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Research Article

Dedifferentiation of cancer cells following recovery from a potentially lethal damage is mediated by H_2S–Nampt

Elena A. Ostrakhovitch[a], *Shin Akakura*[a], *Reiko Sanokawa-Akakura*[a], *Scott Goodwin*[b], *Siamak Tabibzadeh*[a,b,*]

[a]*Frontiers in Bioscience Research Institute in Aging and Cancer, Irvine, CA 92618, USA*
[b]*Department of Radiological Sciences, University of California, Irvine, CA 92868, USA*

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ABSTRACT

Recently, we reported that cancer cells that recover from a potentially lethal damage gain new phenotypic features comprised of mitochondrial structural remodeling associated with increased glycolytic dependency and drug resistance. Here, we demonstrate that a subset of cancer cells, upon recovery from a potentially lethal damage, undergo dedifferentiation and express genes, which are characteristic of undifferentiated stem cells. While these cells are competent in maintaining differentiated progeny of tumor, they also exhibit transdifferentiation potential. Dedifferentiation is characterized by accumulation of hydrogen sulfide (H_2S), which triggers up-regulation of nicotinamide phosphoribosyltransferase (Nampt) accompanied by changes in the redox state. The molecular events triggered by Nampt include elevated production of NAD^+ and up-regulation of H_2S producing enzymes, cystathionine beta synthase (CBS) and cystathionase (CTH) with 3-mercaptopyruvate sulfurtransferase (MST) being detectable only in 3D spheroids. Suppression of Nampt, or inactivation of H_2S producing enzymes, all reduce H_2S production and reverse the ability of cells to dedifferentiate. Moreover, H_2S induced stem cell markers in parental cancer cells in a manner similar to that observed in damage recovered cells. These data suggest of existence of a positive feedback loop between H_2S and Nampt that controls dedifferentiation in cancer cells that recover from a potentially lethal damage.

Abbreviations: CBS, Cyctathionine beta synthase; CTH, Cystathionase; MST, 3-mercaptopyruvate sulfurtransferase; PLDR, potentially lethal damage recovery; CHH, O-(carboxymethyl) hydroxylamine hemihydrochloride, inhibitor of Cyctathionine beta synthase; PAG, DL-propargylglycine, inhibitor of cystathione gamma-lyase; DR^{G-}, Glucose deprivation induced DR cells; DR^{H2O2}, Hydrogen peroxide induced DR cells; DR^{H}, Hypoxia induced DR cells; NAD^+, Nicotinamide adenine dinucleotide; Nampt, Nicotinamide phosphoribosyltransferase; Pc, Parental control tumor cells; DR, Damage-recovered cells; SEM, Standard error of the mean; Sp, spheroidal colonies; T^v, Viable tumor derived cells isolated within the first 24 h from in vivo generated tumors

*Corresponding author at: Frontiers in Bioscience Research Institute in Aging and Cancer, 16471 Scientific Way, Irvine, CA 92618 USA.
E-mail address: fbs@bioscience.org (S. Tabibzadeh).

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Introduction

In the past few years, the idea that cancers arise, progress, and initiate new tumors from a small number of cells with the capability of infinite self-renewal and which harbor the potential to differentiate has got a great momentum [1]. It is now recognized that the molecular signature of such cells is similar to that of embryonic stem cells (ESC). The expression of key factors that control stem cell identity such as *OCT4* has been reported in a variety of cancers including hepatocellular, gastric and cervical carcinomas [2–4]. It has been suggested that *OCT4*$^+$ cancer cells might be tumor-initiating stem cells, a concept that is consistent with the role of this transcription factor in the maintenance of stem cell pluripotency. However, the presence of embryonic *OCT4A* in tumor tissue and tumor cell lines was doubted by some who attributed the observed *OCT4* expression to the expression of *OCT4* pseudogene [5–6]. On the other hand, the expression of another important member of the pluripotency regulatory network *SOX2* (Sex-determining region Y (SRY)-Box2) was also reported in various cancers and cancer cell lines [7–9]. It has been suggested that *SOX2* contributes to the dedifferentiation of cancer cells, endowing them with highly aggressive properties [7,10]. *OCT4* and *SOX2* cooperatively stimulate the transcription of several downstream target genes such as *NANOG*, *DPPA2* (developmental pluripotency-associated two), and *FBXO15*, which also show oncogenic transformation activity [11–13].

The stem cell theory of cancer proposes two major concepts. The first concept suggests that cancers arise from malignant stem cells that are present in somatic tissues, whereas the second concept stresses the importance of dedifferentiation of somatic cells due to factors in their microenvironment [14–16]. However, the origin of cancer stem cells remains the subject of an intense ongoing research; whether such cells give rise to tumors or are generated within an existing tumor still remains an unanswered question. Some view that tumors originate from mutant forms of progenitor cells, adult stem cells or adult progenitor cells that acquire stem cell properties. Others favor the idea that mutation in the stem cells within their niche during development initiates tumors [17]. According to a different conceptual framework, the tumor is made up of several types of stem cells. Among these, only one remains viable in the specific microenvironment that harbors them while others, under the same conditions, are less successful lines. These other lines, however, can become more successful in adapting to other microenvironments or prevail in response to treatment [18]. Another appealing idea is that acquisition of stem cell like characteristics results from dedifferentiation of cancer cells. Such a notion is supported by a growing number of reports showing that inflammation or damage can cause dedifferentiation and generation of cells that exhibit a stem like phenotype [19–20].

It is well known that tumor cells can recover from various types of damage including X-radiation, oxidative stress, and hypoxia. These early studies suggested that the pH and level of glucose are the primary factors that play a crucial role in recovery of cancer cells from Potentially Lethal Damage (PLD) [21–22]. Reduced thiols and particularly glutathione are involved in cellular repair mechanisms [23] and there is compelling evidence that hydrogen sulfide (H_2S) plays a prominent role in cell survival by regulating intracellular redox homeostasis through cysteine-GSH connection [24]. H_2S is a gasotransmitter and its intracellular production is enzymatically regulated. There are three enzymes, which control the production of H_2S, namely, cystathionine β-synthase (CBS), Cystathionase (CTH) also known as cystathione γ-lyase (CSE) [25–26], and 3-mercaptopyruvate sulfurtransferase (MST) [27–28]. H_2S is produced in many different cell types and due to its lipophilic nature, can easily diffuse without the need for transporters [29]. In normoxic tissues, exogenous H_2S is rapidly metabolized, whereas in anoxic tissues, H_2S is unaffected, protecting cells against hypoxia-induced injury [30–31].

Recently, we reported that cancer cells that recover from a potentially lethal damage display mitochondrial reorganization and increased drug resistance [32] similar to that which is found in induced pluripotent stem cells (iPS). Here, we demonstrate that upon recovery from a potentially lethal damage induced by oxidative stress, hypoxia, or glucose deprivation, cancer cells undergo dedifferentiation characterized by reactivation of expression of a repertoire of stem cell genes and plasticity to transdifferentiate into another cell phenotype. This phenomenon is dependent on the production of H_2S and up-regulation of Nampt. H_2S acts as an inducer of Nampt, whereas Nampt, controls the expression level of H_2S generating enzymes and, thus, it fine tunes the level of intracellular H_2S. We also show that cancer cells that are exposed to exogenous H_2S express stem cell markers in a manner similar to that exhibited by cancer cells that recover from a potentially lethal damage. These data show that cancer cells modify their phenotype and gene expression pattern and dedifferentiate by a molecular switch comprised of H_2S and Nampt.

Materials and methods

Materials

Chemicals were purchased from Sigma-Aldrich Company (St Louis, MO) or Fisher Scientific (Pittsburgh, PA). RNeasy Mini Kit for total RNA isolation was purchased from Sigma-Aldrich. Nampt, SSEA1 and SSEA4 antibodies were from Abcam (Cambridge, MA), CBS and CTH antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA), β-actin antibody was from Millipore (Billerica, MA) and secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (Baltimore, PA). H_2S fluorescent probe HSN2 was a kind gift from Professor Michael D. Pluth, University of Oregon, Department of Chemistry, Eugene, Oregon. *NAMPT*-specific siRNA was purchased from Santa Cruz Biotechnology.

Cell culture

CT26 mouse colon carcinoma cell line (CRL-2639), HepG2, MDA-MB-231 and -435S cell lines were obtained from ATCC (Manassas, VA). CT26 cells were cultured in RPMI 1640 with 2 mM glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 25 mM glucose, 1.5 g/L sodium bicarbonate, whereas HepG2, MDA-MB-231 and -435S were maintained in DMEM with 2 mM glutamine and 25 mM glucose, 0.1 mM non-essential amino acids and 10% fetal bovine serum, in a 37°C incubator with 5% CO_2. Parental control (Pc) and damage recovered (DR) cells were maintained in the same media supplemented with 10% FBS. Pc cells were also maintained in α-MEM medium supplemented with 5% FBS on either adherent or ultra-low attachment suspension culture plates. Spheroid colonies