ARTICLE
Journal of Cellular Biochemistry 112:2089–2096 (2011)

Journal of Cellular Biochemistry

Sp1 Is a Transcription Repressor to Stanniocalcin-1 Expression in TSA-Treated Human Colon Cancer Cells, HT29

Alice Y.S. Law, B.H.Y. Yeung, L.Y. Ching, and Chris K.C. Wong*

Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong

ABSTRACT

Our previous study demonstrated that, stanniocalcin-1 (STC1) was a target of histone deacetylase (HDAC) inhibitors and was involved in trichostatin A (TSA) induced apoptosis in the human colon cancer cells, HT29. In this study, we reported that the transcriptional factor, specificity protein 1 (Sp1) in association with retinoblastoma (Rb) repressed STC1 gene transcription in TSA-treated HT29 cells. Our data demonstrated that, a co-treatment of the cells with TSA and Sp1 inhibitor, mithramycin A (MTM) led to a marked synergistic induction of STC1 transcript levels, STC1 promoter (1 kb)-driven luciferase activity and an increase of apoptotic cell population. The knockdown of Sp1 gene expression in TSA treated cells, revealed the repressor role of Sp1 in STC1 transcription. Using a protein phosphatase inhibitor okadaic acid (OKA), an increase of Sp1 hyperphosphorylation and so a reduction of its transcriptional activity, led to a significant induction of STC1 gene expression. Chromatin immunoprecipitation (ChIP) assay revealed that Sp1 binding on STC1 proximal promoter in TSA treated cells. The binding of Sp1 to STC1 promoter was abolished by the co-treatment of MTM or OKA in TSA-treated cells. Re-ChIP assay illustrated that Sp1-mediated inhibition of STC1 transcription was associated with the recruitment of another repressor molecule, Rb. Collectively our findings identify STC1 is a downstream target of Sp1. J. Cell. Biochem. 112: 2089–2096, 2011.

KEY WORDS: APOPTOSIS; LUCIFERASE REPORTER; ChIP; IMMUNOPRECIPITATION

The mammalian glycoprotein, stanniocalcin-1 (STC1) is ubiquitously expressed in different cell-types and is found to be regulated in numerous developmental and pathophysiological processes, including intestinal Ca^{2+}-transport, organogenesis, and carcinogenesis [Ishibashi and Imai, 2002; Chang et al., 2003; Yoshiko and Aubin, 2004; Gerritsen and Wagner, 2005; Wagner and DiMattia, 2006]. The broad expression profile of STC1 in various tissues as well as their low or undetectable level in mammalian blood, have suggested their autocrine and/or paracrine role in the regulation of biological functions [De Niu et al., 2000; Ishibashi and Imai, 2002]. Human STC1 is localized at the metastatic susceptibility locus, 8p, which is known to be a hot-spot for the disruption of tumor progression in colorectal-liver metastases [Chang et al., 1998; Blaker et al., 1999; Chughtai et al., 1999; Knosel et al., 2004; Diep et al., 2006; Macartney-Coxson et al., 2008]. The loss of 8p in colorectal cancers mostly implicates in disease advancement and metastasis [Blaker et al., 1999; Knosel et al., 2004; Diep et al., 2006; Macartney-Coxson et al., 2008]. Intriguingly the 5′-untranslated region of STC1 gene showed long stretches of CAG trinucleotide repeats, which may also relate to genetic instability and transcriptional silencing [Chang et al., 1998; Varghese et al., 1998; Parniewski and Staczek, 2002].

Our previous and other studies have revealed that, STC1 is a hypoxia-inducible factor-1 (HIF-1) target gene [Eisenhofer et al., 2004; Yeung et al., 2005; Westberg et al., 2007a,b; Law et al., 2010], probably expressed in tumor microenvironment and was involved in the process of apoptosis [Zhang et al., 2000; Wu et al., 2006; Lai et al., 2007; Law et al., 2008; Block et al., 2009; Nguyen et al., 2009] and cell proliferation [Daniel and Lange, 2009]. The transcriptional factor specificity protein 1 (Sp1) is known to be important in the transcriptional regulation of genes involved in cancer cell growth, differentiation, and apoptosis [Black et al., 2001; Deniaud et al., 2006, 2009]. Sp1 is also known to regulate several hypoxia-responsive genes such as erythropoietin (EPO) and vascular endothelial growth factor (VEGF) [Sanchez-Elsner et al., 2004; Safe and Abdelrahim, 2005; Song et al., 2009]. Using ChIP bioinfomatics' Mapper, five putative Sp1 binding sites are identified to be located on human STC1 gene 4 kb promoter whereas one is

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: Faculty Research Grant; Grant number: FRG2/09/10/053; Grant sponsor: Hong Kong Baptist University.

*Correspondence to: Prof. Chris K.C. Wong, Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong. E-mail: ckcwong@hkbu.edu.hk

Received 2 January 2011; Accepted 24 March 2011 • DOI 10.1002/jcb.23127 • © 2011 Wiley-Liss, Inc.
Published online 4 April 2011 in Wiley Online Library (wileyonlinelibrary.com).

found in the proximal promoter (−682/−691), in association with a putative elongation factor 2 (E2F) site (−714/−721). The interaction of Sp1 with E2F is known to be important for repression or transactivation of growth-related genes [Johnson and Schneider-Broussard, 1998; Doetzlhofer et al., 1999; Chang et al., 2001]. In respective to the available transcriptional and functional information, in this study we were interested in deciphering the involvement of the transcriptional factor Sp1 in the regulation of STC1 expression. Using pharmacological approach and re-ChIP assay, our data revealed that Sp1, in association with hypophosphorylated Rb formed a repressor complex to inhibit STC1 gene expression in HT29 cells. We also showed that, the co-treatment of the cells with TSA and Sp1 inhibitor (mithramycin A, MTM) significantly and concomitantly increased apoptotic populations and STC1 expression. The data of the present study revealed the first time that, the involvement of Sp1 in the regulation of STC1 gene transcription and supported our previous findings of STC1 being involved in the apoptotic process of human cancer cells.

MATERIALS AND METHODS

EFFECTS OF Sp1, Sp3, AND PP2A INHIBITORS ON STC1 mRNA EXPRESSION

The human colon adenocarcinoma cells HT-29 were grown in McCoy5A supplemented with 10% FBS (HyClone®, Perbio) and antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin) (Invitrogen) at a density of 5×10^4 cells per well in 12-well plates (Falcon). The cells were incubated in 5% CO_2 at 37°C. After overnight incubation, the cells were treated with one of the following treatments: (a) 500 nM trichostatin A (TSA, Calbiochem), (b) 300 nM MTM (an inhibitor for Sp1 binding, Sigma), or (c) TSA + MTM. The cells were pre-incubated with MTM for 30 min before the addition of TSA. Total RNA was extracted, reverse-transcribed, and STC1 mRNA levels were measured by real-time PCR. To test the effects of the PP2A inhibitor (okadaic acid, OKA, Calbiochem) to STC1 mRNA expression, HT-29 cells were incubated with one of the following treatments: (a) 500 nM of TSA, (b) 30 nM of OKA or, (c) TSA + OKA. The cells were pre-incubated with OKA for 30 min before the addition of TSA. Total RNA and protein lysates were collected for real-time PCR and Western blot analysis, respectively.

Sp1 AND STC1 REPORTER ASSAY

The day before transfection, HT-29 cells were plated into six-well tissue culture dishes at a density reaching 70–80% confluence by the time of transfection. Transfection was performed using LipofectAMINE™ 2000 reagent (Invitrogen) with 750 ng of pSp1-luc reporter, which contains six Sp1-binding sites (cloned from human p21 gene promoter (−133/+16)), upstream of the firefly luciferase gene (luc) of pGL3-SV40 plasmid (Promega). The luciferase construct was co-transfected with an internal control, 50 ng of pRL-SV40 plasmid (Promega) for the normalization of the transfection efficiency. Six hours after transfection, the transfection medium was replaced by a complete medium, and the culture was then incubated overnight. The transfected cells were exposed to one of the following treatments: (a) 500 nM of TSA, (b) 300 nM MTM, (c) TSA + MTM, followed by 24 h incubation in 5% CO_2 at 37°C. The cells were then lysed in a passive lysis buffer (Promega). Twenty microliters of the supernatant were used to assay the luciferase activities. Firefly and *Renilla* luciferase activities were sequentially measured from a same sample using the Dual-Luciferase reporter assay system (Promega) and Infinite™ F200 luminometer (TECAN).

TABLE I. The Sequences of Small Interference RNA

Small interference RNA	Target sequence
$siRNA_{ctrl}$	#1: GGCUACGUCCAGGAGCGCA
$siRNA_{sp1}$	#1: GCCAAUAGCUACUCAACUA
	#2: GAAGGGAGGCCCAGGUGUA
	#3: GGGCAGACCUUUACAACUC
	#4: CUACAGAGGCACAAACGUA
$siRNA_{sp3}$	#1: GGUAUUCACUCUAGCAGUA
	#2: GAAAUUUGUUUGUCCAGAA
	#3: GAUAGGAACUGUUAAUACU
	#4: GCGAGAUGAUACUUUGAUU

RNA INTERFERENCE

One day before transfection, HT-29 cells were plated at a density of 5×10^4 cells per well in 12-well plates (Falcon), then mock-transfected or transfected with 20 nM of siCONTROL® Non-Targeting siRNA duplex (siCtrl), human Sp1-specific siRNA duplex ($siRNA_{Sp1}$) (Dharmacon) or human Sp3-specific siRNA duplex ($siRNA_{Sp3}$) (Dhamacon) (Table I), using Dharma*FECT*™ according to the manufacturer's instructions (Dharmacon). The transfected cells were then treated with 500 nM TSA for 24 h. Total RNA was collected for real-time PCR analysis.

CHROMATIN IMMUNOPRECIPITATION (ChIP) AND RE-ChIP ASSAYS

ChIP assay was conducted using the ChIP assay kit according to the manufacturer's instruction (Upstate) and rabbit antibody against Sp1, Sp3 (Santa Cruz) as described in our previous studies [Law et al., 2008]. Re-ChIP assays were performed with modifications of the procedure described by Metivier et al. [2003]. Briefly Sp1 ChIP complexes were eluted by incubation for 30 min at 37°C in 25 μl of 10 mM dithiothreitol (Calbiochem). After centrifugation, the supernatant was diluted with a re-ChIP buffer (20 mM Tris–HCl, 150 mM NaCl, 2 mM EDTA, and 1% Triton X-100, pH 8.0). The diluted complexes were then subjected to immunoprecipitation (IP) by rabbit against retinoblastoma (Santa Cruz). The immunoprecipitated chromatin was analyzed in triplicate by PCR using the primers (GAGTAAAACTGCTGTAAGCAGG-forward and GCCCAAGAGCTGCACCCA-reverse) on proximal region of human STC1 promoter.

IMMUNOPRECIPITATION ASSAYS (IP)

For each IP assay, 1 mg whole-cell lysates in 1 ml IP buffer (20 mM Tris–HCl pH 8.0, 100 mM Nacl, 1 mM EDTA, 0.5% NP-40, and 1× protease inhibitor cocktail) was used. The lysate was initially pre-cleared with 0.5 μg of normal rabbit immunoglobulin G (IgG) (Millipore) and 40 μl protein A/G PLUS agarose beads (Santa Cruz) at 4°C for 20 min. The pre-cleared supernatant was incubated with 2 μg of the rabbit against human Sp1 at 4°C overnight with gentle shaking. This was followed by an addition of 40 μl of protein A/G PLUS agarose beads and was incubated for another 1 h at 4°C. The

protein–antibody–agarose complex was recovered by brief centrifugation and was washed three times with 1 ml IP buffer for 5 min each time at 4°C. The complex was re-suspended in 80 μl of 1× SDS sample buffer containing 2.5% β-mercaptoethanol and boiled for 5 min before being subjected to Western blotting using rabbit anti-phosphorylated protein (Pan) (Zymed, Invitrogen).

RNA EXTRACTION, PCR PRODUCT VERIFICATION, AND REAL-TIME PCR

Cells were dissolved in TRIZOL Reagent. Total RNA was extracted according to the manufacturer's instructions. The RNA A_{260}/A_{280} ratios were between 1.6 and 1.8. The primers for human STC1 [CACACCCACGAGCTGACTTC-forward, TCTCCCTGGTTATG-CACTCTCA-reverse], human Sp1 [TGGTGGTGCCTTTTCACAGG-forward, TTGCTGTTCTCATTGGGTGA CT-reverse], human Sp3 [ACAGCATCTACAACTTCAAGAGTC-forward, TGGATTGTCTGT GGTGTAATCCT-reverse], and actin [GACTACCTCATGAAGATCCT-CACC-forward, TCTCCTTAATGTCACGCACGATT-reverse] were used as described in our previous study [Yeung et al., 2005; Lai et al., 2007]. Briefly, cDNA was synthesized from 1 μg of total cellular RNA using the iScript™ cDNA synthesis kit (Bio-Rad Pacific Ltd.). Quantitated standards (10^4–10^8) and sample cDNAs were analyzed with the iCycler iQ real-time PCR detection system using iQ™ SYBR® Green Supermix (Bio-Rad Pacific Ltd). The copy number for each sample was calculated and all the data were normalized to actin. The PCR conditions were 95°C for 3 min and 40 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 1 min. Fluorescent signal was captured at 82°C, and the occurrence of primer–dimer and secondary products were inspected using melting curve analysis. Control amplifications were conducted either without RT or without RNA. Following PCR amplification, the reaction products were resolved at 100 V on 1% agarose gels with 0.5 μg/ml ethidium bromide (Sigma). All glass- and plastic-ware were treated with diethyl pyrocarbonate (Amersham Biosciences) and autoclaved.

STATISTICAL ANALYSIS

Drugs treatments were performed in triplicate in the same experiments and individual experiments were repeated at least four times. All data are represented as the mean ± SEM. Statistical significance was assessed with a Student's *t*-test or one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. Groups were considered significantly different if $P < 0.05$.

RESULTS

AN INHIBITION OF TSA-INDUCED Sp1 ACTIVITY WAS ACCOMPANIED WITH AN ACTIVATION OF STC1 GENE EXPRESSION AND CELLULAR APOPTOSIS

Our previous study revealed the association of histone deacetylation and NFκB activation in the stimulation of STC1 expression in TSA treated cells [Law et al., 2008]. To identify the involvement of other transcriptional factors in the regulation, the transcriptional activities of the three common TSA-activated transcription factors were screened by reporter assays. NFκB, p53, and Sp1-driven luciferase activities were measured in the cells (Fig. 1A, left panel). The data showed that, TSA treatment induced the activities of both NFκB and Sp1 reporters. The identification of an increase of NFκB transcriptional activity is consistent with our previous finding [Law et al., 2008]. However, the role of Sp1 in STC1 gene transactivation was not known. To illustrate the involvement of Sp1 in STC1 gene expression, the Sp1 inhibitor MTM was used to inhibit Sp1 transcriptional activity in the TSA-treated cells (Fig. 1A, right panel). The inhibition was found to associate with a synergistic induction of STC1 gene transcription (Fig. 1B, left panel), STC1-promoter driven luciferase activity (Fig. 1B, right panel) as well as the percentage of apoptotic cell population (Fig. 1C) in the TSA treated cells.

TRANSCRIPTIONAL ROLE OF Sp1 AND Sp3 IN STC1 GENE EXPRESSION

Since the transcription factor Sp3 shares the similar DNA binding sites with Sp1, we decided to verify the contribution of Sp1 and Sp3 in TSA-induced STC1 gene expression. Using small interference RNA (siRNA), Sp1 and/or Sp3 was selectively knocked down for the demonstration of their roles in STC1 gene transactivation (Supplementary Fig. S1). In TSA treated cells, the knockdown of Sp1 prompted a further activation of STC1 gene expression (Fig. 2). Sp3 silencing showed no significant effect on the TSA-induced STC1 transcript levels.

EFFECTS OF Sp1 HYPERPHOSPHORYLATION ON STC1 TRANSCRIPT LEVELS AND Sp1-BINDING TO HUMAN STC1 GENE PROMOTER

Transcriptional activity of Sp1 is mostly regulated by post-translational modification, which affects the DNA binding activity of the proteins [Fojas et al., 2001; Milanini-Mongiat et al., 2002]. To induce Sp1 hyperphosphorylation, a protein phosphatase inhibitor OKA was used in this study. IP data showed that the OKA treatment increased the level of Sp1 phosphorylation and STC1 expression as compared to the TSA treatment alone (Fig. 3A). ChIP assay was conducted to illustrate the binding of Sp1 to STC1 promoter. TSA treatment increased Sp1 binding to the promoter. The co-treatment with either OKA or MTM significantly reduced the binding of Sp1 to the promoter (Fig. 3B). A re-ChIP assay illustrated Sp1 binding was associated with Rb and this co-binding was abolished in OKA treatment (Fig. 3C).

DISCUSSION

Current evidences support the notion that STC1 is involved in human cancer development [Chang et al., 2003; Eisenhofer et al., 2004; McCudden et al., 2004; Tohmiya et al., 2004; Koide and Sasaki, 2006; Nakagawa et al., 2007; Joensuu et al., 2008; Macartney-Coxson et al., 2008; Klopfleisch and Gruber, 2009]. Our previous studies have revealed that, STC1 is a HIF-1 target gene and was epigenetic regulated by histone deacetylation [Yeung et al., 2005; Law et al., 2008, 2010]. The induction of STC1 expression was shown to be regulated by p53 and NFκB signaling [Lai et al., 2007; Law et al., 2008]. Although the functional role of STC1 is not fully elucidated, considerable number of studies has highlighted its

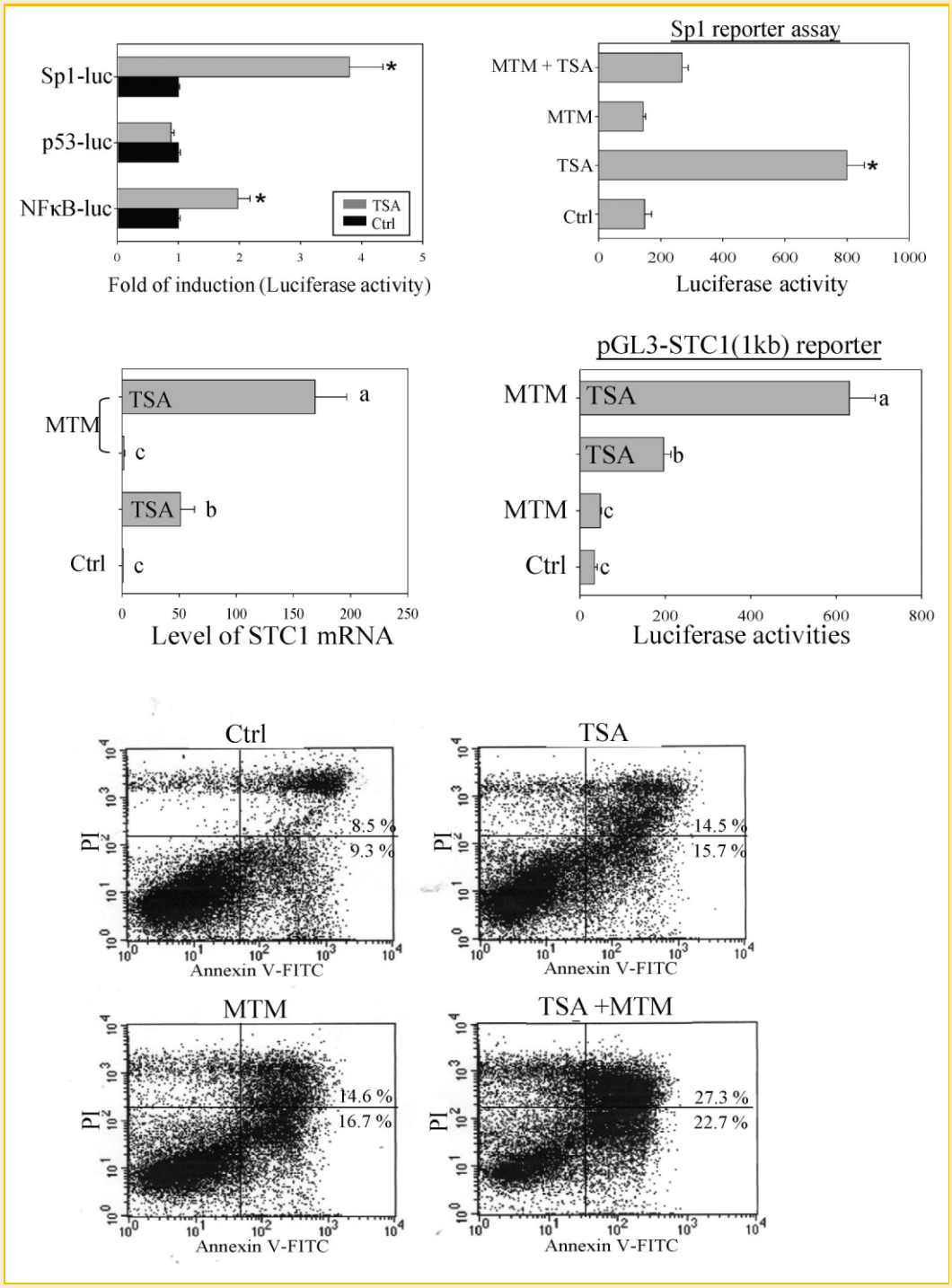

Fig. 1. Trichostatin A (TSA) induced Sp1 activity and co-treatment with mithramycin A (MTM) induced both STC1 gene expression and apoptosis in HT29 cells. A: Luciferase reporter assay showed TSA induced both NFκB and Sp1 reporter activities. No observable effect of TSA on p53 reporter activity was noted. On the right panel, The TSA induced Sp1-driven luciferase activity was abolished in a co-treatment with 300 nM of MTM. B: The stimulatory effects of TSA to STC1 mRNA level (left panel) and STC1 promoter-driven reporter activity (right panel) were significantly enhanced by MTM co-treatment. C: Flow cytometric analysis of annexin V/PI stained cells treated for 24 h with TSA (500 nM), and/or MTM (300 nM). Significant induction of annexin V^+ cells was observed at 24 h of both TSA and MTM treatment. Co-treatment of TSA and MTM further induced annexin V^+ cells. Results shown were obtained from four independent experiments. Asterisks (*) denotes $P < 0.05$ compared with the respective control. Bars with the same letter are not significantly different according to the results of one-way ANOVA followed by Duncan's multiple range tests ($P < 0.05$).

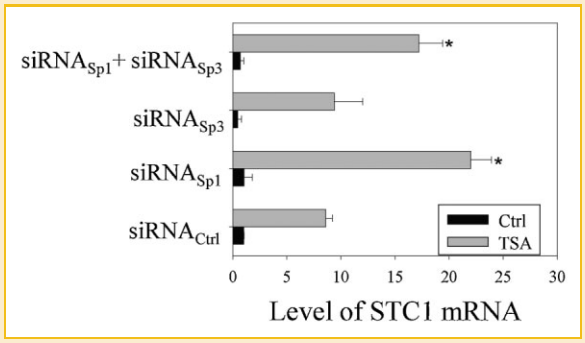

Fig. 2. Effect of Sp1 and/or Sp3 silencing to the transcript levels of STC1. HT29 cells were transfected with siCONTROL nontargeting siRNA, human Sp1, Sp3, or simultaneous transfected with both Sp1 and Sp3. The cells were lysed for real-time PCR analysis. TSA induced STC1 expression was further enhanced in Sp1 silenced cells; while Sp3 silenced cells did not shown significant changes. Asterisks (*) denotes $P < 0.05$ compared with the control.

possible involvement in cell proliferation and apoptosis [Zhang et al., 2000; Wu et al., 2006; Lai et al., 2007; Law et al., 2008; Block et al., 2009; Daniel and Lange, 2009; Nguyen et al., 2009]. Inhibitors of histone deacetylase (HDAC) are known to be the promising drugs for cancer treatment in pre-clinical models and have been investigated in clinical trials. The regulation of gene transcription by acetylation is attributed by the global chromatin remodeling and the activity of chromatin associated non-histone proteins. Of which, the acetylation of the non-histone proteins (i.e., transcriptional factors) attributes considerably to this highly selective transcriptional regulation, leading to an alternation of less than 10% of global gene expression profile. Owing to the selectivity in gene activation, the treatment of cancer cells with HDAC inhibitors can lead to different biological consequences, like cell growth, metastasis, angiogenesis, and apoptosis. The most common transcriptional factors that are known to be regulated by acetylation include p53, Sp1, and NFκB. Using the HT29 cell model, TSA-induced Sp1 transcriptional activity was detected. The Sp1 inhibitor MTM is known to block the binding of Sp1 to GC-rich elements in

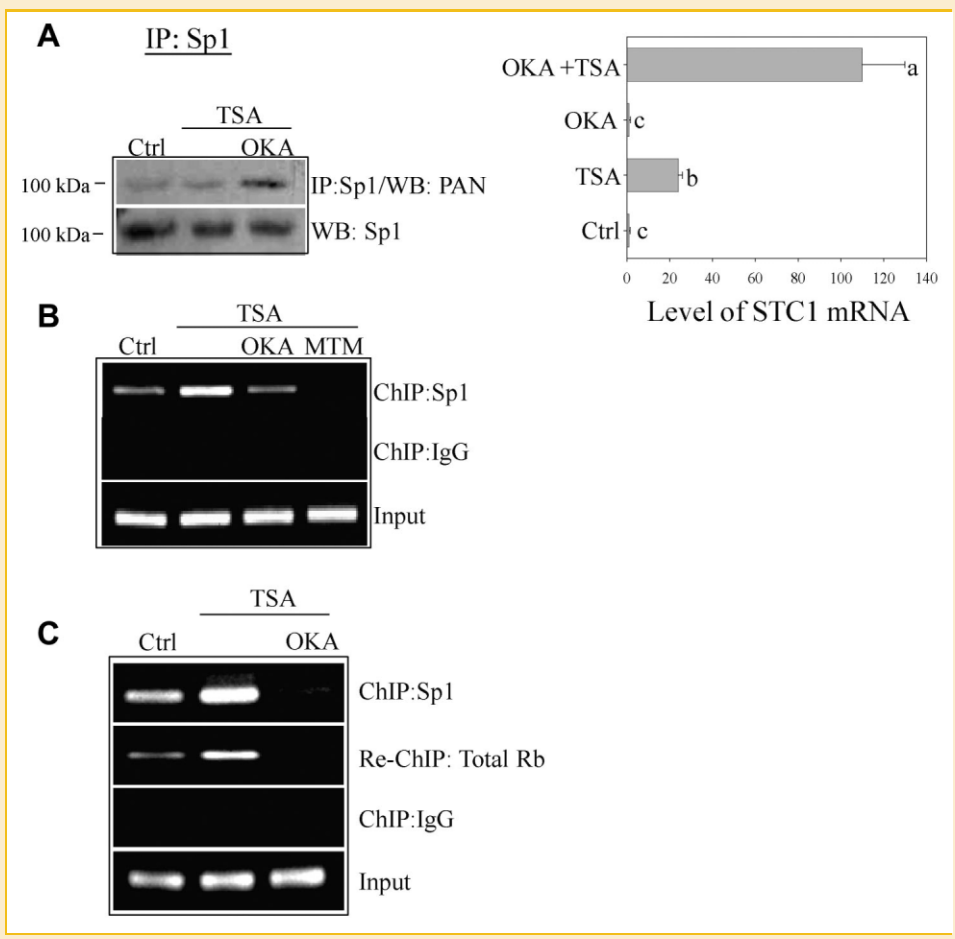

Fig. 3. Effect of a protein phosphatase inhibitor, okadaic acid (OKA) on TSA-induced STC1 expression and the binding of Sp1 and Rb to STC1 gene promoter. HT29 cells incubated with TSA were co-treated with 30 nM OKA for 24 h. The cells were lysed for immunoprecipitation (IP) and real-time PCR analysis. A: IP assay showed that OKA caused hyper-phosphorylation of Sp1 (left panel) and was accompanied with a synergistic induction of STC1 expression (right panel). B: ChIP assay showed the bindings of Sp1 to STC1 gene promoter. The binding was significantly reduced in OKA or MTM co-treatment. C: Re-chip assay showed the binding of Rb to STC1 promoter.

gene promoter [Sastry and Patel, 1993; Albertini et al., 2006]. Consistently the co-treatment of the cells with Sp1 inhibitor, MTM reduced Sp1 transcription activity. The displacement of Sp1 binding on STC1 gene promoter caused the increase of both STC1 gene expression and STC1-promoter driven luciferase activity. Since the DNA binding activity of Sp1 was mostly affected by phosphorylation, whereas five phosphorylation sites (i.e., Ser59, Ser131, Thr453, Thr579, and Thr739) have been confirmed [Armstrong et al., 1997; Chun et al., 1998; Black et al., 2001; Fojas et al., 2001; Milanini-Mongiat et al., 2002; Samson and Wong, 2002; Chu and Ferro, 2005]. The phosphorylation of the specific sites on Sp1 can be modulated by kinases and phosphatases (i.e., casein kinase-II, cyclin-dependent kinase, extracellular signal-regulated kinase, protein phosphatase-1 (PP1), and phosphatase-2A (PP2A)), leading to functional changes in DNA binding and gene transactivation [Chu and Ferro, 2005]. In this study, the specific sites for Sp1 phosphorylation have not been identified, however the dephosphorylation of Sp1 by PP1 and PP2A mostly upregulates Sp1 transcriptional activity [Daniel et al., 1996; Schafer et al., 1997; Lacroix et al., 2002]. OKA is a specific inhibitor for PP1 and PP2A, the induction of Sp1 hyperphosphorylation by OKA might presumably reduce Sp1 binding to STC1 proximal promoter. Yet again the OKA co-treatment significant enhanced TSA-induced STC1 expression. Furthermore, using RNA interference and ChIP assays, our data confirmed that Sp1-mediated transcriptional repression via its binding, together with Rb on human STC1 gene promoter.

The binding site for Sp1 is generally present in promoters of a number of genes, those are involved in multiple aspects of tumorigenesis, like cell growth, apoptosis and angiogenesis [Black et al., 2001; Deniaud et al., 2006, 2009]. With the benefit of hindsight, biological roles of STC1 have been related to these aspects. The identification of growth-related properties of STC1 gene was firstly revealed in the cDNA microarray screening of human fibroblast in response to serum supplement [Iyer et al., 1999]. STC1 mRNA expression was induced by 6–8 folds at 6 h of post-serum stimulation. In a recent study, the knockdown of STC1 expression was found to be related to growth inhibition in human breast cancer cells, expressing SUMO-deficient mutant progesterone receptor [Daniel and Lange, 2009]. A number of studies have highlighted the possible involvement of STC1 in cellular apoptosis [Zhang et al., 2000; Wu et al., 2006; Law et al., 2008; Nguyen et al., 2009]. In addition to cell growth and apoptosis, STC1 was suggested to be a downstream target of VEGF/Wnt2 and was involved in angiogenic responses [Kahn et al., 2000; Wary et al., 2003; Zlot et al., 2003; Holmes and Zachary, 2008; Klein et al., 2009]. Retrospectively the literatures mirror the putative STC1 functions to the tumorigenic effects of Sp1-signalling. More importantly, putative Sp1 and E2F binding sites were found to be located in STC1 proximal promoter. Interestingly the number and the pattern of Sp1 sites identified at proximal promoters of both mouse and human STC1 and STC2 genes were highly conserved, suggesting an intimate association between Sp1 and STC-1 and -2 genes regulation [Bouras et al., 2002]. Moreover, Sp1 is known to be able to act as both negative and positive regulator of gene transcription [Doetzlhofer et al., 1999]. One of the key factors that determine the outcome of Sp1-dependent

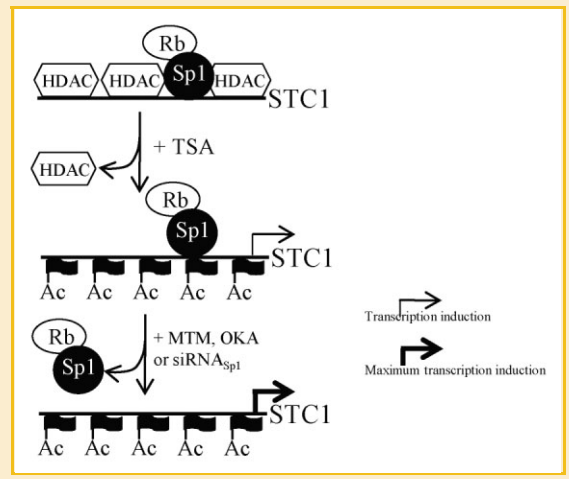

Fig. 4. Model of transcriptional activation of STC1 gene expression by TSA and/or co-treatment with MTM, OKA, or siRNA$_{Sp1}$. In the absence of TSA, the STC1 gene promoter is repressed by HDAC and Sp1/Rb complex. TSA treatment induced histone hyper-acetylation at STC1 gene promoter and gene transcription. The co-treatment reduced the binding of the Sp1/Rb complex on the gene promoter. Maximum induction of STC1 gene expression was observed. HDAC, histone deacetylase; Ac, acetylated histones.

gene regulation involves a competition between the transcriptional repressor HDAC1 and the transactivating factor E2F1 [Doetzlhofer et al., 1999; Zhang et al., 2006]. In the presence of HDAC1, hypophosphorylated Rb interact with Sp1 to form the transcriptional repressor complex [Chang et al., 2001]. Using both pharmacological and ChIP studies, our data illustrated that Sp1 interacted with STC1 gene promoter and may function as a transcriptional repressor to STC1 gene expression (a model is shown in Fig. 4). Our data suggested that, Sp1 inhibited STC1 gene expression by the formation of a repressor complex, including Rb. A similar repressor complex was reported to bind on the promoter region of the cell proliferation-regulated gene (i.e., dihydrofolate reductase) in serum-starved cells [Chang et al., 2001]. Collectively this is the first study to demonstrate the involvement of Sp1 in the regulation of STC1 gene transcription. The data support the notion that, the biological functions of STC1 may relate to cell proliferation and apoptosis.

ACKNOWLEDGMENTS

This work was supported by the Faculty Research Grant (FRG2/09/10/053), Hong Kong Baptist University (C.K.C. Wong).

REFERENCES

Albertini V, Jain A, Vignati S, Napoli S, Rinaldi A, Kwee I, Nur-e-Alam M, Bergant J, Bertoni F, Carbone GM, Rohr J, Catapano CV. 2006. Novel GC-rich DNA-binding compound produced by a genetically engineered mutant of the mithramycin producer *Streptomyces argillaceus* exhibits improved transcriptional repressor activity: Implications for cancer therapy. Nucleic Acids Res 34:1721–1734.

Armstrong SA, Barry DA, Leggett RW, Mueller CR. 1997. Casein kinase II-mediated phosphorylation of the C terminus of Sp1 decreases its DNA binding activity. J Biol Chem 272:13489–13495.

Black AR, Black JD, Azizkhan-Clifford J. 2001. Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer. J Cell Physiol 188:143–160.

Blaker H, Graf M, Rieker RJ, Otto HF. 1999. Comparison of losses of heterozygosity and replication errors in primary colorectal carcinomas and corresponding liver metastases. J Pathol 188:258–262.

Block GJ, Ohkouchi S, Fung F, Frenkel J, Gregory C, Pochampally R, Dimattia G, Sullivan DE, Prockop DJ. 2009. Multipotent stromal cells are activated to reduce apoptosis in part by upregulation and secretion of stanniocalcin-1. Stem Cells 27:670–681.

Bouras T, Southey MC, Chang AC, Reddel RR, Willhite D, Glynne R, Henderson MA, Armes JE, Venter DJ. 2002. Stanniocalcin 2 is an estrogen-responsive gene coexpressed with the estrogen receptor in human breast cancer. Cancer Res 62:1289–1295.

Chang AC, Jeffrey KJ, Tokutake Y, Shimamoto A, Neumann AA, Dunham MA, Cha J, Sugawara M, Furuichi Y, Reddel RR. 1998. Human stanniocalcin (STC): Genomic structure, chromosomal localization, and the presence of CAG trinucleotide repeats. Genomics 47:393–398.

Chang YC, Illenye S, Heintz NH. 2001. Cooperation of E2F-p130 and Sp1-pRb complexes in repression of the Chinese hamster dhfr gene. Mol Cell Biol 21:1121–1131.

Chang AC, Jellinek DA, Reddel RR. 2003. Mammalian stanniocalcins and cancer. Endocr Relat Cancer 10:359–373.

Chu S, Ferro TJ. 2005. Sp1: Regulation of gene expression by phosphorylation. Gene 348:1–11.

Chughtai SA, Crundwell MC, Cruickshank NR, Affie E, Armstrong S, Knowles MA, Takle LA, Kuo M, Khan N, Phillips SM, Neoptolemos JP, Morton DG. 1999. Two novel regions of interstitial deletion on chromosome 8p in colorectal cancer. Oncogene 18:657–665.

Chun RF, Semmes OJ, Neuveut C, Jeang KT. 1998. Modulation of Sp1 phosphorylation by human immunodeficiency virus type 1 Tat. J Virol 72:2615–2629.

Daniel AR, Lange CA. 2009. Protein kinases mediate ligand-independent derepression of sumoylated progesterone receptors in breast cancer cells. Proc Natl Acad Sci USA 106:14287–14292.

Daniel S, Zhang S, DePaoli-Roach AA, Kim KH. 1996. Dephosphorylation of Sp1 by protein phosphatase 1 is involved in the glucose-mediated activation of the acetyl-CoA carboxylase gene. J Biol Chem 271:14692–14697.

De Niu P, Radman DP, Jaworski EM, Deol H, Gentz R, Su J, Olsen HS, Wagner GF. 2000. Development of a human stanniocalcin radioimmunoassay: Serum and tissue hormone levels and pharmacokinetics in the rat. Mol Cell Endocrinol 162:131–144.

Deniaud E, Baguet J, Mathieu AL, Pages G, Marvel J, Leverrier Y. 2006. Overexpression of Sp1 transcription factor induces apoptosis. Oncogene 25:7096–7105.

Deniaud E, Baguet J, Chalard R, Blanquier B, Brinza L, Meunier J, Michallet MC, Laugraud A, Ah-Soon C, Wierinckx A, Castellazzi M, Lachuer J, Gautier C, Marvel J, Leverrier Y. 2009. Overexpression of transcription factor Sp1 leads to gene expression perturbations and cell cycle inhibition. PLoS ONE 4:e7035.

Diep CB, Kleivi K, Ribeiro FR, Teixeira MR, Lindgjaerde OC, Lothe RA. 2006. The order of genetic events associated with colorectal cancer progression inferred from meta-analysis of copy number changes. Genes Chromosomes Cancer 45:31–41.

Doetzlhofer A, Rotheneder H, Lagger G, Koranda M, Kurtev V, Brosch G, Wintersberger E, Seiser C. 1999. Histone deacetylase 1 can repress transcription by binding to Sp1. Mol Cell Biol 19:5504–5511.

Eisenhofer G, Huynh TT, Pacak K, Brouwers FM, Walther MM, Linehan WM, Munson PJ, Mannelli M, Goldstein DS, Elkahloun AG. 2004. Distinct gene expression profiles in norepinephrine- and epinephrine-producing hereditary and sporadic pheochromocytomas: Activation of hypoxia-driven angiogenic pathways in von Hippel–Lindau syndrome. Endocr Relat Cancer 11:897–911.

Fojas dB, Collins NK, Du P, Azizkhan-Clifford J, Mudryj M. 2001. Cyclin A-CDK phosphorylates Sp1 and enhances Sp1-mediated transcription. EMBO J 20:5737–5747.

Gerritsen ME, Wagner GF. 2005. Stanniocalcin: No longer just a fish tale. Vitam Horm 70:105–135.

Holmes DI, Zachary IC. 2008. Vascular endothelial growth factor regulates stanniocalcin-1 expression via neuropilin-1-dependent regulation of KDR and synergism with fibroblast growth factor-2. Cell Signal 20:569–579.

Ishibashi K, Imai M. 2002. Prospect of a stanniocalcin endocrine/paracrine system in mammals. Am J Physiol Renal Physiol 282:F367–F375.

Iyer VR, Eisen MB, Ross DT, Schuler G, Moore T, Lee JC, Trent JM, Staudt LM, Hudson J, Jr., Boguski MS, Lashkari D, Shalon D, Botstein D, Brown PO. 1999. The transcriptional program in the response of human fibroblasts to serum. Science 283:83–87.

Joensuu K, Heikkila P, Andersson LC. 2008. Tumor dormancy: Elevated expression of stanniocalcins in late relapsing breast cancer. Cancer Lett 265:76–83.

Johnson DG, Schneider-Broussard R. 1998. Role of E2F in cell cycle control and cancer. Front Biosci 3:d447–d448.

Kahn J, Mehraban F, Ingle G, Xin X, Bryant JE, Vehar G, Schoenfeld J, Grimaldi CJ, Peale F, Draksharapu A, Lewin DA, Gerritsen ME. 2000. Gene expression profiling in an in vitro model of angiogenesis. Am J Pathol 156:1887–1900.

Klein D, Demory A, Peyre F, Kroll J, Geraud C, Ohnesorge N, Schledzewski K, Arnold B, Goerdt S. 2009. Wnt2 acts as an angiogenic growth factor for non-sinusoidal endothelial cells and inhibits expression of stanniocalcin-1. Angiogenesis 12:251–265.

Klopfleisch R, Gruber AD. 2009. Derlin-1 and stanniocalcin-1 are differentially regulated in metastasizing canine mammary adenocarcinomas. J Comp Pathol 141:113–120.

Knosel T, Schluns K, Stein U, Schwabe H, Schlag PM, Dietel M, Petersen I. 2004. Chromosomal alterations during lymphatic and liver metastasis formation of colorectal cancer. Neoplasia 6:23–28.

Koide Y, Sasaki T. 2006. Stanniocalcin-1 (STC-1) as a molecular marker for human cancer. Rinsho Byori 54:213–220.

Lacroix I, Lipcey C, Imbert J, Kahn-Perles B. 2002. Sp1 transcriptional activity is up-regulated by phosphatase 2A in dividing T lymphocytes. J Biol Chem 277:9598–9605.

Lai KP, Law AY, Yeung HY, Lee LS, Wagner GF, Wong CK. 2007. Induction of stanniocalcin-1 expression in apoptotic human nasopharyngeal cancer cells by p53. Biochem Biophys Res Commun 356:968–975.

Law AY, Lai KP, Lui WC, Wan HT, Wong CK. 2008. Histone deacetylase inhibitor-induced cellular apoptosis involves stanniocalcin-1 activation. Exp Cell Res 314:2975–2984.

Law AY, Ching LY, Lai KP, Wong CK. 2010. Identification and characterization of the hypoxia-responsive element in human stanniocalcin-1 gene. Mol Cell Endocrinol 314:118–127.

Macartney-Coxson DP, Hood KA, Shi HJ, Ward T, Wiles A, O'Connor R, Hall DA, Lea RA, Royds JA, Stubbs RS, Rooker S. 2008. Metastatic susceptibility locus, an 8p hot-spot for tumour progression disrupted in colorectal liver metastases: 13 candidate genes examined at the DNA, mRNA and protein level. BMC Cancer 8:187.

McCudden CR, Majewski A, Chakrabarti S, Wagner GF. 2004. Co-localization of stanniocalcin-1 ligand and receptor in human breast carcinomas. Mol Cell Endocrinol 213:167–172.

Metivier R, Penot G, Hubner MR, Reid G, Brand H, Kos M, Gannon F. 2003. Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell 115:751–763.

Milanini-Mongiat J, Pouyssegur J, Pages G. 2002. Identification of two Sp1 phosphorylation sites for p42/p44 mitogen-activated protein kinases: Their implication in vascular endothelial growth factor gene transcription. J Biol Chem 277:20631–20639.

Nakagawa T, Martinez SR, Goto Y, Koyanagi K, Kitago M, Shingai T, Elashoff DA, Ye X, Singer FR, Giuliano AE, Hoon DS. 2007. Detection of circulating tumor cells in early-stage breast cancer metastasis to axillary lymph nodes. Clin Cancer Res 13:4105–4110.

Nguyen A, Chang AC, Reddel RR. 2009. Stanniocalcin-1 acts in a negative feedback loop in the prosurvival ERK1/2 signaling pathway during oxidative stress. Oncogene 28:1982–1992.

Parniewski P, Staczek P. 2002. Molecular mechanisms of TRS instability. Adv Exp Med Biol 516:1–25.

Safe S, Abdelrahim M. 2005. Sp transcription factor family and its role in cancer. Eur J Cancer 41:2438–2448.

Samson SL, Wong NC. 2002. Role of Sp1 in insulin regulation of gene expression. J Mol Endocrinol 29:265–279.

Sanchez-Elsner T, Ramirez JR, Sanz-Rodriguez F, Varela E, Bernabeu C, Botella LM. 2004. A cross-talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SP1 and Smads. J Mol Biol 336:9–24.

Sastry M, Patel DJ. 1993. Solution structure of the mithramycin dimer-DNA complex. Biochemistry 32:6588–6604.

Schafer D, Hamm-Kunzelmann B, Brand K. 1997. Glucose regulates the promoter activity of aldolase A and pyruvate kinase M2 via dephosphorylation of Sp1. FEBS Lett 417:325–328.

Song Y, Wu J, Oyesanya RA, Lee Z, Mukherjee A, Fang X. 2009. Sp-1 and c-Myc mediate lysophosphatidic acid-induced expression of vascular endothelial growth factor in ovarian cancer cells via a hypoxia-inducible factor-1-independent mechanism. Clin Cancer Res 15:492–501.

Tohmiya Y, Koide Y, Fujimaki S, Harigae H, Funato T, Kaku M, Ishii T, Munakata Y, Kameoka J, Sasaki T. 2004. Stanniocalcin-1 as a novel marker to detect minimal residual disease of human leukemia. Tohoku J Exp Med 204:125–133.

Varghese R, Wong CK, Deol H, Wagner GF, DiMattia GE. 1998. Comparative analysis of mammalian stanniocalcin genes. Endocrinology 139:4714–4725.

Wagner GF, DiMattia GE. 2006. The stanniocalcin family of proteins. J Exp Zoolog A Comp Exp Biol 305:769–780.

Wary KK, Thakker GD, Humtsoe JO, Yang J. 2003. Analysis of VEGF-responsive genes involved in the activation of endothelial cells. Mol Cancer 2:25.

Westberg JA, Serlachius M, Lankila P, Andersson LC. 2007a. Hypoxic preconditioning induces elevated expression of stanniocalcin-1 in the heart. Am J Physiol Heart Circ Physiol 293:H1766–1771.

Westberg JA, Serlachius M, Lankila P, Penkowa M, Hidalgo J, Andersson LC. 2007b. Hypoxic preconditioning induces neuroprotective stanniocalcin-1 in brain via IL-6 signaling. Stroke 38:1025–1030.

Wu S, Yoshiko Y, De Luca F. 2006. Stanniocalcin 1 acts as a paracrine regulator of growth plate chondrogenesis. J Biol Chem 281:5120–5127.

Yeung HY, Lai KP, Chan HY, Mak NK, Wagner GF, Wong CK. 2005. Hypoxia-inducible factor-1-mediated activation of stanniocalcin-1 in human cancer cells. Endocrinology 146:4951–4960.

Yoshiko Y, Aubin JE. 2004. Stanniocalcin 1 as a pleiotropic factor in mammals. Peptides 25:1663–1669.

Zhang K, Lindsberg PJ, Tatlisumak T, Kaste M, Olsen HS, Andersson LC. 2000. Stanniocalcin: A molecular guard of neurons during cerebral ischemia. Proc Natl Acad Sci USA 97:3637–3642.

Zhang Y, Liao M, Dufau ML. 2006. Phosphatidylinositol 3-kinase/protein kinase czeta-induced phosphorylation of Sp1 and p107 repressor release have a critical role in histone deacetylase inhibitor-mediated derepression [corrected] of transcription of the luteinizing hormone receptor gene. Mol Cell Biol 26:6748–6761.

Zlot C, Ingle G, Hongo J, Yang S, Sheng Z, Schwall R, Paoni N, Wang F, Peale FV, Jr., Gerritsen ME. 2003. Stanniocalcin 1 is an autocrine modulator of endothelial angiogenic responses to hepatocyte growth factor. J Biol Chem 278:47654–47659.