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The high mobility group A1 molecular switch: turning on cancer – can we turn it off?

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Abstract

Introduction—Emerging evidence demonstrates that the high mobility group A1 (HMGA1) chromatin remodeling protein is a key molecular switch required by cancer cells for tumor progression and a poorly differentiated, stem-like state. Because the *HMGA1* gene and proteins are expressed at high levels in all aggressive tumors studied to date, research is needed to determine how to ‘turn off’ this master regulatory switch in cancer.

Areas covered—In this review, we describe prior studies that underscore the central role of HMGA1 in refractory cancers and we discuss approaches to target HMGA1 in cancer therapy.

Expert opinion—Given the widespread overexpression of *HMGA1* in diverse, aggressive tumors, further research to develop technology to target HMGA1 holds immense promise as potent anticancer therapy. Previous work in preclinical models indicates that delivery of short hairpin RNA or interfering RNA molecules to ‘switch off’ *HMGA1* expression dramatically impairs cancer cell growth and tumor progression. The advent of nanoparticle technology to systemically deliver DNA or RNA molecules to tumors brings this approach even closer to clinical applications, although further efforts are needed to translate these advances into therapies for cancer patients.

Keywords

high mobility group A1; high mobility group A1 protein; molecular switch; therapeutic target; tumor progression

1. Introduction

Recent studies provide compelling evidence that the high mobility group A1 protein (HMGA1) is a key molecular switch that turns on cancer growth and drives tumor progression [1,2]. Research is now needed to determine how to turn off this fundamental ‘cancer switch’. Initially discovered in extraordinarily proliferative, aggressive cervical cancer cells (HeLa) [3], HMGA proteins are highly expressed during embryogenesis, with low or undetectable levels in most adult tissues [4]. The *HMGA1* gene is also overexpressed in all poorly differentiated tumors studied to date [5-17] and high expression portends a poor outcome in many tumor types [5-9,11,15-17]. *HMGA1* is located on chromosome 6p21 and encodes the HMGA1a and HMGA1b protein isoforms, which result from alternatively spliced mRNA [18,19]. HMGA1 proteins are small, non-histone, chromatin remodeling proteins named after their rapid movement through polyacrylamide gel (thus, high mobility group). They are members of the HMGA family of proteins, which includes the HMGA1 protein isoforms (HMGA1a, HMGA1b) and HMGA2 [5-7,20-24]. HMGA proteins are distinguished from other HMG proteins by the AT-hook DNA binding domains that facilitate binding to AT-rich regions in the minor groove of DNA [25-28]. Although HMGA1 proteins lack intrinsic transcriptional activity, they modulate gene expression by altering chromatin structure and orchestrating the assembly of additional transcription factors to DNA, forming a higher order transcriptional complex or ‘enhanceosome’ [29-35]. HMGA1 proteins also globally activate gene expression by displacing histone H1 proteins, which maintain chromatin in a tightly wound, inactive state [36-40]. In fact, both HMGA1 and histone H1 proteins bind to the same AT-rich sequences in DNA [41]. Sequence analysis in plants demonstrates significant homology between HMGA1 and histone H1, indicating that they may have evolved from the same ancestral protein [38], although their functions have since diverged. The unique expression pattern of *HMGA1* in aggressive cancers, but not in normal tissues, suggests that it could serve as a useful biomarker and effective target for anticancer therapy [16].

Remarkably, *HMGA1* is overexpressed in poorly differentiated cancers originating from all three germ layers, thus suggesting that it plays a fundamental role in tumorigenesis, regardless of where the tumor starts (Table 1) [16]. For example, high *HMGA1* gene or protein levels are found in cancers of the thyroid [42-44], lung [11,12,45], breast [1,8,10,46-48], bladder [8], prostate [49-51], colon [2,52-55], pancreas [14,15,56-59], uterine corpus [60], uterine cervix [61], kidney [62], head and neck [63], nervous system [8,9,64-68], stomach [69,70], liver [71] and hematopoietic system [13,17,72-78]. Global gene and protein expression studies reveal that *HMGA1* overexpression correlates with adverse clinical outcomes. The first such study found that the *HMGA1* gene expression is among a signature of genes that predict treatment failure in primary medulloblastomas [8]. In lung carcinomas, HMGA1 protein-staining is inversely correlated with patient survival

[11]. Higher *HMGA1* mRNA and protein levels were discovered in hepatocellular carcinoma with intrahepatic metastases as compared to those without intrahepatic metastases [71]. In breast cancer, HMGA1 protein levels correlate with high-grade/poor differentiation [48]. *HMGA1* gene expression was also associated with high grade in uterine cancers [60]. In pancreatic cancer, HMGA1 protein levels correlate with both poor differentiation status and decreased survival [15]. *HMGA1* expression is also higher in blasts from patients at relapse in a study of pediatric B-lineage acute lymphoblastic leukemia [17]. Moreover, an analysis of global gene expression profiles from independent studies found that *HMGA1* is among a core 'signature' composed of nine transcription factor genes enriched in embryonic stem cells and high grade/poorly differentiated cancers (breast, bladder and brain) [8]. Importantly, overexpression of this signature was associated with poor survival in patients with these cancers. HMGA1 also induces oncogenic transformation in cultured cells and causes aggressive tumors in transgenic mouse models [74,78-80]. Collectively, these studies suggest that HMGA1 drives tumor progression in diverse tumors.

Although it is well established that *HMGA1* is overexpressed in most, if not all, aggressive tumors studied to date [5-7], the basis for overexpression is not well understood. We first discovered that *HMGA1* expression is induced by the oncogenic transcription factor, cMYC, which provided early evidence that *HMGA1* may function in neoplastic transformation [72]. Nonetheless, *HMGA1* is enriched in tumors that do not have amplification or overexpression of cMYC, suggesting that *HMGA1* expression is induced through other pathways. Two prior studies subsequently demonstrated that AP1 transcription factors also upregulate *HMGA1* expression [81,82]. Recent work suggests that microRNAs may regulate oncogenes such as *HMGA2*, although microRNA regulation of *HMGA1* has not been clearly established [24]. In addition, promoter methylation studies show that the *HMGA1* 5' untranslated region is hypomethylated in embryonic stem cells but methylated in differentiated fibroblasts, thereby suggesting that this may be another means to regulate *HMGA1* expression [80,83]. Another recent study found that Tcf4 in the Wnt- β -catenin pathway induces expression of *HMGA1* [84]. In contrast to *HMGA2*, there are very few reports of translocation events that lead to aberrant expression of *HMGA1*. Further work is needed to elucidate the molecular underpinnings of *HMGA1* overexpression in cancer.

1.1 Results from recent work and significance

Recent work has uncovered a unique role for HMGA1 in normal embryonic stem cells in addition to tumor-initiator/ cancer stem cells [1,2,80]. In human embryonic stem cells, HMGA1 maintains a poorly differentiated, pluripotent state through epigenetic remodeling and regulating stem cell transcriptional networks [80]. Forced expression of HMGA1 prevents differentiation in embryonic stem cells by maintaining high expression of stem cell genes involved in pluripotency and self-renewal, such as *OCT4* and *cMYC*. Moreover, HMGA1 is required for reprogramming somatic cells into induced pluripotent stem cells (iPSCs) by the Yamanaka factors (Oct4, Sox2, Klf4, cMyc). Inhibiting *HMGA1* expression or function prevents the derivation of iPSCs by the Yamanaka factors. In contrast, including HMGA1 with the Yamanaka factors enhances the reprogramming process, resulting in larger and more abundant iPSC colonies [80]. During the reprogramming process, HMGA1 induces expression of *LIN28*, *cMYC* and *SOX2*. Perhaps not surprisingly, studies also show

that *HMGA1* is highly expressed in adult stem cells, such as hematopoietic stem cells and intestinal stem cells [75,76,83,85-87], which could complicate efforts to target *HMGA1* in cancer. A study of mice null for *HMGA1* reported aberrant hematopoiesis, with a paucity of T-lymphoid cells and an expansion in B-lymphoid cells and myeloid cells [87], whereas transgenic mice overexpressing murine *Hmg1a* or human *HMGA1b* developed T-lymphoid malignancies [74,88]. In addition, tumor cells from the *HMGA1a* transgenics can be serially transplanted, indicating that they have long-term self-renewal, like stem cells [74]. During lymphoid tumorigenesis, HMGA1 induces genes that are involved in inflammation, stem cells and cell cycle progression [89]. The *HMGA1a* mouse also develops marked proliferative changes in the intestine, suggesting that there is enhanced intestinal stem cell function [2]. Female *HMGA1a* transgenic mice also develop intestinal polyps and uterine sarcomas [2,60]. Together, these studies suggest that HMGA1 plays a central role in normal stem cell function during development, and perhaps, in adult stem cells postnatally.

Recently published studies report the discovery that ‘switching off’ *HMGA1* expression results in striking antitumor effects [1,2]. Using a potent, viral-mediated delivery of short hairpin RNA (shRNA) to silence *HMGA1*, cell growth was abruptly halted in aggressive breast cancer cells within a few days after silencing *HMGA1* [1]. Surprisingly, the cells also underwent dramatic changes in appearance from spindle-shaped, mesenchymal cells to cuboidal cells with more differentiated, epithelial cell characteristics [1]. In addition, the epithelial gene (*E-CADHERIN*) was induced, while mesenchymal genes (*VIMENTIN*, *SNAIL*) were repressed in the cells with knockdown of *HMGA1*, indicating that HMGA1 regulates epithelial-mesenchymal transition (EMT) genes [1]. Silencing *HMGA1* also disrupts properties needed for tumor initiation and progression, including anchorage-independent cell growth, mobility and invasion. Remarkably, breast cancer cells engineered to knockdown *HMGA1* expression no longer metastasize to the lungs in mouse models [1,2]. Moreover, studies investigating tumor initiator/cancer stem cell properties in triple-negative breast cancer cells [1] found that silencing *HMGA1* impairs growth as three-dimensional spheres, which is a defining property of epithelial stem cells. Switching off *HMGA1* also depletes the tumor initiator cells/cancer stem cells such that tumors no longer formed when a small number of cells were injected. Gene expression analysis of the *HMGA1* knock-down cells showed that the *HMGA1* gene signature was highly enriched in genes expressed in embryonic stem cells [1]. In high-grade colon cancer cells, knocking down *HMGA1* using a plasmid-mediated delivery of shRNA to decrease *HMGA1* expression resulted in similar phenotypes. Oncogenic properties, such as anchorage-independent cell growth, migration, invasion and metastatic progression were disrupted. In addition, cancer stem cell properties were impaired, including growth as three-dimensional spheres and limiting dilution tumorigenesis [2]. Our studies in colon cancer also showed that EMT genes are regulated by HMGA1, including downregulation of *E-CADHERIN* and upregulation of *VIMENTIN* and *TWIST*. Silencing *HMGA1* reprograms aggressive carcinoma cells arising from the pancreas, and other tissues, resulting in dramatic changes in appearance and behavior within days of knocking down *HMGA1* expression (Resar L, unpublished data). Together, these exciting results suggest that targeting HMGA1 could be an effective cancer therapy that targets cancer stem cells [90].

2. Current strategies to target HMGA1

Prior attempts to target HMGA1 have yielded promising results in preclinical models, although further research is needed before these approaches can be effectively translated to the clinic (Figure 1, Table 2). Compounds that bind to AT-rich regions in DNA have been proposed as agents that could impair HMGA1 function. Actinomycin D, for example, is an antibiotic that binds to AT-rich regions in DNA, although its effects on HMGA1 have not been studied [91]. It also binds to DNA duplexes and thereby blocks replication. Two other AT-binding agents, FR900482 and FR66979 form covalent crosslinks with DNA-- drug--HMGA1 complexes in the minor groove of chromatin [92-94]. Of the two, FR900482 has undergone more extensive analysis. FR900482 forms crosslinks with other minor groove-binding proteins *in vivo* (HMGB1, HMGB2), but does not associate with major groove binding factors (Elf-1, NFκB). This agent inhibits proliferation of human T-cell acute lymphoblastic leukemia cells (Jurkat T-ALL cells) at lower concentrations (1 μM) with cell death at higher concentrations (2 μM); TUNEL assays showed apoptosis. In clinical trials, however, FR900482 induced vascular leak syndrome (VLS), a deleterious side effect characterized by enhanced vascular permeability with increased extravasation of fluids and proteins, resulting in interstitial edema and organ failure in severe cases. Clinical manifestations include increased weight, peripheral edema, pleural and pericardial effusions, ascites and anasarca [95]. Although chromatin immunoprecipitation experiments demonstrate that FR900482 effectively crosslinks HMGA1 to the *IL-2* and *IL-2α* gene promoters, it actually induces their expression (rather than blocking it), which could contribute to the development of VLS. FK973 is a structurally similar semisynthetic agent that crosslinks proteins to DNA but also causes VLS [96,97]. In contrast, a benzmethoxy derivative of FK973, FK317, retains antitumor characteristics without inducing VLS [94,98]. Cell death occurred in Jurkat cells at low concentrations (0.5 – 1.5 μM) via necrosis, whereas apoptosis was observed at higher concentrations (10 – 100 μM) [97,98]. FK317 also crosslinks HMGA1 to chromatin, but without inducing *IL-2* and *IL-2α*. As with FR900482, this agent crosslinks other minor groove-binding proteins and is not specific to HMGA1. Based on these promising results, however, additional studies of these agents and their derivatives are warranted.

Antibiotics have also been found to interfere with HMGA1 function. For example, distamycin [99] and netropsin [100] are minor groove-binding drugs that inhibit HMGA1 binding by blocking AT-rich regions located within minor grooves of DNA. Netropsin binds to an AT-rich region in the *nitric oxide synthase-2* promoter [100]. Distamycin was also tested in a murine model of endotoxemia where it blocks expression of the HMGA1 transcriptional target, *P-selectin* [99]. Distamycin attenuates the inflammatory response *in vivo* and blocks HMGA1 binding to the *P-selectin* promoter *in vitro*. While distamycin has not been tested in tumor models, these results suggest that distamycin and similar agents could have antitumor effects. Like other crosslinking agents, however, minor groove-binding drugs are not specific for HMGA1 because they interfere with the activity of multiple minor groove-binding proteins.

Doxorubicin (or adriamycin) is an approved cancer chemotherapeutic agent with antitumor effects that could be mediated, at least partially, through a repressive interaction with the

HMGA1 promoter [101]. Adriamycin is used in treating a wide range of cancers, including leukemias, carcinomas and sarcomas. It acts primarily as a DNA intercalating agent that blocks topoisomerase II, which relaxes DNA prior to replication. It also forms a stable complex with the 21RY promoter region of *HMGA1* [101,102]. This region is positioned between -304 and -284 from the transcription start site of the *HMGA1* promoter and just upstream of a proximal regulatory region. *In vitro* studies show that binding to 21RY represses *HMGA1* transcription, which could contribute to its anticancer effects.

Another small molecule that impairs *HMGA1* function is flavopiridol [75,76]. Derived from the Indian plants, *Dysoxylum binectariferum* or *Amoora rohituka*, flavopiridol was recently shown to downregulate *HMGA1* expression. Flavopiridol has antitumor efficacy in hematologic malignancies, such as acute myeloid leukemia, acute lymphoblastic leukemia and lymphoma, and is currently undergoing clinical trials. It also has cytotoxic effects in preclinical models of solid tumors, including breast and colon cancers. Flavopiridol is a pleiotropic agent, functioning through at least four distinct mechanisms. First, it potentially inhibits pan cyclin-dependent kinases (cdk), thereby blocking cell cycle progression. In the setting of cdk inhibition, the E2 promoter binding factor 1 (E2F1) transcription factor, which normally promotes proliferation, is released and drives apoptosis. Second, by blocking cdk 7 and 9, flavopiridol prevents phosphorylation and activation of RNA polymerase II, thus globally repressing gene transcription, including genes involved in proliferation and survival. Third, flavopiridol interferes with signal transducer and activator of transcription 3 (STAT3) DNA binding, thereby blocking STAT3 oncogenic pathways. Fourth, as noted above, flavopiridol represses *HMGA1* expression, as well as that of *STAT3*, *E2F1* and *POLR2A*, which encodes the major subunit of RNA polymerase II [75,76]. Like *HMGA1*, *STAT3* is overexpressed or activated in diverse tumors and promotes proinflammatory and stem cell pathways. Interestingly, *STAT3* is a direct transcriptional target of *HMGA1* and repressing *HMGA1* and *STAT3* expressions as well as STAT3 DNA-binding activity could synergize to block tumor growth and progression [13,103]. Previous studies found that E2F1 is downstream of *HMGA1* [104], which could contribute to E2F-driven apoptosis in the setting of flavopiridol in tumors with high levels of *HMGA1* and E2F proteins. Repression of *HMGA1* and *E2F1* could also inhibit proliferation pathways induced by these genes. Clinical trials demonstrate that flavopiridol-based therapies induce long-term remissions in elderly patients with refractory leukemia, suggesting that flavopiridol targets leukemic stem cells. Additional studies are warranted to further investigate the role of flavopiridol in tumors expressing high levels of *HMGA1*.

Synthetic oligonucleotide sequences that competitively bind to oncogenic transcription factors represent another promising approach that has been proposed to target *HMGA1* [105,106]. Two prior studies tested AT-rich oligonucleotides as decoys or 'sponges' for *HMGA1* to prevent DNA binding [105,106]. In the first study, RNA molecules were generated with L-ribose enantiomers (a synthetic mirror image of naturally occurring D-ribose) to make up the sugar-phosphate backbone, and were called spiegelmers (which means mirror in German) [105]. This modification serves to stabilize the molecules *in vivo*. The AT-rich spiegelmers prevent recombinant *HMGA1b* from binding to AT-rich double-stranded DNA *in vitro* in a dose-dependent fashion. Spiegelmers were conjugated with

polyethylenimine (PEI) to compact the DNA into positively charged DNA–PEI polyplexes. Because these complexes are attracted to negatively charged cell membranes, they enter cells by endocytosis. The spiegelmers are then released by osmotic lysis once inside a cell. This approach was tested in xenograft tumors derived from injection of cultured pancreatic adenocarcinoma cells (PSN-1 cells). When spiegelmers were injected subcutaneously near the tumors, there was a decrease in tumor size. The tumors were not analyzed further to determine if there was evidence for cell death, apoptosis or other cytotoxic changes. Analysis of tissue distribution, however, showed concentration of the spiegelmers in the tumor with minimal levels in the liver or kidney tissue. Further studies are needed to investigate the antitumor responses and whether HMGA1 proteins are sequestered in the cytoplasm by these agents.

Aptamers generated with phosphorothioate DNA and designed to bind and sequester HMGA1 have also been tested in cultured pancreatic adenocarcinoma cell lines [106]. Phosphorothioate DNA is a synthetic oligonucleotide in which one sulfur atom replaces a non-bridging oxygen in the phosphate group of the phosphodiester bond [106] to enhance stability by conferring resistance to DNaseI nuclease degradation. The aptamers caused cell death in pancreatic cancer cell lines that express high levels of HMGA1 (MiaPaca-2, AsPC-1). In MiaPaca-2 cells (which express only *HMGA1*), but not AsPC-1 cells (which express both *HMGA1* and *HMGA2*), cytotoxicity was enhanced by subsequent treatment with gemcitabine, a drug used in treating pancreatic cancer. The lack of enhanced cytotoxicity by gemcitabine in AsPC-1 cells could be secondary to high levels of HMGA2, which is also oncogenic and has redundant functions with *HMGA1*. It is not clear from studies thus far if the aptamers were specific only to HMGA1. Of note, aptamers have been used in clinical Phase II trials to sequester VEGF and prevent abnormal blood vessel growth in the retina following local, intraocular injections with promising results. Further studies are needed to determine if aptamers can be adapted for systemic delivery or even localized, intratumor delivery, although the latter approach would have more limited clinical utility [107].

Another approach to target HMGA1 is to interrupt downstream oncogenic pathways induced by HMGA1. Because HMGA1 regulates the genes involved in distinct pathways that are involved in tumor progression, such as proliferation, invasion, EMT, angiogenesis and inflammation, inhibitors to these pathways could be used to disrupt at least a subset of HMGA1 oncogenic activities. For example, the *COX-2* gene is directly upregulated by HMGA1 in uterine sarcomas in the *HMGA1a* transgenic model [21,108] and in pancreatic cancer cells [109], and several COX-2 inhibitors are available for use in the clinic. Sulindac, a COX-2/COX-1 inhibitor, was effective in slowing uterine sarcoma growth [108]. Similarly, xenograft tumors derived from poorly differentiated, human uterine sarcoma cell lines with high expression of *HMGA1* responded to COX-2 inhibitors (sulindac or celecoxib). Of note, there were no consistent antitumor effects observed in the lymphoid tumors in the transgenic model [109]. Gene expression analysis indicates that the HMGA1-COX-2 pathway was not consistently upregulated in these tumors, which could account for the lack of efficacy in the lymphoid tumor [89]. Studies in pancreatic xenografts from cell lines with high levels of HMGA1 and COX-2, however, showed significant tumor responses

with sulindac or celecoxib [109]. As noted, *STAT3* is directly induced by HMGA1, and *STAT3* inhibitors have antitumor effects in lymphoid tumors from the HMGA1 transgenic mouse model, both *in vitro* [13] and *in vivo* [103]. As discussed previously, flavopiridol targets *HMGA1* as well as the downstream effectors, E2F and *STAT3*. This agent has potent cytotoxic effects in diverse cancer cells that overexpress *HMGA1* [75,76]. Genes encoding MMPs, such as *MMP-2* [12,16,49] and *MMP-9* [14], are also downstream of HMGA1 and are implicated in tumor progression. Because these proteins degrade multiple components of the extracellular matrix, they are thought to contribute to tumor invasion and metastatic progression. Unfortunately, early trials with inhibitors have been disappointing, possibly because the inhibitors lacked specificity [16]. Nonetheless, studies to identify druggable pathways downstream of HMGA1 could lead to effective antitumor therapies, although this approach is not specific for HMGA1 and will likely miss a subset of oncogenic pathways induced by HMGA1.

Prior studies also suggest that high levels of HMGA1 can enhance cytotoxicity of DNA damaging agents [110,111]. HMGA1 was reported to directly repress the *BRCA1* gene, which functions in DNA repair. It was, therefore, proposed that cells expressing high levels of *HMGA1* would be more sensitive to DNA-damaging agents, such as cisplatin or bleomycin. Studies to test this were performed with MCF-7 breast cancer cells engineered to express high levels of *HMGA1* compared to controls and wild-type murine embryonic stem cells compared to murine embryonic stem cells null for *Hmga*. In both cases, there was diminished *BRCA1* expression and decreased survival following exposure to cisplatin in the cells expressing higher levels of *HMGA1*. In the MCF-7 breast cells, both proliferation and colony formation in soft agar were inhibited by cisplatin and bleomycin, whereas markers of DNA damage were increased [110]. Another group found that cisplatin treatment of mouse skin squamous cell carcinomas induced by exposure to the intercalating agent and carcinogen, benzo(a)pyrene, is associated with decreasing *HMGA1* gene expression and protein levels and cancer cell death [110]. These reports suggest that DNA-damaging agents such as cisplatin and bleomycin could be effective agents in tumors, expressing high levels of *HMGA1* [109,110].

3. Conclusion

As summarized above, prior studies in experimental models demonstrate that targeting *HMGA1* will be an effective antitumor strategy [1,2,112]. Recent work indicates that HMGA1 normally acts as a master regulator of developmental pathways during embryogenesis, but becomes reactivated in cancer where it reprograms cells back to a stem-like, de-differentiated state with accelerated growth rates and the ability to alter cell shape and mobility to spread to distant sites. Although there are therapeutic agents available that function, at least in part, by interfering with HMGA1 activity (Table 2), further research is needed to develop more specific HMGA1 inhibitors.

4. Expert opinion – how can we turn off HMGA1?

An exciting technology that has reached the forefront of research and could turn off HMGA1 is nanoparticle delivery of shRNA or interfering RNAs to block gene transcription

[1,2]. This approach is advantageous because highly specific shRNAs can be designed to target a single gene. As described above, both viral- and plasmid-mediated delivery of shRNA to *HMGA1* has potent antitumor activity in preclinical models of diverse tumor types [1,2]. A major challenge in the field, however, is to circumvent the rapid degradation of shRNA and similar molecules *in vivo*. Nanoparticle delivery can enhance survival of shRNA vectors *in vivo* and are typically composed of lipid layers surrounding DNA plasmids expressing the shRNA. Efforts are currently underway to optimize the lipid composition and structure to enhance both stability and delivery [113,114]. In addition, research is needed to develop approaches that effectively deliver these agents to tumor cells, although the enhanced permeability and retention effect appears to promote delivery of nanoparticles to solid tumor cells due to abnormal tumor vasculature and larger spaces between endothelial cells comprising tumor blood vessels. Current clinical trials suggest that the side-effect profile of lipid-based nanoparticles is reasonable and limited to hepatic toxicity attributed to the lipid loads. Because hematologic tumors, such as acute leukemia, lack a discrete tumor blood supply, approaches to direct nanoparticle delivery to tumor cells may be necessary before this technology can be effective in tumors without an aberrant vascular supply. Nanoparticles can also be adapted to the delivery of drugs, aptamers, spiegelmers or even microRNAs [114-116] that interfere with *HMGA1* expression. Recent studies demonstrated that nanoparticles targeting DNA binding of the *HMGA1* target, STAT3, have antitumor efficacy in murine tumor models [102]. Emerging evidence also suggests that nanoparticle delivery of tumor suppressor microRNAs could be effective in repressing *HMGA1* and other oncogenic pathways, particularly since microRNAs target multiple genes and oncogenic pathways [115,116]. Given the widespread overexpression of *HMGA1* in diverse, aggressive tumors, further research to develop effective approaches to specifically target *HMGA1* holds immense promise as a potent anticancer strategy. Approaches with specificity for *HMGA1*, such as nanoparticle delivery of short hairpin to *HMGA1*, are particularly desirable because they can be designed to target *HMGA1* without interfering with AT-binding proteins that may dampen *HMGA1* activities *in vivo*.

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Article highlights

- High mobility group A1 (*HMGA1*) is a potent oncogene that is highly expressed during development and in all aggressive cancers studied to date.
- *HMGA1* expression correlates with poor clinical outcomes in diverse malignancies.
- Silencing *HMGA1* in preclinical tumor models blocks oncogenic properties, including invasion and metastatic progression.
- Silencing *HMGA1* depletes cancer stem cells and blocks cancer stem cell properties.
- Therapeutic approaches to target HMGA1 are needed and should have profound antitumor effects in diverse malignancies.
- Nanoparticles to deliver short hairpin RNA to *HMGA1* are particularly promising because they have the potential to specifically target *HMGA1* in cancer.

Figure 1. Targeting HMGA1 in cancer therapy: available agents and their sites of action

Gray dashed arrows indicate normal pathways through which HMGA1 functions; these same pathways become dysregulated in cancer. The *HMGA1* gene is transcribed in the nucleus and translated in the cytoplasm before the HMGA1 protein translocates back into the nucleus where it binds to AT-rich regions in the minor groove of DNA. There, HMGA1 alters chromatin structure and recruits other transcriptional regulators to form an enhanceosome that influences the expression of diverse downstream genes. The red lines show points in this process that can be inhibited by the agents listed.

HMGA1: High mobility group A1; shRNA: short hairpin RNA; STAT3: Signal transducer and activator of transcription 3.

Table 1
Cancers overexpressing *HMGA1*

<i>HMGA1</i> expression studies	Ref.
Bladder	[8]
Breast	[1,8,10,48]
Colorectal	[2,52-55]
Head and Neck	[63]
Hematopoietic system (leukemia/lymphoma)	[13,17,72-77]
Kidney	[62]
Liver	[71]
Lung	[11,12,45]
Nervous system (medulloblastoma/neuroblastoma/glioblastoma)	[8,9,64-68]
Pancreas	[14,20,56-59,104,108]
Prostate	[48,50,51]
Stomach (Gastric)	[69,70]
Thyroid	[42-44]
Uterine cervix	[61]
Uterine corpus	[60,105,109]

HMGA1: High mobility group A1.

Table 2
Compounds that target HMGA1 in preclinical studies

Compound	Putative mechanism	Potential limitations	Model system*	Main ref./source
<i>AT-rich crosslinking agents</i>				
FK973	Crosslinking agent	Not specific for HMGA1, toxicity (vascular leak syndrome)	Cultured murine L1210 leukemia cells	Beckerbauer <i>et al.</i> [93] Nakamura <i>et al.</i> [97]
FR66979	Crosslinking agent	Not specific for HMGA1, toxicity (vascular leak syndrome)	<i>In vitro</i> biochemical assays	Fedele <i>et al.</i> [88] Rajski and Williams [92]
FR900482	Crosslinking agent	Not specific for HMGA1, toxicity (vascular leak syndrome)	Jurkat T-cell acute lymphoblastic leukemia cells	Schuldenfrei <i>et al.</i> [89] Yanagisawa and Resar [90] Beckerbauer <i>et al.</i> [93,94]
FK317	Crosslinking agent	Not specific for HMGA1	Jurkat T-cell acute lymphoblastic leukemia cells, Phase II trials	Yanagisawa and Resar [90] Beckerbauer <i>et al.</i> [94]
<i>Antibiotics</i>				
Actinomycin D [‡]	Intercalating agent – blocks transcription and replication, AT-rich DNA binding	Not specific for HMGA1; Chemotherapy side effects (bone marrow suppression, oral ulcers or mucositis, gastrointestinal toxicity/diarrhea)	<i>In vitro</i> biochemical assays	Fedele <i>et al.</i> [87] Wadkins <i>et al.</i> [91]
<i>Netropsin</i>				
Distamycin	Minor groove-binding	Not specific for HMGA1	Mouse model of endotoxemia	Pazdur <i>et al.</i> [96] Grant <i>et al.</i> [100]
Adriamycin	Minor groove-binding	Not specific for HMGA1	Mouse model of endotoxemia	Baluna and Vitetta [95] Baron <i>et al.</i> [99]
	Intercalating agent – blocks replication and transcription, complexes with the 21RY <i>HMGA1</i> promoter region, leading to downregulation of <i>HMGA1</i> transcription;	Not specific for HMGA1, chemotherapy side effects (bone marrow suppression, oral ulcers or mucositis, gastrointestinal toxicity/diarrhea and significant cardiac toxicity)	A431 squamous cell skin carcinoma cells	Nakamura <i>et al.</i> [97] Akhter <i>et al.</i> [101]
Bleomycin	Radiomimetic (stimulates effect of radiation), induces double-strand DNA breaks	Not specific for HMGA1	MCF-7 invasive breast ductal carcinoma cells, murine embryonic stem cells	Baldassare <i>et al.</i> [110]; Sharma <i>et al.</i> [111]
<i>Aptamer and aptamer-like agents</i>				
NOX-A50 Spiegelmer	Direct HMGA1 binding to AT-rich artificial substrate	Clinical delivery	Xenograft mice with pancreatic cancer cells	Grant <i>et al.</i> [100] Maasch <i>et al.</i> [105]
Aptamer technology (synthetic, biostable oligo-binding targets)	Direct HMGA1 binding to AT-rich artificial substrate	Clinical delivery	Pancreatic adenocarcinoma cancer cell lines (Panc-1, Miapaca-2 and AsPC-1)	Akhter <i>et al.</i> [101], Watanabe <i>et al.</i> [106]
<i>Cyclin-dependent kinase inhibitor/pleiotropic agent</i>				
Flavopiridol	Pleiotropic effects: Pan cyclin-dependent kinase inhibition, repression of RNA Pol II, global repression of gene expression, blocks STAT3 DNA-binding activity	Not specific for HMGA1, tumor lysis syndrome	Clinical trial of adult patients with relapsed or refractory acute myeloid leukemia	Nelson <i>et al.</i> [76] Karp <i>et al.</i> [75]

Compound	Putative mechanism	Potential limitations	Model system*	Main ref./source
<i>Anticancer platinum-based drug</i>				
Cisplatin	Chromatin damage, DNA-drug adducts	Not specific for HMGA1, chemotherapy side effects (bone marrow suppression, organ toxicity, nausea, vomiting)	Squamous cell carcinoma induced <i>in vivo</i> in Swiss-albino mice, MCF-7 invasive breast ductal carcinoma cells	Baldassare <i>et al.</i> [110]; Sharma <i>et al.</i> [111] Hillion <i>et al.</i> [108] Scala <i>et al.</i> [112]
<i>RNA interference</i>				
Antisense technology (antisense or RNA interference with siRNA or shRNA) MicroRNAs	Inhibits HMGA1 transcription or translation	Clinical delivery	Adenovirus delivery of antisense cDNA into lung, colon, breast, thyroid, pancreatic cells, antisense RNA delivery into Burkitt's lymphoma, breast cells, si/shRNA delivery into pancreatic, uterine and lung cancer cells	Di Cello <i>et al.</i> [109] Boylan <i>et al.</i> [113] Wood <i>et al.</i> [72] Dolde <i>et al.</i> [10] Liao <i>et al.</i> [14] Tesfaye <i>et al.</i> [60] Hillion <i>et al.</i> [12] Belton <i>et al.</i> [2] Shah <i>et al.</i> [1]

* Studies where HMGA1 was targeted

[‡] Actinomycin-D may compete with HMGA1, although this has not been directly tested

HMGA1: High mobility group A1; shRNA: Short hairpin RNA; STAT3: Signal transducer and activator of transcription 3.