Imaging mitochondrial redox potential and its possible link to tumor metastatic potential

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Abstract Cellular redox states can regulate cell metabolism, growth, differentiation, motility, apoptosis, signaling pathways, and gene expressions etc. A growing body of literature suggest the importance of redox states for cancer progression. While most studies on redox state were done on cells and tissue lysates, it is important to understand the role of redox state in a tissue in vivo/ex vivo and image its heterogeneity. Redox scanning is a clinical-translatable method for imaging tissue mitochondrial redox potential with a submillimeter resolution. Redox scanning data in mouse models of human cancers demonstrate a correlation between mitochondrial redox state and tumor metastatic potential. I will discuss the significance of this correlation and possible directions for future research.

Keywords Cancer aggressiveness · Fluorescence · Redox scanning · NADH · FAD or flavoprotein

Introduction

As a hallmark of cancer, abnormal metabolism has taken the center stage of research in recent years (Pedersen 2007; Christofk et al. 2008; Hsu and Sabatini 2008; Cairns et al. 2011; Hanahan and Weinberg 2011). Most cancers exhibit the Warburg effect – increased glucose consumption even in the presence of oxygen, on which FDG-PET (fluorine-18-2-D-deoxyglucose positron emission tomography) is based to stage tumors and monitor treatment response (Quon and Gambhir 2005; Mac Manus and Hicks 2008). In addition, mitochondrial bioenergetic/genetic abnormalities have been shown to mediate carcinogenesis and tumor progression (King et al. 2006; Modica-Napolitano et al. 2007; Mayevsky 2009; Kaelin and Thompson 2010). Genetic mutations have been identified in cancer patients for certain mitochondrial metabolic enzymes in the TCA cycle including isocitrate dehydrogenase, succinate dehydrogenase and fumarase (Thompson 2009). The expressions of genes or activities of proteins known to drive tumor progression such as Myc/HIF1α/p53 have been shown to regulate cellular metabolism including mitochondrial metabolism (Dang 1999; Semenza 2010; Cairns et al. 2011). On the other hand, tumor microenvironment and metabolism may be upstream regulators of signaling pathways (Hsu and Sabatini 2008). Therefore, it has become increasingly important to understand the interwined relationship among tumor signaling pathways, metabolism, and microenvironment.

Maintenance of redox state homeostasis has been regarded as important for cancer cells (Dorward et al. 1997; Grek and Tew 2010; Cairns et al. 2011; Locasale and Cantley 2011). As a matter of fact, tremendous research studies (Puppi and Dely 1983; Dorward et al. 1997; Adler et al. 1999; Nkabyo et al. 2002; Weir et al. 2002; Cook et al. 2004; Olschewski et al. 2004; Agarwal and Auchus 2005; Ido 2007; Sattler et al. 2007; Banerjee 2008; Ying 2008; Gough 2009; Maccarrone and Brune 2009; Pani et al. 2009; Sarsour et al. 2009; Grek and Tew 2010; Ishimoto et al. 2011) have demonstrated or implicated redox state as a key mediator of many cellular functions and activities including metabolism, growth, differentiation, cell cycle, motility/invasion, apoptosis, survival, immunological response, oxidative stress, gene transcription, and signaling (Fig. 1). Some studies have implied a connection between the redox potentials (or NADH levels) and the metastatic potential of cancers (Zhang et al. 2006; Ishikawa et al. 2008b; Pan et al. 2009; Pelicano et al. 2009; Grek and Tew 2010). Reactive oxygen species (ROS) are known to cause oxidative stress on proteins, lipids, DNA/RNAs and also act as signaling molecules to drive cancer cell motility/invasion and tumor progression. ROS can
induce a higher risk of metastasis either by causing more DNA mutagenesis or regulating tumor progressions directly by enhancing cell invasion and metastasis. A mitochondrial DNA mutation encoding a subunit of NADH dehydrogenase (complex I) was shown to control the development of metastasis in animal models by generating more ROS, which, in turn, directly regulates certain nuclear genes that promote metastasis (Ishikawa et al. 2008a, b). However, a high level of ROS or oxidants does not necessarily indicate more oxidized redox potential. It has been known that tumors with high levels of ROS are often counter-balanced with high levels of reductants such as vitamin C, reduced glutathione (GSH) and NADPH (Hyodo et al. 2006; Pelicano et al. 2009; Pani et al. 2010; Keshari et al. 2011). It is the balance between oxidants and reductants that define the cellular redox potential. Still, redox potential is a complex issue due to multiple intracellular redox systems and their dependence on subcellular compartments (cytosol, nuclear, mitochondrion, etc.). Currently the relationship between cellular redox potential and cancer metastatic potential is far from clear.

Most prior work on redox status was done on the molecular and cellular levels under in vitro conditions or on tissue lysates. To investigate the role of redox potential in tumor progression, it is necessary to image the redox status and its spatial distribution in tissue. The tissue heterogeneity in functional/metabolic/genomic status has been regarded as an important characteristic for malignancy (Gaustad et al. 2005; Schroeder et al. 2005; Gerlinger et al. 2012; Shah et al. 2012). Intra-tumor heterogeneity has been shown to be an important factor for studying tumor metastasis (Nowell 1976; Fidler and Kripke 1977; Fidler and Hart 1982). The heterogeneity in a tumor metabolic microenvironment can occur on a small distance <1 mm (Mueller-Klieser et al. 1991; Li et al. 2009b; Xu et al. 2010). Therefore, effective sub-millimeter imaging methods are needed to measure the tumor redox state in vivo/ex vivo. Redox imaging on the basis of the fluorescence signals from NADH and flavoproteins is the only clinically-translatable method that can achieve 3D imaging of the tissue mitochondrial redox state at a submillimeter resolution.

In this mini-review we will cover some basic biological roles of NAD(H) and flavins, and the principles and methodology of mitochondrial redox imaging. We will then review the work studying the link of mitochondrial redox potential to tumor metastatic potential using the redox imaging. In the end, we will discuss the significance of these studies in terms of basic research and clinical management for cancer.

**NAD(H), flavins and mitochondrial redox imaging**

As universal free energy carriers in bioenergetics, NAD+ (oxidized nicotinamide adenine di-nucleotides) and NADH mediate a number of oxidation-reduction reactions along pathways of energy metabolism. By controlling glycolysis in the cytosol and the Krebs cycle in mitochondria, the redox potential NAD+/NADH is linked to the phosphorylation potential [ATP]/([ADP]·[Pi]) in living tissues and provides a key parameter for the metabolic control of normal and diseased phenotypes (Veech 2006). In addition, NAD+/NADH is a key component in cellular redox homeostasis as NAD(H) is coupled to NADP(H) by transhydrogenase activity (Lemasters and Nieminen 2001) and, thus, can indirectly affect the oxidation-reduction couples of glutathione and thioredoxin systems as well (Banerjee 2008). These redox couples and related redox-sensitive enzymes may affect almost all major signaling pathways including p53, PI3K and MAPK (Adler et al. 1999; Olovnikov et al. 2009). Accumulating evidence has shown that NAD+ is also a key signaling molecule serving as a precursor to calcium-releasing agents and a substrate for protein modification of transcription factors by PARP (poly-ADP-ribosylation polymerase) (Banerjee 2008). NAD+ can mediate many cellular activities including signaling, reactive oxygen species (ROS) generation, growth, differentiation, survival, and apoptosis (Ziegler 2005; Orrenius et al. 2007; Ying 2008).

In addition to NAD(H), another group of redox-important molecules flavin nucleotides including flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) also play important roles in various biological processes including metabolism and signaling events (Lehninger et al. 1993; Taylor et al. 2001; Senda et al. 2009; Becker et al. 2011) FAD or FMN are coenzymes or prosthetic groups for various flavoproteins including the NADH dehydrogenase (complex I) and pyruvate dehydrogenase in mitochondria. These flavoproteins are quite often coupled with NAD+/NADH. FADH2 is also a free energy carrier in electron transport and the FAD-coupled redox potential FAD/FADH2 regulates key reactions in the TCA cycle, oxidative...