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Hitting the Bull's Eye: Targeting HMGA1 in Cancer Stem Cells

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Summary

Emerging evidence suggests that when cancer cells hijack normal stem cell properties, they acquire the ability to invade, metastasize to distant sites, and evade therapy. Thus, eliminating cancer cells with stem cell properties, or cancer stem cells, is of prime importance for the successful treatment of cancer, regardless of the tissue of origin. Previous efforts to target cancer stem cells, however, have been largely unsuccessful. Recent studies led to the discovery of a novel role for the high mobility group A1 protein as a master regulator in both cancer stem cells and normal embryonic stem cells. Here, we present exciting new work unveiling the HMGA1 as a promising target for therapies directed at eradicating cancer stem cells.

Keywords

High mobility group A1 protein; HMGA1; cancer stem cells; epithelial-mesenchymal transition; embryonic stem cells; chromatin remodeling proteins

Cancers become deadly when tumor cells develop the ability to evade therapy and spread to distant sites. Thus, understanding why some cells acquire these properties and how to effectively target resistant, metastatic cancer cells are the most important hurdles confronted by cancer biologists and clinicians treating cancer patients today. While several proposed mechanisms for drug resistance exist, the cancer stem cell theory has gained momentum with emerging knowledge of both normal embryonic development and tumor progression [1-4]. The cancer stem cell theory proposes that cancers exist as a cellular hierarchy, which is maintained by cells known as cancer stem cells (CSCs) or tumor-initiating cells (TICs; Figure 1A). Unlike the clonal evolution theory, which contends that all cells within the tumor have an equal likelihood of acquiring mutations that confer a selective advantage, the cancer stem cell theory holds that only CSCs are capable of reconstituting the tumor. Like normal stem cells, CSCs/TICs have unique “stem cell” properties, including: 1.) long-term self-renewal, which enables them to divide for long periods of time, and, 2.) plasticity, or the

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ability to alter their appearance, growth patterns, and function. In embryonic stem cells (ESCs), these properties are essential for normal development, while in CSCs, these properties are required for invasion, metastatic progression, and survival following exposure to therapy.

Increasing evidence suggests that some tumors have a small population of CSCs that are responsible for maintaining the tumor, with a larger population of “bulk” tumor cells that respond to therapy [1-2]. For example, many hematologic tumors can be eliminated by induction chemotherapy alone, but will invariably relapse if no further treatment is given. This behavior is consistent with the CSC model whereby the bulk tumor responds to therapy, while a small number of CSCs are refractory to therapy and maintain the tumor unless additional, effective therapy is administered. Alternatively, some tumors appear to be comprised predominantly of refractory, poorly differentiated stem-like tumor cells at presentation. To illustrate, most patients with pancreatic ductal adenocarcinomas will succumb to this malignancy, even when the tumors are small and surgically resectable at the time of diagnosis [5-6]. This suggests that pancreatic ductal adenocarcinoma cells are refractory, metastatic, and “stem-like” prior to clinical presentation in virtually all cases. In addition, recent studies indicate that remarkable heterogeneity exists within a single tumor, as evident by the varying tumorigenic potential of cells [1-4] and varied gene expression profiles from the same tumor [7]. It is plausible that CSCs, clonal evolution, and tumor heterogeneity all contribute to relapse and resistant disease, even within a single tumor. Regardless of the mechanism for resistance, each scenario requires anticancer treatment to be effective, not only at removing the bulk tumor burden, but also at preventing relapse by eliminating cells that can initiate and maintain the tumor (CSCs/TICs). Complicating this issue further is the fact that CSCs, similar to their normal counterparts (ESCs and adult stem cells), have inherent properties that confer resistance to many drugs commonly used to treat cancer, including specialized channels and enzymes to remove toxins, and the ability to remain quiescent or dormant [1,8]. As such, there is an urgent need to discover treatment approaches to target CSCs and/or stem-like cancer cells.

Attempting to better characterize and ultimately target CSCs has therefore been an area of active investigation. Several groups have identified cell surface markers or enzyme activities found in CSCs/TICs, and not surprisingly, many of these markers are shared by normal ESCs and adult stem cells [1-2]. Unfortunately, the CSC/TIC markers can be transient and non-specific, and therefore have remained elusive therapeutic targets [8]. An alternative approach to target CSCs is to modulate key regulators in signaling cascades that maintain the CSC/TIC phenotype. Recent work has identified the *high mobility group A1 (HMGA1)* gene as such a potential target [9-12; Figure 1B and Table 1]. This gene encodes the HMGA1a and HMGA1b protein isoforms that result from alternatively spliced mRNA [13-18]. HMGA1 was first discovered in highly proliferative, widely metastatic cervical cancer cells (HeLa) over 30 years ago [19] and has since been identified as a master regulator of transcriptional networks in normal ESCs and poorly differentiated, stem-like cancer cells [9-15, 20-22].

HMGA1 proteins belong to the class of chromatin remodeling proteins which modulate gene expression and cellular function by altering chromatin structure [13-34]. In fact, they are

among the most abundant, nonhistone chromatin binding proteins found in cancer cells [13-16, 19]. HMGA1 proteins are named in part based on their small size (~10 kD) and thus rapid mobility (or highly mobility group) when separated on polyacrylamide electrophoretic gels. They also bind to chromatin at AT-rich regions (thus HMGA) in regulatory regions upstream of many genes important in development and cancer. After binding to DNA, HMGA1 bends chromatin and recruits additional transcription factors, forming a higher order transcriptional complex or “enhanceosome” that modulates gene expression. *In vitro* studies found that HMGA1 remodels chromatin by changing the rotational setting of DNA on the surface of isolated nucleosomes [35]. Elegant work subsequently demonstrated that HMGA1 plays a fundamental role in repositioning nucleosomes to facilitate gene expression with T cell activation [36-38]. HMGA1 also recruits additional chromatin remodeling complexes to DNA. For example, HMGA1 proteins are required for the recruitment of SWI/SNF chromatin remodeling complex to the HIV promoter, which results in histone acetylation and transcription from the LTR promoter [39]. Although HMGA1 proteins do not appear to have transcriptional activity alone, they alter gene expression by orchestrating the assembly of transcription factor complexes to DNA. Because histone H1 proteins maintain chromatin in a tightly bound, inactive state, HMGA1 proteins can globally activate gene expression by dislodging repressive histone H1 proteins [30-34]. In fact, sequence homology analysis of HMGA1 in plants suggests that histone H1 and HMGA1 evolved from the same ancestral protein.

HMGA1 is highly expressed during embryogenesis, with low or undetectable levels in most tissues postnatally [40]. *HMGA1* is also highly expressed in adult stem cells, such as hematopoietic stem cells [41-44] and intestinal stem cells [45]. Mice deficient in *HMGA1* develop aberrant hematopoiesis and a cardiomyopathy [46-48], while mice overexpressing *HMGA1* develop diverse tumors including hematopoietic malignancies as well as pituitary, gastrointestinal, and uterine tumors [49-51]. HMGA1 was identified among a stem cell signature comprised of 9 genes highly expressed in embryonic stem cells that encode transcription factors [9]. This signature was derived by comparing multiple gene expression profiles from independent studies of ESCs. Interestingly, the HMGA1 stem cell signature predicts refractory disease in diverse solid tumors, including breast, bladder, and brain cancers [9]. In normal ESCs, HMGA1 maintains stem cells in an undifferentiated, pluripotent state by regulating key stem cell genes, including *Lin28*, *Nanog*, *cMyc*, and *Sox2* [10]. In fact, HMGA1 is required for cellular reprogramming of somatic cells to induced pluripotent stem cells (iPSCs). These studies suggest that HMGA1 could drive stem cell properties in CSCs through molecular pathways active in normal ESCs. In keeping with the shared transcription factor signature in normal ESCs and resistant cancer cells or CSCs, previous studies have also uncovered stem cell transcriptional networks and signaling pathways activated during tumor progression [10-12, 20-22]. For example, stem cell pathways are activated by HMGA1 in a transgenic model of lymphoid tumorigenesis [22]. Deletion of tumor suppressor genes (*INK4A/ARF*) that are normally silenced in stem cells leads to accelerated lymphoid tumorigenesis in an *HMGA1* transgenic model [52]. An HMGA1 gene signature of 63 genes was also identified in poorly differentiated, triple-negative breast cancer cells. Strikingly, over half of the genes in this signature are highly enriched in embryonic stem cells [12]. Although there are limited published reports of CSC

gene expression profiles, *HMGA1* was identified in a leukemic stem cell signature identified in a murine model of acute myeloid leukemia [53]. In unpublished studies, we found that *HMGA1* is enriched in the leukemic stem cell fraction of myeloid leukemias. Together, these studies indicate that *HMGA1* regulates fundamental pathways in normal embryonic developmental, which become reactivated and dysregulated in cancer. Indeed, *HMGA1* is not only up-regulated in diverse cancers, but it also portends a poor prognosis in many tumors [6, 9, 11-15, 49, 51-65].

CSCs behave like “corrupted” normal stem cells during tumor initiation and progression. Although long-term self-renewal and pluripotency, or the ability to differentiate into other lineages, are hallmarks of normal ESCs, these properties are distorted in CSCs. For example, untethered self-renewal could account for high proliferative rates observed in some CSCs and aggressive tumors. While pluripotency, per se, is uncommon in most cancers and CSCs outside of some germ cell tumors, most resistant cancer cells have some degree of plasticity, or the ability to alter their appearance and behavior, including growth patterns, metabolism, and other fundamental properties. Increasing evidence suggests that epithelial tumor progression occurs, at least in part, from changes resembling an epithelial-mesenchymal transition (EMT) whereby cuboidal, immobile epithelial cells become elongated, invasive and mobile (mesenchymal) [11-12, 66-68]. EMT occurs normally during embryonic development with gastrulation when embryos develop from a single-layered spherical collection of cells (blastula) to a three-layered structure (gastrula) [66-68]. Many epithelial tumors (carcinomas) undergo changes consistent with EMT during tumor invasion and migration prior to metastatic progression to distant sites. Although less well-studied, metastatic cancer cells must subsequently undergo additional alterations to establish residence at the metastatic site, some of which resembles a mesenchymal-epithelial transition (or MET).

Emerging evidence indicates that *HMGA1* is a master regulator of EMT and tumor progression in diverse tumor models [11-12, 20]. For example, experimental models of tumor progression demonstrate that inhibiting *HMGA1* expression blocks proliferation, anchorage-independent cell growth, migration, invasion, and metastatic progression [11-12, 20, 51, 54-55, 59-64, 69-70]. More recently, we found that silencing *HMGA1* rapidly and dramatically reprograms aggressive, poorly differentiated breast cancer cells into cells with a more normal appearance and behavior [12]. Uncontrolled cell growth was halted in cancer cells after viral-mediated delivery of a short hairpin RNA to “switch off” *HMGA1*. Within a few days of silencing *HMGA1*, there were striking changes in appearance from spindle-shaped, mesenchymal tumor cells to more differentiated-appearing, cuboidal-shaped, epithelial cells. The tumor cells with knock-down of *HMGA1* no longer metastasized from mammary fat pads to the lungs. Moreover, the cells with *HMGA1* “switched-off” could no longer form foci in the lungs following tail vein injections [12]. Applying this same technology to colorectal and pancreatic cancer cells also disrupted cell growth and morphology, inducing changes consistent with MET (unpublished data). *HMGA1* is also important in CSCs. Inhibiting *HMGA1* expression in poorly differentiated cancer cells blocks 3-dimensional sphere formation, a defining property of CSCs and normal epithelial stem cells. In poorly differentiated breast cancer cells, not only is primary sphere

formation impaired, but silencing *HMGA1* also prevents the formation of secondary and tertiary sphere formation. In addition, tumors do not form when lower numbers of cancer cells were injected, indicating that the CSC/TIC population was depleted in cells with *HMGA1* knock-down [11-12]. In both colon and breast cancer, downstream transcriptional targets of HMGA1 that function in stem cells and EMT were identified [11-12]. Interestingly, a study from over a decade earlier identified *CD44* as an HMGA1 gene target, long before it was known that *CD44* is an important marker of some CSCs [71]. The ever increasing list of genes regulated by HMGA1 indicates that it orchestrates transcriptional networks important in normal development and in resistant cancer cells. Together, these findings demonstrate that targeting HMGA1 in cancer reprograms poorly differentiated, metastatic cancer cells into cells with a more differentiated appearance and slower growth rates. These results also suggest that disrupting *HMGA1* expression will also target cancer stem cell properties, and even the CSC/TICs population in tumors with this hierarchical organization.

Expert Commentary & Five-Year View

Because recent work has identified a unique role for HMGA1 as a master regulator of tumor progression and a stem cell phenotype, future research is needed to develop approaches to target HMGA1 in cancer therapy. A few compounds have been described that block HMGA1 function by crosslinking it to DNA, although none have proven to be specific and some are associated with significant toxicity that could result from disrupting the function of other AT DNA binding proteins, such as AKNA [72-75]. Of note, one crosslinking agent resulted in toxicity by enhancing expression of HMGA1 target genes [73]. Some studies have attempted to block transcriptional targets or pathways downstream of HMGA1 with promising results [51,63-64,76-77]. For example, both the *cyclo-oxygenase-2* (*COX-2*) and *signal transducer and activator of transcription 3* (*STAT3*) genes are induced by HMGA1 and they have been targeted pharmacologically in preclinical studies of tumors overexpressing *HMGA1* [51,63,76-77]. While the results are promising, this approach will affect only a subset of HMGA1 downstream pathways. Recent technology to deliver short hairpin RNA plasmids is an exciting approach because it should be highly specific for HMGA1, as observed in our studies with *in vitro* cell culture models or murine preclinical models [11-12,78-79]. Results from early clinical trials with nanoparticles to alter gene expression have been encouraging and suggest that this approach could be used to deliver shRNA to modulate HMGA1 expression in tumors [77-79]. Another approach to target *HMGA1* will be to deliver microRNAs that repress its expression and/or translation [80-82]. Because *HMGA1* is highly expressed and likely plays an important role in adult stem and progenitor cells, future efforts will need to elucidate the molecular underpinnings that distinguish normal adult progenitor/stem cells from CSCs with the goal of targeting only the abnormal, cancer cells. Our recent work identifying HMGA1 as a key factor in orchestrating gene expression required for normal development and tumor progression is an important first step in this direction. Developing technology to target HMGA1 in resistant cancer cells promises to be an important strategy in the war on cancer.

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Key Issues

- *HMGA1* encodes the HMGA1a and HMGA1b chromatin remodeling proteins, which alter chromatin structure and modulate gene expression.
- *HMGA1* is highly expressed in all aggressive, poorly differentiated tumors studied to date and high levels portend a poor prognosis in diverse tumors
- *HMGA1* is enriched in normal embryonic stem cells, adult stem cells, and cancer stem cells.
- *HMGA1* induces oncogenic transformation in cultured cells and aggressive tumors in transgenic mice
- Silencing *HMGA1* results in a dramatic reprogramming of proliferative, invasive, spindle-shaped, mesenchymal cancer cells into non-invasive cells with slow proliferation rates and a more differentiated, cuboidal, epithelial appearance.
- Switching off *HMGA1* also blocks tumor progression and depletes cancer stem cells/tumor-initiator cells in murine models
- HMGA1 is required for cellular reprogramming of somatic cells into induced pluripotent stem cells by the Yamanaka factors.
- HMGA1 induces stem cell transcriptional networks during development in normal, pluripotent stem cells and with tumor progression
- Recent work suggests that targeting HMGA1 will eliminate cancer stem cells in diverse tumors

Figure 1. HMGA1 in Cancer Stem Cells

A.) Traditional therapies target the more common “bulk” tumor cells, leaving therapy-resistant, stem-like cells that express high levels of *HMGA1* and other stem cell genes. With time, the CSC/TICs repopulate the tumor and lead to relapse. However, therapies that target the stem-like *HMGA1* expressing cells would eliminate those cells which are capable of repopulating the tumor and could result in cures when the bulk tumor cells are eliminated with traditional therapies.

B.) *HMGA1* drives cancer stem cell phenotypes, and targeting *HMGA1* disrupts these properties, including: (1) growth as 3-dimensional spheres (2) a de-differentiated (high-grade), plastic state, (3) anchorage independent growth, (4) long-term self-renewal and proliferation, (5) an epithelial-mesenchymal transition (EMT), (5) invasion/migration, (6) intravasation/migration, (7) extravasation and metastatic potential, after which the process could repeat itself from the metastatic site.

Table 1
HMGA1 in Normal Stem Cells and Cancer Stem Cells

	Model System	Finding	Citation
<i>HMGA1 in normal embryogenesis, ESCs, and adult stem cells</i>	Murine embryonic stem cells and postnatal tissues	<i>HMGA1</i> is highly expressed in embryonic development and silenced after birth	Chiappetta <i>et al</i> , 1996 [40]
	Heterozygous and homozygous <i>HMGA1</i> -null mice	<i>HMGA1</i> -null mice develop aberrant hematopoiesis, a myeloproliferative disorder, and a cardiomyopathy	Fedele <i>et al</i> , 2006 [46]
	Human embryonic stem cells and primary tumors	<i>HMGA1</i> is among a 9 gene transcription factor stem cell signature	Ben-Porath <i>et al</i> , 2008 [9]
	Human hematopoietic stem cells	CD34+ hematopoietic stem cells express <i>HMGA1</i>	Zhou <i>et al</i> , 2001 [41] Karp <i>et al</i> , 2011 [43], Nelson <i>et al</i> , 2011 [44], Chou <i>et al</i> , 2011 [42]
	<i>Lgr5</i> -knock-in (<i>Lgr5</i> -ki) mice	<i>HMGA1</i> mRNA and protein are enriched in <i>Lgr5</i> + intestinal stem cells	Munoz <i>et al</i> , 2012 [45]
	Human embryonic stem cells and induced pluripotent stem cells	<i>HMGA1</i> is highly expressed in ESCs and iPSCs, and expression falls with differentiation	Shah <i>et al</i> , 2012 [10]
<i>HMGA1 in CSCs</i>	Murine model of AML	<i>HMGA1</i> is among the leukemia stem cell signature	Somervaille <i>et al</i> , 2009 [53]
	Colon cancer cell lines	<i>HMGA1</i> is required for tumor progression and colon cancer stem cells/tumor-initiator cells	Belton <i>et al</i> , 2012 [11]
	Breast cancer cell lines	<i>HMGA1</i> is required for tumor progression and breast cancer stem cells/tumor-initiator cells	Shah <i>et al</i> , 2013 [12]