that DCs play an important role in the radiation associated immune response in cancer therapy: for example, in combination experiments of photon irradiation with toll like receptor agonists 7 (TLR7) we and other groups have shown in several tumor models including pancreatic cancer, colon cancer, sarcoma and lymphomas that DC activity is essential to convey a strong immunogenic antitumor effect of radiation [27–29]. Nevertheless, DCs that are present in cancer patients may have a different sensitivity to radiation than DCs generated with cells of healthy subjects given another limitation of the experiments performed in this research work.

To our current knowledge, no research group has ever investigated effects of particle therapy (e.g., proton and/or C12) on DC function. Although it is known that particle therapy offers beneficial characteristics regarding higher LET and higher relative biological effectiveness and therefore induces cancer cell killing more than conventional photon RT, our results indicate, that particle therapy might have a rather stimulatory effect on immune cells like DCs, as we demonstrated in migration assays for proton irradiation (Fig. 5).

All together our data show that irradiation with photons, protons and carbon ions do not markedly alter the phenotypic maturation of MO-DCs, nor does irradiation alter their basic functionality including phagocytosis, migration ability and cytokine secretion. The data support the notion that DCs can play a robust role in the immune response after radiotherapy of cancer.

5. Conclusions

In summary, our results show that different fractionation regimens and doses do not negatively impact differentiation and functionality of DCs and therefore we assume that their potential for inducing an adaptive immune response is not significantly impaired. Although using a higher LET, particle therapy with protons and carbon ions did not reduce DC function compared to photon RT, but might be stimula-

tory regarding migrational capacity of iMO-DCs.

Author contributions

LK, AH and LO performed data collection. LK and AH were responsible for writing and original draft preparation. LK, AH and JH-R performed the statistical analysis. LK, AH, PEH, JD and SR conceived of the analysis and participated in its design and coordination. SB performed radiobiological dose calculations for particle therapy. All the authors were responsible for data interpretation, participated in manuscript revisions, and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/FBS/14/1/10.31083/j.fbs1401002.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, *et al*. Immunobiology of dendritic cells. Annual Review of Immunology. 2000; 18: 767–811.

[2] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998; 392: 245–252.

[3] Steinman RM. Decisions about Dendritic Cells: Past, Present, and Future. Annual Review of Immunology. 2012; 30: 1–22.

[4] Nussenzweig MC, Steinman RM, Gutchinov B, Cohn ZA. Dendritic cells are accessory cells for the development of anti-trinitrophenyl cytotoxic T lymphocytes. The Journal of Experimental Medicine. 1980; 152: 1070–1084.

[5] Witmer MD, Steinman RM. The anatomy of peripheral lymphoid organs with emphasis on accessory cells: light-microscopic immunocytochemical studies of mouse spleen, lymph node, and Peyer's patch. The American Journal of Anatomy. 1984; 170: 465–481.

[6] Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. Journal of the National Cancer Institute. 2013; 105: 256–265.

[7] Hatfield P, Merrick A, Harrington K, Vile R, Bateman A, Selby P, *et al*. Radiation-induced cell death and dendritic cells: potential for cancer immunotherapy? Clinical Oncology. 2005; 17: 1–11.

[8] Zheng H, Ban Y, Wei F, Ma X. Regulation of Cytokine Gene Expression in Immunity and Diseases (pp. 117–138) Netherlands: Springer. 2016.

[9] Chouaib S, Chehimi J, Bani L, Genetet N, Tursz T, Gay F, *et al*. Interleukin 12 induces the differentiation of major histocompatibility complex class I-primed cytotoxic T-lymphocyte precursors into allospecific cytotoxic effectors. Proceedings of the National Academy of Sciences of the United States of America. 1994; 91: 12659–12663.

[10] Cao M, Chen Z, Xing Y. Gamma irradiation of human dendritic cells influences proliferation and cytokine profile of T cells in autologous mixed lymphocyte reaction. Cell Biology International. 2004; 28: 223–228.

[11] Reuben JM, Korbling M, Gao H, Lee B. The effect of low dose gamma irradiation on the differentiation and maturation of monocyte derived dendritic cells. Journal of Gravitational Physiology. 2004; 11: P49–P52.

[12] Rodel F, Frey B, Gaipl U, Keilholz L, Fournier C, Manda K, *et al*. Modulation of Inflammatory Immune Reactions by Low-Dose Ionizing Radiation: Molecular Mechanisms and Clinical Application. Current Medicinal Chemistry. 2012; 19: 1741–1750.

[13] Formenti SC. Immunological aspects of local radiotherapy: clinical relevance. Discovery Medicine. 2010; 9: 119–124.

[14] Donnelly EH, Nemhauser JB, Smith JM, Kazzi ZN, Farfán EB, Chang AS, *et al*. Acute radiation syndrome: assessment and management. Southern Medical Journal. 2010; 103: 541–546.

[15] Pecaut MJ, Nelson GA, Gridley DS. Dose and dose rate effects of whole-body gamma-irradiation: I. Lymphocytes and lymphoid organs. In Vivo. 2001; 15: 195–208.

[16] Liu S. Nonlinear Dose-Response Relationship in the Immune System following Exposure to Ionizing Radiation: Mechanisms and Implications. Nonlinearity in Biology, Toxicology, Medicine. 2003; 1: 154014203908444.

[17] Liu SZ, Jin SZ, Liu XD, Sun YM. Role of CD28/B7 costimulation and IL-12/IL-10 interaction in the radiation-induced immune changes. BMC Immunology. 2004; 2: 8.

[18] Jahns J, Anderegg U, Saalbach A, Rosin B, Patties I, Glasow A, *et al*. Influence of low dose irradiation on differentiation, maturation and T-cell activation of human dendritic cells. Mutation Research. 2011; 709-710: 32–39.

[19] Formenti SC, Demaria S. Systemic effects of local radiotherapy. The Lancet Oncology. 2009; 10: 718–726.

[20] MOLE RH. Whole body irradiation; radiobiology or medicine? The British Journal of Radiology. 1953; 26: 234–241.

[21] Guan F, Peeler C, Bronk L, Geng C, Taleei R, Randeniya S, *et al*. Analysis of the track- and dose-averaged LET and LET spectra in proton therapy using the geant4 Monte Carlo code. Medical Physics. 2015; 42: 6234–6247.

[22] Haberer T, Becher W, Schardt D, Kraft G. Magnetic scanning system for heavy ion therapy. Nuclear Instruments and Methods in Physics Research Section a: Accelerators, Spectrometers, Detectors and Associated Equipment. 1993; 330: 296–305.

[23] Paganetti H, Niemierko A, Ancukiewicz M, Gerweck LE, Goitein M, Loeffler JS, *et al*. Relative biological effectiveness (RBE) values for proton beam therapy. International Journal of Radiation Oncology, Biology, Physics. 2002; 53: 407–421.

[24] Suzuki M, Kase Y, Yamaguchi H, Kanai T, Ando K. Relative biological effectiveness for cell-killing effect on various human cell lines irradiated with heavy-ion medical accelerator in Chiba (HIMAC) carbon-ion beams. International Journal of Radiation Oncology, Biology, Physics. 2000; 48: 241–250.

[25] Yamauchi Y, Safi S, Orschiedt L, Gardyan A, Brons S, Rieber J, *et al*. Low-dose photon irradiation induces invasiveness through

the SDF-1α/CXCR4 pathway in malignant mesothelioma cells. Oncotarget. 2017; 8: 68001–68011.

[26] Merrick A, Errington F, Milward K, O'Donnell D, Harrington K, Bateman A, *et al*. Immunosuppressive effects of radiation on human dendritic cells: reduced IL-12 production on activation and impairment of naive T-cell priming. British Journal of Cancer. 2005; 92: 1450–1458.

[27] Schölch S, Rauber C, Tietz A, Rahbari NN, Bork U, Schmidt T, *et al*. Radiotherapy combined with TLR7/8 activation induces strong immune responses against gastrointestinal tumors. Oncotarget. 2015; 6: 4663–4676.

[28] Adlard AL, Dovedi SJ, Telfer BA, Koga-Yamakawa E, Pollard C, Honeychurch J, *et al*. A novel systemically administered toll-like receptor 7 agonist potentiates the effect of ionizing radiation in murine solid tumor models. International Journal of Cancer. 2014; 135: 820–829.

[29] Dovedi SJ, Melis MHM, Wilkinson RW, Adlard AL, Stratford IJ, Honeychurch J, *et al*. Systemic delivery of a TLR7 agonist in combination with radiation primes durable antitumor immune responses in mouse models of lymphoma. Blood. 2013; 121: 251–259.