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hMTH1 is required for maintaining migration and invasion potential of human thyroid cancer cells

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ABSTRACT

Cancer cells, including thyroid cancer cells, suffer from oxidative stress damaging multiple cellular targets, such as DNA and the nucleotide pool. The human MutT homologue 1 (hMTH1) controls the oxidative DNA damage load by sanitizing the nucleotide pool from the oxidized DNA precursor, 8-oxodGTP. It has previously been shown that hMTH1 is essential for cancer cell proliferation and survival, therefore hMTH1 inhibition has been proposed as a novel anticancer therapeutic strategy. Here we show that thyroid cancer cells respond to siRNA mediated hMTH1 depletion with increased DNA damage load and moderately reduced proliferation rates, but without detectable apoptosis, cell-cycle arrest or senescence. Importantly, however, hMTH1 depletion significantly reduced migration and invasion potential of the thyroid cancer cells. Accordingly, our results allow us to propose that hMTH1 may be a therapeutic target in thyroid malignancy, especially for controlling metastasis.

1. Introduction

Oxidative stress-induced damage has been implicated in several human pathologies, including thyroid tumorigenesis. The thyroid tissue is a very good model to study oxidative stress, as active hydrogen peroxide (H_2O_2) production is required for thyroid hormone synthesis by thyroid peroxidase (TPO). In addition to being a substrate for hormone synthesis, H_2O_2 is a source of reactive oxygen species (ROS) that might contribute to pathology [1]. Increased oxidative stress in thyreocytes, and thus oxidative damage, was shown in several studies, including thyroid adenoma, as well as papillary (PTC) and follicular (FTC) thyroid carcinomas [2,3]. Thus, oxidative stress-induced mutagenesis may play an important role in the thyroid tumorigenesis. However, more recent analysis revealed that the most prevalent mutations in human thyroid cancer samples probably do not arise through oxidative stress-induced DNA lesions [4,5].

PTC and FTC constitute two major variants of differentiated thyroid carcinoma (DTC), the most common human endocrine malignancy. PTC is the most frequent type (80% of all DTC cases) and is associated with a favorable prognosis [6]. In DTC, a high 10-year survival reaches 90% [7], however disease progression with lymph node metastases is seen in up to 50% of PTC patients, with further spread to distant locations that reduces survival [8,9]. Another form of thyroid cancer (TC), undifferentiated/poorly differentiated anaplastic thyroid carcinoma (ATC), constitutes less than 5% of all TC cases but it is the most aggressive thyroid malignancy with a median survival less than one year.

Abbreviations: ATC, anaplastic thyroid cancer; BER, base excision repair; CREB, cAMP response element binding protein; DAPI, 4′,6-diamidine-2-phenylindole dihydrochloride; DDR, DNA damage response; dNTP, deoxynucleoside triphosphate; DSBs, double-stand breaks; DTC, differentiated thyroid cancer; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; ERK, extracellular signal-regulated protein kinase; Fpg, formamidopyrimidine DNA-glycosylase; FTC, follicular thyroid cancer; H_2O_2, hydrogen peroxide; MAP kinase, mitogen-activated protein kinase; MAPK, MAP kinase; MEK, MAPK/ERK kinase; MMP, matrix metalloproteinases; (h)MTH1, (human) MutT homologue 1; OIS, oncogene-induced senescence; 8-oxoG, 8-oxoguanine; PTC, papillary thyroid cancer; ROS, reactive oxygen species; TC, thyroid cancer; TIMPs, tissue inhibitors of metalloproteinases

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Currently there is no effective therapeutic strategy available for ATC [10,11].

Nucleic acid bases are prone to oxidation, not only when present in DNA, but also in the deoxynucleoside triphosphate (dNTP) pool [12,13]. Oxidized dNTPs display miscoding properties leading to mutagenesis. 8-oxodGTP, for example, mispairs with adenine when incorporated into DNA leading to AT to CG transversions (reviewed in [14–16]). In mammalian cells, 8-oxodGTP incorporation is prevented by the MTH1 enzyme (MutT homologue 1; also known as nudix hydrolase 1, NUDT1) dephosphorylating 8-oxodGTP to 8-oxodGMP [17]. The importance of this DNA damage avoidance mechanism is highlighted by the fact that MTH1 deficiency in mammalian [18] and other systems [19–21] leads to increased spontaneous mutation rates and increased tumor formation was seen in MTH1 knockout mice [18]. The role of MTH1 in human tumorigenesis appears, however, to be complex. On one hand, suppression of human MTH1 (hMTH1) leads to increased DNA damage levels accompanied with reduced proliferation, *RAS* oncogene-induced senescence (OIS), G_1/S cell cycle arrest, inhibition of cancerous transformation and cancer cell death [22–32]. Conversely, hMTH1 overexpression, which appears to be one feature of cancer tissue [33–36], was shown to reduce DNA damage and mutation rates, but also to support proliferation and survival of transformed cells leading to tumor progression [26,27,37,38]. Thus, cancer cells appear to be more vulnerable to hMTH1 depletion or inhibition than untransformed cells [23–25,38]. Moreover, functional MTH1 was shown to be required for maintenance of xenograft tumors in mouse models [24,25,27,28,32].

In spite of the strong evidence supporting hMTH1 as a target for anticancer therapy, use of small molecule inhibitors targeting hMTH1 as a therapeutic strategy is currently under debate [29–31,39–42]. This shows that further assessment of hMTH1 function in carcinogenesis is required. Moreover, the absence of effective therapeutic approaches in anaplastic thyroid carcinoma calls for further search for the new therapeutic targets. To date hMTH1 function has not been studied in cancer cell lines of the thyroid origin. We hypothesize that hMTH1 inhibition might be particularly effective in thyroid cancer cells as they experience persistent oxidative stress. Here, we provide evidence that hMTH1 supports cancerous phenotypes of several thyroid cancer cell lines, including ATC. Thus we propose hMTH1 as a new potential target for therapeutic intervention in thyroid malignancy.

2. Materials and methods

2.1. Reagents

All reagents were of high purity and were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck-Millipore (Temecula, CA, USA) unless otherwise indicated. Enzymes were from A&A Biotechnology (Gdynia, Poland). All media and cell culture reagents were from Life Technologies or Lonza, unless otherwise indicated.

2.2. Cell culture and depletion of hMTH1 expression

Nthy-ori 3-1 (referred herein as NTHY) and FTC133 cells were from European Collection of Authenticated Cell Cultures (ECACC, UK), BCPAP form German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). 8505C and TPC1 cells were kindly provided by Dr. C. Hoang-Vu (Martin-Luther-University Halle, Germany) and Dr. M. Santoro (The University of Naples Federico II, Italy), respectively.

All cell lines were maintained in RPMI-1640, except from FTC133, which was cultured in DMEM:F12 both supplemented with 10% fetal bovine serum in humidified 5% CO_2 atmosphere at 37 °C. Viable cells numbers were estimated by Trypane Blue exclusion method. All cell lines were tested for mycoplasma contamination on a regular basis.

hMTH1 expression knockdown experiments were performed on cells treated with 5 nM Silencer® Select Validated *hMTH1* siRNA (referred herein as *hMTH1*si) or Silencer® Select Negative Control No. 2 siRNA (referred herein as controlsi) (Ambion, Foster City, CA, USA) and Lipofectamine 2000 (Life Technologies, USA) in Opti-MEM medium according to the manufacturer's instructions. Before further processing cells were silenced for 72 h, unless otherwise indicated. Each time *hMTH1* knockdown was verified by qPCR.

2.3. RNA isolation and quantitative PCR

Total RNA from cultured cells was isolated with GeneMATRIX Universal RNA Purification Kit (EURx, Gdansk, Poland) before cDNA synthesis using the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Life Technologies, USA). Quantitative PCR (qPCR) was performed with Maxima SYBR Green/Fluorescein qPCR Master Mix (Fermentas, Vilnius, Lithuania) on CFX Connect real-time PCR instrument (BioRad, USA). All transcript levels were estimated using standard curve generated by serial dilution of pooled cDNA samples, normalized to 18S RNA and presented as fold changes relative to control. The following primer sequences were used: *18 s RNA* 5′−CCAGTAAGTGCGGGTCATAAG-3′ and 5′−CCATCCAATCGGTAGTAGCG-3′; *hMTH1* 5′-CAGATCGTGTTTGAGTTCGTG-3′ and 5′-CGCATTTCGTCGCTCTC-3′; *GLB1* 5′−CCTACATCTGTGCAGAGTGG-3′ and 5′-TTCATCTTGGGCAGAAGGAC-3′; *P16INK4 A* 5′-CAACGCACCGAATAGTTACG-3′ and 5′-CAGCTCCTCAGCCAGGTC-3′; *MMP1* 5′-CTGGCCACAACTGCCAAATG-3′ and 5′-CTGTCCCTGAACAGCCCAGTCCTTA-3′; *MMP2* 5′-CAGGGAATGAGTACTGGGTCTATT-3′ and 5′-ACTCCAGTTAAAGGCAGCATCTAC-3′; *MMP9* 5′-GCACGACGTCTTCCAGTACC-3′ and 5′-CAGGATGTCATAGGTCACGTAGC-3′.

2.4. Quantitative Western blot analysis

Standard Western blot analysis was performed on whole cell crude lysates extracted in RIPA buffer (ThermoFisher Scientific, Rockford, USA) using primary antibodies rabbit anti-p21, rabbit anti-phospho-Erk1/2 (T202/Y204), rabbit anti-Erk1/2, rabbit anti-phospho-MEK1/2 (S217/S221), rabbit anti-MEK1/2, rabbit anti-phospho-Akt (S473), rabbit anti-Akt, rabbit anti-phospho-CREB (S133), rabbit anti-CREB, rabbit anti-p38 from Cell Signaling Technology Inc. (Danvers, MA, USA), rabbit anti-phospho-p38 (T180/Y182) from R&D Systems (Minneapolis, MN, USA), rabbit anti-hMTH1 from Novus Biologicals (Littleton, CO, USA) or mouse anti-beta-actin from Abcam (Cambridge, UK), followed by HRP-conjugated secondary goat anti-rabbit IgG (Dako, Glostrup, Denmark) or goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, USA). Specific protein signals were detected using SuperSignal West Pico Chemiluminescent Substrate or SuperSignal West Dura Extended Duration Substrate (ThermoFisher Scientific, Rockford, USA) on ChemiDoc XRS$^+$ Imaging System (BioRad, Hercules, CA, USA) and quantified using Quantity One software (BioRad, Hercules, CA, USA). To estimate relative level of protein phosphorylation same amounts of protein samples on two separate membranes were probed with antibodies against phosphorylated or non-phosphorylated protein and obtained signals, normalized to beta-actin levels, were divided.

For MAP kinase pathway inhibition experiments, hMTH1 knockdown was performed for 72 h, followed by addition of specific MEK1/2 inhibitor U0126 (Cell Signaling Technology Inc.; Danvers, MA, USA) at 50 μM in RPMI-1640 with 2.5% FBS and incubation for 4 h. Next, cell lysates were prepared and standard Western blot analysis was performed.

2.5. Alkaline comet assay

DNA damage (single- and double strand breaks, as well as abasic sites) was determined by the alkaline version of the "comet" assay, performed as described by Singh et al. [43], with modifications by