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TIF1β is phosphorylated at serine 473 in colorectal tumor cells through p38 mitogen-activated protein kinase as an oxidative defense mechanism

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ABSTRACT

TIF1β is a pleiotropic regulator of a diverse range of cellular processes such as DNA repair or gene repression in stem cells. This functional switch depends on phosphorylation at serine residue 473 and multiple pathways exist to accomplish this. However, the effects of exogenous reactive oxygen species (ROS) generated by bacterial flora and dietary metabolites in the colonic lumen or chemotherapy on TIF1β have not been determined. We report here that exposure of colorectal cancer (CRC) cell lines DLD-1 and HCT116 to hydrogen peroxide specifically induces TIF1β Ser473 phosphorylation. Hydrogen peroxide also induces primarily p38 MAPK and some p42/44 MAPK phosphorylation. Chemical inhibition of p38 MAPK and p42/44 MAPK reduced phosphorylation of TIF1β serine 473 and increased CRC cell death upon peroxide exposure. Taken together, this suggests that it is primarily peroxide-induced p38 MAPK that mediates Ser473 phosphorylation and activation of TIF1β to enable more efficient DNA repair to assist in tumor cell survival against exogenous ROS.

1. Introduction

The protein Transcription Intermediary Factor 1 beta (TIF1β) is an epigenetic modulator that represses errant gene expression via complexing with heterochromatin protein 1 (HP1), allowing it to interact with the Krüppel-associated box (KRAB) repressor domain found in zinc-finger transcription factors [1]. It is highly expressed in hematopoietic stem cells (as a repressive maintenance factor) [2] as well as several types of cancers [3–5] and high expression of TIF1β is associated with poor prognosis in colorectal cancer patients [6]. A mediator of multiple homeostatic processes, TIF1β has another key function where it influences DNA repair through HP1 proteins [7] and mediates cellular survival after DNA damage in MCF-7 and HCT116 cells [8]. Whether TIF1β serves as a repressor or a repair modulator seems to be site-dependent, as the phosphorylation site of TIF1β at serine 473 (TIF1β-S473) has been found to regulate TIF1β-HP1 interaction [9] and activate its role in efficient DNA repair and cell survival [10].

Bacterial flora and dietary metabolites produce ROS in the colon and rectum [11] and inadequate antioxidant defense could result in loss of tissue homeostasis and subsequent tumor formation. To this end, several reports have been published that point to ROS involvement in the pathogenesis of colorectal cancer (CRC) [12–14]. The ROS hydrogen peroxide (H_2O_2) can modify proteins and nucleic acids and it is thought to work at low levels as a signaling molecule regulating cell survival and growth through modulation of transcription factors [15]. However, even if compensatory mechanisms are activated, microsatellite instability from excessive H_2O_2 would induce DNA damage [16] associated with colorectal cancer development. Therefore, peroxides are implicated in both the regulation of and progression of diseases and the role they play is dependent on their concentration [17,18]. Although the cellular damage caused by ROS in colorectal cancer

Abbreviations: TIF1β, Transcription Intermediary Factor 1 beta; ROS, reactive oxygen species; CRC, colorectal cancer; MAPK, mitogen-activated protein kinase; HP1, heterochromatin protein 1.

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has been well studied, less well known is the role of these potential second messengers in regulating gene transcription via phosphorylation of repressor proteins.

In the case of TIF1β, several factors have been reported to induce phosphorylation on the primary activation site Ser473. For example, mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 (MK2) regulates phosphorylation of TIF1β-S473 in Kaposi's sarcoma-associated herpes virus (KSHV) infection [19]. In early S-phase under normal culture conditions, protein kinase Cδ (PKCδ) regulates TIF1β-S473 phosphorylation for cell cycle progression and cellular proliferation [9]. Temporally dynamic TIF1β-S473 phosphorylation has been found upon growth factor stimulation [20]. On the other hand, TIF1β-S473 phosphorylation may also be induced by nutrient depletion in breast cancer [21]. This diverse pool of regulatory elements establishes Serine 473 as the common activation hub for TIF1β to fulfill its roles of chromatin regulation (DNA damage response) and cell survival and growth. However, little is known about the effect of oxidative stress as an additional modulator of phosphorylation on this crucial site and if activation of the DNA repair mechanism by basal or exogenous ROS could upregulate TIF1β to protect tumor cells by increasing efficiency of DNA repair.

In this study, we hypothesized that, at physiologically relevant concentrations, TIF1β phosphorylation will increases, blunting peroxide-induced cell damage. We found that, in fact, H_2O_2-induced CRC cell death was intensified in TIF1β knockdown conditions. We then discovered that H_2O_2 dose induce phosphorylation of TIF1β-S473. In addition, activation of p38 MAPK and secondary activation of p42/44 MAPK by ROS may regulate TIF1β-S473 phosphorylation in response to oxidative stress to protect CRC cells from ROS-mediated death.

2. Material and methods

2.1. Cell lines and cell culture

Human colorectal adenocarcinoma HCT116 cells and human embryonic kidney (HEK) 293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum and 1% of a penicillin-streptomycin solution (Gibco) at 37 °C in a 5% CO_2-humidified atmosphere. Human colorectal adenocarcinoma cells (DLD-1) were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum and 1% of a penicillin-streptomycin solution at 37 °C in a 5% CO_2-humidified atmosphere. Multiple frozen aliquots were established upon the acquisition and all experimental cells were passaged for fewer than 30 passages after reviving from liquid N2. Cells were washed once after cell growth to a subconfluent state and then made quiescent by incubation in serum- and supplement-free medium for 18 h (hr) before further experiments.

2.2. H_2O_2 treatment

A 1 M stock solution of H_2O_2 was initially made by diluting a 30%

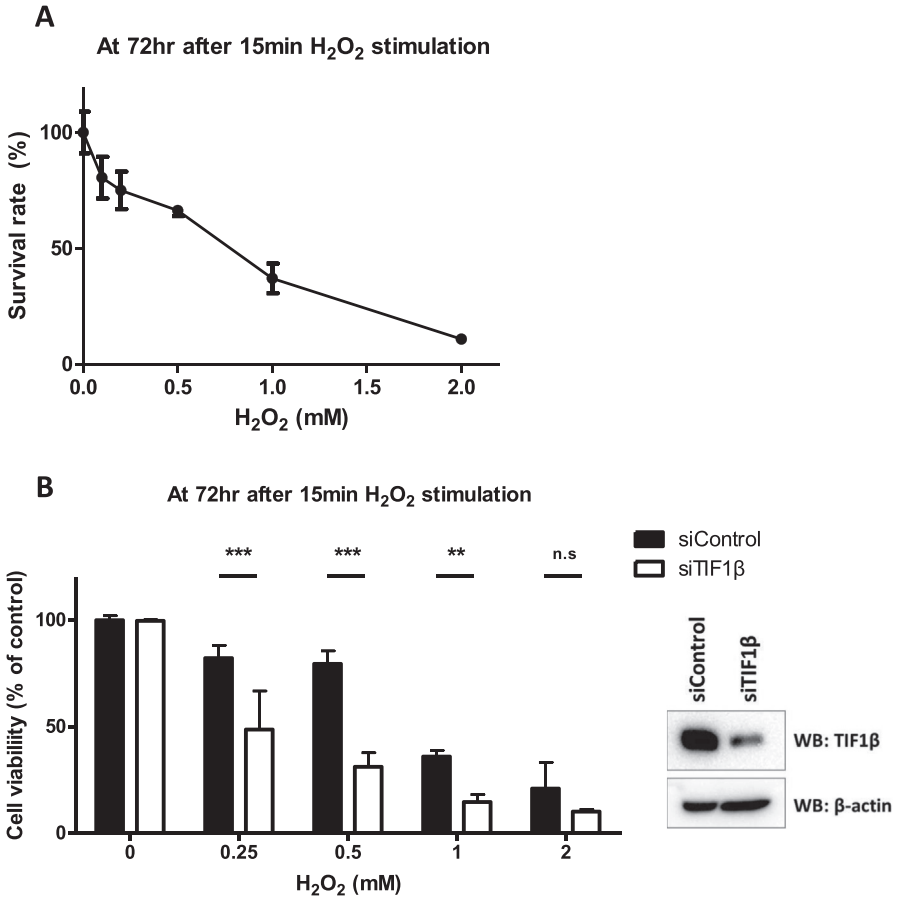

Fig. 1. H_2O_2-induced cell death is enhanced by TIF1β knockdown in DLD-1 cells. (A) DLD-1 cells were treated for 15 min with differing concentrations of H_2O_2 as indicated. Cell viability was determined at 72 h after H_2O_2 treatment by MTT assay. Data are presented as mean ± SD, n = 3. (B) DLD-1 cells were transfected with siRNA (control and TIF1β). After 48 h, cells were challenged with H_2O_2 for 15 min. Cell viability was determined at 72 h after H_2O_2 treatment by MTT assay. Data are presented as mean ± SD, n = 3. **P < 0.01, ***P < 0.001. Right panel shows Western blot data indicating total TIF1β after siRNA transfection.