Redox Biology 12 (2017) 499–504

Contents lists available at ScienceDirect

Redox Biology

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

Research Paper

Hypoxia and hydrogen sulfide differentially affect normal and tumor-derived vascular endothelium

Serena Bianco[a], Daniele Mancardi[d], Annalisa Merlino[a], Benedetta Bussolati[c], Luca Munaron[a,b,*]

[a] Department of Life Sciences & Systems Biology, University of Torino, Italy
[b] Nanostructured Interfaces and Surfaces Centre of Excellence (NIS), University of Torino, Italy
[c] Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy
[d] Department of Clinical and Biological Sciences, University of Torino, Italy

ARTICLE INFO

Keywords:
Hydrogen sulfide
Human microvascular endothelial cells
Human breast carcinoma-derived EC
Human renal carcinoma-derived EC
Tumor angiogenesis

ABSTRACT

Background: endothelial cells play a key role in vessels formation both under physiological and pathological conditions. Their behavior is influenced by blood components including gasotransmitters (H_2S, NO and CO). Tumor cells are subjected to a cyclic shift between pro-oxidative and hypoxic state and, in this scenario, H_2S can be both cytoprotective and detrimental depending on its concentration. H_2S effects on tumors onset and development is scarcely studied, particularly concerning tumor angiogenesis. We previously demonstrated that H_2S is proangiogenic for tumoral but not for normal endothelium and this may represent a target for antiangiogenic therapeutical strategies.

Methods: in this work, we investigate cell viability, migration and tubulogenesis on human EC derived from two different tumors, breast and renal carcinoma (BTEC and RTEC), compared to normal microvascular endothelium (HMEC) under oxidative stress, hypoxia and treatment with exogenous H_2S.

Results: all EC types are similarly sensitive to oxidative stress induced by hydrogen peroxide; chemical hypoxia differentially affects endothelial viability, that results unaltered by real hypoxia. H_2S neither affects cell viability nor prevents hypoxia and H_2O_2-induced damage. Endothelial migration is enhanced by hypoxia, while tubulogenesis is inhibited for all EC types. H_2S acts differentially on EC migration and tubulogenesis.

Conclusions: these data provide evidence for a great variability of normal and altered endothelium in response to the environmental conditions.

1. Introduction

During physiological tissue development, vasculature organization and sprouting varies to meet blood demand. In solid tumors, however, the abnormal cell proliferation and vascular disorganization cause an insufficient blood supply, leading to glucose deprivation and hypoxia [1]. The hypoxic environment of tumors is heterogeneous, both spatially and temporally, and can change in response to cytotoxic therapy. Carcinomas usually support their growth by stimulating blood vessel development (angiogenesis) [2,3]. Blood flow within these new vessels is often chaotic, causing alternating periods of hypoxia followed by re-oxygenation. Reperfusion is well established to cause generation of reactive oxygen species (ROS) exacerbating the ischemic injury especially after myocardial or cerebral ischemia [4,5]. Oxygen radicals production during reperfusion may, therefore, represent a source of ROS also within breast carcinomas and other solid tumors. In addition, in breast tumor, glucose deprivation rapidly induces cellular oxidative stress, as demonstrated in MCF-7 breast carcinoma cell line, although it does not cause oxidative stress in non-transformed cell lines [6]. This may be due to glucose deprivation, which depletes intracellular pyruvate within the breast carcinoma cell, preventing the decomposition of endogenous oxygen radicals.

In recent years increasing evidences emerged on the role of the bioactive gaseous molecule H_2S in normal and tumor vascularization [7,8]. In vascular endothelium H_2S may act as vasorelaxant and anti-inflammatory agent as well as a powerful stimulator of angiogenesis. Indeed, H_2S promotes mitosis, migration and tubulogenesis in human umbilical vein EC (HUVEC) and in the retinal endothelial cell (EC) line RF/6A [9,10].

Oxidative stress promotes cell death in response to a variety of pathophysiological conditions and an excessive amount of ROS, including hydrogen peroxide (H_2O_2), leads to lethal vascular endothe-

* Corresponding author at: Department Life Sciences & Systems Biology University of Torino, Via Accademia Albertina 13, 10123 Torino, Italy.
E-mail addresses: serena.bianco@unito.it (S. Bianco), daniele.mancardi@unito.it (D. Mancardi), benedetta.bussolati@unito.it (B. Bussolati), luca.munaron@unito.it (L. Munaron).

http://dx.doi.org/10.1016/j.redox.2017.03.015
Received 15 December 2016; Received in revised form 13 March 2017; Accepted 15 March 2017
Available online 18 March 2017

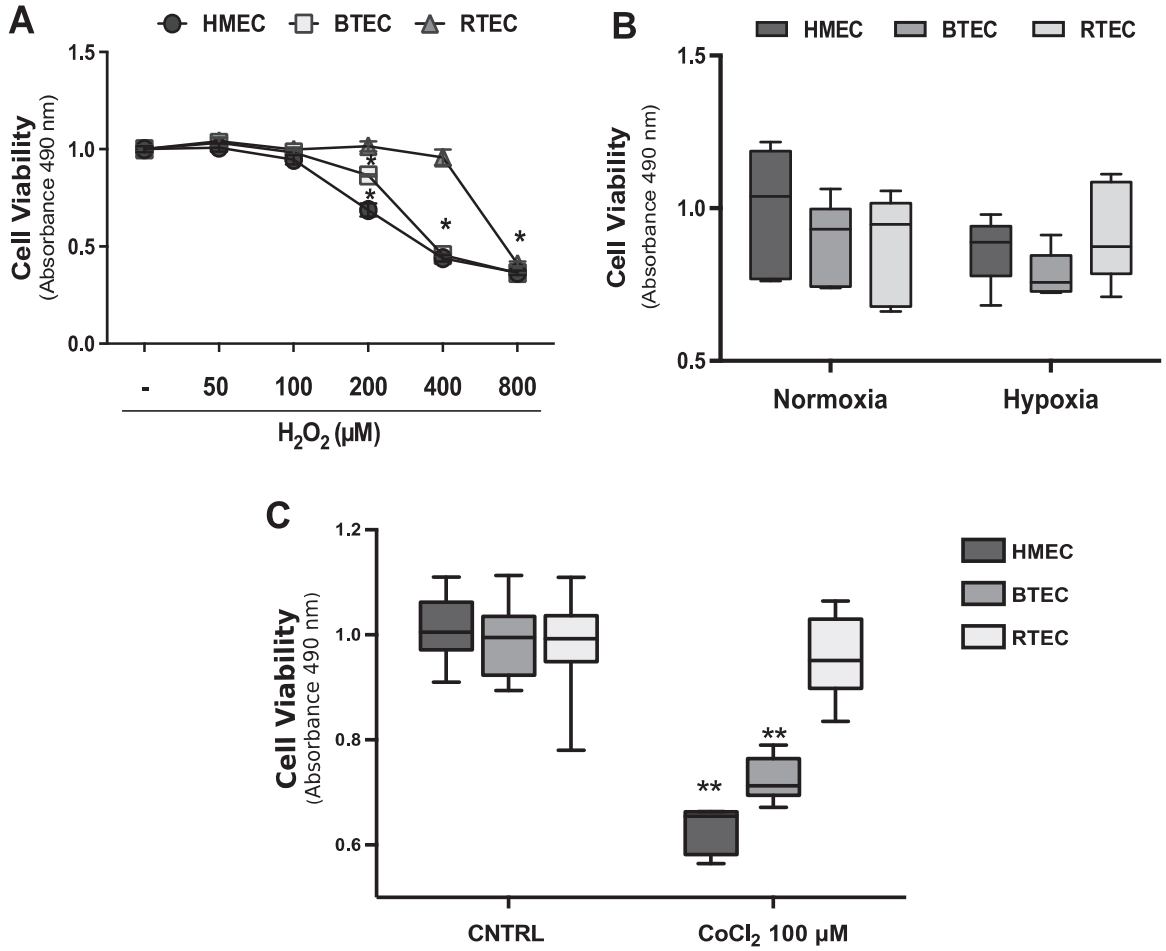

Fig. 1. High H_2O_2 and chemical hypoxia are toxic for EC. A) Representative dose-response curve of cytotoxicity assay on HMEC, BTEC and RTEC upon treatment with H_2O_2 (50–800 μM; 24 h). Data are expressed as the mean ± S.E.M Wilcoxon test: *p < 0.0001 vs CNTRL. B) Cytotoxicity experiment (24 h) performed on EC under normoxic or hypoxic conditions (see Section 2), in DMEM 1% FCS medium. Data from a representative experiment are expressed as the mean ± S.E.M. Wilcoxon test. C) Viability assay on EC treated with $CoCl_2$ (100 μM; 24 h). Data from a representative experiment are expressed as the mean ± S.E.M. Wilcoxon test: **p < 0.001 vs CNTRL. CNTRL: DMEM 1% FCS medium.

lial cell injury [11]. Hydrogen sulfide has been shown to be protective against H_2O_2-induced oxidative stress: for example, 25–100 μM NaHS enhances cell survival upon H_2O_2 treatment in H9c2 cardiomyoblasts [12].

We recently described the effects of H_2S on microvascular EC obtained from human breast carcinoma (BTEC). Ca^{2+} imaging and patch-clamp experiments revealed that acute perfusion with NaHS (a saline H_2S donor) induces [Ca_i] increases, as well as [K^+_i] and non-selective cationic currents [13]. Stimulation with NaHS, at the same concentration range (1 nM–200 μM), evoked Ca_i signals also in normal human microvascular EC (HMVEC), although the amplitude was significantly lower. Conversely, doses lower than 10 μM of NaHS did not evoke any detectable elevation in Ca_i in the excised endothelium of rat aorta. Moreover, NaHS failed to promote either migration or proliferation on HMVEC, while BTEC migration was enhanced at low-micromolar NaHS concentrations (1–10 μM) [13]. Remarkably, pretreatment with an inhibitor of endogenous H_2S production (PAG), drastically reduced migration and Ca_i signals induced by Vascular Endothelial Growth Factor (VEGF) in BTEC. These data suggest that H_2S plays a role in proangiogenic signaling of tumor-derived but not normal human EC pointing to a potential differential sensitivity of normal EC compared to TEC that could be an interesting tool for tumor angiogenesis treatment. Furthermore, its ability to interfere with BTEC responsiveness to VEGF suggests that it could be an interesting target for antiangiogenic strategies in tumor treatment.

Here we unveil a great variability in the functional responses to hypoxia and oxidative stress and their regulation by exogenous H_2S in endothelial cells derived from two different human carcinomas as well as normal microvascular endothelium.

2. Materials and methods

2.1. Cell cultures

Breast tumor-derived endothelial cells (BTEC) and renal tumor-derived endothelial cells (RTEC) from human breast lobular-infiltrating carcinoma biopsy and renal carcinoma respectively, were isolated and periodically characterized in the laboratory of Professor Benedetta Bussolati (Department of Internal Medicine, Molecular Biotechnology Center and Research in Experimental Medicine Center, University of Torino, Italy). BTEC and RTEC were grown in EndoGRO-MV-VEGF Complete Media Kit, composed of EndoGRO Basal Medium and EndoGRO-MV-VEGF Supplement Kit (Merck Millipore). EndoGRO Basal Medium is a low-serum culture media for human microvascular endothelial cells, supplemented with a kit containing rhVEGF (5 ng/ml), rhEGF (5 ng/ml), rhFGF (5 ng/ml), rhIGF-1 (15 ng/ml), L-glutamine (10 mM), hydrocortisone hemisuccinate (1.0 μg/ml), heparin sulfate (0.75 U/ml), ascorbic acid (50 μg/ml), fetal bovine serum FCS 5%. Human microvascular endothelial cells (HMEC) are dermal-derived cells purchased from Lonza. HMEC, as well as BTEC, were grown in EndoGRO-MV-VEGF Complete Media Kit.

All cell cultures were maintained in normoxic (37 °C, 21% O_2 and 5% CO2) and, when needed, hypoxic (37 °C, 5% O_2 and 5% CO2) incubator (InVIVO2 200 equipped with a Ruskinn Gas Mixer Q) using