Radiotherapy and Oncology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Review

Toward A variable RBE for proton beam therapy

Henning Willers [a,*], Antino Allen [b], David Grosshans [c], Stephen J. McMahon [d], Cläre von Neubeck [e], Claudia Wiese [f], Bhadrasain Vikram [g]

[a] Department of Radiation Oncology, Massachusetts General Hospital, Boston; [b] Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock; [c] Department of Radiation Oncology, M.D. Anderson Cancer Center, Houston, USA; [d] Centre for Cancer Research & Cell Biology, Queen's University Belfast, Belfast, UK; [e] OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Helmholtz-Zentrum Dresden – Rossendorf, Dresden and German Cancer Consortium (DKTK), Partner Site Dresden and German Cancer Research Center (DKFZ), Heidelberg, Germany; [f] Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins; and [g] Radiation Research Program/Division of Cancer Treatment & Diagnosis, National Cancer Institute, Bethesda, USA

ARTICLE INFO

Article history:
Received 28 March 2018
Received in revised form 9 May 2018
Accepted 17 May 2018
Available online xxxx

Keywords:
Relative biological effectiveness
Protons
Carbon ions
DNA repair

ABSTRACT

In the clinic, proton beam therapy (PBT) is based on the use of a generic relative biological effectiveness (RBE) of 1.1 compared to photons in human cancers and normal tissues. However, the experimental basis for this RBE lacks any significant number of representative tumor models and clinically relevant endpoints for dose-limiting organs at risk. It is now increasingly appreciated that much of the variations of treatment responses in cancers are due to inter-tumoral genomic heterogeneity. Indeed, recently it has been shown that defects in certain DNA repair pathways, which are found in subsets of many cancers, are associated with a RBE increase *in vitro*. However, there currently exist little *in vivo* or clinical data that confirm the existence of similarly increased RBE values in human cancers. Furthermore, evidence for variable RBE values for normal tissue toxicity has been sparse and conflicting to date. If we could predict variable RBE values in patients, we would be able to optimally use and personalize PBT. For example, predictive tumor biomarkers may facilitate selection of patients with proton-sensitive cancers previously ineligible for PBT. Dose de-escalation may be possible to reduce normal tissue toxicity, especially in pediatric patients. Knowledge of increased tumor RBE may allow us to develop biologically optimized therapies to enhance local control while RBE biomarkers for normal tissues could lead to a better understanding and prevention of unusual PBT-associated toxicity. Here, we will review experimental data on the repair of proton damage to DNA that impact both RBE values and biophysical modeling to predict RBE variations. Experimental approaches for studying proton sensitivity *in vitro* and *in vivo* will be reviewed as well and recent clinical findings discussed. Ultimately, therapeutically exploiting the understudied biological advantages of protons and developing approaches to limit treatment toxicity should fundamentally impact the clinical use of PBT.

Introduction

There exists a great potential for therapeutic benefits of proton beam therapy (PBT) in several cancer types [1]. PBT has superior physical characteristics compared to standard photon radiation in many anatomical sites, but its biological properties have been thought to be similar to photons [2,3]. This is reflected by the use of a generic relative biological effectiveness (RBE) of 1.1 for both cancer and normal tissues (Fig. 1). However, there exists a scarcity of data on RBE variations in human tumors. In a comprehensive review from 2002, the average RBE was estimated as ~1.2 *in vitro* and ~1.1 *in vivo*. However, most of the 20 cell lines were of Chinese hamster ovary (CHO) origin resulting in a somewhat higher *in vitro* RBE, and only 7 human cancer cell lines were included [2]. In a recent update, the number of cancer cell lines remained limited, with an average RBE of ~1.15 [3]. However, there remains considerable variability related to both experimental conditions (incl. dose, beam characteristics) and cell biology (incl. DNA repair status, α/β ratio).

This experimental basis for the current clinical use of a generic RBE of 1.1 is a major limitation, given the considerable genomic heterogeneity across cancers even for the same type and histology as unraveled by recent genomic studies. Moreover, it is increasingly appreciated that much of the variations in treatment sensitivity observed clinically are due to inter-tumoral heterogeneity, which includes alterations in the DNA damage response (DDR) [4–9]. Indeed, several reports have now demonstrated that defects

* Corresponding author.
E-mail address: hwillers@partners.org (H. Willers).

https://doi.org/10.1016/j.radonc.2018.05.019
0167-8140/

A

$$RBE_{(Dose,\ Endpoint,\ Beam\ properties)} = \frac{Dose_{(Endpoint/Photons)}}{Dose_{(Endpoint/Protons)}}$$

B

Types of Low-LET Ionizing Radiation

Radiation	Energy	LET (keV/μm)	RBE
Co60 γ-rays	~1.2 MV*	~0.3	1.0
X-rays	~250 kVp	~2	1.1-1.15
Protons	65-250 MeV	~2.5 (mid-SOBP)	~1.1

*representative of linac-based patient treatments

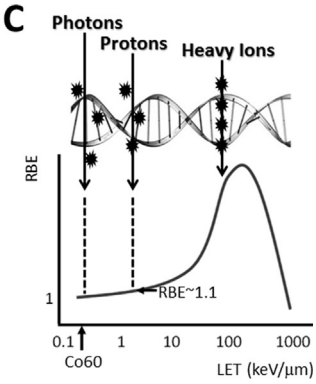

Fig. 1. Relative Biological Effectiveness (RBE) of different ionizing radiation qualities. (A) RBE is the ratio of doses to reach the same level of effect (Endpoint) when comparing two radiation modalities, i.e., reference photons (Cobalt-60, Co60) vs. protons. The radiation dose in PBT is prescribed as Gy[RBE] according to ICRU. For example, for a RBE = 1.1 and a desired Co60 equivalent (Co60Eq) dose of 2 Gy, the corresponding physical proton dose would be 1.82 Gy (1.82 Gy × 1.1 = 2 Gy[RBE]). (B) Examples of ionizing radiation types and representative dose-average LET (Linear Energy Transfer) and RBE values [10,103,104]. LET of γ-rays refers to secondary electrons. (C) Illustration of how protons induce DNA damage that is slightly more clustered than photons (or Co60 γ-rays), which in DNA repair-proficient cells yields a RBE of 1.1.

in the homologous recombination (HR) and Fanconi Anemia (FA) DDR pathways are associated with RBE values of 1.3 or more *in vitro* [10–13]. However, there currently exist few pre-clinical *in vivo* or clinical data to demonstrate the existence of increased RBE values in human cancers, and evidence with regard to RBE variations in normal tissues remains sparse as well [2,14–16].

Importantly, we lack an in-depth understanding of the mechanisms that underlie RBE variations in tumors and normal tissues, and we are currently unable to identify individual cancer patients whose tumors and/or normal tissues exhibit increased sensitivity to PBT. These shortcomings constitute critical barriers to fully harnessing the potential superiority of PBT and to avoiding unnecessary toxicity. Here, we review our current knowledge of and approaches to understanding RBE variations in tumors and normal tissues. In the not too distant future, therapeutically exploiting the understudied biological advantages of protons and developing approaches to limiting treatment toxicity are expected to fundamentally impact the clinical use of PBT in the increasing number of proton centers worldwide.

DNA damage caused by particle radiation and its repair

DNA repair of double-strand breaks and clustered damages

Although the use of charged particle therapy has increased rapidly over the last few decades, the contribution and the interplay of specific DNA repair pathways to the repair of DNA lesions induced by these radiation modalities is incompletely understood. Particle radiations such as proton or carbon ion beams induce more highly localized and clustered DNA damage than X- and γ-rays. Clustered DNA damage includes abasic sides, base damages, single- (SSBs) and double-strand breaks (DSBs) that are in close proximity to each other [17]. The complexity and yield of radiation-induced clustered DNA damage increases with ionization density [18–21]. Hence, for a given dose, therapeutic carbon beams (200–430 MeV/n; ~>10–80 keV/μm linear energy transfer (LET)) are expected to induce more clustered DNA lesions than therapeutic proton beams (65–250 MeV/n; ~2–10 keV/μm). Clustered DNA damage represents a considerable obstacle to efficient repair, and DSBs within clustered lesions rejoin with slower kinetics and less completely than frank DSBs [20,22], likely contributing to the observed higher RBEs for cell killing after charged particle- compared to photon-irradiated cells [18,23]. A major question is whether the mechanisms of repairing DNA damages caused by PBT resemble those triggered by photons or those operating in response to heavy ion exposure.

When potentially lethal DSBs occur, cells repair these DNA ends mainly by two distinct pathways, non-homologous end joining (NHEJ) and HR. These two pathways differ biochemically, have different substrate requirements, and are used differently throughout the cell cycle (for review, see [24]). Briefly, NHEJ is the main pathway of ionizing radiation-induced DSB repair in G1- and early S-phase cells while both HR and NHEJ contribute to DSB repair in late S-/G2-phase cells [25,26]. Importantly, HR also is the predominant pathway for the repair of stalled and damaged DNA replication forks [27,28]. Notably, mutations in HR genes increase cellular sensitivity to photon radiation and also to replicative and transcriptional stress [29,30]. During HR a DSB, or a DNA replication fork encountering a DNA lesion, undergoes nucleolytic resection to yield 3′ single-stranded (ss) DNA ends which are immediately covered by the ssDNA binding protein replication protein A (RPA). RPA is then replaced by the RAD51 recombinase forming a nucleoprotein complex termed the presynaptic filament. The presynaptic filament searches for, engages, and invades a homologous duplex target DNA to form the displacement loop (D-loop). DNA synthesis and resolution of DNA intermediates follows to complete HR repair [31]. During NHEJ, the KU70/80 heterodimer, which has high affinity for free DNA ends [13], initiates the pathway, whereby nucleolytic processing of DNA ends is blocked. KU70/80 recruit DNA-PKcs, and DNA-PKcs immobilizes the two DNA ends and facilitates the rejoining reaction [32–34], in which ligation is carried out by the XRCC4-DNA ligase IV complex [35]. NHEJ is the major repair pathway for DSBs induced by photon radiation including X-rays (for review, see [36]).

Repair pathways for high-LET radiations

To date, only a limited number of studies have addressed the relative contributions of NHEJ, HR and resection-mediated repair pathways to removing complex DSBs induced by different charged particle radiation types. Evidence is accumulating that shows that NHEJ is less capable of removing clustered DSBs induced by high-LET radiations as compared to low-LET radiations [37–42]. Yajima *et al.* [42] investigated the propensity of human and mouse cells to undergo DNA resection after low-LET (X- or γ-rays) versus high-LET radiations (70 keV/μm carbon (290 MeV/n) or 250 keV/μm iron ions (500 MeV/n)). Their study showed that >80% of the DSBs induced by heavy ions were subjected to end resection, which is significantly more than what was observed after low-LET radiations [41,43]. Interestingly, Yajima *et al.* [42] also reported on DNA resection occurring in G1 phase cells after heavy ion treatment and suggested that microhomology-mediated end-joining

Please cite this article in press as: Willers H et al. Toward A variable RBE for proton beam therapy. Radiother Oncol (2018), https://doi.org/10.1016/j.radonc.2018.05.019