Photon, proton and C12 irradiation influences maturation and functionality of dendritic cells

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Introduction

Radiotherapy (RT) induces DNA-damage that either causes induction of tumor cell death or inhibition of the proliferating capacity of these cells. Furthermore, considerable evidence emerges that antineoplastic effects extend beyond these mechanisms. Those secondary effects contribute to anti-tumor responses in a local but also systemic manner via activation of the immune system: The role of dendritic cells (DCs) is well described to be essential for priming effective radiation-induced adaptive immunity. Through increased release of tumor-associated antigens (TAA) after RT, DCs are recruited and cross-presentation of TAA leads to activation of B- and T-lymphocytes, therefore playing a pivotal role in adaptive immune response and immunogenic cell death. However, there are still many hypotheses regarding the influence of RT on activation of the immune system. The aim of our experiments is to further characterize the impact of different radiation types and dosages on differentiation and functionality of DCs.

Methods

Human CD14-positive monocytes were isolated from peripheral blood mononuclear cell samples. After cytokine stimulation with Interleukin-4 (IL-4) and granulocyte macrophage colony-stimulating factor (GM-CSF) monocytes were induced into immature DCs (iDCs) and later mature DCs (mDCs). Monocytes were irradiated with different radiation doses (1x15 Gy, 5x2 Gy, 1x0.5 Gy) and radiation types (photons, protons, carbon ions) on day 0. Maturation to mDCs was induced on day 7 by adding tumor necrosis factor alpha (TNFα) to the culture medium. Differentiation and maturation of DCs was assessed by staining of cell surface molecules CD14, CD83, CD80, CD86, CD209 und HLA-DR via flow cytometry. Functional analysis of irradiated DCs was performed through FITC-labelled phagocytosis assay, migrational assays and IL-12 ELISA.

1. Generation of human DCs

Flow cytometry

Gating Strategy

Results

Fig. 1: Cells were irradiated on day 0 with three different radiation doses (1 x 15 Gy, 5 x 20 Gy, 1 x 0.5 Gy) and expression of cell surface marker was analyzed on day 2, 4, 7, and day 14 using the designated panel of antibodies. Indicated in red are cell surface markers expressed at high levels in monocytes, immature DC and mature DC.

Fig. 3: No major significant changes in the immune profile during differentiation of monocytes into iDCs and mDCs were seen after treatment with different radiation types

Fig. 4 and 5: Functional analysis revealed maintenance of phagocytic capacity and secretion of IL-12 of irradiated DCs

Fig. 6: Proton-RT induced migrational capacity of iDCs.

Conclusion

Our experiments reveal that after irradiation with different doses and types maturation of DCs was unchanged compared to the control group. Capability for phagocytosis was unaffected after irradiation of DCs, indicating persistent functionality of the immune system. An additional RT-induced effect of particle therapy on the immunogenic potential of DCs is possible due an increase of IL-12 secretion and migrational capacity.