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Review

Cancer Prevention Research

Specificity Protein Transcription Factors and Cancer: Opportunities for Drug Development

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Abstract

Specificity protein (Sp) transcription factors (TFs) such as Sp1 are critical for early development but their expression decreases with age and there is evidence that transformation of normal cells to cancer cells is associated with upregulation of Sp1, Sp3, and Sp4, which are highly expressed in cancer cells and tumors. Sp1 is a negative prognostic factor for pancreatic, colon, glioma, gastric, breast, prostate, and lung cancer patients. Functional studies also demonstrate that Sp TFs regulate genes responsible for cancer cell growth, survival, migration/invasion, inflammation and drug resistance, and Sp1, Sp3 and Sp4 are also nononcogene addiction (NOA) genes and important drug targets. The mechanisms of drug-induced downregulation of Sp TFs and pro-oncogenic Sp-regulated genes are complex and include ROS-dependent epigenetic pathways that initially decrease expression of the oncogene cMyc. Many compounds such as curcumin, aspirin, and metformin that are active in cancer prevention also exhibit chemotherapeutic activity and these compounds downregulate Sp TFs in cancer cell lines and tumors. The effects of these compounds on downregulation of Sp TFs in normal cells and the contribution of this response to their chemopreventive activity have not yet been determined. *Cancer Prev Res; 11(7); 371–82. ©2018 AACR.*

Background

Tumor development and cancer chemoprevention

Cancer is a complex disease in which there are remarkable differences between and within tumor types and these differences contribute to the difficulties in developing effective therapeutic regimens for some types of cancer. However, despite this heterogeneity among cancers, the "hallmarks of cancer" are relatively well-defined and provide a tentative framework for developing and improving strategies for cancer treatment and for overcoming difficulties associated with tumor heterogeneity (1). The phenotypic and genotypic differences in tumors is related, in part, to the equally complex pathways that contribute to development of a tumor which in classical terms was associated with initiation, promotion, progression, and invasion (2). These stages of cancer development have been linked to specific events (e.g., carcinogen-induced initiation) and have been characterized by identification of intermediary cell types between a normal and a fully transformed cancer cell and these include polyps (colon cancer), various intraepithelial lesions, moles, papillomas, atypical hyperplasias and nodules. The multiple stages of cancer development are closely linked to temporal-dependent mutations, chromosomal translocations and other events which lead to activation of oncogenes and inactivation of tumor suppressor genes, and the order of these events has been well-documented for many cancers (1).

In contrast to cancer chemotherapy and the development and application of targeted chemotherapeutic drugs, the parallel development of targeted chemopreventive agents that may specifically block one or more genes (e.g., oncogenes) to inhibit formation of a transformed cancer cell has not been extensively investigated. There is ample evidence that lifestyle modifications such as increased exercise, avoidance of excess weight, decreased smoking and drinking alcohol, and dietary factors provide some protection from developing cancer (3–6). Moreover, laboratory animal and some human studies demonstrate that many traditional medicines and their active components and some pharmaceuticals exhibit cancer chemoprevention activities. Some of these chemoprotective compounds include anti-inflammatory drugs such as aspirin, sulindac and its metabolites, the antidiabetic drug metformin, curcumin, active components of cruciferous vegetables such as phenethylisothiocyanate (PEITC), triterpenoid natural products, and synthetic analogues including celastrol, betulinic acid and

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bardoxolone, resveratrol, and several polyphenolics (7–13). These compounds exhibit a diverse spectrum of activities that contribute to their efficacy as cancer chemopreventive agents; however, all of these compounds exhibit one common activity as cancer chemotherapeutic agents, namely they induce downregulation of specificity protein (Sp) transcription factors (TF) Sp1, Sp3, and Sp4 and pro-oncogenic Sp-regulated genes (14–27). There is strong evidence that Sp TFs are nononcogene addiction (NOA) genes in cancer (28) and this review will focus on identification and the mechanism of action of anticancer agents that target Sp TFs. In addition, there may also be a hitherto unrecognized role for targeting Sp TFs for chemoprevention and this will also be discussed.

Sp TFs and Development of Cancer

Sp TFs and their role in early development

Specificity proteins (Sp) are members of the Sp/Krüppel-like factor (KLF) family of 25 transcription factors that play multiple roles in maintaining cellular homeostasis and some members are critical factors in diseases including cancer (29). Sp1 was the first transcription factor identified and characterized, and $Sp1^{-/-}$ mouse embryos exhibit retarded development and many other abnormalities and embryolethality is observed around day 11 (30). Knockout mouse models show that Sp (Sp1–Sp9) transcription factors (TF) are required for early development. Sp1–Sp9 exhibit domain structures, which contain a highly conserved C2H2 zinc finger DNA binding domain, which dictates high binding affinity to GC-boxes and lower binding affinities for CT and GT boxes. Sp1–Sp4 also contain an N-terminal glutamine rich transactivation domain (TAD), whereas Sp5–Sp9 lack this domain and these and other Sp-specific structural differences dictate the different functions of these TFs. Research on the structure and function of Sp TFs has focused primarily on Sp1 showing that Sp1 and its modifications (phosphorylation, acetylation, glycosylation) are critical *trans*-acting factors that activate multiple genes and also play a critical role in the basal transcriptional machinery (29, 31). Sp1 also interacts with many other nuclear factors to modulate gene expression. In contrast, the functions of other Sp TFs have not been as extensively investigated and it is possible that Sp3 and Sp4 that exhibit many of the same structural and DNA binding affinities observed for Sp1 may also exhibit as yet unknown functions similar to Sp1.

Sp TFs and normal cell transformation

Although Sp1 is critical for embryonic development, there is evidence in rodents and humans that Sp1 expression in normal tissues decreases with age (32, 33), whereas Sp1 and also Sp3 and Sp4 are highly expressed in various tumors. Differences in the expression and functions of Sp1 in human fibroblasts and patient-derived fibrosarcoma cell lines were investigated by knockdown studies. For example, carcinogen- or oncogene-induced transformation of human fibroblasts resulted in an 8- to 18-fold increase in expression of Sp1 protein, whereas knockdown of Sp1 in fibrosarcoma cell lines decreased their ability to form tumors in athymic mice (34), and the role of Sp1 and Sp3 in malignant transformation of human skin fibroblasts has been reviewed (35). EGF-mediated transformation of bladder epithelial cells is due to RING-domain-dependent induction of Sp1 and Sp-regulated miR-4295 (36), and Kras-induced transformation of MCF10a mammary cells is also due to Sp1-dependent repression of miR-200 family miRNAs (37). APOBEC3G expression in peripheral blood mononuclear cells increases cell-cycle progression and expression of Sp1 and Sp1-regulated genes, suggesting a role for Sp1 in transformation of these cells (38). Stable transduction of human skeletal muscle fibroblasts with PAX3-FOXO1, telomerase, and NMyc resulted in formation of transformed cell lines that resemble alveolar rhabdomysarcoma (ARMS) cells, and both the genetically transformed and patient-derived ARMS cells expressed high levels of Sp1, Sp3, and Sp4 (39). Interestingly, genetic transformation of the muscle fibroblasts dramatically increased expression of Sp1 and Sp3 but Sp4 protein was highly expressed in both the nontransformed and transformed cells (39). Most of these studies have focused on Sp1 (and not Sp3 or Sp4) and it is clear that increased or activated Sp1 and possibly other Sp TFs are important in normal cell transformation and thus represent a potential target for chemopreventive agents. Drugs that specifically inhibit Sp TFs during transformation have not previously been investigated; however, as indicated above, many chemopreventive compounds that also exhibit cancer chemotherapeutic activity act, in part, through downregulation of Sp TFs and this will be thoroughly examined in this review.

Sp TFs and Cancer

Sp overexpression as a negative prognostic factor

Studies in several laboratories have detected high levels of Sp1, Sp3, and Sp4 in pancreatic, bladder, esophageal, breast, prostate, lung, colon, multiple myeloma, epidermal, thyroid, and RMS cell lines, and Table 1 summarizes the prognostic significance of Sp1 for several human cancers (40–57). The expression of Sp1 and, in some cases Sp3, in most tumors was higher than in nontumor tissue and high expression of Sp1 in tumors was a negative prognostic factor for patient survival or correlated with a higher grade of malignancy. However, there were at least two tumor types (lung and breast) where there were differences between studies. In lung cancer patients, it was reported that Sp1 levels decreased with increasing tumor stage and higher levels correlated with increased survival, and this was confirmed in cell culture experiments (50). In contrast, a second study showed a parallel expression of Sp1 and CD147 in lung tumors (51), and CD147 expression has

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Table 1. Summary of Sp1 expression and prognostic significance in tumors

Tumor (ref.)	High/low	Prognosis (high Sp) and expression
Pancreatic (40, 57)	17/25	Increased Sp1 in higher grade (decreased survival)
	Variable	Poor prognosis – vascular invasion
Glioma (41, 42)	130/92	Decreased survival
	27/28	Increased expression in higher grade
Colon (43, 60)	—	Sp1/Sp3 elevated and decreased survival (binding to μPAR)
	61/25	Increased depth of invasion (inversely correlated with miR-149
Gastric (44–47)	Variable	Increased expression in high grades and poor survival
	Variable (?)	Survival decreased in diffuse type by Sp1; low in most intestinal types
	Variable	Increased with stage; decreased survival (correlated with VEGF)
	35/30	Increased with stage; decreased survival
Head and neck (48)	26/26 (Sp3)	Increased Sp3 predicted poor survival
Prostate (49)	Variable	Sp1/Sp3/FLIP combination predicted increase recurrence
Lung (50, 51, 58)	40/6 (I and II)	Lower Sp1 with increasing stage; low Sp1 correlated with poor prognosis
	29/43 (IV)	
	Variable	Sp1 correlated with CD147 (invasive factor)
		Increase Sp1 in tumors
Breast (52–54)	43/17	Increased invasion, stage and decreased survival
	Variable (?)	Lower Sp1 with increasing stage
	Variable	Sp1 correlated with CD147
		Sp1 predicts poor prognosis
Rhabdomysarcoma (39)	—	Highly overexpressed in tumors
Hepatocellular (55, 56)	34/50	Overexpressed in tumor
	15/25	Higher Sp1 – poor prognosis

been linked to tumor invasion and metastasis and poor prognosis for lung cancer patients and another study also reported high levels of Sp1 in lung cancer versus normal lung tissue (58). In breast cancer patients, one study indicated that Sp1 decreased with increasing tumor stage and low levels of Sp1 correlated with increased tumor metastasis (52), where other reports indicate that overexpression of Sp1 in breast tumors is associated with poor prognosis (53, 54). Clearly differences in the prognostic value of Sp1 in breast and lung tumors (and cells) requires further investigation, and future studies should also evaluate whether Sp3 and Sp4 are prognostic factors for various cancers.

Regulation of Sp TF expression by miRNAs

The mechanisms associated with high levels of Sp1 and other Sp proteins in tumors are not well defined; however, one possible pathway may be related to the inverse relationships between the expression of Sp1 and several miRNAs in normal versus tumor tissue (59). miRNAs that inhibit Sp1 expression include miR-200b/200c, miR-335, miR-22, miR-23b, miR-29c, miR-145/miR-133a/miR-133b, miR-137, miR-149, miR-223, miR-330, miR-375, miR-29b, and miR-429, and for some of these miRNAs, high and low expression are positive and negative prognostic factors, respectively, in various cancer types. For example, miR-149 directly targets and represses expression of Sp1 in colon cancer, and tumors from patients with high expression of miR-149 exhibit increased survival, whereas Sp1 expression was increased in tumors exhibiting increased invasion (60). Transfection of colon cancer cells with miR-149 mimics decreased growth, colony formation and invasion, and in colon cancer cells and colon tumors the expression of miR-149 and Sp1 was inversely related (60). Some miRNAs overexpressed in cancer cells including members of the miR-17-92 (miR-20a/miR-17-50) and miR-239∼27a∼24-2 (miR-27a) clusters indirectly help to maintain high expression of Sp1, Sp3, and Sp4 in cancer cells (61, 62). ZBTB proteins are transcriptional repressors that bind GC-rich motifs and inhibit expression of Sp1, Sp3, Sp4, and Sp-regulated genes by competitively displacing *trans*-acting Sp transcription factors from promoter DNA-binding sites (62). It was initially shown that miR-27a inhibited expression of ZBTB10 in breast cancer cells (61) and subsequent studies have identified miR-27a as an inhibitor of ZBTB34 (14). miR-20a and miR-17-5p inhibit expression of ZBTB4 and studies in multiple cancer cell lines demonstrate that repression of the ZBTBs results in high expression of Sp1, Sp3, and Sp4 which can be decreased either by transfection of cells with the miRNA antagomirs or ZBTB overexpression (14, 61, 62). Thus, miRNAs regulate Sp protein expression by direct sequence-specific interactions with 3′-UTR sequences in Sp genes and by miRNA-dependent suppression of Sp-repressors such as ZBTB4, ZBTB10, and ZBTB34. It is possible that the increase in expression of Sp1, Sp3, and Sp4 induced by cell transformation may also be regulated by changes in miRNA expression; however, this has not yet been investigated.

Sp transcription factors as NOA genes

Sp1 has been extensively characterized as a pro-oncogenic factor that regulates genes required for cell growth, survival, migration, and metastasis and this has been extensively reviewed (59, 63, 64). These results correlate with the overexpression of Sp1 in tumors and cancer cell lines and its role as a negative prognostic factor. Sp3 and Sp4 are overexpressed in cancer cell lines and results of RNA interference studies showed that individual

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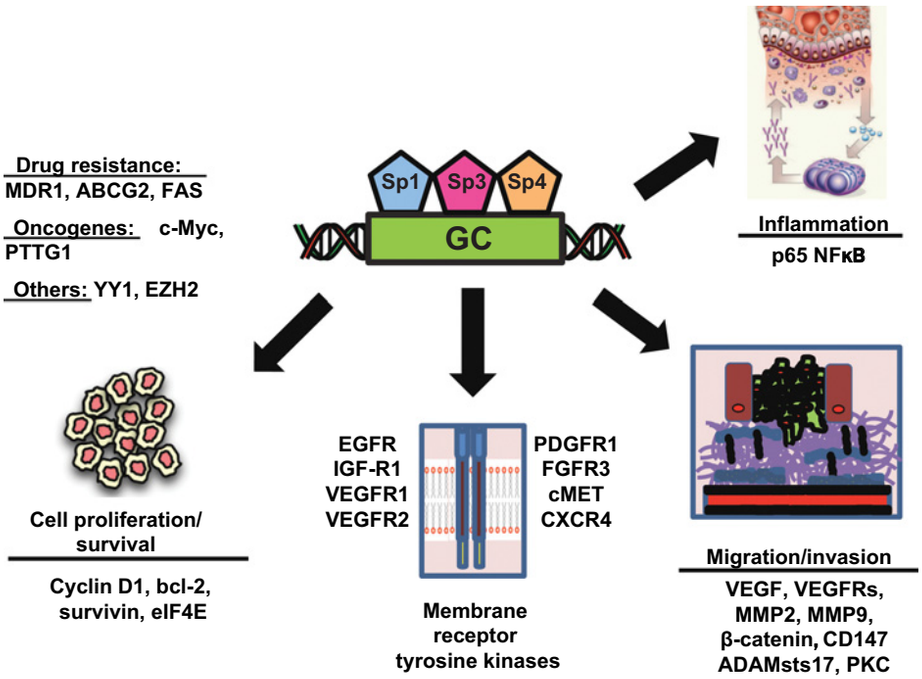

Figure 1.
Sp-regulated genes/pathways. Sp TFs factors regulate expression of proliferation, survival, migration/ invasion, inflammatory, and drug resistance pathway and related genes in cancer cells (14, 15, 18–20, 22, 23, 28, 59, 61–63, 66, 69, 70, 84).

knockdown of Sp1, Sp3, and Sp4 indicated that these TFs exhibited similar pro-oncogenic functions with only cell context–dependent differences in potency. These results were consistent with the designation of Sp TFs as NOA genes which are defined as essential genes that "support the oncogenic phenotype of cancer cells but are not required to the same degree for the viability of normal cells" (65). A recent report directly compared the effects of individual knockdown of Sp1, Sp3, and Sp4 by RNAi in A549 lung, SW480 colon, 786-O kidney, SKBR3 and MDA-MB231 breast, and Panc1, L3.6pL, and MiaPaca2 pancreatic cancer cells on cell proliferation, survival, and migration (28). These results and a comparable study in Rh30 rhabdomyosarcoma cells (66) demonstrated that individual knockdown of Sp1, Sp3, and Sp4 significantly decreased cell growth and migration and induced apoptosis in nine cancer cell lines derived from 6 different tumors (28). Moreover, knockdown of Sp1 or Sp1/Sp3/Sp4 (combined) in L3.6pL cells also decreased tumor growth *in vivo* in a xenograft model and both *in vitro* and *in vivo* studies showed that Sp knockdown decreased several pro-oncogenic Sp-regulated genes including survivin, bcl-2, VEGF, and EGFR (ref. 66; Fig. 1).

It was recently suggested that there were concerns regarding development of anticancer drugs that target Sp1 as Sp1 also regulates genes that protect against cancer (64). Highly invasive Panc1 pancreatic cells were used as a model to investigate genes regulated by Sp1, Sp3, and Sp4 and their functions. Results of RNAi coupled with microarray analysis showed that Sp1, Sp3, and Sp4 regulated unique and common sets of genes associated with cell growth, survival, and migration/invasion with the maximum gene overlap between Sp3 and Sp4 (28). Moreover, Ingenuity Pathway Analysis (IPA) confirmed that all three Sp TFs regulate both cancer-promoting and cancer-inhibiting genes and this was confirmed by real-time PCR. However, examination of the gene set by causal IPA which quantitatively integrates all of the changes in gene expression strongly predicted that Sp1, Sp3, and Sp4 were associated with cell proliferation, survival, migration, and invasion and not the reverse pathways (28). These results are consistent with the designation of Sp1, Sp3, and Sp4 as NOA genes and their importance as targets for anticancer drugs (65).

Sp TFs as Targets for Cancer Chemotherapeutic Drugs

Small-molecule drugs and natural products that target Sp TFs

There are an increasing number of small-molecule anticancer agents that decrease expression of Sp1 in cancer cells and this includes well known cancer chemopreventive agents metformin, aspirin, sulindac, isothiocyanates, polyphenolics, and other natural products and their synthetic analogues, anticancer drugs such as bortezomib and retinoids (7–27, 59, 66–93; Table 2). These compounds were not developed to block Sp1/Sp1-regulated genes but this activity may contribute to their efficacy as anticancer drugs. Initial studies showed that the NSAID and COX-2 inhibitor celecoxib decreased angiogenesis and the angiogenic gene *VEGF* in pancreatic cancer cells (67), suggesting that NSAIDs may target Sp TFs as *VEGF* is an Sp-regulated

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Table 2. Small molecules that downregulate Sp transcription factors in cancer cells

		References
1.	Natural products and synthetic analogues: PEITC, BITC, celastrol, curcumin, betulinic acid, resveratrol, polyphenolics, bardoxolone-Me (CDDO-Me), CDODA-Me, isorhapontigenin, triptolide, β-lapachone, honokiol, piperlongumine	(14, 15, 18–22, 25, 26, 39, 70, 74, 81, 85–92)
2.	NSAIDs: Aspirin, tolfenamic acid, celecoxib, GT-094, diclofenac, salicylate	(16, 17, 67, 68, 83)
3.	Other drugs: Metformin, HDAC inhibitors, retinoids, α-tocopherol, thiazolidinediones, proteasome inhibitors	(23, 24, 66, 75–80)
4.	Other compounds: Arsenic trioxide, ascorbic acid, *t*-butyl hydroperoxide, hydrogen peroxide, zinc depletion, WIN 55,212-12, penfluridol	(70–73, 82, 84, 93)

gene in many cancer cell lines. An initial screening of several different structural classes of NSAIDs showed that tolfenamic acid used as an antiinflammatory drug and structurally-related biaryl derivatives (diclofenac and diflunisal) decrease expression of Sp1, Sp3, Sp4 and VEGF in pancreatic cancer cells (68). The antineoplastic activity of tolfenamic acid has been confirmed in multiple tumor models (16) and would seem to be an excellent drug candidate alone and in combinations for human clinical trials. Moreover, in an orthotopic mouse model of pancreatic cancer, tolfenamic acid decreased tumor growth and metastasis to the liver and was more effective and potent than gemcitabine which is the current drug of choice for pancreatic cancer chemotherapy (68). Other NSAIDs including aspirin and salicylate, and a nitro NSAID (GT-094) also downregulate Sp1, Sp3, Sp4, and Sp-regulated genes in colon cancer cells, suggesting that this pathway contributes to the anticancer activity of aspirin (17, 69).

Although tolfenamic acid was the first drug identified that decreased expression not only of Sp1 but also Sp3 and Sp4, subsequent studies showed that many compounds including several currently used anticancer agents also decreased Sp1, Sp3 and Sp4 gene products in cancer cell lines. These include cannabinoids, metformin, triterpenoids such as betulinic acid, celastrol, the synthetic compound methyl 2-cyano-3,11-dioxo-18β-olean-1,13-dien-30-oate (CDODA-Me) and methyl 2-cyano-3,12-dioxo-oleana-1,9-dien-28-oate (CDDO-Me, bardoxolone) derived from glycyrrhetinic and oleanolic acids, respectively. In addition, several reactive oxygen (ROS)-inducing agents including CDDO-Me, curcumin (in some cell lines), the nitro-aspirin GT-094, phenethylisothiocyanate (PEITC), HDAC inhibitors, benzylisothiocyanate (BITC), penfluridol, piperlongumine, and arsenic trioxide also induce ROS-dependent downregulation of Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated genes (15–20, 66, 70–73, 91–93). Moreover, hydrogen peroxide, *t*-butyl hydroperoxide, pharmacologic doses of ascorbate that induce hydrogen peroxide also decrease expression of Sp1, Sp3, and Sp4 in cancer cells (71, 73), indicating that the efficacy of many ROS-inducing anticancer agents may be linked to their downregulation of Sp TFs.

Mechanisms of drug-induced Sp1 degradation and repression

Anticancer drugs downregulate expression of Sp TFs by activation of protein degradation pathways (e.g., proteasomes and caspases), repression of Sp gene expression or a combination of both pathways and activation of these pathways is both compound- and cell context-dependent (59). For example, betulinic acid induces proteasome-dependent degradation of Sp1, Sp3, and Sp4 in prostate cancer cells (21), ROS-dependent repression of Sp1, Sp3, and Sp4 in colon cancer, and in breast cancer cells these effects are both ROS- and cannabinoid (CB) receptor-dependent (74). Betulinic acid binds the CB1 and CB2 receptors and in breast cancer cells, CB receptor antagonists or knockdown partially reverse the drug-induced effects on Sp proteins. Curcumin also decreases expression of Sp1, Sp3, and Sp4 by activation of proteasomes in bladder cancer cells and by ROS-induced gene repression in colon and pancreatic cancer cell lines (19, 22, 72).

There are several reports that investigate the mechanism of Sp1 degradation. Retinoid-induced degradation of Sp1 is caspase-dependent (75), and subsequent studies suggest that this may also involve parallel induction of transglutaminase that crosslinks (and inactivates) Sp1. An orally active proteasome inhibitor K-7174 used for the treatment of bortezomib-resistant multiple myeloma also induces caspase-8–dependent degradation of Sp1 (76), whereas the retinoid-induced response is caspase-3–dependent (77) and anti-IgM–induced B-cell apoptosis also results in caspase-3–dependent cleavage of Sp1 (78).

Vitamin E succinate–mediated proteasome-dependent degradation of Sp1 (Fig. 2B) is due to increased protein phosphatase 2A (PP2A), which in turn inactivates JNK kinase activity (79), whereas thiazolidinediones-induced proteasome degradation of Sp1 is due to upregulation of the E3 ligase $SCF^{\beta\text{-}TrCP}$ and the β-transducin repeat-containing protein (β-TrCp) directly bound Sp1 to enhance ubiquitination (80). Proteasome-dependent degradation of Sp1 by betulinic acid in HeLa, liver, and lung cancer cells is due to decreased expression of the Sumo protease SENP1, resulting in increased levels of sumoylated Sp1, which is rapidly exported into the cytosol and ubiquitinated by the RING finger protein 4 E3-ligase (81).

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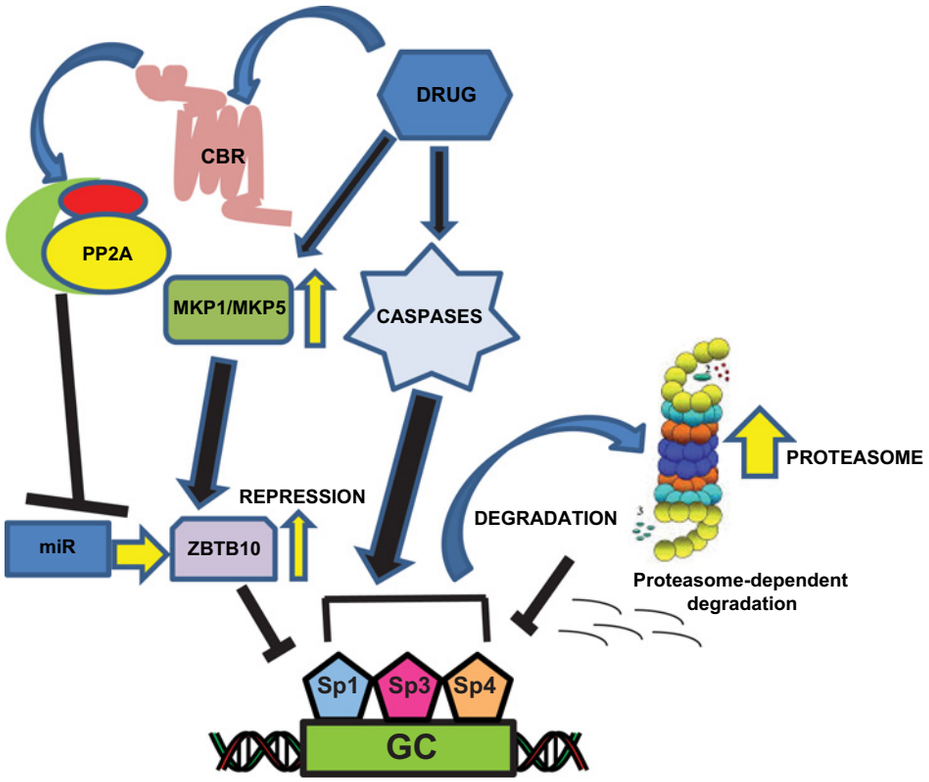

Figure 2.
ROS-independent degradation/repression of Sp1, Sp3, and Sp4. Sp1 or all three Sp proteins are degraded by drug-induced activation of caspases or proteasomes (17, 21, 22, 68) or repressed via activation of phosphatases (23).

Degradation and suppression of Sp1, Sp3, and Sp4: ROS-independent

The zinc chelator TPEN induces caspase-3–dependent proteolysis of Sp1, Sp3, and Sp4 and, in Jurkat and HeLa cells, this can be rescued by addition of excess zinc (82). Aspirin also induces caspase-dependent degradation of nuclear Sp1, Sp3, and Sp4 proteins in RKO and SW480 colon cancer cells (Fig. 2). Similar responses are observed for TPEN, and addition of zinc sulfate blocks the effects of both aspirin and TPEN (17). There is some evidence that aspirin can sequester zinc and this would disrupt the zinc finger DNA binding domains of Sp1, Sp3, and Sp4 and possibly enhance their rate of degradation. In colon cancer cells, tolfenamic acid induces caspase-dependent degradation of nuclear Sp1, Sp3, and Sp4 and this response is only partially rescued by zinc in SW480 but not RKO cells (83), suggesting another as yet unknown mechanism for caspase-dependent Sp degradation in cancer cells. Several reports show that betulinic acid, celastrol, tolfenamic acid, curcumin, and metformin induce cancer cell–specific degradation of Sp1, Sp3, and Sp4 by activated proteasomes; however, the mechanisms have not been determined.

A major pathway for drug-induced repression of Sp1, Sp3, and Sp4 gene expression in cancer cells is due to induction of ZBTB transcriptional repressors that competitively displace Sp TFs from *cis*-acting promoter GC-boxes found in the Sp1, Sp3, and Sp4 and Sp-regulated gene promoters. For example, the cannabinoid (CB) receptor ligand WIN 55,212-2 activates PP2A in colon cancer cells and induces ZBTB10 via downregulation of miR-27a (84). Induction of mitogen-activated protein kinase-1 (MPK-1) and MPK-5 by metformin, curcumin, and rosiglitazone also induces ZBTB10 and downregulates miR-27a, resulting in repression of Sp1, Sp3, and Sp4 gene expression (23); however, the detailed mechanisms of phosphatase induction (PP2A, MKP-1, and MKP-5) and their effects on miR-27a and possibly other miRNAs have not been determined.

Mechanism of action of ROS-inducing anticancer agents

ROS-inducing anticancer drugs such as buthionine sulphoximine, β-lapachone, imexon, and methoxyestradiol that directly target antioxidant pathways have been developed for clinical applications (94). Moreover, many clinically used drugs such as arsenic trioxide, taxol, paclitaxel, doxorubicin, and platinum-derived drugs also induce ROS and this contributes to their overall anticancer activity (rev. in ref. 94). Although ROS-inducing anticancer agents induce DNA damage and both oxidative and ER stress through initial mitochondrial damage, there are also reports that these compounds induce many of the same responses and genes observed after Sp knockdown (Fig. 1). Moreover, initial studies showed that pro-oxidants such as hydrogen peroxide, *t*-butyl hydroperoxide, pharmacologic doses of ascorbate (generates hydrogen peroxide), and arsenic trioxide downregulated Sp1, Sp3, Sp3, and Sp-regulated genes (71–73). Similar ROS inducers such as curcumin, BITC, HDAC inhibitors, celastrol, CDDO-Me,

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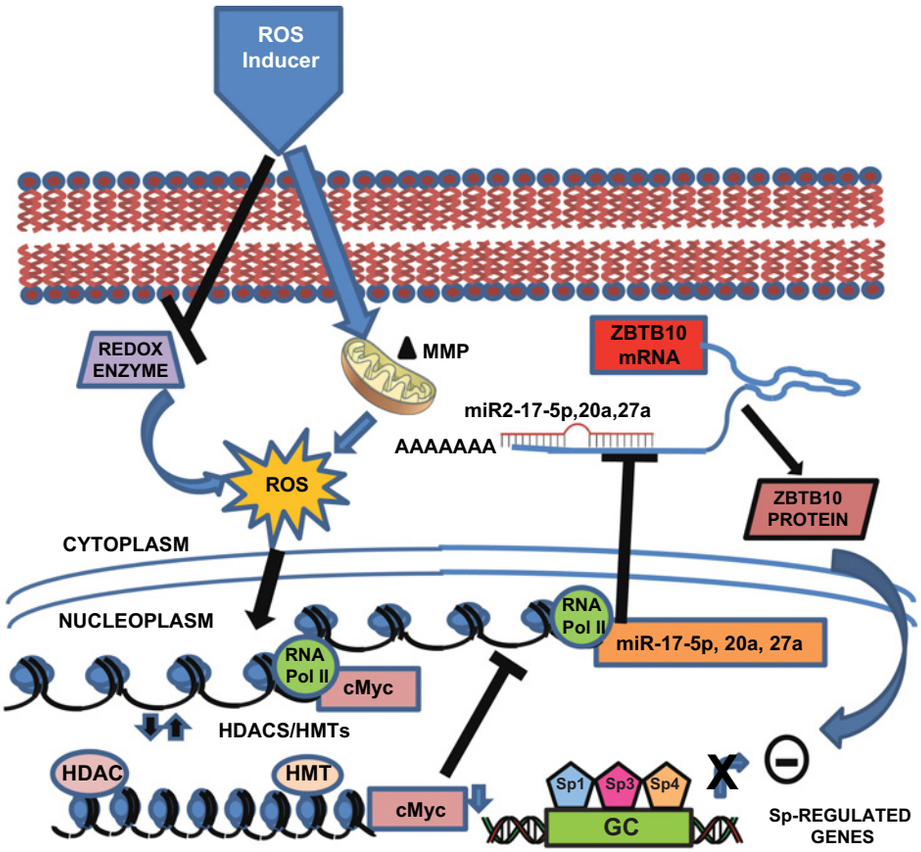

Figure 3.
ROS-dependent repression of Sp1, Sp3 and Sp4. ROS initially induces epigenetic downregulation of cMyc, which in turn decreases expression of cMyc-regulated miRNAs and induction of the miRNA-regulated ZBTB transcriptional repressors (14, 15, 18, 19, 66, 69).

GT-094, betulinic, and PEITC also induced ROS-dependent repression of Sp gene expression, which was significantly attenuated by cotreatment with antioxidants such as glutathione (15–20, 66, 70–73). Since both ROS-dependent repression of Sp and Sp-regulated genes and also growth, survival, and migration/invasion are attenuated after cotreatment with glutathione (GSH) and as direct Sp knockdown results in similar responses (28), then the antineoplastic activities of ROS/ROS-inducing agents must be due, in part, to downregulation of Sp TFs.

A key study in determining the mechanism of action of ROS inducers was a report showing that treatment of SW480 colon cancer cells with hydrogen peroxide induced a genome-wide shift of chromatin-modifying complexes from non-GC-rich to GC-rich sites, and one of the genes downregulated by hydrogen peroxide was *cMyc* (95). It has also been reported that cMyc regulates expression of miR-27-92 and miR-23a∼27a∼24-2 clusters containing miR-27a and miR-20a/miR-17-5p, respectively (96, 97). A subsequent study reported that the ROS inducer PEITC decreased expression of cMyc in pancreatic cancer cells and ChIP analysis showed that this was accompanied by loss of cMyc from the miRNA promoters (14). Moreover, the rapid (≤3 hour) PEITC-induced downregulation of cMyc was accompanied by a decrease in *Pol*II and the activation mark H4K16Ac on the cMyc promoter. In Panc1 (but not Panc28 or L3.6pL) cells, Sp1 was also rapidly decreased (≤3 hours) after treatment with PEITC and the histone activation marker H3K4me3 was decreased, whereas H4K16Ac was unchanged. Thus, the overall mechanism of action of ROS-inducing anticancer agents involves ROS-mediated epigenetic downregulation of cMyc, decreased expression of cMyc-regulated miRNAs, and induction of miRNA-suppressed ZBTBs (ZBTB10, ZBTB34, and ZBTB4), which repress Sp1, Sp3, and Sp4 gene expression (Fig. 3). These reports not only demonstrate a novel mechanism for repression of Sp TFs and Sp-regulated genes but also show that the oncogene *cMyc* regulates the high expression of the NOA Sp genes and ROS simultaneously targets both oncogene and NOA genes in cancer cells.

Summary

High expression of Sp1 is a negative prognostic factor for multiple tumors (Table 1) and with few exceptions, Sp1 exhibits pro-oncogenic functions and plays a role in cancer cell proliferation, survival, angiogenesis, migration, and invasion. Sp1 clearly fits the description of a NOA gene. RNAi studies show that both Sp3 and Sp4 also exhibit pro-oncogenic functions and, in the future, the focus on Sp1 should be expanded to include Sp3 and Sp4 which are coexpressed (highly) along with Sp1 in cancer cells and

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tumors. There is now strong evidence that multiple antineoplastic drugs target degradation or repression of Sp transcription factors through several different pathways (Figs. 2 and 3), and activation of one or more pathways is both drug- and cell context–dependent. ROS-inducing anticancer agents also decrease expression of Sp1, Sp3, and Sp4 in cancer cells and this involves epigenetic downregulation of c*Myc* and c-*Myc*–regulated miRNAs and induction of ZBTB repressors. Upregulation of Sp TFs during the transformation of a precancerous to a transformed cell also provides an opportunity for applications of drugs that target Sp TFs as cancer chemopreventive agents and this is an area that should be further investigated. Results of several laboratory animal studies show the advantages of drug combination chemotherapies that include an agent specifically targeting Sp transcription factors (98–100) and this approach should also be used in human clinical studies to improve outcomes and overcome drug resistance in cancer chemotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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