Electrochimica Acta 179 (2015) 65–73

Contents lists available at ScienceDirect

Electrochimica Acta

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

Scanning electro-chemical microscopy reveals cancer cell redox state

Stefania Rapino [a,b,*,1], Raluca Marcu [a,2], Alessandra Bigi [a], Alice Soldà [a,b,1], Massimo Marcaccio [b,1], Francesco Paolucci [b,1], Pier Giuseppe Pelicci [a], Marco Giorgio [a,**]

[a] Experimental Oncology Department, European Institute of Oncology, Via Adamello 16, 20139, Milan, Italy
[b] Chemistry Department "G. Ciamician", University of Bologna, Via Selmi 2, 40126, Bologna, Italy

ARTICLE INFO

Article history:
Received 24 December 2014
Received in revised form 5 April 2015
Accepted 8 April 2015
Available online 9 April 2015

Keywords:
Scanning Electrochemical Microscopy
Cancer Diagnostics
Ras transformation
Redox balance
Glutathione

ABSTRACT

Cancer cells show an abnormal balance of reduced/oxidised species and the detection of cancer redox balance can be exploited for diagnostic purposes. In particular, it is known that the expression of oncogenic Ras changes the intracellular oxidised/reduced glutathione balance. Electrochemical imaging of living cells is currently feasible by scanning electrochemical microscopy and was used here, using ferrocenemethanol as redox mediator, to measure the oxidised/reduced glutathione balance in human breast epithelial MCF10A cells expressing constitutively active Ha-Ras Val12 mutant compared to normal MCF10A cells. Oxidized ferrocenemethanol is reduced by glutathione and the resulting maps of current over cell cultures were different for transformed cells compared to normal cells. Furthermore, scanning electrochemical microscopy using ferrocenemethanol redox mediator was able to distinguish human lung carcinoma cells from the surrounding normal epithelium of clinical specimens. We propose that scanning electrochemical microscopy analysis using specific redox mediator such as ferrocenemethanol may become a useful tool in cancer diagnostics.

1. Introduction

Cancer is characterized by an abnormal metabolism [1,2], O_2 utilisation [3] and increased levels of reactive oxygen species (ROS) (see [4] for review) resulting in a change of the balance between reduced/oxidised species (i.e. redox balance) [5]. Changes in cellular redox balance affect proliferation, differentiation and survival, contributing to disease progression [6–8]. The expression of oncogenes, such as Myc [9], Bcl-2 [10] or Ras [11], has been reported to affect redox balance. In particular, oncogenic Ras was found both to increase [12] and to decrease [13] the level of reduced glutathione (GSH) depending on the cell line, whereas antioxidant treatment has been shown to prevent oncogene-induced transformation [14] and senescence [15].

GSH is an ubiquitous tripeptide, present in the millimolar range in cells, which takes part in several cellular reaction pathways [16] and is critical to maintain the intracellular redox balance [17]. GSH is oxidized to glutathione disulfide (GSSG) by glutathione peroxidase and peroxiredoxins during hydrogen peroxide scavenging, and by glutaredoxins during the reduction of protein disulfide bonds. GSSG is reduced back to GSH by the glutathione reductase while oxidizing pyrimidine dinucleotides and by glutathione dehydrogenase while oxidizing ascorbate. Therefore, the GSH/GSSG ratio acts as an indicator for a broad range of the cellular redox balance species. The GSH/GSSG ratio has been found altered in tumors where high GSH concentration would favor the escape of the senescence tumor-suppressive barrier induced by Ras activation [11].

Redox balance can be measured by means of scanning electrochemical microscopy (SECM), which can monitor the release of redox species from living cells or the interaction of exogenously added redox species (i.e. redox mediators) with living cells providing information on cellular metabolism and/or topography (on MCF10A [18], Huvec [19], NRK52E [20], PC12 [21], HeLa [22], and HepG2 [23] cells, and see the chapters 11 and 12 of [24] for a comprehensive review). In case the redox mediator does not interact with the living cell, the recorded SECM current decreases while approaching the cell resulting in a purely negative feedback. Alternatively, an increase of current - with respect to the purely negative feedback - may be recorded when the redox mediator, previously converted at the UME, reacts with some cellular component capable of regenerating the redox mediator back to its pristine form (regeneration current). A number of redox

* Corresponding author.
** Corresponding author at: Experimental Oncology Department, European Institute of Oncology, Via Adamello 16, 20139, Milan, Italy. Tel.: +390294375040.
E-mail addresses: stefania.rapino3@unibo.it (S. Rapino), marco.giorgio@ieo.eu (M. Giorgio).
[1] ISE member.
[2] Present address: Mitochondria and Metabolism Center, 815 Mercer Street, Seattle, USA.

http://dx.doi.org/10.1016/j.electacta.2015.04.053
0013-4686/

probes was previously reported for the investigation of normal and metastatic living cells by SECM [25,26]. SECM was also used for the real time investigation of cell membrane permeability [27]. The use of ultramicroelectrodes was reported for the detection of reactive oxygen species (ROS) and neurotransmitters from single cells [28] and nanoelectrodes were used for the investigation of ROS inside living cells [29].

Herein, we report the results of SECM experiments aimed at investigating the glutathione disulfide/glutathione balance, which is directly correlated to the overall cell redox balance, in normal and transformed/cancer cells to assess the cancer related alterations. As depicted in the scheme in Fig. 1, we used ferrocenemethanol to mediate the sensing of glutathione disulfide/glutathione balance at the SECM ultramicroelectrode.

2. Experimental

2.1. Cellular Metabolism

Respiration of intact cells was measured using a Clark-type oxygen electrode (Hansatech Instruments) as described in [30]. Lactate release expressed in μmol/mL/hour/mg protein was measured with an EnzyChrome Lactate Assay kit (BioAssay Systems). Glucose consumption expressed in μM/ml/hour/mg protein was measured with Assay kit (Bioassay Systems).

2.2. Measurement of the GSH/GSSG ratio

For the measurement of glutathione redox ratio (GSH/GSSG) we used the Grx1-roGFP2 fluorescent sensor [31]. MCF10A cells expressing Grx1-roGFP2 were analysed by fluorescence activated cell sorting (FACS) using an excitation wavelength of 488 nm. The fluorescence was normalised to control untreated cells infected with the empty vector.

2.3. SECM measurements

SECM measurements were performed using an experimental setup coupling a 910B SECM (CH Instruments) with a Nikon ECLIPSE 200 inverted optical microscope. The stepper motors and the piezoelectric component of the 910B CHI instrument for the microelectrode displacement were removed from the original stage and mounted on the plate of the inverted microscope. The apparatus modifications for the optical/electrochemical microscope coupling were accomplished in house in our workshop [32,33].

All electrochemical measurements were carried out in a 35 mm Petri dish located on the plate holder of the inverted microscope and used for the cell culturing. The SECM probes were 10 μm Pt diameter ultra-microelectrodes with a ratio of the glass sheath and the active electrode radius, RG = 10. The tips were polished and cleaned prior the use with polishing clothes with alumina (0.05 mm) followed by sonication for some seconds in a bath sonicator. A platinum wire was used as the counter electrode and all the potentials are referred to the (Ag/AgCl/3 M KCl) reference electrode. Ruthenium hexaammine ($[Ru(NH_3)_6]Cl_3$, Ruhex), Ferrocenemethanol and ferrocenecarboxylic acid were purchased from SIGMA-Aldrich (Sigma-Aldrich) and were used as received. Feedback mode measurements for the topographical characterization were performed in 0.5 mM Ruhex at −0.35 V for control and RasV12 transformed cells, in 0.5 mM of Fc(COOH) at 0.5 V for lung cells from patient, these concentrations of the redox mediator were not affecting the cell viability.

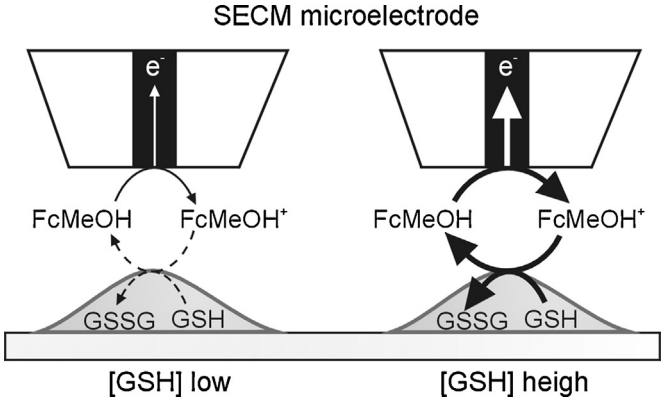

Fig. 1. Scheme of the strategy adopted. The overall intracellular redox balance is investigated using SECM with $FcCH_2OH$ as redox mediator.

Using the negative feedback signal over the plastic dish, the UME was positioned over a cell free region of the plastic dish at a controlled tip/Petri dish distance. SECM images were accomplished at a tip/Petri dish distance of 11 μm, ensuring that the microelectrode did not get in mechanical contact with the cells during lateral scanning. The translational rate of the UME for the SECM images was 20 μm/s. The selected area for SECM imaging was flat as confirmed by SECM approach curves experiments. Approach curves on single cells were accomplished until the UME/cell zero-separation point was established. A sudden change in the recorded current approach curve slope indicated the contact point. The approaches were also followed online by optical microscopy revealing a slight change in the cell shape as soon as the mechanical contact was achieved.

2.4. Cell culture

MCF10A cells (ATCC: crl-10317) were cultured in (1:1) Dulbecco's Modified Eagle's Medium (DMEM)/Nutrient Mixture F-12Ham (that do not contain glutathione) (Gibco-Life Technologies Corporation) supplemented with 5% horse serum, 20 ng/ml epidermal growth factor (EGF), 50 ng/ml cholera toxin, 500 ng/ml hydrocortisone and 0.01 mg/ml insulin (Gibco-Life Technologies Corporation) [34]. Cells were infected using a puromycin-resistant retroviral construct containing an oncogenic form of Ras (pBabe-RasV12) or using the empty vector (pBabe) as described in [35]. Forty-eight hours post infection cells were selected using 2 μM puromycin for 4 days.

Primary cells were obtained from the IEO Biobank in agreement with the current policy for clinical specimen acquiring and following the notification to the legal representative of the ethical committee of the hospital. Cells were prepared by collagenase type I digestion of fresh biopsies and then cultured in (1:1) F-12Ham/DMEM supplemented with 1% fetal bovine serum, glutamine, penicillin-streptomycin, gentamicin, amphotericin, transferrin, 1 μg/ml insulin, 1 μg/ml hydrocortisone, 10 mM Hepes pH 7.5, 50 μg/ml ascorbic acid, selenite, ethanolamine, 50 ng/ml cholera toxin, 10 nM EGF, 35 μg/ml bovine pituitary extract, 10 nM T3 and 10 nM β-Estradiol (from Gibco-Life Technologies Corporation and Sigma-Aldrich). Cells were passed upon trypsin digestion. Passage 3 of these cells was used for biochemical and SECM analysis.

2.5. Protein extraction, SDS-PAGE and Western blotting

Cells were washed with ice-cold PBS and lysed in RIPA buffer, 1 mM PMSF, and both protease and phosphatase inhibitors. After lysis on ice for 30 min, cells were centrifuged at 18,000 g for 15 min, and supernatants analysed for protein content. Protein concentration of lysates was determined by the Bradford assay (Bio-Rad). Equal amounts of protein were separated on Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis (SDS-PAGE) and