Biochimica et Biophysica Acta 1820 (2012) 104–115

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Trichostatin A and sirtinol suppressed survivin expression through AMPK and p38MAPK in HT29 colon cancer cells

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ARTICLE INFO

Article history:
Received 6 July 2011
Received in revised form 4 November 2011
Accepted 21 November 2011
Available online 1 December 2011

Keywords:
Trichostatin A
Sirtinol
Histone deacetylase
Survivin
Colon cancer

ABSTRACT

Background: Elevated levels of survivin and histone deacetylases (HDACs) are often found over-expressed in human cancers, including colorectal cancer, and have been implicated in tumorigenesis. HDAC inhibition induces growth arrest and cell death in various transformed cell; however, the mechanisms by which this reduces cell viability in colorectal cancer cells remain unexplained.
Methods: We explored the actions of two HDAC inhibitors, trichostatin A (TSA) and sirtinol, in HT29 colon cancer cells.
Results: TSA and sirtinol induced apoptosis and inhibited cell proliferation in HT29 cells. These results are associated with the modulation of survivin. Survivin promoter luciferase activity and Sp1, a transcription factor that contributes to survivin expression, were suppressed in cells exposed to TSA or sirtinol. TSA and sirtinol also activated p38 mitogen-activated protein kinase (p38MAPK) and AMP-activated protein kinase (AMPK). Inhibitors of p38MAPK or AMPK signaling abrogated TSA and sirtinol's effects of decreasing cell viability. Survivin promoter luciferase activity in the presence of TSA or sirtinol was restored by AMPK dominant negative mutant or p38MAPK inhibitor. Furthermore, Sp1 binding to the survivin promoter region decreased while p63 binding to the promoter region increased after TSA or sirtinol exposure.
Conclusions: We report a p38MAPK- and AMPK-mediated downregulation of survivin, and its functional correlation with decreased colon cancer cell viability in the presence of HDAC inhibitor. p63 and Sp1 may also contribute to TSA and sirtinol actions.
General significance: This study delineates, in part, the underlying mechanisms of TSA and sirtinol in decreasing survivin expression and subsequent colon cancer cell viability.

1. Introduction

Interactions between DNA and histones regulate the activation or repression of gene transcription. Several chemical modifications, particularly the acetylation of lysine residues, may change the status of histones and impact gene transcription. Excessive de-acetylation of histones has been linked to cancer pathologies as they promote the repression of tumor regulatory genes. Histone acetylation is regulated by two opposing enzymes: histone acetylases and histone de-acetylases (HDACs). HDACs can be divided into four classes: class I consists of HDAC 1, 2, 3 and 8, class II consists of HDAC 4, 5, 6, 7, 9 and 10, class III consists of sirtuins (SIRT1–7), and class IV consists of HDAC 11, which exhibits features of both classes I and II HDACs. HDAC inhibitors may cause an increase in the acetylation of histones, leading to the re-expression of silenced tumor regulatory genes [1,2]. Importantly, HDACs de-acetylate not only histones but also non-histone substrates, which contribute to a variety of cellular responses [1]. HDAC inhibitors have the ability to induce cell cycle arrest, cell differentiation, and apoptosis, as well as the ability to attenuate metastasis in numerous cancer cell types including colorectal cancer cells [3,4]. However, the molecular mechanisms underlying HDAC inhibitor's actions in arresting cell cycle and decreasing cell viability have not been delineated. Surgery, chemo-therapy, and radio-therapy have failed to significantly improve the prognosis of patients with advanced colorectal cancer. In addition, few patients benefit from modern target therapy. Therefore, we used trichostatin A

Abbreviations: HDAC, histone deacetylase; TSA, trichostatin A; AMPK, AMP-activated protein kinase; SIRT, sirtuin; IAP, inhibitor-of-apoptosis; ChIP, chromatin immunoprecipitation.

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doi:10.1016/j.bbagen.2011.11.011

(TSA) and sirtinol, two structurally and functionally different HDAC inhibitors, to elucidate the mechanisms of HDAC inhibition in decreasing colon cancer cell viability.

Aberrantly increased cell survival in cancer cells is typically attributed to Bcl-2 [5] or inhibitor-of-apoptosis (IAP) cytoprotective proteins. IAPs have recently emerged as broader regulators of cellular homeostasis with functions extending beyond apoptosis inhibition [6]. For instance, the IAP family protein, survivin, also plays essential roles in mitosis [7]. An overexpressed survivin gene is observed in most cancer cell types [8]. Survivin expression might decrease the survival rates in patients with colorectal cancer [9]. Survivin, thus, might play a crucial role in tumor progression [7,10,11]. Recent studies further suggest that survivin may be an independent prognostic factor and may serve as a new target for cancer therapy [12]. The therapeutic potential is particularly important in light of the relative ineffectiveness of surgery, chemotherapy, radiotherapy, and target therapy in treating advanced colorectal cancer. The regulation of survivin gene expression largely occurs at the transcription level [13]. The promoter region of the survivin gene contains many transcription factor binding sites. These transcription factors include Sp1, HIF-1α, c-myc, Stat3, and the tumor suppressors, p53. In particular, Sp1 may play a crucial role in inducing survivin expression [13–15]. However, whether HDAC inhibitors affect Sp1-survivin cascade in colorectal cancer cells is unknown.

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that acts as a cellular stress sensor [16]. Downstream effectors of AMPK signaling, such as tuberous sclerosis complex (TSC) and the mammalian target of rapamycin (mTOR), play important roles in cell cycle progression and tumor formation [17]. Several lines of evidence have demonstrated that AMPK activation inhibits the proliferation while inducing apoptosis in neuroblastoma cells [18], glioblastoma cells [19], and colon cancer cells [20]. This inhibition occurs through various mechanisms, including the activation of the p38MAPK pathway [17], increased expression of the cell cycle regulatory protein, $p21^{cip/Waf1}$ [21], inhibition of NF-κB activity [20], and inhibition of the Akt-mTOR pathway [19]. AMPK may play a causal role in regulating cell survival and growth. We wanted to determine whether AMPK or p38MAPK signaling cascades contribute to HDAC inhibitor's negative effect on colon cancer cell viability. Results from the present study provide experimental evidence to support the contention that TSA and sirtinol decrease survivin expression and colon cancer cell viability via activation of p38MAPK and AMPK signaling cascades. Negative regulation of Sp1 may also be involved in the actions of TSA and sirtinol.

2. Materials and methods

2.1. Reagents

DMEM, optiMEM, RPMI medium 1640, McCoys 5A medium, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Invitrogen (Carlsbad, CA). Antibodies specific for α-tubulin were purchased from Novus Biologicals (Littleton, CO). Normal IgG, anti-mouse and anti-rabbit IgG conjugated alkaline phosphatase antibodies, rabbit polyclonal antibodies specific for Sp1 and HDAC3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against acetylated-lysine, AMPKα phosphorylated at Thr172 or p38MAPK phosphorylated at Thr180/Tyr182 were purchased from Cell Signaling (Beverly, MA). Antibodies against myc tag, DDDDK (flag), AMPK, p38, p63 and survivin were obtained from GeneTex Inc (Irvine, CA). Trichostatin A (TSA) and sirtinol were bought from Calbiochem (San Diego, CA). Turbofect™ in vitro transfection reagent was purchased from Upstate Biotechnology (Lake Placid, NY). A Cell Proliferation ELISA, BrdU, assay kit was acquired from Roche Applied Science (Indianapolis, IN). Survivin promoter luciferase construct was purchased from Health Research Inc. (Roswell Park Cancer Institute Division, NY). Sp1-luciferase construct was provided by Dr. Toshiyuki Sakai (Kyoto Prefectiral University of Medicine, Japan). AMPK dominant negative mutant (AMPKDN) was provided by Dr. Morris Birnbaum (HHMI, PA, U.S.A). HDAC3-flag (Addgene plasmid 13819) and HDAC4-flag (Addgene plasmid 13821) constructs as described previously [22] were provided by Dr. Eric Verdin (Department of Medicine, University of California, San Francisco, USA). Renilla-luc, and the Dual-Glo luciferase assay system were purchased from Promega (Madison, WI). All materials for immunoblotting were purchased from Bio-Rad (Hercules, CA). All other chemicals were obtained from Sigma (St. Louis, MO).

2.2. Cell culture

HT29, HCT116, and colo205 cell lines were obtained from the American Type Culture Collection (Livingstone, MT). The cells were maintained in DMEM (HT29), McCoy5A (HCT116), or RPMI1640 (colo205) containing 10% FCS, 100 U/ml of penicillin G, and 100 μg/ml streptomycin in a humidified 37 °C incubator.

2.3. Immunoblot analysis

Immunoblot analyses were performed as described previously [23]. Briefly, cells were lysed in an extraction buffer containing 10 mM Tris (pH 7.0), 140 mM NaCl, 2 mM PMSF, 5 mM DTT, 0.5% NP-40, 0.05 mM pepstatin A, and 0.2 mM leupeptin. Samples of equal amounts of protein were subjected to SDS-PAGE and transferred onto a NC membrane which was then incubated in a TBST buffer containing 5% non-fat milk. Proteins were visualized by specific primary antibodies and then incubated with alkaline phosphatase-conjugated secondary antibodies. Immunoreactivity was detected using NBT/BCIP following the manufacturer's instructions. Quantitative data were obtained using a computing densitometer with a scientific imaging system (Kodak, Rochester, NY).

2.4. Cell viability assay

Cell viability was measured by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as described previously [24].

2.5. Transfection in HT29 cells

HT29 cells (10^5 cells per well) were either transfected with survivin-luc (1 μg) or Sp1-luc (1 μg) plus renilla-luc (0.5 μg) for reporter assay or transfected with pcDNA (1 μg), HDAC3-flag (1 μg) or HDAC4-flag (1 μg) for MTT assay using Turbofect reagent (Upstate Biotechnology, Lake Placid, NY) according to manufacturer's instructions.

2.6. Dual luciferase reporter assay

Cells were transfected with survivin-luc or Sp1-luc plus renilla-luc using Turbofect reagent. Cells with or without treatments were then harvested, and the luciferase activity was determined using a Dual-Glo luciferase assay system kit (Promega) according to manufacturer's instructions, and was normalized on the basis of renilla luciferase activity.

2.7. Cell proliferation assay

Cells underwent mitogenic quiescence by serum starvation for 24 h. After starvation, cells were subsequently stimulated with serum (10% FBS) in the presence or absence of TSA or sirtinol for another 48 h. Cell proliferation was then determined using a Cell Proliferation ELISA assay kit (Roche Applied Science, Indianapolis, IN) based on