Clinical Oncology 26 (2014) 289–300

Contents lists available at ScienceDirect

Clinical Oncology

journal homepage: www.clinicaloncologyonline.net

Overview

Redox Proteins and Radiotherapy

Y. Zhang, S.G. Martin

Academic Unit of Clinical Oncology, University of Nottingham, School of Medicine, Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham, UK

Received 4 November 2013; received in revised form 28 January 2014; accepted 7 February 2014

Abstract

Although conventional radiotherapy can directly damage DNA and other organic molecules within cells, most of the damage and the cytotoxicity of such ionising radiation, comes from the production of ions and free radicals produced via interactions with water. This 'indirect effect', a form of oxidative stress, can be modulated by a variety of systems within cells that are in place to, in normal situations, maintain homeostasis and redox balance. If cancer cells express high levels of antioxidant redox proteins, they may be more resistant to radiation and so targeting such systems may be a profitable strategy to increase therapeutic efficacy of conventional radiotherapy. An overview, with exemplars, of the main systems regulating redox homeostasis is supplied and discussed in relation to their use as prognostic and predictive biomarkers, and how targeting such proteins and systems may increase radiosensitivity and, potentially, improve the radiotherapeutic response.

Key words: Radiosensitivity; radiotherapy; reactive oxygen species; redox homeostasis; redox imaging; redox proteins

Statement of Search Strategies Used and Sources of Information

The review was based on PUBMED database searches of key influential literature and the recent relevant papers concerning advances of the topic. The search terms used were redox protein, reactive oxygen species, antioxidant, radiotherapy, radiosensitivity, glutathione, thioredoxin, redox imaging. Full articles were retrieved when the abstract was deemed relevant. The bibliographies of retrieved papers were also searched and relevant articles included.

Introduction

Conventional radiotherapy (low-linear energy transfer (LET) radiation) exerts its cytotoxic effects by a variety of means, including damage to DNA and other organic molecules. Such damage can occur via the radiation directly ionising the biological molecules or indirectly by interacting with other molecules, mostly water molecules, surrounding such 'targets', creating ions and free radicals, which can migrate to and damage critical targets in the cell, such as DNA, lipids and proteins [1]. About two-thirds of the low-LET radiation-induced damage to DNA in mammalian cells is attributed to the hydroxyl radical [2], one of several types of reactive oxygen species (ROS) created when the radiolysis of water occurs. ROS play a dual role, either deleterious [3–5] or beneficial [6], in living systems. Intracellular ROS levels are regulated by a number of redox buffering systems, including non-enzymatic antioxidant and redox proteins to maintain intracellular redox homeostasis. Tumour cells are normally under increased oxidative stress (due to elevated intracellular ROS levels) by virtue of their increased cellular metabolic rate required to sustain their increased proliferation. Redox buffering systems in tumour cells are often deregulated (increased activity and/or overexpressed) compared with their normal counterparts — such deregulation can often lead to increased radioresistance [7–9]. As such, targeting redox proteins may be a useful strategy to increase the therapeutic efficacy of conventional radiotherapy. In this review, we summarise the role of redox proteins in regulating the radiosensitivity of tumour cells as well as their potential to predict therapeutic outcome and

Author for correspondence: S.G. Martin, Academic Unit of Clinical Oncology, University of Nottingham, School of Medicine, Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham NG5 1PB, UK. Tel: +44-115-823-1846; Fax: +44-115-823-1849.

E-mail address: stewart.martin@nottingham.ac.uk (S.G. Martin).

0936-6555/$36.00
http://dx.doi.org/10.1016/j.clon.2014.02.003

increase prognostication. Several types of redox modulator and their potential for use in radiotherapy are introduced.

Reactive Oxygen Species and Redox Homeostasis

'Redox' is the name given to describe two important processes: 'reduction' and 'oxidation'. Reduction is a decrease in oxidation state — the gain of electrons; whereas oxidation is an increase in oxidation state — the loss of electrons. Redox reactions represent the energy currency of living cells. ROS are a natural by-product of the normal cellular metabolism of oxygen. Common ROS include superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2). These are chemically reactive molecules containing oxygen with free radicals carrying one or more unpaired orbital electrons in their outer shell.

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad \text{(Reaction 1)}$$

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{(Reaction 2)}$$

$$H_2O_2 + e^- \rightarrow {}^{\bullet}OH + OH^- \quad \text{(Reaction 3)}$$

Mitochondria are a major source of ROS production, accounting for about 90% of the total cellular ROS generation. During mitochondrial oxidative metabolism, the primary free radical produced is the superoxide anion (reaction 1), which can be converted to hydrogen peroxide by superoxide dismutases (SODs) [10] (reaction 2). The hydrogen peroxide that is produced is subsequently converted to the hydroxyl radical with the assistance of partially reduced metal ions, in particular iron (reaction 3). A number of other endogenous ROS sources exist in mammalian cells, including dinucleotide phosphate (NADPH) oxidases (NOX) and dual oxidase in cell membranes, peroxisomes and a group of intracellular enzymes that produce ROS during their enzymatic functioning, including xanthine oxidase, cyclooxygenases, cytochrome p450 enzymes and lipoxygenases. ROS can also be induced by exogenous factors, such as ultraviolet light, pollutants, tobacco, drugs, xenobiotics and ionising radiation [11].

As indicated above, ROS play a dual role, either deleterious or beneficial, in living systems, with their local concentration dictating which is followed. At low/moderate concentrations, ROS can be beneficial: they are used by the immune system for killing pathogens [12] and can induce mitogenic responses [13]. ROS also play important roles in a number of cellular signalling pathways through covalent modification of specific cysteine residues in certain redox-sensitive proteins (reviewed in [6]). However, at high concentrations, ROS become detrimental and a threat to cells for a number of reasons. They can damage DNA (both the purine and pyrimidine bases and the deoxyribose backbone) (reviewed in [3–5]), oxidise polyunsaturated fatty acids in lipids [14] and also amino acids in proteins. The potential biological damage caused by increased ROS levels is termed oxidative stress [7] (Figure 1) and is, as discussed later, one of the major ways by which radiotherapy (conventional low-LET radiations) exerts its cytotoxic effects on cells. Intracellular ROS levels are regulated by antioxidant systems, which scavenge ROS in either an enzymatic or non-enzymatic manner. The delicate balance between ROS production and their clearance by various types of scavenger (i.e. antioxidants) is 'redox homeostasis' (Figure 1). Its maintenance is crucial to living organisms and prevents the damage caused by oxidative stresses. Alterations to redox homeostasis can directly influence the radioresponse of cells.

Antioxidants and Redox Proteins

Cells have a number of systems to regulate ROS levels and maintain redox homeostasis. They can be broadly categorised as non-enzymatic antioxidant and antioxidant enzymes (redox proteins) and are shown in Figure 2. The functions and regulation of each are briefly discussed below; their involvement in the regulation of the radiotherapy response is discussed later.

Non-enzymatic Antioxidants

As mentioned above, intracellular antioxidants can protect cells from the damage induced by ROS, as well as regulating redox-sensitive signalling pathways. Non-enzymatic antioxidant molecules include the water-soluble antioxidants glutathione (GSH), ascorbic acid (vitamin C), flavonoids and uric acid and lipid-soluble antioxidants α-tocopherol (vitamin E), carotenoids and vitamin A, α-Lipoic acid/dihydrolipic acid and ubiquinol/ubiquinone (coenzyme Q_{10}) (Figure 2).

Water-soluble Antioxidants

GSH and ascorbic acid are the most abundant non-enzymatic cellular antioxidants. As both are water soluble they perform their ROS scavenging activity primarily in the aqueous intracellular environment. The antioxidant function of GSH, and the GSH system as a whole, is discussed later. Ascorbic acid is the most effective water-soluble antioxidant found in human plasma and can scavenge reactive species such as hydroxyl, alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals. The ability to reduce the tocopheroxyl radical to tocopherol is another important characteristic of ascorbic acid.

Lipid-soluble Antioxidants

Lipid-soluble antioxidants provide defence against the peroxidation of lipids in the hydrophobic environment of cells and organelle membranes. α-tocopherol and ubiquinol scavenge reactive free radicals by becoming stable radicals themselves (i.e. tocopheroxyl radical and ubiquinone, respectively), which is a common mechanism among many radical-scavenging antioxidants. However, unlike many other antioxidants, both the oxidised (α-lipoic acid) and reduced dihydrolipic acid can serve as antioxidants, which can also reduce dehydroascorbic acid to generate lipoic acid and ascorbate.