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Sp transcription factor family and its role in cancer

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Abstract

Specificity protein 1 (Sp1) and other Sp and Krüppel-like factor (KLF) proteins are members of a family of transcription factors which bind GC/GT-rich promoter elements through three C_2H_2-type zinc fingers that are present at their C-terminal domains. Sp1–Sp4 proteins regulate expression of multiple genes in normal tissues and tumours. There is growing evidence that some Sp proteins play a critical role in the growth and metastasis of many tumour types by regulating expression of cell cycle genes and vascular endothelial growth factor. Sp/KLF proteins are also potential targets for cancer chemotherapy.

Keywords: Sp1; Sp2; Sp3; Sp4; Transcription factors; Cancer; VEGF

1. Introduction

Specificity protein 1 (Sp1) was the first transcription factor identified and cloned, and shown to be a sequence-specific DNA-binding protein that activated a broad and diverse spectrum of mammalian and viral genes [1–5]. Sp1 protein recognises GC/GT boxes and interacts with DNA through three C_2H_2-type zinc fingers located at the C-terminal domain [6–8]. Based on results of crystal structure and NMR studies, each of the three zinc fingers in Sp1 recognises three bases in one strand, and a single base in the complementary strand of the GC-rich elements where the consensus Sp1 binding site is 5′-(G/T)GGGCGG(G/A)(G/A)(C/T)-3′ [9,10]. A recent NMR study now shows that the more C-terminal zinc finger 1 has reduced specificity and can also bind only two bases in the recognition sequence [11]. This may account for the interactions of Sp1 with diverse GC-rich promoter sequences and for Sp1-dependent regulation of a large number of mammalian genes in normal and transformed cells [12–14]. Although Sp1 binding affinities to non-consensus GC-rich motifs may be lower than for consensus sequences, their functional interactions in regulating gene expression may be highly significant.

2. Sp family of transcription factors and their expression in tumours

Sp1 is a member of a growing family of nuclear proteins that modulate gene transcription and the Sp/Krüppel-like factors (KLFs) are categorised by their similar modular structures [reviewed in [15–20]]. Sp1–Sp4 form a subgroup (Fig. 1) which contain several distinct overlapping features/regions which include activation domains (AD), the C-terminal zinc finger DNA-binding region, and an inhibitory domain (ID) in Sp3 that is involved in the suppressive activity of Sp3. Sp5–Sp8 are structurally similar and appear to be truncated forms of Sp1–Sp4 in which portions of the N-terminal regions have been deleted. The chromosomal locations of Sp1–Sp8 are adjacent to a HOX gene cluster. At least 15 KLFs have been characterised, and these proteins also

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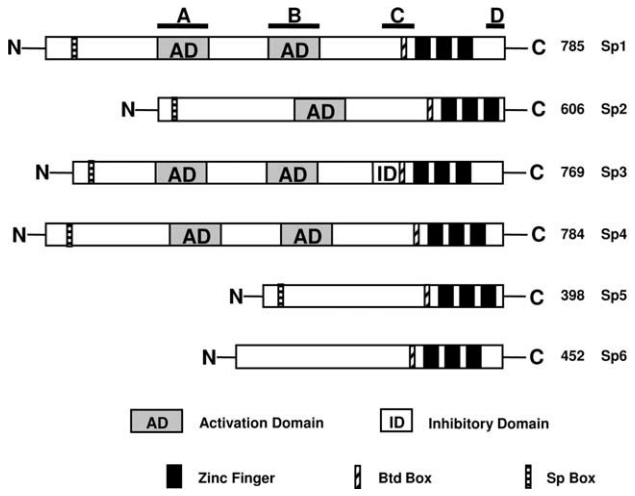

Fig. 1. Structural features of Sp proteins. Sp1–Sp6 proteins contain several common domains in their C-terminal region, whereas Sp5 and Sp6 exhibit a truncated N-terminal structure [15–20]. Buttonhead (Btd) and Sp boxes are conserved regions in all Sp proteins [17].

contain the three zinc finger motifs but exhibit considerable structural variability. KLF subfamilies include the basal transcription element binding (BTEB) proteins and transforming growth factor β (TGFβ)-inducible early gene (TIEG) proteins. The function of individual Sp/KLF proteins is continually being defined and depends, in part, on the temporal and tissue-specific patterns of individual gene expression. Sp1 is widely expressed in many tissues/cells; however, the relative quantitative expression is not well defined and may be highly variable. For example, studies on the developmental expression of Sp1 in the mouse showed that Sp1 mRNA levels in different tissues varied by at least 100-fold [21]. Sp1 directly interacts with TATA-binding protein associated factors (TAFs) and other nuclear cofactors which comprise the basal transcription factors [22–26]. Sp1-mediated transcription also involves a cofactor required for Sp1 (CRSP) complex which has been identified in HeLa cervical adenocarcinoma cells [27–29]. The CRSP complex contains multiple proteins and resembles, in part, protein complexes associated with other transcription factors similar to the yeast/mediator protein complex [30]. Interactions of mediator-like complexes with other Sp proteins have not been reported; however, it is likely that these occur and may play a role in the differential regulation of Sp-dependent gene expression.

Although the specific physiological functions of Sp proteins have not been determined, results of gene knockout studies in mice have provided valuable insights on some critical functions of these genes. For example, $Sp1^{-/-}$ embryos exhibit multiple abnormalities and retarded development and embryolethality on day 11 of gestation [31]. $Sp2^{-/-}$ mice have not been reported; however $Sp3^{-/-}$ mice exhibit growth retardation, defects in late tooth formation, and the animals die at birth [32,33]. $Sp4^{-/-}$ mice either die shortly after birth or survive with significant growth retardation. In addition, male (but not female) $Sp4^{-/-}$ mice do not reproduce and have abnormal reproductive behaviour [34]. It is clear from these and other Sp/KLF gene knockout studies that this family of transcription factors plays critical roles in normal development of tissues/organs.

There is also emerging evidence that Sp protein expression may be a critical factor in tumour development, growth and metastasis; however, most of these studies are limited and have focused primarily on Sp1 protein/mRNA expression. Wang and coworkers investigated Sp1 and vascular endothelial growth factor (VEGF) expression in gastric tumours [35]. Sp1 protein was highly expressed in nuclei of gastric tumour cells, whereas minimal to non-detectable levels were detected in stromal or normal glandular cells within or surrounding the tumour. The results also showed that the survival of patients with high Sp1 expression was significantly decreased compared to patients with weak to non-detectable Sp1 expression. Since Sp1 also regulates VEGF expression, there was a positive correlation between Sp1 and VEGF expression in gastric cancer patients, and patients with high VEGF levels also had decreased survival times [36]. Shi and coworkers [37] showed that Sp1 was overexpressed in pancreatic tumours compared to normal tissues, and overexpression of Sp1 in tumours and pancreatic cancer cell lines correlated with elevated VEGF levels. These results were consistent with molecular biology studies showing that Sp1 plays a major role in regulation of VEGF. Sp1 protein expression was elevated in 11 out of 14 breast carcinomas, whereas only 1 in 5 benign breast lesions expressed detectable Sp1 [38], and Sp1 was overexpressed in thyroid tumours compared to normal tissues [39]. DNA-dependent protein kinases Ku70 and Ku80 are upregulated in colon tumours compared to adjacent normal tissues, and this also correlated with increased levels of Sp1 expression in these tumours [40]. Moreover, promoter analysis studies confirm that constitutive expression of these kinases is regulated by Sp1 interaction with GC-rich promoters in these genes. These data link elevated Sp protein expression in tumours to upregulation of genes that are involved in tumour growth and metastasis. Additional research is required to determine direct linkages between overexpression of Sp1 and other Sp family members in various tumour types since Sp proteins may be important prognostic factors and therapeutic targets.

3. Regulation of growth promoting and cell survival genes by sp proteins in cancer cells

Sp family proteins regulate basal/constitutive expression of genes involved in multiple functions in both normal and cancerous tissues [18]. Genes that regulate

growth and cell cycle progression frequently contain proximal GC-rich promoter sequences, and their interactions with Sp proteins and other transcription factors are critical for their expression. For example, several studies show that VEGF expression in cancer cell lines is regulated through Sp protein interactions with several proximal GC-rich motifs [37,41–44]. In pancreatic cancer cells, there was a correlation between expression of Sp1 and VEGF protein and reporter gene activity in cells transfected with constructs (pVEGF) containing VEGF promoter inserts [37]. Results of deletion mutational analysis of the VEGF promoter demonstrated that four proximal GC-rich sites between −109 to −61 contributed to constitutive activity of transfected pVEGF constructs. The results showed that Sp1 clearly regulated expression of VEGF in pancreatic cancer cells; however, several of the cell lines expressed low Sp1 protein, and this correlated with low transactivation in cells transfected with pVEGF constructs suggesting that Sp1-mediated angiogenesis through induction of VEGF was cell context dependent.

A recent study in this laboratory [44] further investigated the role of Sp1 and other Sp proteins in regulation of VEGF and proliferation of Panc-1 cells that expressed Sp proteins. The results of the VEGF studies confirm that the proximal GC-rich sites in Panc-1 cells are required for expression of VEGF; however, the results of RNA interference studies showed that multiple Sp proteins are involved in VEGF regulation (Fig. 2). Sequential knockdown of Sp1, Sp3 and Sp4 showed that all three proteins regulated transactivation in cells transfected with pVEGF1, pVEGF2 and pVEGF3. Sp4 knockdown decreased activity ($\geqslant 50\%$) in cells transfected with all three constructs, whereas Sp1 and Sp3 differentially regulated pVEGF1 and pVEGF3, respectively. This suggests that there were differential DNA interactions and/or functions of Sp1 and Sp3 on the VEGF promoter, and this may reflect the promoter position-dependent assembly of Sp1, Sp3 and Sp4 complexes. Sp4 expression has not been extensively investigated in cancer cells, and these results suggest that the metastatic and growth potential of pancreatic tumours may also be dependent on Sp4 levels.

RNA interference using small inhibitory RNAs for Sp1, Sp3 and Sp4 was also used to investigate the role of these proteins in growth of Panc-1 cells [44]. Results summarised in Fig. 3 show that transfection of Panc-1 cells with small inhibitory RNA for luciferase (iGL2) did not affect distribution of Panc-1 cells in G_0/G_1, G_2/M or S phases of the cell cycle, whereas small inhibitory RNA for Sp1 (iSp1) decreased the percentage of cells in G_2/M (5.52%) and S (5.74%) phase and increased the percentage in G_0/G_1 (10.26%). This response was accompanied by decreased Rb phosphorylation and reflects the overall decrease in multiple genes associated with Sp1-dependent growth. In contrast, the most dramatic response was observed in cells transfected with small inhibitory RNA for Sp3 (iSp3) in which the percentage of cells in G_0/G_1 and S phase increased by 20.14% and decreased by 17.39%, respectively. Subsequent analysis of several cell cycle genes showed that transfection with iSp3 in Panc-1 and other pancreatic cancer cell lines resulted in upregulation of the cyclin-dependent kinase inhibitor p27. Moreover, similar

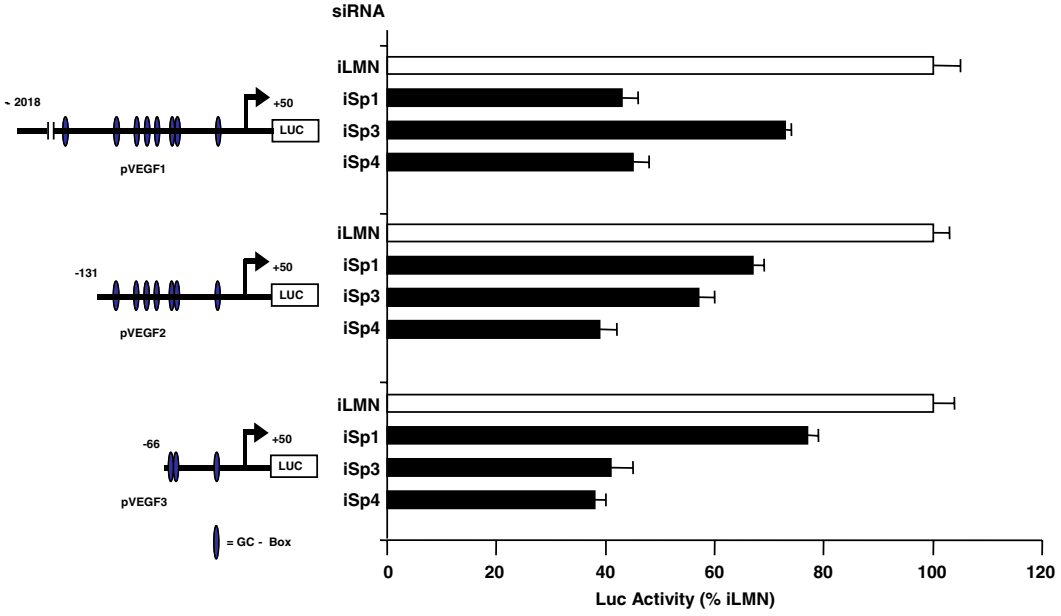

Fig. 2. Regulation of VEGF expression in Panc-1 pancreatic cancer cells. Cells were transfected with various VEGF promoter constructs, transfected with small inhibitory RNAs for lamin (iLMN) (non-specific), Sp1 (iSp1), Sp3 (iSp3) or Sp4 (iSp4), and luciferase activity determined as previously reported [44].

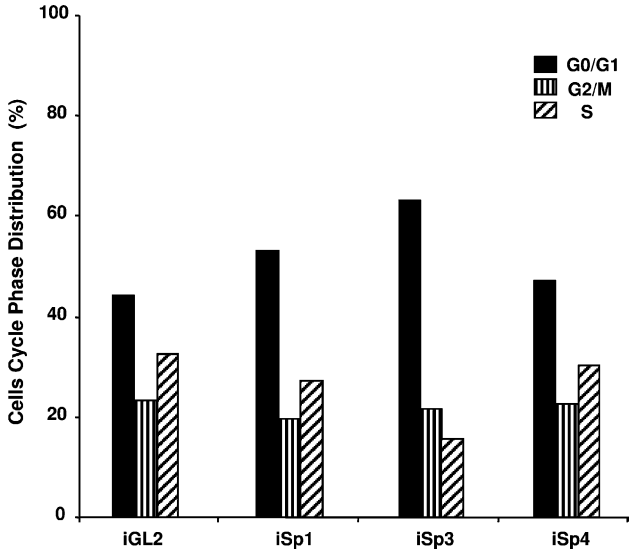

Fig. 3. Effects of Sp proteins on cycle phase distribution in Panc-1 cells. Cells were transfected with small inhibitory RNA for luciferase (iGL2), iSp1, isp3 or iSp4, and the percentage distribution of Panc-1 cells in G_0/G_1, G_2/M and S phases of the cell cycle were determined by FACS analysis [44]. *Sp3* knockdown increased G_0/G_1 and decreased S phase due to increased expression of p27.

results were obtained in cells transfected with p27 promoter-reporter construct which contain four GC-rich elements that bind Sp3 and other Sp proteins. Thus, Sp3 enhances growth of pancreatic cancer cells by suppressing p27 mRNA and protein expression through interaction with GC-rich promoter elements.

The role of Sp proteins in regulating expression of transforming growth factor β (TGFβ)/TGFβR receptor (TGFBR) signalling in cancer cell lines has also been reported [45–49]. The loss of this important growth inhibitory pathway is critical for oncogenesis in some tumours. Estrogen receptor-positive MCF-7 breast cancer cells (late passage MCF-7L) are insensitive to TGFβ and do not express TGFBRI or TGFBRII. Overexpression of the latter receptor restores TGFβ-responsiveness [50]. TGFBRII contains proximal GC-rich sites that are required for expression of this gene [45–48], and levels of Sp1 and Sp3 expression are critical for regulation of TGFBRII. A comparison of Sp1/Sp3 levels and ratios in TGFβ-responsive early passage MCF-7 cells (MCF-7E) and TGFβ-nonresponsive MCF-7L cells demonstrates lower levels of Sp1 and higher Sp3 expression (decreased Sp1/Sp3 ratios) in MCF-7L compared to MCF-7E cells. Thus, Sp3 acts as a repressor of TGFBRII [49] and thereby inhibits an important growth inhibitory pathway in breast cancer cells, and this is functionally similar to the suppression of p21 by Sp3 in pancreatic cancer cells.

Sp1 also plays a role in regulating several genes in breast cancer cells associated with cell growth and cycle progression (cyclin D1, E2F1, c-*fos*, transforming growth factor α), purine/pyrimidine synthesis and metabolism [thymidylate synthase, adenosine deaminase, DNA polymerase α, carbamylphosphate synthetase/aspartate carbamyltransferase/dihydroorotase (CAD)]; angiogenesis (VEGF); and anti-apoptosis (bcl-2) [12,13,43,51–58]. Moreover, estrogen-induced proliferation of estrogen receptor (ER)-positive MCF-7 or ZR-75 breast cancer cells is accompanied by induction of the genes indicated above by a novel non-classical mechanism of estrogen action that involves Sp1 protein and GC-rich promoter sites [59–62]. ER binds Sp1 in the presence or absence of estrogen, and hormone-induced transactivation involves ERα/Sp1 interactions with specific GC-rich promoter sequences. This response does not require the DNA-binding domain of ERα [59]. The importance of Sp1 in mediating hormone-induced MCF-7 cell proliferation was confirmed by RNA interference using iSp1 (Fig. 4) [63]. The results show that Sp1 knockdown increases the percentage of cells in G_0/G_1 (from 75.3% to 78.3%) and decreases the percentage in S-phase (from 15.1% to 12.1%) in solvent-treated cells. In hormone-treated MCF-7 cells the percentage of cells in G_0/G_1 and S phase were 66.1% and 23.7%, respectively; however, after cotransfection with iSp1, these values were 71.9% and 17.3%, respectively. The pivotal role of Sp1 in G_1 phase has been confirmed in HBL-100 and HeLa cells [41]. In synchronised HBL-100 cells, Sp1 protein was more highly expressed in G_1 compared to S and G_2/M phases of the cell cycle. The differences in Sp protein levels were due to proteosome-dependent degradation pathways which were activated in S and G_2/M phases. Moreover, expression of dominant negative Sp1

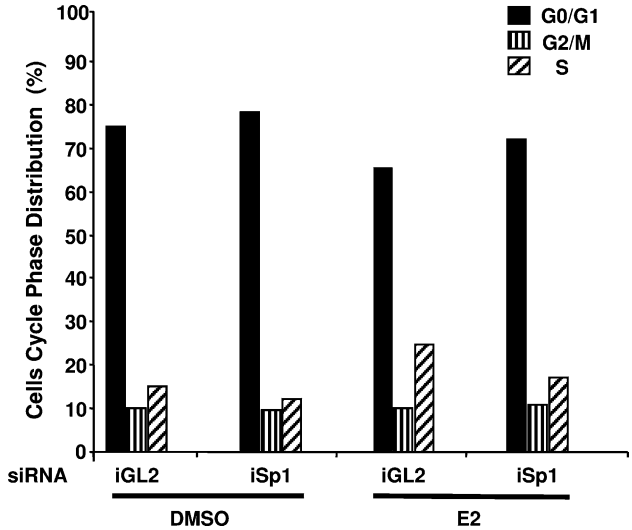

Fig. 4. Effects of *Sp1* knockdown on cell cycle phase distribution in MCF-7 breast cancer cells treated with DMSO or 10 nM E2 [63]. Cells were transfected with iGL2 or iSp1, treated with DMSO or 10 nM E2, and the percentage distribution of the cells in G_0/G_1, G_2/M and S phases of the cell cycle were determined by FACS analysis. iSp1 decreased cells in S phase and increased cells in G_0/G_1 in both treatment groups.

in HeLa cells increased the percentage of cells in G_1 phase, decreased expression of cyclin D1 and increased p27. These results contrast to the report showing that Sp3 protein suppressed p27 protein expression in pancreatic cancer cells [44], and the rationale for these differences in Sp protein-dependent regulation of p27 in different cancer cell lines requires further investigation. Nevertheless, it was apparent that Sp proteins regulate transcription of genes involved in the growth and metastasis of cancer cell lines and must be considered as prognostic factors for this disease and as potential targets for cancer chemotherapy.

4. Strategies for targeting Sp protein pathways in cancer cells

For many human cancers, Sp protein overexpression is a negative prognostic factor for survival and, not surprisingly, these transcription factors contribute to the proliferative and metastatic tumour phenotype. Strategies for inhibiting Sp-dependent pathways have focused on several approaches which include drugs that inactivate GC-rich DNA motifs, oligonucleotides and peptide nucleic acid–DNA chimeras that specifically interact with Sp1 binding motifs (decoys), and chemicals that modulate Sp protein expression. Mithramycin binds GC-rich motifs, and several studies show that treatment of cells with this compound decreases Sp1–DNA binding and Sp1-dependent gene expression [41,64,65]. For example, induction of the angiogenic factor thymidine phosphorylase by tumour necrosis factor α (TNFα) in colon cancer cells is dependent on enhanced Sp1–DNA binding, and this response was blocked in cells cotreated with mithramycin [64]. The DNA binding antitumour drug hedamycin also complexes with G-rich DNA, and this compound inhibits *survivin* transcription through interactions with a proximal GC-rich Sp1 binding site (−115 to −95) in the *survivin* promoter [66]. Other compounds such as thalidomide and the daunomycin-derived bisanthracycline WP631 also act through blocking G-rich sites and inhibit Sp1-activated transcription [67,68]. Double stranded oligodeoxynucleotides containing a consensus GC-rich Sp1 binding site (Sp1 decoys) have been developed for inhibiting Sp1-dependent gene expression [69–71]. For example, Ishibashi and coworkers [69] transfected Sp1 decoys into human lung A549 adenocarcinoma and human glioblastoma multiform U251 cancer cell lines and inhibited TNFα-induced VEGF, TGFβ1 and tissue factor gene/protein expression. The mutant Sp1 decoy also induced cellular responses including decreased cell proliferation, *in vitro* invasiveness, and urokinase-type plasminogen activator mRNA levels. Sp1 decoys also suppress cytokine transcription and proliferation of rat mesengial cells treated with high levels of glucose [70], suggesting a possible role for this approach, not only in cancer cell growth inhibition, but in prevention of renal hypertrophy. Other variations on Sp1/transcription factor decoys are being developed [72,73], and there may be clinical potential for applications of this technology in treatment of specific cancers.

Cyclooxygenase 2 (COX-2) inhibitors such as celecoxib are being developed as anticancer drugs, and there is considerable evidence showing that these compounds exhibit anti-angiogenic activity [74–76]. A recent study has linked the anti-angiogenic effects of celecoxib in pancreatic cancer cells to modulation of Sp1 transcription factor activity [77]. This study showed that celecoxib inhibited pancreatic tumour growth and decreased liver metastasis in an athymic nude mouse model, and this was paralleled by growth inhibition and decreased VEGF protein expression in Panc-1 cells. Subsequent analysis of the *VEGF* promoter identified proximal GC-rich motifs (−104 to −60) that were critical for celecoxib-induced downregulation of VEGF, and this was accompanied by decreased Sp1 protein phosphorylation and decreased P-Sp1/Sp1 ratios. These observations suggest that the anti-angiogenic activity of celecoxib in pancreatic cancer cells is linked to targeted dephosphorylation of Sp1. Ongoing research in this laboratory [78] has also investigated the mechanisms of action of COX-2 inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs) and their effects on cancer cell proliferation and VEGF expression in colon, pancreatic and other cancer cell lines. The growth inhibitory effects of these compounds are accompanied by downregulation of activity in SW-480 colon cancer cells transfected with various pVEGF constructs (Fig. 5), and this was accompanied by decreased expression of Sp1 and Sp4 but not Sp3 proteins. Since regulation of VEGF is dependent on Sp1, Sp3 and Sp4, our results show that decreased expression of VEGF in SW-480 and other colon cancer cells treated with celecoxib, nimesulfide or NS-398 is due to downregulation of Sp1 and Sp4. It was also shown that decreased Sp1/Sp4 by COX-2 inhibitors/NSAIDs in colon cancer cells was COX-2-independent and due to activation of proteosomes which specifically target degradation of Sp1 and Sp4 [78]. These results suggest that further development of this degradation pathway and its specificity may be an important new approach for drug-induced cancer cell growth arrest and inhibition of angiogenesis.

5. Mechanisms of Sp protein action in cancer cells

The primary mechanism of Sp protein-dependent transactivation in cancer and non-cancer cell lines involves initial binding to GC-rich promoter sequences and subsequent interactions with components of the basal transcription machinery to activate gene expression.

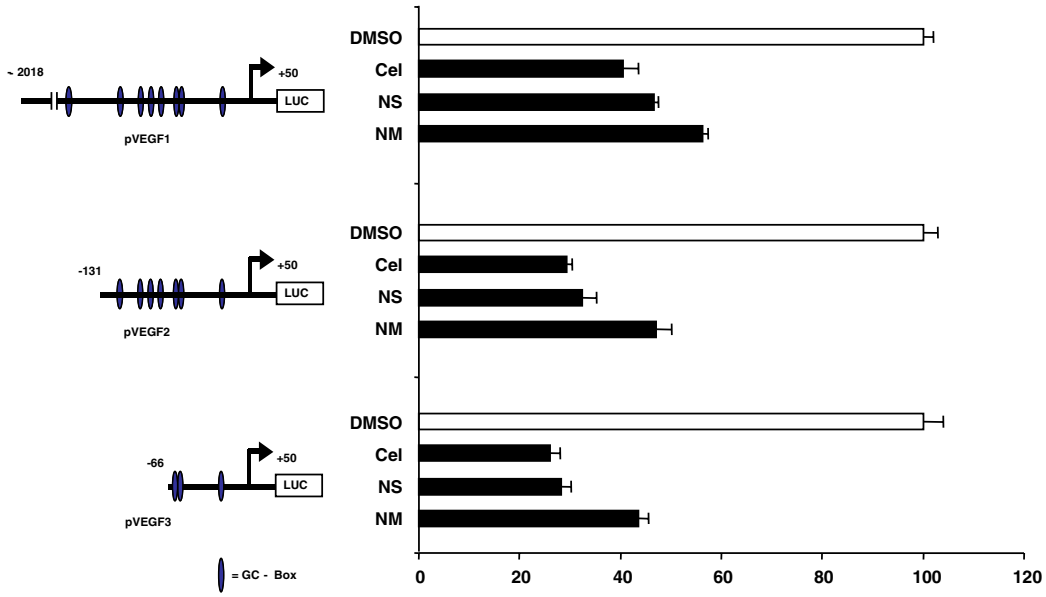

Fig. 5. Regulation of VEGF expression by COX-2 inhibitors in SW480 colon cancer cells [78]. SW480 colon cancer cells were transfected with *VEGF* promoter constructs, treated with the COX-2 inhibitors celecoxib (Cel), nimesulfide (NM), or NS-398 (NS), and luciferase activity was determined [78]. Decreased activity was observed in all treatment groups for the three constructs compared to the solvent (DMSO control).

This sequence of events is common to many transcription factors; however, Sp-dependent activation of genes is highly complex, dependent on both gene promoter and cell context and on interactions with other nuclear proteins as discussed below.

5.1. Promoter DNA-dependent interactions of Sp proteins with other nuclear factors

Sp-dependent activation of genes has been extensively investigated primarily using Sp1 and Sp3 proteins as models. Courey and coworkers [79] first reported synergistic interactions of Sp1 on GC-rich promoters where it has been hypothesised that four Sp1 proteins cooperatively bind to form a homooligomeric complex [80]. Subsequent attenuation of Sp1-dependent regulation of GC-rich promoters has been reported by interactions of Sp1 and other factors to the same elements. Sp1 and Sp3 both bind GC-rich sequences, and these interactions can be cooperative or Sp3 can decrease Sp1-dependent transactivation. For example, it was reported that basal expression of the 15-lipoxygenase-2 gene in human prostate epithelial cells is dependent on interactions of Sp1 with GC-rich sites, whereas Sp3 decreases activity from the same sites [81]. Expression of the human activator protein 2γ gene in breast tumour cells is also regulated by Sp1 (induction) and Sp3 (inhibition) interactions with GC-rich sites, and the cellular Sp1/Sp3 ratios is a determinant factor in expression of this gene in breast cancer cell lines [82]. KLFII and Sp1 bind the same GC-rich site in the caveolin-1 gene reporter, and relative expression of these proteins (Sp1 induces/KLFII inhibits) regulates caveolin-1 [83]. In contrast, expression of CYP3A7 in human liver cancer cell lines is cooperatively activated by both Sp1 and Sp3 through interactions with a G-rich NFκB-like element [84]. Thus, interaction of Sp1, other Sp/KLF proteins, and other transcription factors that bind GC-rich sites (egr-1, NFκB) is an important pathway for regulating gene expression through differential cellular expression of these proteins.

Sp1 and/or Sp3 cooperatively activate several genes through interactions with other DNA-bound transcription factors including E2F proteins, NF-Y, the aryl hydrocarbon receptor (AhR) complex, SMADs, NFκB, GATA proteins, jun and other transcription factors [reviewed in [15–20]]. Genes regulated by Sp1 and these transcription factors may contain their respective *cis*-element; however, direct Sp1-protein interactions have also been observed. For example, Sp1 and NF-Y cooperatively activate the major histocompatibility complex class II-associated invariant chain in cancer cell lines [85]. *In vitro* studies suggest that Sp1 stabilises binding of NF-Y to an adjacent CCAAT box and NF-Y stabilises binding of Sp1 to a GC-rich promoter [86]. Research in this laboratory also observed interactions between NF-Y and Sp1 interactions on the E2F1 promoter where Sp1 did not affect the off-rate of NF-Y dissociation from a DNA complex but Sp1 significantly increased the on-rate of NF-Y–DNA binding [52].

5.2. Interactions of Sp proteins with other nuclear factors

Sp protein-dependent regulation of gene expression also involves direct protein–protein interactions with

other nuclear factors in which only the Sp protein is bound to promoter DNA. Many early studies characterised interactions of Sp1 with several nuclear cofactors associated with the basal transcription machinery, and these include the TATA binding protein (TBP), and several TBP-associated factors (TAFs) including Drosophila TAF110 and its human equivalent (hTAF130), hTAFII55 and hTAFII250 [86–92]. Sp1-dependent transactivation is also dependent on an additional protein complex designated as cofactors required for Sp1 coactivation (CRSP) [27–29]. The CRSP complex and other multiprotein complexes associated with various transcription factors resemble Mediator in yeast which binds Pol II. These complexes contain both unique and overlapping proteins, and the 700 kDa CRSP complex contains at least nine different subunits, including CRSP200, CRSP150, CRSP130, CRSP100, CRSP85, CRSP77, CRSP70, CRSP34 and CRSP30. The precise role of these proteins in mediating Sp-dependent transactivation is not completely defined; however, CRSP200 may be an ''anchor'' cofactor since this protein directly binds with other ligand-activated nuclear receptors and is required for interactions of the complexes with these receptors [93,94].

Sp-dependent transactivation through interactions with GC-rich sites is also modulated by direct Sp1 interactions with many other nuclear factors [reviewed in [20]]. For example, Sp1-interacting proteins include the AhR, Arnt, several GATA transcription factors, p53, MEF2C, SMAD2, SMAD3, SMAD4, Msx1, several viral proteins (c-rel, p50, p52, rel A, tat and BPV-E2), El1, Rb, p107 (Rb-like), DNMT1, ZBP-89, GATA-3, NF-YA, VHL, MyOD, HDAC1, PML, HTLF, E2F1, YY1, MDM2, c-jun, AP-2, myc, NFAT-1, HD protein, cyclin A, Oct-1, TBP, HNF3, BRCA1, and other proteins. In addition, Sp1 also binds directly to several ligand-activated and orphan nuclear receptors, and these include the estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), retinoic acid receptor (RAR), retinoic X receptor (RXR), peroxisome proliferator-activated receptor γ (PPARγ), vitamin D receptor (VDR), steroidgenic factor-1 (SF-1), chicken ovalbumin upstream promoter transcription factor-II (COUP-TFII) and HNF-4 [reviewed in [20]]. Although domain-specific interactions between Sp1 and other proteins have not been completely defined, several studies indicate that the C-terminal C/D domain of Sp1 is the major site for protein interactions. Protein exchange at this site may be mechanistically important, and current studies in this laboratory are investigating the mechanisms of inhibitory AhR-ERα/Sp1 crosstalk which may involve displacement of ERα by the AhR complex since both AhR and ERα bind Sp1 at the C/D domain [95,96].

Interactions between Sp1 and other proteins can differentially affect Sp1-dependent transactivation and thereby provide flexible pathways for gene expression that depends on both gene promoter and cell context. For example, overexpression of c-jun in human HepG2 cancer cells results in increased activation of the cyclin-dependent kinase inhibitor p21 through interactions of c-jun with Sp1 bound to proximal GC-rich sites in the p21 promoter [97]. c-Jun interacts with multiple domains of Sp1 and the glutamine-rich B domain of Sp1 (aa 424–542) is sufficient for c-jun-dependent transactivation. In contrast, c-jun inhibits p21 transactivation in human embryonic epithelial 293 cells through interactions with Sp1 bound to GC-rich site 3 (-77 to -83) in the p21 promoter (note: the region contains six GC-rich sites) [98]. It has been suggested that c-jun-induced hyperphosphorylation of Rb may also be involved in this response. These results demonstrate the important role of Sp1 and possibly other Sp proteins as multifunctional modulators of gene expression through direct interactions with different nuclear proteins (DNA-independent) or by interacting with DNA-bound transcription factors.

5.3. Modification of Sp proteins

Several reports indicate that phosphorylation of Sp1 by various kinase pathways is important for Sp1-dependent activation of some genes, and this adds another layer of complexity to the function of this transcription factor. Regulation of VEGF in several prostate cancer cell lines is dependent on phosphatidylinositol-3-kinase (PI3-K) activity, and this is linked to increased phosphorylation of Sp1 and enhanced binding to the proximal GC-rich $-88/-66$ promoter sequence [99]. In contrast, mitogen-activated protein kinase (MAPK)-dependent phosphorylation of Sp1 is important for induction of VEGF in fibroblasts and *Drosophila* cells [100] and for induction of α6-integrin gene expression in prostate cancer cells [101]. Cyclin A-cyclin-dependent kinases also increase phosphorylation of Sp1 and Sp1-dependent responses/gene expression, and this is also associated with increased DNA binding of Sp1 [102–104]. Atypical protein kinase C and protein kinase A (PKA)-dependent phosphorylation of Sp1 is required for increased platelet-derived growth factor B-chain and *p*27 gene expression, respectively, [105,106] and phosphorylation of Sp1 also enhanced binding to GC-rich elements. Insulin and glucagon also differentially modulate Sp1 in rat hepatoma H4IIE cells. Insulin acts through PI3-K and also enhances *O*-glycosylation of Sp1, whereas glucagon induces phosphorylated Sp1 through the cAMP/PKA pathway [107].

Acetylation of Sp1 and Sp3 have also been linked to increased transactivation associated with these transcription factors [108–110]. For example, Sp3 acts as a transcriptional repressor of TGFBR in late passage MCF-7 cells, whereas induced acetylation of Sp3 results

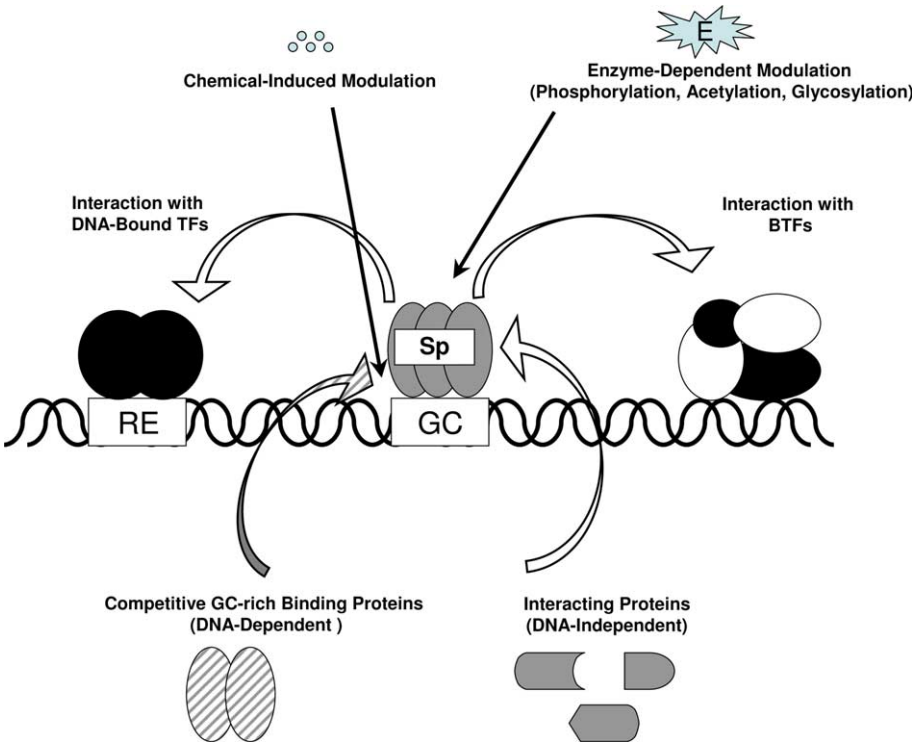

Fig. 6. Proposed model for regulating Sp-dependent transactivation and pathways that modulate this response.

in activation of TGFBR in these cells [109,110]. Similarly, topoisomerase II inhibitors activate the SV40 promoter in cancer cell lines through acetylation of Sp1 [111], and both Sp1 and Sp3 dependent acetylation is dependent on the coregulator p300 which exhibits histone acetyltransferase activity. In contrast, serine/threonine O-linkage with *N*-acetylglucosamine inhibits Sp1-dependent transactivation, and this represents another factor that modifies the function of Sp1 [112].

6. Conclusions

Transcription factors are now recognized as targets for development of new anticancer drugs [113], and this review outlines the important role of Sp-dependent gene expression in tumour development, growth and metastasis. The complexity of Sp-dependent regulation of genes in cancer has primarily been reported for Sp1 and to a lesser extent Sp3; however, based on recent reports it is conceivable that Sp4 protein may also be important in some cases. The complexity of Sp protein-dependent regulation of genes is illustrated in Fig. 6 and includes domain-specific interactions of Sp1 with other nuclear factors (DNA-independent), DNA-bound transcription factors, and chemical- and enzyme-induced modifications. This complexity may be highly advantageous for treating some cancers since combined targeting of different pathways that modulate Sp protein expression and activity may enhance the selectivity of this type of therapy. The importance of Sp proteins and KLFs as tissue-specific drug targets will also require more comprehensive research on their distribution and function in normal and tumour tissues.

Conflict of interest statement

None declared.

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