Image segmentation of alveolar macrophages reveals chronic inflammation in carbon ion irradiated rat lungs*

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Introduction

Radiation-induced pneumonitis represents a severe side effect in lung tissue after radiotherapy. The chronic form of pneumonitis, which in some cases persists over many months after irradiation, is discussed to be an inducer of fibrosis, another severe side effect. One potential mechanism leading to fibrosis is the release of certain cytokines by invading immune cells (especially macrophages), which stimulate fibroblast proliferation and overproduction of extracellular matrix [1].

In view of the increased application of carbon ions in radiotherapy, we aimed to assess whether carbon ions are able to induce chronic inflammation in lung tissue. We quantified alveolar macrophages present in slices of rat lung tissue 42 weeks post irradiation. To achieve a better precision compared to the commonly used scoring of bright field images, we established immunofluorescence stainings of macrophages with subsequent image segmentation.

Materials and Methods

Adult male albino Wistar rats were housed as described [2]. Whole lungs were irradiated with carbon ions at the SIS facility (270 MeV/u, 13 keV/μm) at doses between 7.7–12.5 Gy. 42 weeks after irradiation, lungs were extracted and embedded in paraffin. 4 μm slices were cut and stained with a macrophage-specific antibody (ED1, AbD Serotec) followed by an Alexa Fluor 488-conjugated secondary antibody (Life Technologies) and counter-stained with DAPI. 40 images were recorded per slice with an epifluorescence microscope (Leica, $20\times$ objective). Image segmentation was performed using the CellProfiler software [3]. Cells were segmented via the DAPI channel by OTSU thresholding. Cells were counted as ED1 positive if their median fluorescence intensity in the ED1 channel was ≥ 15 standard deviations higher than the median fluorescence intensity of the whole image. Data were analysed using a pipeline custom-written in the R language. Percentage of ED1 positive nuclei in relation to all nuclei was used to calculate per-slice values. Significance level was set to $\alpha = 0.01$.

Results and Discussion

The chronic inflammatory response did not yield a clear dose response (not shown). This is possibly due to the 42 week time span between irradiation and processing, during which dose responses might be obfuscated by ongoing physiological processes. For this reason we combined all doses and compared the results with unirradiated animals (Fig. 1). Unirradiated animals had a median of 10% macrophages in the lung tissue. This number increased to 15% in irradiated animals 42 weeks post irradiation. The irradiated animals demonstrated a significantly increased percentage of macrophages compared to unirradiated animals (WILCOXON test, $p = 0.0006$). We conclude that carbon ions are able to induce a chronic inflammatory response that persists at least 10 months post irradiation, which is in agreement with a study using X-rays [4].

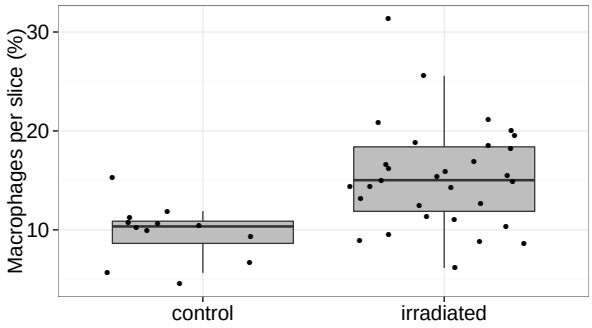

Figure 1: Percentage of macrophages per slice relative to whole cell count. Single dots represent single tissue slices of 4 control or 15 irradiated animals.

References

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