Seminars in Cancer Biology 25 (2014) 23–32

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

The role of reactive oxygen species and metabolism on cancer cells and their microenvironment

Ana Costa[a,b,1], Alix Scholer-Dahirel[a,b,1], Fatima Mechta-Grigoriou[a,b,*]

[a] Stress and Cancer Laboratory, Institut Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, Paris France
[b] Inserm, U830, Paris, F-75248, France

ARTICLE INFO

Article history:
Received 30 July 2013
Received in revised form
22 December 2013
Accepted 30 December 2013

Keywords:
ROS
Oxidative stress
Mitochondria
Stroma
Myofibroblast
CAF heterogeneity
Actin
PDGFR
Caveolin

ABSTRACT

Compelling evidence show that reactive oxygen species (ROS) levels are finely regulated in the cell and can act as "second messengers" in response to diverse stimuli. In tumor epithelial cells, ROS accumulate abnormally and induce signaling cascades that mediate the oncogenic phenotype. In addition to their impact on tumor epithelial cells, ROS also affect the surrounding cells that constitute the tumor microenvironment. Indeed, ROS production increases tumor angiogenesis, drives the onset of inflammation and promotes conversion of fibroblast into myofibroblasts. These cells, initially identified upon wound healing, exhibit similar properties to those observed in fibroblasts associated with aggressive adenocarcinomas. Indeed, analyses of tumors with distinct severity revealed the existence of multiple distinct co-existing subtypes of carcinoma-associated fibroblasts (CAFs), with specific marker protein profiling. Chronic oxidative stress deeply modifies the proportion of these different fibroblast subtypes, further supporting tumor growth and metastatic dissemination. At last, ROS have been implicated in the metabolic reprogramming of both cancer cells and CAFs, allowing an adaptation to oxidative stress that ultimately promotes tumorigenesis and chemoresistance. In this review, we discuss the role of ROS in cancer cells and CAFs and their impact on tumor initiation, progression, and metastasis.

1. Introduction

ROS cellular levels are crucial for the cell fate. Accumulation of intracellular ROS in normal cells contributes to the oxidation of various components, including nucleic acids, proteins, and lipids [1]. These various oxidative reactions cause multiple damages, which usually promote apoptosis in case of overwhelming damages, but can also drive to abnormal proliferation and lead to transformation. Anti-oxidant mechanisms that can be either enzymatic (including catalases, dismutases, and peroxidases) or non-enzymatic (such as vitamin A, C, or E) are critical to protect cells against ROS-induced damages both at steady state and upon acute oxidative stress. In addition, low levels of intracellular ROS are recognized to be signaling molecules. Indeed both ROS concentration and sub-cellular compartmentalization are important for effective signaling. Interestingly, ROS were found in redox-active endosomes (redoxosomes) that may contribute to regulate their spatial and temporal regulation [2]. ROS accumulation in tumors has a wide and severe impact on various biological processes, eliciting proliferation, genomic instability, inflammation, resistance to apoptosis, and metabolic shift to glycolysis (Warburg effect). Ultimately, high levels of ROS in tumors contribute to tumorigenesis and tumor progression. Considering that the variation of ROS levels is a key early event in tumor initiation, understanding how ROS are generated is relevant and will be addressed in this review as a starting point. It is recognized that tumor microenvironment can drive the tumor aggressiveness and metastasis. In this regard, the reactivity of stromal components, such as CAFs, towards ROS plays a pivotal role in tumor initiation and sustains tumor growth and dissemination. This review outlines the role of ROS as signaling molecules, their role in myofibroblast conversion, metabolic reprogramming and implications for tumor growth, and progression.

2. ROS sources in cancer

ROS comprise a group of oxygen derivatives resulting from distinct oxidation status of O_2, including radical forms (having free or unpaired electrons), such as superoxide radical anion ($O_2^{\bullet -}$), carbon dioxide free-radical ($CO_2^{\bullet -}$), or hydroxyl free-radical (OH^-) and non-radical forms (such as hydrogen peroxide, H_2O_2) [3]. ROS are usually generated through a cascade of reactions that starts

* Corresponding author at: Stress and Cancer Laboratory, Institut Curie, Inserm U830, 26 rue d'Ulm, 75248 Paris Cedex 05, France. Tel.: +33 1 56 24 6653; fax: +33 1 56 24 6650.
E-mail address: fatima.mechta-grigoriou@curie.fr (F. Mechta-Grigoriou).
[1] These authors contributed equally to this work.

1044-579X/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.semcancer.2013.12.007

with the production of superoxide anions ($O_2^{\bullet -}$), which rapidly dismutate into H_2O_2 either spontaneously, at low pH for instance, or through catalysis by superoxide dismutase (SOD). Most of the damage caused by H_2O_2 and $O_2^{\bullet -}$ result from their conversion to even more reactive and toxic species, such as OH^-, generated from H_2O_2 through the Fenton reaction. Yet, because of its long-life and high permeability across membranes, H_2O_2 still remains the most reactive form of ROS for promoting signaling at long distance inside one single cell or even between two different cells. ROS molecules have a wide range of chemical reactivity and signaling properties, which will be addressed in this review regarding their role on CAFs and on tumorigenesis.

2.1. Methods to measure ROS

ROS measurement in cells and tissues is of great interest. However, caution should be taken as ROS can be compartmentalized at subcellular levels. Likewise, ROS scavengers can also distort the assessment of ROS levels in the cells [4]. The detection method to use depends on the form of ROS to be detected. One of the most common methods to detect intracellular ROS levels relies on the use of cell-permeant ROS-sensitive fluorescent dyes, such as 2′,7′-dichlorofluorescein (DCF). The carboxy-derivative of DCF, 5,6-chloromethyl 2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) carries additional negative charges that improve its retention compared to noncarboxylated forms. After diffusion into the cell, DCFDA is deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS. DCF is a highly fluorescent compound, which can be detected by fluorescence spectroscopy with maximum excitation and emission spectra of 495 nm and 529 nm, respectively. DCF compounds being trapped into the cells by deacetylation immediately after passive diffusion into the cytoplasm, their use ensures specific labeling of intracellular ROS contents, and thus strictly excluding staining of ROS produced by the cells in the extracellular medium. DCF compounds are oxidized by various ROS, including HO• (Hydoxyl radical), superoxide anion ($O_2^{\bullet -}$), peroxyl radical (ROO•), peroxynitrite anion ($ONOO^-$), and thus allow specific quantification of these species. The increase in dye-fluorescence and thus quantification of ROS production are the most often assessed using a flow cytometer, as shown in [5,6], but can also be evaluated, although with less precision, by fluorescence microscopy [7]. Another method commonly used for ROS measurements is based on electron paramagnetic resonance spectroscopy (ESR). ESR detects the absorption of microwave energy, which occurs on transition of unpaired electrons in an applied magnetic field. ESR is thus the method of choice for detecting compounds with unpaired electrons, such as $O_2^{\bullet -}$ or OH•. These species are paramagnetic molecules and are highly reactive when submitted to a magnetic field. Thus, they can be quantified by this methodology, as shown in [8]. Finally, in contrast to the DCF-based method, which detects a variety of ROS, the Amplex Red assay allows the specific detection of H_2O_2. This assay is based on the oxidation of the Amplex Red molecule by horseradish peroxidase and H_2O_2 to resorufin, which is fluorescent. One caveat of this technique is the instability of the Amplex Red dye. Finally, $O_2^{\bullet -}$ levels can be detected by reduction of ferricytochrome C to ferrocytochrome C by $O_2^{\bullet -}$ Ferricytochrome reduction results in increased spectrophotometric absorbance in proportional to superoxide levels. However, because other enzymes, such as xanthine oxidase, can also participate in the cytochrome C reduction, this assay must be performed in the presence and absence of SOD [9]. Overall, ROS are short-lived molecules. This property makes them difficult to measure and the use of various methods to determine ROS levels is advisable.

2.2. Main sources of ROS

Chronic oxidative stress can be the result of an imbalance between radical-generating and radical-scavenging systems. Endogenous ROS are generally produced as a byproduct of biological reactions involved in energy metabolism and occurring mainly within mitochondria or peroxisomes. In addition, the $NADPH^-$ oxidases (NOX) were the first enzymes identified as being able to generate ROS by themselves. Initially isolated from phagocytes, this family of enzymes was rapidly found in many other tissues. In this review, due to their importance regarding cancer regulation, we will focus on two main sources of intracellular ROS: mitochondria and NOX. Mitochondrial ROS are generated as a byproduct of the electron transport chain. In the mitochondria two sites (site IQ- complex I and site IIIQo- complex III) have been recognized as the predominant producers of ROS, such as superoxide anions [10]. It is generally considered that two major conditions lead to significant $O_2^{\bullet -}$ production: when the electron transport chain is defective in ATP production and levels of NADH/NAD^+ are high [11]. While other sites have been shown to release superoxide anions to the mitochondrial matrix, mainly complex III produces superoxide anions to both matrix and inter-membrane space. Superoxide anions located in the inter-membrane space of the mitochondria have been proposed to have implications to signaling in the cytoplasm [12] as they might access to cytoplasm. Regardless the mitochondrial site of production, superoxide anions are rapidly converted to H_2O_2 by superoxide dismutase 2 (SOD2), located in the mitochondrial matrix. Other SOD enzymes, namely SOD1 and SOD3 (located respectively in the cytoplasm and in the extracellular matrix) also play an important role in regulating mitochondrial ROS. The second main source of intracellular ROS in tumor cells is the family of $NADPH^-$ oxidases (NOX1-5 and DUOX1-2) [13]. All NOX enzymes are transmembrane proteins (plasma membrane, endoplasmatic reticulum and mitochondria) that transport electrons across biological membranes to reduce oxygen into superoxide anions, which are further a target of SOD1 for the production of H_2O_2. Due to place restriction, we will focus below on these two major sites of ROS production in cancer cells, the mitochondria and the NOX enzymes.

2.3. Origins of oxidative stress in cancers

Tumor epithelial cells produce high levels of ROS resulting in constant exposition to oxidative stress. Indeed, high levels of ROS have been detected in several cancers, such as in breast, ovarian, liver, or colon cancers. ROS in tumor epithelial cells can derive from various causes including increased metabolism associated with dysfunctional mitochondria, oncogene activity, abnormal expression of NOX enzymes (especially NOX1 and NOX4), dysfunction of cycloxygenases (COX), lipoxygenases (LOX), and thymidine phosphorylase or altered anti-oxidant defenses (Fig. 1) [14]. One of the main sources of ROS in tumor epithelial cells arises from mitochondrial dysfunction coupled with the metabolic readjustment to generate ATP. The highly proliferative rates exhibited by tumor cells require high levels of energy and building blocks for biomass production. Despite this high energy requirement, cancer cells shift their oxidative metabolism to aerobic glycolysis, a process referred to as the Warburg effect, which is observed regardless the oxygenation levels. Indeed, aerobic glycolysis is favored compared to oxidative phosphorylation despite the low ATP amount being produced and independently of the presence of O_2. This process is referred to as the Warburg effect and is one of the main hallmarks of cancer cells. In this context, the electron transport is compromised, but the complex I and complex III of the respiratory chain are still active. This condition is associated with a high membrane potential ($\Delta\Psi$), leading to an increased leakage of electrons and an abnormal NADH/NAD^+ ratio, which result into significant $O_2^{\bullet -}$ production