The High Mobility Group A1 (*HMGA1*) Transcriptome in Cancer and Development

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Abstract

Background & Objectives—Chromatin structure is the single most important feature that distinguishes a cancer cell from a normal cell histologically. Chromatin remodeling proteins regulate chromatin structure and high mobility group A (HMGA1) proteins are among the most abundant, nonhistone chromatin remodeling proteins found in cancer cells. These proteins include HMGA1a/HMGA1b isoforms, which result from alternatively spliced mRNA. The *HMGA1* gene is overexpressed in cancer and high levels portend a poor prognosis in diverse tumors. *HMGA1* is also highly expressed during embryogenesis and postnatally in adult stem cells. Overexpression of *HMGA1* drives neoplastic transformation in cultured cells, while inhibiting *HMGA1* blocks oncogenic and cancer stem cell properties. *Hmga1* transgenic mice succumb to aggressive tumors, demonstrating that dysregulated expression of *HMGA1* causes cancer *in vivo*. *HMGA1* is also required for reprogramming somatic cells into induced pluripotent stem cells. HMGA1 proteins function as ancillary transcription factors that bend chromatin and recruit other transcription factors to DNA. They induce oncogenic transformation by activating or repressing specific genes involved in this process and an HMGA1 "transcriptome" is emerging. Although prior studies reveal potent oncogenic properties of *HMGA1*, we are only beginning to understand the molecular mechanisms through which *HMGA1* functions. In this review, we summarize the list of putative downstream transcriptional targets regulated by HMGA1. We also briefly discuss studies linking *HMGA1* to Alzheimer's disease and type-2 diabetes.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

Conclusion—Further elucidation of *HMGA1* function should lead to novel therapeutic strategies for cancer and possibly for other diseases associated with aberrant *HMGA1* expression.

Keywords

High mobility group A1; HMGA; chromatin; cancer; tumor progression; metastasis; genes; cancer stem cells; embryonic stem cells

1. INTRODUCTION

The structural organization of the nucleus and its chromatin is the single most important histologic feature that distinguishes a cancer cell from a normal cell. Chromatin consists of nuclear DNA and proteins, and chromatin structure dictates whether genes will be actively transcribed or silent. Not surprisingly, chromatin structure is central to normal cell function, both in undifferentiated embryonic stem cells during development and in differentiated, mature cells. Eukaryotic chromatin exists in a condensed form as depicted in the "beads-on-a-string model" in which repeating units of DNA tightly wound around small basic histone proteins, called nucleosomes (the bead), are joined by linker DNA sequences (the string) to form chromatin fibers. This compact, yet malleable structure, not only provides an intricately organized framework for DNA, but also endows eukaryotes with the ability to repress or activate gene expression. As such, induction or repression of gene expression requires transient reorganization of the highly-ordered chromatin structure in which that gene resides, a process known as chromatin remodeling. Increasing evidence underscores the importance of aberrant chromatin remodeling in cancer and many other diseases.

Chromatin structure is maintained largely by chromatin binding proteins, which include both histone and nonhistone proteins. While many groups have investigated histones and chromatin binding proteins that enzymatically modify histones or DNA, few have focused on the high mobility group A (HMGA) chromatin remodeling proteins. In fact, HMGA proteins are among the most abundant, nonhistone chromatin binding proteins found in the nucleus of cancer cells [1–3]. They are members of the high mobility group (HMG) superfamily of proteins comprised of diverse, low molecular weight proteins named by their rapid migration or high mobility in polyacrylamide gel. HMGA1 proteins are primarily located in the nucleus and function in chromatin remodeling, although they lack an identifiable enzymatic activity. This superfamily of proteins was first discovered over 30 years ago in highly proliferative human cervical HeLa cancer cells [1–3]. Within the next two decades, members of the HMGA family (HMGA1a, HMGA1b, and HMGA2) were isolated from cancer cells and their genes were cloned [3–30]. All HMG proteins share an acidic, carboxyl terminus and associate with chromatin, but are distinguished by unique functional domains that confer distinct DNA binding motifs and biologic activities [21]. The HMGA family is defined by the AT-hook DNA binding domains [13–30], which mediate binding to nuclear chromatin at AT-rich regions in the minor groove, where HMGA1 proteins bend DNA to allow other transcription factors to bind [13–31]. Prior studies also show that HMGA1 proteins bind to mitochondrial DNA at AT-rich regions [32, 33; reviewed in 34]. The HMGA1 subfamily – *the subject of this review* - consists of both the HMGA1a and HMGA1b protein isoforms (formerly HMG-I and HMG-Y), which result from

alternative splicing of the *HMGA1* mRNA [6–8]; HMGA1a differs from HMGA1b by an additional 11 internal amino acids upstream of the second AT hook [6–8] (Fig. 1). The biological significance of two distinct isoforms is not yet clear, as functional studies indicate many overlapping roles [28, 29].

To date, HMGA1 proteins are known to participate in a myriad of cellular processes [1–119] including transcriptional regulation [17–24], neoplastic transformation [22–25, 28–30, 50, 54, 60, 68, 70–73, 75, 77, 86, 88, 90, 91, 100, 117–119], embryogenesis [98], anoikis [71, 77], metastatic progression [28, 29, 53, 54, 68, 70, 117], cell cycle regulation [100–108], repair of DNA damage [109–112], cellular senescence [113–115], mitochondrial function [32–34], and retroviral integration [116]. Most of these varied biological activities of HMGA1 are thought to result from its ability to alter chromatin structure and modulate gene expression, leading to different molecular pathways depending upon the cellular context. Promoter analyses and gene expression profile studies have uncovered downstream gene targets and an HMGA1 "transcriptome" is emerging (Fig. 2). In this review, we outline prior studies that reveal a central role of *HMGA1* in diverse, aggressive cancers and normal development. We focus on the transcriptional targets regulated by HMGA1 in cancer and stem cells. In addition, we briefly consider studies that implicate *HMGA1* in the pathogenesis of diabetes [35–38] and Alzheimer's disease [39–42].

2. *HMGA1* IS UP-REGULATED IN RAPIDLY PROLIFERATING CELLS & CANCER

The first evidence linking HMGA1 proteins to cancer was their discovery as abundant chromatin binding proteins in HeLa cells, the aggressive human cervical carcinoma cells with a remarkable proliferative capacity [3]. Subsequent studies showed high levels of HMGA1 proteins in rat and mouse cells after oncogenic transformation by retroviral transduction [4, 5]. HMGA1 proteins are also elevated in spontaneous mouse tumors and tumors induced by either carcinogens or viral oncogenes compared to normal tissue [4, 5, 8]. High levels of HMGA1 proteins are found in rapidly proliferating tissues and neoplastic cells, with absent or low levels in normal, differentiated, adult tissues [43–46]. The *Hmga1* gene was identified early on as a gene induced by serum or individual growth factors in quiescent murine fibroblasts, an experimental model that facilitated the discovery of several key oncogenic transcription factors [44]. In this model, *Hmga1* is a "delayed-early" gene whose expression follows the initial wave of "immediate-early" genes [44]. Many immediate- and delayed-early genes are required by cells to traverse the G1/S boundary of the cell cycle and function as oncogenes when aberrantly expressed. Further studies uncovered high levels of *HMGA1* expression at the mRNA or protein level in human cancer cells or primary tumors from diverse tissues, including thyroid [45–48], lung [49–51], breast [52–59, 117], bladder [58], prostate [60–62], colon [63–68], pancreas [69–74], uterine corpus [75], uterine cervix [76], kidney [77], head and neck [78], nervous system [58, 79–84], stomach [85, 86], liver [87], and hematopoietic system [88–93, 118, 119]. *HMGA1* expression is low or undetectable in normal tissue counterparts. Taken together, these findings suggest a central role for *HMGA1* in neoplastic transformation.

Subsequent studies found that high levels of *HMGA1* expression correlate with adverse clinical outcomes and more advanced disease in cancer [reviewed in 24]. For example, high expression of *HMGA1* at the mRNA or protein level was found in cultured cells derived from metastatic tumors compared to localized tumors, including breast [29, 54, 117], colon [63–65, 68], prostate [60], and pancreatic [69, 70, 74, 94] cancers. Further evidence that *HMGA1* overexpression portends a poor prognosis in diverse cancers came with the advent of global gene and protein microarray technology. The first such study found that *HMGA1* gene expression correlates with poor prognosis in primary medulloblastomas [79]. In squamous cell carcinoma and adenocarcinoma lung, an inverse correlation was observed for HMGA1 protein staining by immunohistochemical analysis and survival [49]. Higher *HMGA1a* mRNA and protein levels were discovered in hepatocellular carcinoma with intrahepatic metastases compared to those without intrahepatic metastases [87]. In breast cancer, HMGA1 protein levels correlate with high-grade/poor differentiation [55] and recurrent disease [56]. *HMGA1* gene expression also associates with high-grade in uterine cancers [75]. In pancreatic cancer, HMGA1 protein levels are positively correlated with both poor differentiation status and decreased survival [74]. In pediatric B-lineage acute lymphoblastic leukemia, *HMGA1* expression is higher in patients at relapse [119]. Moreover, a study comparing global gene expression profiles from many independent studies found that *HMGA1* is among a core "signature" comprised of 9 transcription factor genes enriched in embryonic stem cells and high-grade/poorly differentiated cancers (breast, bladder and brain) [58]. Importantly, overexpression of this signature was associated with poor survival in patients with these cancers. Collectively, these studies suggest that *HMGA1* promotes tumor progression and is a biomarker and potential target for more advanced disease.

3. *HMGA1* IS HIGHLY EXPRESSED IN PLURI-POTENT STEM CELLS

Normally, *HMGA1* genes are expressed at high levels during embryogenesis, with low or absent levels in most adult, differentiated murine tissues [23, 95, 98]. Similarly, *HMGA1* is enriched in human and murine embryonic stem cells as well as induced pluripotent stem cells and adult stem cells, such as CD34+ hematopoietic stem cells and intestinal stem cells [58, 92, 93, 96–98, 120]. As noted above, HMGA1 is a core transcription factor in embryonic stem cells [58]. Because pluripotency factors (NANOG, OCT4, SOX2, KLF4, or cMYC) were not identified in this stem cell signature, it was postulated that HMGA1 and other core factors regulate the same cellular pathways induced by stem cell pluripotency factors [58]. Consistent with this hypothesis, *HMGA1* is a transcripional target of cMYC, an oncogenic protein and pluripotency factor (see below) [88]. In embryonic stem cells, *HMGA1* expression falls with differentiation and its expression parallels that of pluripotency genes, *NANOG, OCT4* and *SOX2* [98]. A global gene expression study in a murine model of leukemia identifed *HMGA1* as a member of the core leukemic stem cell signature [99]. Studies in human embryonic stem cells indicate that HMGA1 regulates key stem cell and pluripotency genes [98]. In fact, a recent landmark paper showed that *HMGA1* is required for cellular reprogramming of somatic cells to induced pluripotent stem cells by the Yamanaka factors [98]. This study also showed that *HMGA1* enhances reprogramming to induced pluripotent stem cells by the Yamanaka factors. These findings suggest HMGA1

orchestrates transcriptional networks that maintain a primitive, poorly differentiated, stem-like state, both in cancer and normal development.

4. HMGA1 PROTEINS FUNCTION IN REGULATING GENE EXPRESSION

Once it became known that *HMGA1* expression is closely linked to aggressive malignancy, several groups began to study its functional role in cancer to uncover novel mechanisms that could be blocked in therapy. Early studies focused on chromatin structure and transcription [121–140]. HMGA1 proteins were found to alter gene expression through at least two separate mechanisms. First, HMGA1 proteins bind to the matrix- and scaffold-associated regions (MARs or SARs) of chromatin, which contain AT-rich sequences and high affinity binding to the nuclear matrix [121–125]. The nuclear matrix is a dynamic structure that maintains nuclear organization and provides sites for transcription, replication, and alternative splicing of mRNA. After binding to MARs/SARs, HMGA1 proteins anchor chromatin to the nuclear scaffold. This interaction serves to topologically organize independent DNA domains that function in both replication and transcription, although a detailed understanding of HMGA1's role in chromatin domains and the organization of the nuclear matrix is lacking. HMGA1 proteins also displace histone H1 proteins, which repress transcription by maintaining a tightly wound, heterochromatic chromatin configuration in experimental models [123]. Thus, HMGA1 proteins could facilitate global activation of gene expression by relieving histone H1-mediated repression of transcription. Interestingly, the homology of HMGA1 proteins to histone H1 in plants and lower organisms suggest that they evolved from the same protein [141], although their transcriptional regulatory functions have since diverged.

In addition to a role in global chromatin structure, HMGA1 proteins also regulate specific target genes. The repertoire of genes induced by HMGA1 is extensive, and likely only beginning to emerge. *Interferon-β* (*IFN-β*) gene is among the most studied HMGA1 targets, which was identified by screening a cDNA expression library with a regulatory region of the *IFN-β* promoter for DNA binding proteins [126–140]. HMGA1 binds to two of four positive regulatory regions (PRD II and IV) in an enhancer region located in the 5′UTR gene upstream of the transcription start site for *IFN-β* [126–140]. After binding to DNA, HMGA1 promotes cooperative DNA binding of additional essential transcription factors through two distinct mechanisms. First, HMGA1 binds to an AT-rich site in the minor groove at the PRDII element, which "unbends" DNA to enable NF-κB (p50/p65) to gain access and bind to the opposite side of the DNA helix. Second, at the PRD IV element, HMGA1 interacts with the ATF-2/c-Jun heterodimer to enhance its affinity to DNA. These protein-protein and protein-DNA interactions result in the assembly of a prototypical transcriptional enhancer complex or "enhanceosome" that includes ATF-2/c-Jun and IRF proteins in a highly cooperative fashion [126–140]. The enhanceosome is required for synergy between the involved transcription factors and results in reversing the intrinsic bend in DNA at this enhancer site. Once bound, the enhanceosome recruits histone acetyltransferase proteins (p300/CBP-associated factor PCAF in mammals or GCN5 in yeast), which acetylate HMGA1 at a critical lysine amino acid (amino acid 71) in addition to modifying histone proteins by acetylation. Acetylation at amino acid 71 stabilizes the enhanceosome and leads to recruitment of the CBP-Pol II (CREB-binding protein-Polymerases II) enzyme complex

that replaces PCAF/GCN5. Next, the SWI/SNF holoenzyme/nucleosome remodeling complex [130, 131] is recruited and induces a conformational change in a nucleosome position that results in binding of TFIID transcription factor to activate transcription. HMGA1-mediated enhanceo-some formation is necessary for induction of *IFN-β* following viral infection of cells. Conversely, acetylation at the HMGA1 amino acid 65 by CBP leads to disruption of the enhanceosome and cessation of transcription [130, 131]. Recent studies suggest that HMGA1 may not be present in the final enhanceosome structure, but rather acts as a molecular chaperone during different stages in the assembly process of the pre-initiation complex, and ultimately dissociates from the final enhancer complex [138–140]. Studies of additional downstream transcriptional targets indicate that an HMGA1 enhanceosome may regulate many other genes and their pathways (discussed in this review). Although HMGA1 proteins do not function like transcription factors alone, they organize the framework of nuclear protein-DNA transcriptional complexes to modulate transcription. Because they alter the conformation of DNA, they have been termed "architectural transcription factors". How these transcriptional activities relate to HMGA1 function in cancer and other diseases is an area of active investigation, which we consider in this review.

5. HMGA1 PROTEINS INDUCE ONCOGENIC AND METASTATIC PROPERTIES IN CULTURED CELLS

Cloning and characterization of the promoter for murine *Hmga1* led to the discovery that *Hmga1* is a direct transcriptional target of the cMyc oncoprotein [88] and provided additional clues that *HMGA1* functions in cancer. HMGA1 proteins also induce potent oncogenic properties in cultured mammalian cells, similar to those of cMYC [22–24, 28–30, 50, 54, 68, 70, 75, 86, 117, 142]. To illustrate, ectopic expression of the murine *Hmga1a* or *Hmga1b* isoform results in colony formation/anchorage-independent cell growth in rat fibroblasts (Rat1a) or human lympho-blastoid cells (CB33) [28, 88]. Similarly, fibroblasts overexpressing *Hmga1a* or *Hmga1b* are tumorigenic in nude mice and metastasize to the lungs. In addition, overexpression of human *HMGA1a* or *HMGA1b* induces anchorage-independent cell growth in a noninvasive, human breast cancer cell line (MCF-7) *in vitro* [29]. The breast cells engineered to overexpress *HMGA1b* metastasize locally following injection into mammary fat pads [29]. Interestingly, the tumors have histopathologic features and express proteins characteristic of an epithelial-mesenchymal transition (EMT), a molecular program whereby cells lose their epithelial characteristics (planar, apical-basal polarity, lack of motility) and acquire mesenchymal features, including motility, invasiveness, and resistance to apoptosis. Although first described during embryonic development, and later in tissue culture models, EMT-like reprogramming is now known to drive tumor progression [29, 68, 117]. These findings demonstrate that *HMGA1* could play a causal role in both tumor initiation and metastatic progression.

Studies that interfered with *HMGA1* function or expression provide further insight into *HMGA1*'s role in tumorigenesis [28, 29 50, 68, 70, 75, 88, 117, 143–146]. Inhibiting *HMGA1* expression dramatically blocks both cellular proliferation and anchorage-independent cell growth (colony formation) in soft agar in Burkitts lymphoma cells [88], which are derived from an aggressive childhood lymphoid malignancy characterized by a

translocation event that leads to deregulated *cMYC* expression. *HMGA1* is also up-regulated in cell lines derived from Burkitts lymphoma or leukemia [88]. Adenovirus-mediated antisense knock-down of *HMGA1* results in apoptotic cell death in cultured human thyroid, colon, lung, and breast cancer cells, but not in cells derived from normal tissue [143]. Interfering with *HMGA1* function through an antisense or dominant-negative approach in human breast cancer cells also blocks proliferation and colony formation in soft agar [29, 54, 117]. In an orthotopic xenograft model of pancreatic cancer, knock-down of *HMGA1* blocks metastatic progression [70]. This group also found that *HMGA1* confers protection from anoikis, the process whereby cells undergo apoptosis when they are deprived of attachment to a matrix or placed in anchorage-independent culture conditions [71]. Inhibition of anoikis is thought to promote metastatic progression since viable, unattached cells are needed to migrate from the primary tumor to a metastatic site. Knock-down of *HMGA1* confers sensitivity to gemcitabine, a nucleoside analogue and first line therapy in pancreatic adenocarcinoma [73]. Another group found that silencing *HMGA1* also blocks anoikis in renal cell carcinoma cells [77]. In colon cancer cells, knock-down of *HMGA1* interferes with metastatic progression in a murine model [68], as well as anchorage-independent cell growth, migration, and invasion. Moreover, the stem cell property, growth in three-dimensional spheres, was blocked [68, 117]. Silencing *HMGA1* also prevents tumorigenesis when limited numbers of cancer cells were injected, indicating that silencing *HMGA1* depletes cancer stem cells. This work further highlights the functional role for *HMGA1* in driving tumor progression.

More recently, studies in poorly differentiated, triple-negative breast cancer cells showed that *HMGA1* functions as a key molecular switch required by cancer cells for oncogenic and stem cell properties. Silencing *HMGA1* using a potent lentivirus to deliver short hairpin to *HMGA1* abruptly halts cell growth [117]. Silencing *HMGA1* also dramatically changed the cancer cell morphology from mesenchymal, spindle-shaped cells to more differentiated-appearing, epithelial, cuboidal cells [117]. In addition, silencing *HMGA1* impaired oncogenic (colony-formation, migration/invasion, xenograft tumorigenesis) and cancer stem cell (growth as spheres, limiting dilution tumorigenesis) properties. Switching-off *HMGA1* also prevented seeding and metastatic progression to the lungs, both from mammary fat pads or tail vein injections. Together, these findings suggest that *HMGA1* is a master regulator of tumor progression, stem cell properties, and resistance to therapy.

6. *HMGA1* ANIMAL MODELS FOR MALIGNANCY & OTHER DISEASES

Genetically engineered mouse models provide the most convincing evidence that *HMGA1* causes cancer *in vivo* [68, 75, 90, 100]. Transgenic mice misexpressing murine *Hmga1a* develop aggressive lymphoid malignancy by 2–10 months with complete penetrance [90]. In this model, the murine *Hmga1a* transgene is driven by the H-2K promoter and immunoglobin μ enhancer, which directs transgene expression in the T and B cell lymphoid compartments. As expected, the transgene is expressed in B and T lymphoid cells, with highest levels in T-cells. All founders develop lymphoid malignancy, with an aggressive T-cell leukemia/lymphoma phenotype that recapitulates salient histopathologic and molecular features of human T-cell leukemia and lymphoma. The transgene is expressed at levels ranging from 2 to 10-fold above that observed in normal murine lymphocytes. This mouse

model mimics human B- and T-cell lymphoblastic leukemia where *HMGA1a* expression is increased by 2 to 10-fold over that observed in normal human T and B cells [90]. When crossed onto an *Ink4a/Arf* null background, leukemogenesis is markedly accelerated [118]. The transgene is also misexpressed in the uterus and all females develop uterine sarcomas by 8–10 months of age [75]. In the uterus, the transgene is expressed at levels of 5 to 15-fold above that found in control uteri [75], which is similar to high-grade human uterine tumors where *HMGA1a* mRNA is 2 to 20-fold higher than levels observed in normal uterine tissue [75]. These *Hmga1* mice also develop hyperproliferative changes in the small and large intestines [68], with polyposis similar to that observed in APC mutated mouse models [147]. In the gut tissue, *Hmga1a* expression is increased by about 3 to 5-fold above that observed in controls [68]. Preliminary studies in the transgenics indicate that there is enhanced intestinal stem cell function and number in the transgenics, further linking *HMGA1* to diverse stem cells (Xian and Resar, unpublished data).

Another transgenic mouse model was engineered which expresses the human *HMGA1b* isoform under the control of a CMV promoter, which drives expression in most tissues [100]. These mice develop natural killer T-cell (NK) lymphomas, although with a lower penetrance and at a later age than the *Hmga1a* mouse model [90]. Females also develop pituitary adenomas with a penetrance of 80% by 16 months; 15% of males develop pituitary adenomas by 22 months. The differences in tumor incidence between male and female mice is intriguing and suggests that gender-specific hormones may influence tumor development. The basis for the different phenotypes in the two transgenic models is not known, but could result from differences in murine or human gene function, levels of expression, or the specific isoforms expressed by the transgene. Nonetheless, these independent mouse models demonstrate that *HMGA1* causes cancer in mammals.

Studies describing embryonic stem cell lines [144] and mice [35, 145] deficient in *Hmga1* were also reported. When cultured under conditions to differentiate into hematopoietic cells, murine embryonic stem cells lacking *Hmga1* were reported to exhibit skewed hematopoietic differentiation, with a decrease in T-cell precursors [145]. Similarly, the same group reported that *Hmga1a* knock-out mice have decreased numbers of T-cells and develop skewed hematopoietic differentiation with leukemic transformation in some cases, which led to the speculation that *Hmga1* could also have tumor-suppressor effects [145]. The lympho-proliferative disease also resembles lymphoproliferative disease in patients with T cell immunosuppression and could reflect a decrease in T cell number and/or function. Although additional studies are needed to elucidate the mechanisms involved in the aberrant hematopoiesis with *Hmga1* deficiency, these studies further support an important role for *Hmga1* in T-cell development and leukemic transformation. Interestingly, a subsequent study reported that mice deficient in *Hmga1* are less susceptible to chemically induced skin cancers, highlighting *Hmga1*'s role in oncogenic transformation [146].

7. *HMGA1*, MITOCHONDRIAL FUNCTION, & THE WARBURG EFFECT

In addition to binding to nuclear chromatin to regulate gene expression, HMGA1 proteins also translocate to the mitochondria where they bind to mitochondrial DNA at AT-rich sequences in the D-loop control region [32–34]. Studies in MCF-7 breast cancer cells found

that forced overexpression of *HMGA1* results in decreased mitochondrial DNA and mitochondrial mass, while simultaneously causing increased reactive oxygen species and sensitivity to glycolytic inhibitors [32–34]. These findings suggest that *HMGA1* promotes aerobic glycolysis, at least in part, by interfering with mitochondrial function while increasing oxidative phosphorylation and the formation of reactive oxygen species in the remaining mitochondria. The dependence of cancer cells on aerobic glycolysis was first described in the 1920s by Otto Warburg when he discovered that cancer cells avidly take up glucose and convert it almost exclusively to lactate in the presence of adequate oxygen (aerobic glycolysis) [148, 149]. Later dubbed the "Warburg Effect", this metabolic alteration and others associated with tumor metabolism have emerged as hallmarks of cancer [150]. More recent studies of global metabolic alterations in intestines of *Hmga1* transgenic mice indicate that aerobic glycolysis is enhanced [151–153]. Indeed, most major tumor suppressors and oncogenes alter cellular metabolism. *cMYC*, for example, promotes aerobic glycolysis in cancer by inducing genes that enhance glucose uptake, while inhibiting oxidative phosphorylation in the mitochondria [154–158]. In MCF-7 cells, *HMGA1* also induces expression of a subset of mitochondrial genes, including *NADH dehydrogenase subunit 2*, *NADH dehydrogenase subunit 6*, *cytochrome c oxidase subunit 1*, and *ATP synthase 6* [32–34]. Importantly, some of these mitochondrial genes are up-regulated in cancer, although their functional roles in malignant transformation are incompletely understood. In addition to increasing sensitivity to glycolytic inhibitors and reactive oxygen species, this study also found that the efficiency of DNA repair decreases with increasing *HMGA1* expression. These studies suggest that *HMGA1* overexpression in cancer cells could produce mutations in genomic and mitochondrial DNA, while disrupting DNA repair. Further studies will be needed to elucidate the roles of *HMGA1* in mitochondrial function and cellular metabolism because recent evidence indicates that targeting cancer metabolism could be an effective therapeutic approach [150–158].

8. TYPE-2 DIABETES MELLITUS

Decreased expression of the *insulin receptor* (*Ir*) gene (discussed below), decreased insulin signaling, hyperglycemia, and a type-2 diabetes-like phenotype [35–38] were also reported in the *Hmga1* deficient mice. This group also described increases in the glucose transporter 3 (Glut3) protein, possibly as a compensatory mechanism in response to decreased *Ir* expression and signaling. Four patients with mutations that decrease expression of *HMGA1* were also described from a screen of 148 patients with type-2 diabetes [35]. Two cases were from the same family and both individuals have a hemizygous deletion of the *HMGA1* gene locus. The remaining two cases have a single nucleotide deletion in the 3′ untranslated regions, which was reported to decrease *HMGA1* gene expression [35]. More recent studies (outlined below) identified additional genes involved in glucose metabolism that are regulated by *HMGA1*. The human correlates suggest that decreased *HMGA1* expression could contribute to the development of type-2 diabetes in a subset of patients [35]. This group also reported cardiac hypertrophy in the knock-out mice, although there are no studies in humans that link genetic alterations affecting *HMGA1* with cardiac hypertrophy [145].

9. ALZHEIMER'S DISEASE

In addition to cancer and diabetes, recent studies implicate *HMGA1* in the pathogenesis of Alzheimer's disease, a neurodegenerative disorder that currently afflicts over 5 million Americans [39–41, 159–162]. Alzheimer's disease is characterized by neuronal loss, glial cell proliferation, aberrant angiogenesis, and accumulation of "senile plaques" composed of amyloid-β [159–161]. The *Presenilin-1* (*PS1*) and *Presenilin-2* (*PS2*) genes generate proteins (PS1 and PS2) involved in the production of amyloid-β [39–42]. An aberrantly spliced form of PS2 was demonstrated in the brains of sporadic Alzheimer's disease, the most common form of this disease. The aberrant splicing results from exon 5 skipping, which causes a frameshift with a premature termination codon and could lead to an accumulation of a deleterious protein (called PSV2) in the brain and the development of Alzheimer's disease [39–42, 159–161]. *In vitro* studies show that HMGA1 binds to a site within exon 5 and inactivates normal splicing, resulting in aberrantly spliced, truncated PSV2 protein [42]. In addition, HMGA1 proteins are elevated in the hippocampus of patients with sporadic Altzheimer's disease and *HMGA1* is induced with hypoxia in neuronal cells [39–42]. Moreover, inhibitory oligonucleotides to *HMGA1* block the abnormal splicing event. Although further work is needed, these studies uncover a potential role for *HMGA1* in Altzheimer's disease and possibly other neurodegenerative diseases associated with abnormal PS2 accumulation.

10. HMGA1 TRANSCRIPTIONAL TARGETS

Despite progress in our understanding of HMGA1 in development, cancer, and other diseases, there remain important questions about the molecular programs that are regulated by HMGA1 as well as the pathways that govern its function and expression. Global genomic studies are beginning to uncover HMGA1 pathways and define an HMGA1 transcriptome (Figs. 2, 3, Table 1).

Of the more than 75 HMGA1 transcriptional targets that have been reported thus far, most include Nuclear Factor-κB (NF-κB) regulatory elements in their promoter regions and many participate in mediating inflammatory pathways. Given the increasing evidence that inflammation is a precursor lesion in diverse cancers, this link between HMGA1 and NF-κB suggests a model whereby HMGA1 and NF-κB proteins cooperate to induce inflammatory signals and drive transformation [23]. Indeed, HMGA1 regulates many genes with roles in both inflammation and cancer. Like HMGA1, NF-κB is also known to play an important role in cancer progression, resistance to therapy, and a stem-like state. HMGA1 could therefore enhance NF-κB function in these processes. Proteomic studies have identified additional proteins that interact with HMGA1 and modify its function [163, 164]. In the following section, we review what is known about HMGA1 transcriptional targets and how their associated pathways could mediate HMGA1 function. We limited our review to reports of putative targets in which HMGA1-dependent function or expression has been validated by more than one experimental approach.

10.1. Inflammatory Cytokine and Cytokine Receptor Genes with Similar Enhancer Regions

10.1.1. IFN-β—*IFN-β* is among the most studied HMGA1 target genes and was first discovered just over two decades ago [126–140]. *IFN-β* expression is up-regulated following viral infection of cells, and *IFN-β*, in turn, induces expression of a large set of genes encoding antiviral gene products. These include chemokines and cytokines that stimulate an inflammatory response to counter viral infections. *IFN-β* plays a role in mediating cell-cell interactions involved in innate and acquired immune function. In addition, some chemokines play a role in metastatic progression and *HMGA1-IFN-β*-chemokine pathways could contribute to tumor progression. *IFN-β* also functions in osteogenesis by inhibiting osteoclast differentiation [165]. Similar pathways in cancer could promote inflammatory pathways and a poorly differentiated state. Moreover, the elegant studies that defined the *IFN-β* enhanceosome uncovered a unique role for HMGA1 as an architectural transcription factor that orchestrates the assembly of transcription factor complexes in enhancer regions to modulate gene expression [125–140].

10.1.2. IFN-γ—Following the identification of *IFN-β* as an HMGA1 transcriptional target, other putative target genes were discovered based on their homology to the *IFN-β* enhancer-promoter regions. The *IFN-γ* gene was also reported to be a downstream gene target regulated by HMGA1 [166]. IFN-γ is produced primarily by CD8+ T cells, and to a lesser extent by CD4+ T cells and NK cells. IFN-γ functions as a key inducer of T helper cells whose expression is increased following TCR stimulation in the setting of specific cytokines, such as IL-2, IL-12, and IL18. Like IFN-β, IFN-γ functions in intercellular communication during innate and acquired immune responses and in tumor surveillance. A prior study reported that transgenic mice expressing *HMGA1b* driven by the *lck* promoter have increased expression of *IFN-γ* mRNA and protein in mature T cells as compared to controls. This group also found that HMGA1 binds directly to 2 AT rich regulatory regions within the promoter of the *IFN-γ* gene using electrophoretic mobility or gel shift experiments. In addition, they reported that HMGA1 induced expression of the promoter in transfection experiments, while a dominant-negative HMGA1 repressed promoter expression. Together, these data are consistent with direct regulation of *IFN-γ* expression by HMGA1.

10.1.3. IL-4 and IL-2—The murine *IL-4* and *IL-2* genes harbor similar AT-rich, transcriptional enhancer sites to *IFN-β* in their upstream regulatory regions [167–171]. *In vitro* studies show that HMGA1 binds to AT-rich sites in the *IL-4* promoter to repress transcription [169], while it enhances *IL-2* transcription [167–169]. IL-2 stimulates T-cell proliferation and differentiation into effector cells [172]. For example, IL-2 promotes the development of T cell immunologic memory by stimulating the growth, differentiation and survival of antigen-specific CD4+ and CD8+ T cells. IL-2 also induces division of T regulatory cells, proliferation and differentiation of NK cells, and immunoglobulin production by B cells. Thus, IL-2 plays an important role in immunologic memory and adaptive immunity. *IL-2* is also expressed in T- and B-cell malignancies [167–170]. In contrast, *IL-4* is involved in B cell proliferation and differentiation [171]. These observations are consistent with the existing mouse models that misexpress *HMGA1* genes, which develop T-cell lymphoid malignancies [90, 100]. In addition to *IL-2*, the *IL-2 receptor α*,

IL-15, and the *IL-15 receptor α*, were reported to be up-regulated in *HMGA1b* transgenic mice [100] (discussed in more detail below). In human T-cell ALL, *HMGA1* is also overexpressed [90, 108, 118]. Thus, the T-cell specific transcriptional targets further support a role for *HMGA1* in T-cell development and malignancy.

10.1.4. E-Selectin (ESEL, ELAM-1)—*ESEL* is up-regulated by HMGA1. The organization and expression of the *ESEL* promoter has similarities to the *IFN-β* enhancer, including AT-rich regions in positive regulatory domains (PRD) I-IV regions [173]. This gene encodes cell adhesion molecules (CAMs) induced by inflammatory cytokines, such as interleukin-1-β and tumor necrosis factor alpha (TNF-α). Similar to the *IFN-β* promoter, HMGA1 binds to an enhancer site and promotes the binding of ATF-2 and NF-κB [173]. Like other selectins, *ESEL* plays an important role in inflammation, although *ESEL* is expressed only in <u>e</u>ndothelial cells (therefore designated <u>E</u>) [174]. It serves to recruit white cells to sites of inflammation by mediating the adhesion of white cells to the vascular lining and is associated with inflammatory processes and disease states [175].

10.1.5. P-Selectin (PSEL)—HMGA1 also induces expression of *PSEL*, another CAM gene that is expressed in <u>p</u>latelets (thus designated *PSEL*) as well as in endothelial cells [176]. HMGA1 binds to the *PSEL* promoter, as demonstrated *in vitro* by gel shift experiments and *in vivo* by chromatin immunoprecipitation in bovine aortic endothelial cells (BAECs) at an AT-rich site in a complex that includes the p50/p65 members of the NF-κB family. In transfection experiments in BAECs, HMGA1 cooperates with p50/p65 to induce expression of *PSEL.* Distamycin, a drug that binds to the minor groove, blocks HMGA1 binding to the *PSEL* promoter in gel shift experiments and decreases *PSEL* expression in transfection experiments without affecting *ESEL* expression. Distamycin also blocks *PSEL* expression in a murine model for inflammation during endotoxemia, with no decrease in *ESEL* expression [176]. The basis for the differential effects on *P-* and *ESEL* are not clear and further studies are needed to determine under what cellular contexts HMGA1 regulates *P-* and *ESEL*. In cancer, P-Selectin has a similar functional role to E-Selectin, recruiting CAMs that mediate the interaction of activated platelets with leukocytes. P-Selectin also facilitates cancer cell invasion into the bloodstream and metastatic progression.

10.1.6. Interleukin 2 Receptor α (IL-2Rα, IL-2RA)—The *IL-2Rα* gene is rapidly and potently induced in T-cells following mitogenic stimuli and it is also overexpressed in Jurkat T-cell leukemia cells. The promoter includes an enhancer region with positive regulatory regions (PRR) I and II, which are essential to activate *IL-2Rα* expression following mitogenic stimulation [177, 178]. The Elf-1 transcription factor binds at PRRII, and HMGA1 interacts with Elf-1 *in vitro.* PRRI contains an NF-κB binding motif and biochemical studies showed that Elf-1 interacts with the NF-κB family members, p50 and c-Rel, bound to PRRI. In this setting, it was proposed that HMGA1 serves as the molecular "glue" bridging the interactions between Elf-1 and NF-κB. Alternatively, HMGA1 could maintain Elf-1 in a transcriptionally active confirmation. In contrast to the *IFN-β* gene, HMGA1 does not appear to enhance Elf-1 binding to PRR II. Like *IL-2*, activation of *IL-2Rα* gene facilitates T-cell proliferation and antigen-induced T-cell immune function. Subsequent studies showed that HMGA1 binds to the *IL-2Rα* promoter following T-cell

activation, and remodels an inhibitory nucleosome, thereby inducing *IL-2Ra* gene expression [178–180]. *IL-2 and IL-2Ra* were reported to be up-regulated in the *HMGA1b* transgenic model and could contribute to the development of the T-cell leukemias [100].

10.1.7. Tumor necrosis factor-β (Tnf-β, lymphotoxin α)—HMGA1 up-regulates expression of the gene encoding the pro-inflammatory cytokine, tumor necrosis factor-β (TNF-β; also known as lymphotoxin α) [181]. The murine *Tnf-β* promoter includes an AT-rich region to which HMGA1 binds by gel shift experiments. In transfection experiments, HMGA1 activates expression of a *Tnf-β* promoter construct with the AT-rich region in murine leukemia cells. TNF-β is a cytokine produced by mitogenic-activated macrophages and monocytes and plays a key role in inflammation. Knock-out mouse models demonstrate that Tnf-β is essential for the formation and maintenance of lymphoid tissues, including lymph nodes, the spleen, and Peyer's patches in the gut [182]. Tnf-β/Lymphotoxin-α is a member of the Tnf family which forms heterotetramers with lymphotoxin-β and serves to anchor Tnf-β to the cell surface. The primary function of Tnf-β is to mediate diverse inflammatory, immunostimulatory, and antiviral processes. It is also involved in NF-κB signaling. Although originally discovered as an anti-tumor factor, increasing evidence suggests that it is involved in inflammatory pathways that can also promote oncogenesis, particularly in settings of prolonged inflammation [183]. *TNF-β* is also constitutively expressed in some human leukemia cell lines [183].

10.1.8. MGSA/GROα/Chemokine (C-X-C Motif) Ligand CXCL1—HMGA1 directly activates expression of the gene encoding the chemokine, *MGSA/GROα/CXCL1*, which is up-regulated as melanocytes progress to melanoma [184]. The *MGSA/GROα/CXCL1* promoter has similar regulatory elements as *ESEL* and HMGA1 was found to bind to an AT-rich region nested within the NF-κB element similar to its binding to the *ESEL* promoter. Transfection experiments with promoter constructs containing mutations in the regulatory elements showed that HMGA1, NF-κB, and SP1 are required for transactivation of this gene. These findings suggest that HMGA1 could mediate progression to melanoma by up-regulating the *MGSA/GROα/CXCL1* cytokine gene. This protein serves as a chemoattractant for neutrophils and thereby has a role in inflammation. Aberrant expression of *MGSA/GROα/CXCL1* is also associated with growth and progression in a subset of tumors [175].

10.2. Additional Cytokine and Chemokine Genes

10.2.1. Interleukin 15 (Il-15 and Il-15Rα)—*Il-15* and *Il-15Rα* were also reported to be up-regulated in the *HMGA1b* transgenic model [100]. Like IL-2, IL-15 is a proinflammatory cytokine that is secreted by macrophages following viral infection [185, 186]. Moreover, both IL-2 and IL-15 bind to common erythropoietin receptor subunits and they may compete for receptor binding, thereby negatively regulating each other. IL-15 also stimulates T-cell proliferation, the generation of cytotoxic T cells, stimulation of immunoglobulin synthesis by B cells, and the generation and persistence of NK cells. IL-15 induces expression of TNF-α, IL-1β, and other inflammatory cytokines [185]. In contrast to IL-2, IL-15 is not required for T regulatory cells [185]. Proliferation of NK cells induced by IL-15 results in downstream JAK-STAT signaling. In murine studies, Il-15 also has anti-apoptotic effects by

inducing expression of apoptosis inhibitors Bcl2/Bcl1/Bcl-x(L) [175, 185, 186]. Abnormalities in IL-15 have been observed in immunologic diseases, such as rheumatoid arthritis, inflammatory bowel disease and colitis, and lymphoid malignancies associated with human T-cell lymphotropic virus 1 infection [185, 186]. More recently, both IL-15 and soluble IL-15Rα were found to be elevated in the serum of patients with large granular lymphocyte (LGL) leukemia, a T-cell lymphoproliferative disorder characterized by clonal expansion of mature T or NK cells [185]. *IL-15Ra* mRNA was also increased in the peripheral blood monocytes of patients LGL leukemia and it was proposed that enhanced signaling of IL-15 pathways contribute to the pathogenesis of the disease. Although it is not known if *HMGA1* is involved in LGL leukemia, these findings suggest that IL-15 induces inflammatory pathways in this lymphoproliferative disorder [185].

10.2.2. IL-10—IL-10 is produced by monocytes, and to a lesser extent lymphocytes, and involved in inflammation and immune-regulation. It is commonly known to be an anti-inflammatory cytokine because it inhibits the synthesis of a pro-inflammatory cytokines, including IFN-gamma, IL-2, IL-3, TNF and GM-CSF produced by activated macrophages and by helper T-cells. IL-10 is also associated with signaling through the JAK-STAT pathway [175]. Gel shift experiments show that HMGA1 enhances binding of the nuclear factory Y (NF-Y) to the *IL-10* promoter in a Burkitts lymphoma cell line [187]. In contrast, antibodies to HMGA1 diminish the intensity of this band, suggesting that HMGA1 is part of the NF-Y complex binding to the *IL-10* promoter. Although promoter expression studies were not performed, it was postulated that HMGA1 and NF-Y cooperate to enhance *IL-10* expression. IL-10 has the ability to suppress T_H1 cell differentiation, which could maintain T cells in an undifferentiated state. IL-10 also promotes B cell proliferation, survival and antibody production which could contribute to malignant transformation in B cells [175, 187]. Studies demonstrating *HMGA1* overexpression in both T and B lymphoid malignancies provide additional evidence for a link between *HMGA1*, IL-10, and lymphoid tumors [90]. Another study found that IL-10 stimulates self-renewal of hematopoietic stem cells *ex vivo,* suggesting that *HMGA1* could contribute to the maintenance of hematopoietic stem cells through IL-10 [188]. Studies in mice indicate that it is an essential immunoregulatory molecule in the intestines [175].

10.2.3. Granulocyte Macrophase Colony Stimulating Factor (GM-CSF) and IL-2—In Jurkat T-cell acute lymphoblastic leukemic cells, HMGA1 induces expression of the *GM-CSF* [189] in addition to *IL-2* cytokine growth factors. HMGA1 binds to the AT-rich sequence within a CD28 response element of the *GM-CSF* and *IL-2* promoters to enhance binding of the NF-κB family member, c-Rel. Antisense RNA targeting *HMGA1* or *c-Rel* abolishes activation of these promoters, suggesting that HMGA1, together with c-Rel, induce expression of *GM-CSF* and *IL-2*. GM-CSF stimulates hematopoietic stem cells to produce granulocytes and monocytes as part of an immune and inflammatory cascade, and could promote inflammatory pathways mediated by *HMGA1* [190]. As detailed above, IL-2 drives T-cell proliferation and is overexpressed in some T-cell malignancies [109, 189]. Targeted disruption of *Il-2* is also associated with colitis in mice [175].

10.2.4. IL-2rb and Il-18r1—Genome-wide analysis of lymphoid tumors from *Hmga1a* transgenic mice identified additional genes that are regulated by HMGA1 during tumorigenesis and involved in inflammation and white blood cell signaling [108]. Genes expressed in lymphoid cells from *Hmga1a* transgenic mice early in tumorigenesis (2 months) and in established tumors (12 months) were compared to control lymphoid cells to identify genes induced by Hmga1 during leukemic transformation [108]. Microarray analysis and quantitative RT-PCR show that the *Il-2 receptor β* (*Il-2rb*) and *Il-18 receptor 1* (*Il-18r1*) are up-regulated in early and late tumorigenesis. The beta subunit of Il-2 receptor (Il2rb) forms a heterodimer with Il-2 receptor α (Il2ra) or heterotrimer with Il2ra and Il-2 receptor γ (Il2rg) to form an Il-2 receptor, which mediates mitogenic stimulation or receptor-mediated endocytosis induced by Il-2. The Il2ra-Il2rb heterodimer is a high affinity receptor, while the heterotrimer (Il2ra, Il2rb and Il2rg) is an intermediate affinity receptor. As noted above, IL-2 signaling is involved in T-cell proliferation, further linking *HMGA1* to T cell function. This study also found that *IL-2RB* is repressed in human Jurkat T-cell leukemia cell lines following knockdown of *HMGA1* expression, suggesting that this pathway is functional in human T-cell acute lymphoblastic leukemia in addition to the T-cell acute lymphoblastic leukemia in the Hmga1 transgenics. The *Il-18 receptor 1* (*Il-18r1*) gene encodes the Il-18 receptor which binds to the Il-18 cytokine. IL-18 belongs to the IL-1 superfamily that induces cell-mediated immunity following infection with microbial products. Following stimulation by IL-18, natural killer and T cells release IFN-γ to activate macrophages and other cells involved in cell-mediated immunity and inflammation [175]. Further studies are needed to determine if the *IL-2 receptor β* (*IL2rb*) and *IL-18 receptor 1* are directly regulated by HMGA1.

10.2.5. Chemokine (CXC-Motif) Receptor 3 (CXCR3)—Early in lymphoid tumorigenesis, the gene encoding the Cxcr3 receptor chemokine is up-regulated in lymphoid cells from the *Hmga1a* transgenics compared to controls, as shown by microarray gene expression profile analysis and quantitative RT-PCR in mouse and human cells [108]. Cxcr3 is a chemokine receptor protein in the CXC chemokine receptor family [175]. Normally expressed in activated T cells and NK T cells, it functions in leukocyte trafficking and recruitment of inflammatory cells. The Cxcr3 could mediate inflammatory pathways that contribute to tumorigenesis. It is not yet known if *Cxcr3* is directly regulated by HMGA1 [108].

10.3. Additional Genes Involved Leukocyte Function and Inflammation

10.3.1. T Cell Receptor α-chain (TCRα, TRA)—HMGA1 represses the *TCRa* gene, which directs tissue- and stage-specific expression and V(D)J recombination of this gene locus [191]. Using an *in vitro* transcription assay system in Jurkat T-cell leukemia cells, investigators found that HMGA1 is part of a T cell-specific repressor complex that is sensitive to DNA topology. These studies suggest that HMGA1 binds to the repressor and inactivates the *TCRa* promoter when DNA is in a supercoiled state. In the absence of the repressor complex, *TCRa* gene expression is not dependent upon the 3′ enhancer and DNA topology. This pathway could also serve to maintain T-cells in a less differentiated state prior to expression of the TCR complex. The overexpression of *HMGA1* in T-cell acute lymphoblastic leukemia is also consistent with a role for *HMGA1* in maintaining an

aberrantly differentiated, leukemogenic phenotype in T cells [90, 100]. Other known pathways associated with TCRa are Class I MHC mediated antigen processing and presentation. *TCRa* is also associated with T-cell acute lymphoblastic leukemia and colitis [175].

10.3.2. Immunoglobulin heavy chain ε (Ighe)—Transcription of the germ-line *immunoglobulin heavy chain ε* enhancer is required for the immuno-globulin isotype switch from the μ heavy chain constant region that encodes IgM to the ε heavy chain constant region that encodes IgE. Transcription from the *ε* enhancer in B-cells is repressed by Hmga1 and induced by IL-4 and lipopolysaccharide [192]. Gel shift and DNAase footprint experiments demonstrate that Hmga1 binds directly to an AT-rich region at the *ε* enhancer and transient transfection experiments in mouse B lymphoma cells show that *Hmga1* represses *immunoglobulin heavy chain ε* transcription. This suggests that Hmga1 could interfere with immunoglobulin isotype switch from IgM to IgE in B cells.

10.3.3. Immunoglobulin Heavy Chain μ, Immunoglobulin Heavy Constant μ, (IGHM)—Subsequent studies showed that HMGA1 co-activates expression of the *IGHM* enhancer in human B cells, although *in vitro* studies suggest that HMGA1 indirectly associates with DNA at this enhancer [192–195]. HMGA1 interacts with the PU.1 transcription factor to enhance PU.1 binding to the μ enhancer and potentiation of the PU.1/ETS-1 interaction at the enhancer. While HMGA1 is not associated with the μ enhancer by chromatin immunoprecipitation, silencing *HMGA1* expression results in repression of the μ enhancer. These findings suggest that HMGA1 orchestrates the transcriptional complex, but is not present when transcription commences. The μ enhancer mediates V(D)J rearrangement of the immunoglobulin heavy chain required for IgM production during B cell development [192]. Repression of *Ighe* enhancer and activation of the *IGHM* by HMGA1 could help to maintain IgM production and repress IgE production in B cells. IgM antibodies are critical for primary defense mechanisms and recognizing external invaders into the body [175].

10.3.4. GP91-PHOX (Cytochrome B-245, CYBB)—HMGA1 also binds upstream of the *GP91-PHOX* gene *in vitro*, which encodes the heme-binding beta subunit of NADPH oxidase (cytochrome b) [195]. This interaction was discovered by screening an expression library with the *GP91-PHOX* promoter CCAAT-box region that includes an AT-rich motif [196]. HMGA1 binds specifically to this AT-rich region; studies were not done to show if gene expression was altered by HMGA1 binding. NADPH oxidase is a mitochondrial enzyme that produces reactive oxygen species (ROS) required by neutrophils to kill bacteria in phagocytic vacuoles. ROS is involved in diverse processes, including cancer, artherosclerosis, and Alzheimer's disease.

10.3.5. CD8β1 (CD8b1)—Gene expression studies in lymphoid tumors from the *Hmga1a* transgenics also show that the *CD8β1* (*CD8b1*) gene is up-regulated in the *Hmga1a*-driven lymphoid tumors [108]. CD8β1 forms a dimer with CD8α (CD8a) to form the CD8 T cell receptor (TCR), which is normally expressed on the surface of cytotoxic T cells. It is also found on natural killer cells, cortical thymocytes, and dendritic cells, where it is involved in

antigen-specific T cell activation [175]. CD8 is also expressed in T-cell acute lymphoblastic leukemia. Knocking-down *HMGA1* in Jurkat T-cell acute lymphoblastic leukemia cells represses *CD8b1* expression, further implicating the *HMGA1-CD8Rb1* pathway in T cell leukemia.

10.3.6. Granzyme M (GZMM)—The *Gzmm* gene, which encodes granzyme M, is also up-regulated in the *Hmga1a* transgenic lymphoid cells, both early and late in tumorigenesis [108]. This was confirmed by qRT-PCR in human and mouse cells. This gene is expressed in natural killer and activated T cells and the protein product is stored in large cytoplasmic vacuoles (called granzymes) together with granzyme A, B, and H. Granzyme M is a serine protease involved in apoptosis through caspase activation [175]. Whether HMGA1 directly regulates *GZMM* remains to be determined.

10.3.7. Spleen Tyrosine Kinase (Syk)—Syk encodes a non-receptor tyrosine kinase which transduces signals downstream of diverse trans- membrane receptors involved in innate and adaptive immunity, proliferation, and survival [197]. Although it was originally identified as an essential factor downstream of B-cell receptor (BCR) signaling, Syk also functions in T-cell receptor signaling. Syk transduces signals involved in cellular adhesion, osteoclast maturation, platelet activation, and angiogenesis [197]. It also induces inflammatory pathways and, together with NF-κB, Syk activates expression of chemokine and cytokines following infection by pathogens. Moreover, Syk is constitutively active in hematologic malignancies and efforts are underway to target its activity in lymphoid malignancies [197]. *Syk* was reported as a gene induced by Hmga1 in mouse embryo stem cells (mESCs), both in gene expression profile analysis and quantitative RT-PCR studies [198].

10.4. Transcription Factor Genes

10.4.1. Gata-1, Gata-4 and Gata-6—A comparison of mouse embryonic stem cells (mESCs) deficient in *Hmga1* to wildtype mESCs led to the identification of target genes involved in endoderm and mesoderm-drived tissues, including blood cell commitment and [144, 198]. When mESC-derived embryoid bodies (EBs) were cultured in methylcellulose under conditions that enable hematopoietic differentiation, the *Hmga1* deficient mESCs were reported to form fewer "hematopoietic EB bodies", defined as EBs that differentiate into colonies with macrophages, granulocytes, and hemoglobin-producing erythroid precursors. This group also reported a decrease in cells expressing the T-cell marker, Thy1.2, with an increase in B cells expressing the pan-B-cell marker, B220. Conversely, these investigators reported a decrease in myeloid and monocytic differentiation, while both erythroid and megakaryocyte differentiation were enhanced. Following treatment of embryoid bodies with retinoic acid to induce mesodermal differentiation, this group reported an increase in *globin* (ζ, α, β) gene expression in the *Hmga1* deficient cells. Because the Gata-1 zinc finger transcription factor regulates globin gene expression, *Gata-1* expression was assessed and both mRNA and protein were reported to be increased in the *Hmga1* deficient cells after culture under conditions that induce hematopoietic differentiation. The Gata family of zinc finger transcription factors regulate differentiation and fate specification in diverse tissues; the first member to be identified, Gata-1, was discovered in hematopoiesis

[175]. A *Gata-1* promoter regulatory region (−3.9 to −2.6 kilobases upstream of the first exon) linked to the luciferase reporter was expressed at higher levels in the *Hmga1* null ES cells compared to the wildtype ES cells. Chromatin immunoprecipitation show that HMGA1 binds to this region as well as a second downstream regulatory region. Electrophoretic mobility shift assays (EMSA) confirmed HMGA1 binding to a 46 base pair AT-rich site in the downstream regulatory region; the upstream regions were not studied. Together, these studies suggest that Hmga1 directly represses *Gata-1* during hematopoietic differentiation.

Both *Gata-4* and *Gata-6* were also reported as genes repressed by Hmga1 in a similar microarray study comparing genes expressed in wildtype mouse embryonic stem cells (mESCs) to mESCs deficient in *Hmga1* [198]. The differential regulation of *Gata-4* was confirmed by RT-PCR in mESCs and mouse embryo fibroblasts (MEFs), respectively. *Gata-4* and *Gata-6* are expressed in diverse mesoderm- and endoderm-derived tissues, including the heart, lung, liver, gut, and gonad [199, 200]. Gata-4 regulates genes involved in embryogenesis, including cardiac muscle differentiation and function, while Gata-6 is thought to regulate terminal differentiation and proliferation. Mutations in *GATA-4* and *GATA-6* are associated with septal defects and other forms of congenital heart disease in humans.

10.4.2. Lung Krupple-like Factor (Lklf)—*Lklf* encodes a member of the Kruppel-like family of zinc finger transcription factors that is highly related to the erythroid kruppel-like factor (EKLF) and recognizes a similar DNA binding motif [201, 202]. Deletion of *Lklf* in mice results in embryonic lethality with retarded growth, craniofacial abnormalities, abnormal bleeding and anemia *in utero* [201]. Although yolk sac erythropoiesis is normal, fetal liver cultures from these embryos fail to generate erythroid cells. Studies in lung epithelia reveal that LKLF is an important anti-inflammatory factor that blocks pro-inflammatory cytokine production [202]. In mESCs, Hmga1 was reported to up-regulate *Lklf* expression in gene expression profile analysis and RT-PCR, although studies in more differentiated MEFs suggest that Hmga1 represses *Lklf* [201].

10.4.3. Zif268, Early Growth Response Protein 1 (Egfr1), Nerve Growth Factor-Induced Protein A (Ngfr-1), or Krox-24—*Zif268* [also known as *early growth response protein 1 (Egfr1), nerve growth factor-induced protein A (Ngfr-1)*, or *Krox-24*] is an immediate-early gene induced by growth factors, cytokines, and stress signals that encodes a zinc finger transcription factor [203]. In the absence of stimulation, *Zif268* expression is low in most tissues, except the brain. In the brain, it is associated with neuronal activity and it may function in neuronal plasticity [175]. Zif268 also regulates genes involved in mitogenesis and differentiation. Although some studies suggest that it has tumor suppressor function, recent work in prostate cancer indicates that *ZIF268* promotes proliferation [203]. A report of gene expression profile analyses and quantitative, RT-PCR in mESCs, MEFs, and liver tissue deficient in *Hmga1* compared to wildtype fibroblasts showed that *Zif268* is induced by Hmga1 [198].

10.4.4. JunB and cFos—*JunB, cFos,* and *cMyc* are additional immediate-early genes that encode proto-oncogenic transcription factors and are up-regulated by Hmga1 [198]. Gene expression profile analysis and qRT-PCR studies reported induction of these genes in

wildtype mESCs compared to mESCs deficient in *Hmga1* [198]. *JunB* and *cFos* are members of the AP1 family with oncogenic properties. Human *cFOS* was also identified as an HMGA1 gene target in vascular smooth muscle cells [discussed below; ref. 204]. As noted previously, *cMYC* encodes a potent oncogenic transcription factor that also regulates *HMGA1* expression [88, 205]. Studies in hESCs and induced pluripotent stem cells also indicate that HMGA1 regulates *cMYC* expression [see below; ref. 98]. This regulatory circuit could orchestrate proliferative and oncogenic pathways in tumors overexpressing *HMGA1, cMYC, JUNB,* and *cFOS*.

10.4.5. NANOG, OCT4, SOX2, cMYC, and LIN28—Studies in hESCs and cellular reprogramming discovered a novel role for *HMGA1* in maintaining a de-differentiated state in pluripotent stem cells [98]. In hESCs induced to differentiate, *HMGA1* expression falls and parallels that of known pluripotency genes. Silencing *HMGA1* in hESCs results in repression of *OCT4*, *SOX2*, *cMYC*, and *LIN28*, while forced expression of *HMGA1* in hESCs cultured under differentiation conditions maintains expression *OCT4*, *SOX2*, *cMYC* and *NANOG*. HMGA1 binds directly to the promoters of *SOX-2*, *cMYC*, and *LIN28 in vivo* in hESCs, as demonstrated by chromatin immunoprecipitation. In experiments of induced pluripotency with the "Yamanka" reprogramming factors (Oct4, Sox2, Klf4, cMyc or OSKM), *HMGA1* enhances cellular reprogramming, yielding an increased number of fully reprogrammed, induced pluripotent stem cell colonies that were larger in size. HMGA1 also up-regulates expression of *SOX2*, *cMYC*, and *LIN28* during cellular reprogramming. Moreover, interfering with *HMGA1* expression (*via* short hairpin RNA) or function (*via* a dominant-negative construct) completely abrogates reprogramming of somatic cells to induced pluripotent stem cells by the Yamanaka factors. These findings demonstrate a novel function for *HMGA1* as a key regulator of the stem cell state by directly inducing networks involved in pluripotency [98].

10.4.6. Signal Transducer and Activator of Transcription 3 (STAT3)—Global gene expression profile analysis in fibroblasts identified *STAT3* as a critical downstream target of HMGA1 [91]. *STAT3* is up-regulated in cultured cells and transgenic mice engineered to overexpress *Hmga1a*. Similar to other HMGA1-regulated promoters, HMGA1 binds to an AT-rich region near an NF-κB binding site and activates *STAT3* expression. HMGA1 occupies this promoter in cultured cells from diverse hematopoietic malignancies, including myeloid and lymphoid leukemia cells. Moreover, a STAT3 small molecule inhibitor induces apoptosis in leukemic cells from *Hmga1* transgenics, but not in normal lymphoid cells, indicating that this pathway could be a rational therapeutic target in some malignancies [91]. STAT3 small molecule inhibitors also result in decreased tumor burdens in the *Hmga1a* transgenic mice crossed onto an *Ink4a/Arf* null background [206]. Recent studies also showed that a small molecule inhibitor to STAT3 disrupted lymphoid tumor cell proliferation *in vitro* and xenograft tumors *in vivo* [206]. Because *STAT3* is a key mediator of inflammatory signals and molecular pathways that contribute to cancer initiation and progression, including proliferation, angiogenesis, metastatic progression, survival, and immune evasion [207–209], this pathway could be important in diverse tumor types.

10.4.7. Fox-P1—*Fox-P1* is repressed in lymphoid cells from *Hmga1* transgenic mice, both early and late in tumorigenesis [108]. This gene is also repressed by HMGA1 in human Jurkat T-cells. Fox-P1 is a member of the "forkhead box or FOX" transcription factors [108]. The P subfamily of Fox transcription factors regulate tissue and cell-type specific genes during development and differentiation [210]. The FOX-P1 protein has both DNA binding and protein-protein binding domains. *FOX-P1* maps to chromosomal region 3p14.1, which is lost in several tumor types, and *FOX-P1* is therefore thought to function as a tumor suppressor [210].

10.4.8. Eomesodermin (EOMES)—In the same gene expression analysis, the *Eomes* gene (also known as *T-box brain protein 1* or *TBR1* gene) was up-regulated both early and late in lymphoid transformation [108]. Knock-down of *HMGA1* in human Jurkat T-cell acute lymphoblastic leukemic cells represses *EOMES* expression, indicating that the *HMGA1-EOMES* network is also activated in human T-leukemia cells. *EOMES* encodes a transcription factor that functions in endodermal and mesodermal specification during development [211–213]. Disruption of the *Eomes* gene in mice demonstrate that it is necessary for trophoblastic development and gastrulation. Studies in human T cells indicate the *EOMES* is critical for IFN-γ production in CD8+ cells, and possibly in CD4+ cells [213, 214]. Although further studies are needed to determine if *EOMES* is directly regulated by HMGA1, this pathway could promote inflammatory signaling, EMT and other stem-like properties in cells overexpressing *HMGA1*.

10.4.9. TWIST—Increasing evidence suggests that cancer cells metastasize by co-opting stem cell transcription networks that mediate changes resembling an epithelial-mesenchymal transition (EMT) during tumor progression [58, 68, 214]. In poorly differentiated colon cancer cells, HMGA1 induces the EMT genes, *TWIST* and *VIMENTIN,* while repressing the epithelial gene, *E-CADHERIN* [68; VIMENTIN and E-CADHERIN are discussed in detail later]*. TWIST* encodes a bHLH transcription factor protein involved in EMT during embryogenesis and in tumor progression. Concurrent with repressing *TWIST* and *VIMENTIN*, and inducing *E-CADHERIN*, silencing *HMGA1* in the high-grade colon cancer cells also blocks three dimensional colonosphere formation, a cancer stem cell property [68]. Moreover, there is depletion in tumor-initiator cells in limiting dilution tumorigenesis experiments in the *HMGA1* knock-down cells. In addition, interfering with *HMGA1* expression blocks anchorage-independent cell growth, migration, and invasion *in vitro* as well as metastatic progression *in vivo* [68]. These results indicate that *HMGA1* promotes tumor progression through transcriptional networks that facilitate EMT, metastatic progression, and a de-differentiated, stem-like state.

10.4.10. HAND1 (Heart and Neural Crest Derivatives Expressed 1)—In addition to activating cellular programs that mediate tumor growth and progression, HMGA1 represses factors required for differentiation. To illustrate, HMGA1 was reported to repress *HAND1 (Heart and Neural Crest Derivatives expressed 1)*, a member of the Twist subfamily of basic helix-loop-helix (bHLH) transcription factors [215]. Studies in *Hand1* null mice indicate that it plays a critical role in placenta formation and cardiac morphogenesis in the ventricular chambers [215] *Hand1* expression was reported to be enhanced in *Hmga1* null

mice and HMGA1 binds to the *Hand1* promoter, both *in vitro* and *in vivo*, as demonstrated by in gel shift analysis and chromatin immunoprecipitation. Transfection experiments showed that HMGA1 represses *HAND1* promoter expression. In addition, there was an inverse correlation between expression of *HMGA1* and *HAND1* in primary thyroid tumors. Moreover*, HMGA1* overexpression was reported to be associated with DNA methylation of the *HAND1* promoter in anaplastic thyroid carcinomas in advanced tumors. *HAND1* is also silenced in gastric, colorectal, and pancreatic cancers [215–218].

10.4.11. Transcription Factor EB (Tfeb)—The *Tfeb* gene is also reported to be induced by Hmga1 in wild-type mESCs compared to those deficient in *Hmga1* in microarray gene expression profile analysis and RT-PCR experiments [198]. This gene encodes a protein that regulates genes involved in lysosomal biogenesis and autophagy [219]. The protein is phosphorylated by ERK2 which responds to levels of extracellular nutrients, suggesting a link between *HMGA1*, TFEB, nutrient sensing, and autophagy [220]. Increasing evidence also suggests an important role for nutrient metabolism and autophagy in tumor cells [155–158]. TFEB activates the expression of *CD40L* in T-cells; thus, it is linked to cell-dependent antibody responses in activated CD4 T-cells and cancers associated with abnormal T-cell growth [175].

10.5. Genes Involved in Repair of DNA Damage and Cell Cycle Progression

10.5.1. Xeroderma Pigmentosa Complementation Group A (XPA)—HMGA1 participates in genomic instability in cancer cells by modulating expression of DNA repair genes [61, 110–112]. The gene encoding the nuclear excision repair factor, XPA, is repressed in MCF-7 cells engineered to overexpress *HMGA1a* [112]. Nuclear excision repair (NER) is responsible for the detection and repair of large, DNA helix distorting lesions, such as cyclobutane pyrimidine, photoproducts, and cisplatin adducts that arise following UV radiation and chemical carcinogens. Chromatin immunoprecipitation experiments show that HMGA1 binds upstream of the *XPA* gene. In transfection experiments, HMGA1a no longer represses the *XPA* promoter when the consensus HMGA1 binding site is mutated. MCF-7-*HMGA1* cells also exhibit an increase in cyclobutane pyrimidine dimers, consistent with the hypothesis that HMGA1 is associated with DNA helix distorting lesions. Another study discovered an increase in unbalanced translocations using spectral karyotype analysis of prostate cancer cells engineered to overexpress *HMGA1* [61]. Together, these studies suggest that cancer cells with high expression of *HMGA1* are predisposed to chromosomal instability, at least in part, by repressing *XPA*.

10.5.2. Breast Cancer 1 (BRCA1)—The *BRCA1* tumor suppressor gene was also reported to be repressed by HMGA1 [110]. *BRCA1* was originally isolated as the gene mutated in familial breast and ovarian cancer [175]. It is important in regulating cell cycle progression, processing DNA damage, and maintaining chromosomal stability, including repair of double-stranded breaks. It associates with other tumor suppressors, DNA damage sensors, and signal transducers to form a large protein complex known as the BRCA1-associated genome surveillance complex (BASC). Mutations in *BRCA1* are associated >40% of inherited breast cancer and >80% of cases of with inherited breast and ovarian cancer. Prior studies report that *Brca1* is up-regulated mESCs null for *Hmga1*, while it was

down-regulated in cells and tissues overexpressing *HMGA1* [110]. This group found that HMGA1 binds directly to the *BRCA1* promoter/enhancer region (gel shift assays; chromatin immunoprecipitation) and represses its expression in transfection experiments. They also reported that HMGA1 blocks induction of the *BRCA1* gene by estrogen. In a pilot study of 14 human breast cell lines and primary tumors, *HMGA1* and *BRCA1* mRNA and expression were inversely correlated [111]. These findings suggest that repression of the *BRCA1* tumor suppressor by *HMGA1* could contribute to tumorigenesis in cases where BRCA1 is not mutated.

10.6. Genes Involved in Pro-Survival/Anti-Apoptotic Pathways

10.6.1. B-Cell Lymphoma 2 Gene (BCL-2)—A prior study reported that HMGA1 promotes a survival/anti-apoptotic response by directly inducing *BCL-2* expression [221]. In breast cancer cells (MCF-7) engineered to overexpress *HMGA1b*, HMGA1b was reported to bind to an AT-rich region, as shown *in vitro* and *in vitro* in gel shift analysis and chromatin immunoprecipitation. Transient transfection experiments showed that *HMGA1* interferes with the repression of the *BCL-2* promoter by p53 and functional studies revealed that *HMGA1* blocks p53-induced apoptosis. HMGA1 was also reported to bind to the Brn-3a transcription factor by co-immunoprecipitation. Brn-3a is known to bind to both p53 and HIPK2 to repress *BCL-2* expression, thereby promoting apoptosis. Co- expression of *HMGA1* with p53 in transfection experiments blocks repression of *BCL-2* and functional studies show that apoptosis is also blocked by *HMGA1*. In addition, the expression of *HMGA1* and *BCL-2* correlates in breast cancer cell lines. Together, these studies suggest that *HMGA1* interferes with p53-mediated transcriptional repression of the anti-apoptotic genes (*BCL-2*), thereby up-regulating its expression.

10.7. Genes Involved in RAS/ERK Signaling

10.7.1. c-KIT Ligand/Stem Cell Factor, SCF—The RAS/ERK signaling pathway is disrupted in many cancers and thought to be a key mechanism that drives uncontrolled cell growth in tumors. This pathway mediates extracellular signaling by mitogens, which result in receptor tyrosine kinase activity and phosphorylation events that promote the removal of GDP from RAS to enable GTP binding and activation, subsequently activating protein kinases and ultimately activating oncogenic transcription factors. Thus, activation of RAS signaling could promote oncogenic transformation by *HMGA1*. Genes involved in RAS/ERK (MAPK) signaling were identified using global gene expression analysis in breast (MCF-7) cells transduced to overexpress *HMGA1a* [222, 223]. The gene encoding c-KIT Ligand (also known as Stem Cell Factor or SCF) was identified in this screen [222]. c-Kit ligand binds to its receptor to activate RAS/ERK signaling. HMGA1 binds to an AT-rich region upstream of the *c-KIT Ligand* gene *in vitro* and *in vivo*. c-Kit ligand is essential for hematopoiesis during embryogenesis and important for maintenance of hematopoietic stem cells in the niche during adult hematopoiesis. Thus, overexpression of *HMGA1* could enforce hematopoietic stem cell-like programs during leukemic transformation through *c-KIT* pathways and also help to maintain leukemic cells in a protective niche.

10.7.2. Caveolin-1 and Caveolin-2 (CAV1 and CAV2)—The *CAV1* and *CAV2* genes are also repressed by HMGA1 in the MCF-7 model [222]. The caveolin protein family

(caveolin-1, -2, -3) are membrane proteins involved in receptor-independent endocytosis. The caveolins form "cave-like" structures that associate with cholesterol and sphingolipids in the cell membrane. Caveolin-1 functions in cellular signaling and accumulating evidence suggests that it is important in survival of androgen-insensitive prostate cancer, by promoting metastases, and the development of multidrug resistance [223]. Caveolin-1 links integrin subunits to the tyrosine kinase FYN to initiate the Ras-ERK pathway in cell cycle progression [224]. *CAV1* and *CAV2* expression are also associated with a basal-like, triple negative phenotype in breast cancer [225]. Caveolins also inhibit some components of RAS/ERK signaling and repression by HMGA1 could enhance RAS/ERK function [222]. Further work is needed to determine if HMGA1 directly down-regulates *CAV1* and *CAV2*.

10.7.3. p96/Differentially Expressed in Ovarian Cancer 2 (DOC2)—The *p96* gene was reported to be repressed in murine embryonic stem cells deficient in *Hmga1* [198]. *p96* is a putative mitogen-responsive gene that is repressed in human ovarian and mouse mammary tumors [226–228]. Expressing the *p96* human homolog, *DOC2*, in human ovarian carcinoma cells inhibits both cell growth and xenograft tumorigenesis, consistent with a tumor suppressor function [228]. In mice, deficiency of *Doc2* (*disabled-2 or Dab2*) results in embryonic lethality with endodermal disorganization. In murine embryonic stem cells, Doc2 appears to repress *cFos* expression and could act as a negative regulator of MAPK/Ras/ERK mitogenic signaling [228].

10.7.4. Gly96 or Immediate Early Response Gene 1 (Iex-1, Ier3)—*Gly96* (*Iex-1* or *Ier3*) was also reported to be repressed by Hmga1 in gene expression studies (microarray and quantitative RT-PCR) comparing wildtype MEFs to those deficient in *Hmga1* [198]. *Gly96* is an immediate-early gene that is rapidly induced following various cellular stressors, including growth factors, irradiation, viral infection, inflammatory cytokines, chemical carcinogens and hormones [229]. *Gly96* encodes a short-lived glycoprotein and is also transcriptionally regulated by NF-κB, cMyc, Ap1, Sp1, and p53. Transgenic mice expressing *Gly96* in T cells develop T-lymphomas [229]. Gly96 can either positively or negatively regulate proliferation, possibly by acting as a substrate of ERK or an inhibitor to ERK phosphorylation and function. Gly96 also plays an indirect role in protecting the cell from Fas- or tumor necrosis factor type alpha-induced apoptosis pathways [175]. It is not yet known if Hmga1 directly regulates *gly96*/*IEX-1.*

10.8 Genes Involved in Vascular Smooth Muscle Proliferation and Function

10.8.1. Inducible Nitric-Oxide Synthase (iNOS or NOS2)—*i-NOS* or *NOS2* is an HMGA1 gene target with a promoter similar to that of the *IFN-β*. In vascular endothelial cells, HMGA1 binds to an AT-rich region in the enhancer, thus facilitating the binding of NF-κB to its cognate DNA binding site to form a complex, which activates transcription of *NOS2* [230–232]. HMGA1 also binds further upstream (at positions −3,506 to −3,375) [230]. Activation of *NOS2* is blunted by a dominant-negative HMGA1 construct, the small molecule inhibitor, distamycin [172], or RNA interference to *HMGA1* [230–232]. The gene product, inducible nitric oxide synthase, is the enzyme that catalyzes the conversion of L-arginine to nitric oxide (NO) following induction by cytokines or other inflammatory signals. NO functions as a gaseous signaling molecule released from the endothelium to

cause vasodilation of blood vessels [232]. It is also generated by granulocytes, monocytes, and macrophages. In cancer, it is thought to contribute to angiogenesis. In addition, NOS2 enhances *Cyclooxygenase-2* (*COX-2*) activity by S-nitrosylation [233–236] which also participates in angiogenesis and many oncogenic pathways. Thus, the *HMGA1-NOS2-COX-2* pathway could serve as another inflammatory signal that promotes angio-genesis and multiple, diverse oncogenic pathways.

10.8.2. SMOOTH MUSCLE SPECIFIC GENE (SM22) TRANSGELIN (TRGLN), and cFOS—Studies in vascular smooth muscle cells showed that HMGA1 proteins are dramatically induced during their proliferation. In addition, HMGA1 enhances expression of both the *SM22, (TRGLN)*, and the *cFOS* proto-oncogene [237]. In gel shift experiments, HMGA1 binds to an AT-rich region within the DNA binding site of the Serum Response Factor (SRF), a protein known to function during proliferation to activate vascular smooth muscle genes. HMGA1 interacts with SRF and enhances its binding to the *SM22* and *cFOS* promoters, thereby augmenting SRF-dependent trans-activation of these genes. The activation of vascular smooth muscle genes could induce angiogenesis during tumor development. *cFOS* functions as a proto-oncogene and regulator of cell proliferation, differentiation, and transformation. It could cooperate with *HMGA1* during transformation. Of note, SRF is also elevated in Alzheimer's disease and a recent study suggests [238] that this results in increased accumulation of amyloid β. Thus, these SRF-dependent smooth muscle genes could work together with *HMGA1* in cancer, Alzheimer's disease, and embryonic development.

10.8.3. Cd44—The *Cd44* gene is a downstream transcriptional target of *HMGA1* that is up-regulated in vascular smooth muscle cells to promote proliferation following arterial wall injury [239]. *CD44* encodes a cell adhesion molecule that is induced during inflammation, wound healing, tumorigenesis, and metastatic progression. Previous studies identified CD44 as a marker for progenitor/stem cells in breast tissue, breast cancer, and other solid tumors [240–242]. More recently, CD44 was found to be a key factor in maintaining acute myeloid leukemic stem cells in the bone marrow niche; HMGA1 could indirectly facilitate this interaction by inducing *CD44* expression [242]. The murine *Cd44* promoter includes an AP-1 site adjacent to an AT-rich HMGA1 DNA binding site and the AP-1 transcription factors, cJun and cFos, along with Hmga1, bind to these sites in gel shift assays [239]. In transfection experiments, HMGA1 potentiates AP-1 transactivation of the murine *Cd44* promoter, suggesting that AP-1 proteins and HMGA1 cooperate in inducing *Cd44*. Interestingly, Cd44/CD44 is subject to proteolytic cleavage by MMPs, which are also regulated by HMGA1 (see below), and this could provide a negative feedback loop whereby HMGA1 induces *CD44* expression and also promotes degradation of the CD44 protein once translated. Given the link between CD44 and stem cell qualities [240–242], the *HMGA1-CD44* pathway could maintain a stem cell phenotype in cancer and normal stem cells.

10.8.4. Vascular Smooth Muscle Actin, Vsm-Actin—*Vascular smooth muscle actin, Vsm-actin* was also reported to be induced by Hmga1 in MEFs, both in microarray gene expression studies and qRT-PCR [198]. The protein encoded by *Vsm-actin* is involved the cytoskeleton, which is responsible for the structure and integrity of the cell [175].

Alterations in cellular cytoskeleton could contribute to cellular mobility and metastatic progression.

10.9. Genes Encoding Helix-Loop-Helix (HLH) Factors Regulated by HMGA1

10.9.1. Id3—The inhibitors of differentiation (Id or inhibitors of DNA binding proteins) represent another class of helix-loop-helix (HLH) proteins that are reported to be regulated by HMGA1 [243]. Id proteins bind cellular proteins to regulate cell growth, angiogenesis, and differentiation of adult and embryonic cells [243, 244]. In contrast to bHLH proteins like HAND1, Id proteins lack the distinctive region of basic amino acids found in other HLH proteins (bHLH) that are critical for DNA binding. As a result, heterodimers between Id proteins and other bHLH transcription factors are not capable of interacting with DNA and the Id protein thereby exerts a dominant-negative effect that results in sequestration of factors including, E2A and RB, to inhibit their transcriptional activity. Similar to *HMGA1*, both *Id1* and *Id3* are overexpressed in proliferating and neoplastic tissues, but decreased in differentiated cells. *Id3* was reported to be a potential HMGA1 target gene in an investigation of gene expression profiles by microarray analysis from mESCs deficient in *Hmga1* [198]. *Id3* gene expression by qRT-PCR was reported to correlate with *HMGA1* expression in MEFs and spleen, but not in liver or heart from the mice with wildtype or deficient (homozygous or heterozygous) *Hmga1*. Id3 protein levels by Western analysis also correlated positively with Hmga1 in the spleen, kidney, and lung, but not in MEFs, liver, brain, or testes from the wildtype or *Hmga1* deficient mice [198]. The basis for the variation in mRNA and protein levels is unclear, but could relate to differentiation status and cellular context. Gel shift experiments showed that HMGA1 binds to an AT rich sequence in the *Id3* promoter *in vitro* and chromatin immunoprecipitation showed that HMGA1 occupies this region *in vivo* in mouse splenocytes. Because Id proteins inhibit differentiation, the *HMGA1-Id3* pathway could help to maintain cells in an undifferentiated state during development and cancer as well as in specific tissues which require undifferentiated progenitors.

10.9.2. T-Cell Leukemia Homeobox 3 (TLX3, HOX11L2) and Nirenberg-KIM 2 Homeobox 5 (NKX2-5, CSX) Genes—HMGA1, together with PU.1, also induce expression of fusion genes that encode HLH proteins involved in T-cell acute lymphoblastic leukemia [245]. These fusion genes encode either the T-cell leukemia homeobox 3 protein (TLX3) or the highly related Nirenberg Kim homeobox protein (NKX2-5, also known as the cardiac-specific homeobox or CSX protein). Dysregulation of the *TLX3* gene results from a translocation event at 5q35 *via* the recurrent t(5;14) (q35;q32) rearrangement, while the neighboring homeobox gene, *NKX2-5*, is activated by a variant t(5;14)(q35;q32) or by t(5;14)(q35;q11.2) involving the the T-cell receptor D locus at 14q11.2. These translocations juxtapose the 3′ noncoding region of the *BCL11B* gene to the *NKX2-5* or *TLX3* genes. *BCL11B* normally encodes a Kruppel family zinc finger gene located at 14q32. To determine how the translocation events lead to dysregulation of the *TLX* or *NKX2-5* genes, the 3′-*BCL11B* noncoding sequence involved in the translocations was investigated and binding sites for both HMGA1 and PU.1 were identified by the TRANSFAC database. Interfering with expression of either *HMGA1* or *PU.1* decreases expression of *TLX3/NKX2-5* fusions, suggesting that HMGA1 and PU.1 bind to the 3′-*BCL11B* noncoding

sequence to enhance expression of the fusion proteins. The TLX and NKX2-5 homeobox domain proteins function as key regulators in cell fate decisions and proliferation and their expression is often cell-type specific. *NKX2-5* is normally expressed during embryogenesis in cardiac progenitors and involved in cardiac development. *TLX3* is expressed in the dorsal and ventral medulla oblongata during development, a region in the brainstem important in controlling respiration. Mice deficient in *TLX3* die within 24 hours after birth due to central respiratory failure. While HMGA1 regulates other embryonic genes involved in malignancy, *TLX* and *NKX2-5* are embryonic genes that become translocated to other genes, and the resultant fusion genes are regulated by HMGA1.

10.10. HMGA1 Target Genes Involved in Adipo-genesis

10.10.1. Obese, Leptin—The *obese/leptin* gene was identified as transcriptional targets induced by Hmga1 and involved in adipocyte differentiation [103, 244]. The first clue linking *Hmga1* to adipogenesis was the observation that *Hmga1* expression increases when cultured, preadipocytes (3T3-L1 cells) are induced to differentiate [244]. Experiments using an antisense approach to inhibit *Hmga1* expression block differentiation of pre-adipocytes while enhancing their proliferation rates. In addition, HMGA1 interacts with the CCAAT/enhancer-binding protein β (C/EBPβ) and potentiates C/EBPβ-mediated transactivation of the *obese* gene [244]. *Obese* is an adipocyte-specific gene that encodes the adipocyte-derived hormone, leptin. Leptin plays a key role in regulating energy intake and expenditure, including appetite and metabolism, suggesting that *Hmga1* is an important regulator in these processes. Prior studies also suggest that leptin is involved in blastocyst development during murine embryogenesis [246].

10.11. Putative Targets Involved in Invasion and Metastatic Progression

10.11.1. Matrix Metalloproteinases

10.11.1.1. *MMP-2, MMP-9, MMP-13* and *Integrin-β1*: Several independent groups studying *HMGA1* identified at least three different matrix *metalloproteinases* (*MMP*) as HMGA1 *transcriptional* targets [29, 50, 60, 70]. This family of zinc-dependent proteinases were originally characterized based on their ability to degrade the extracellular matrix and basement membrane proteins. By degrading the basement membrane, some members of the MMP family are thought to enhance cell mobility in a stationary tumor cell and promote metastases. In fact, MMP activity correlates with cellular invasiveness and metastatic potential in a subset of solid tumors [247]. More recently, MMPs were shown to exert other important biologic effects relevant to cancer by cleaving critical proteins involved in angiogenesis, apoptosis, chemotaxis, cell migration, and cell proliferation [247]. Even tumor suppressor functions have been demonstrated for MMP family members [248]. Thus, it has become clear that MMPs not only function in cancer progression and metastasis, but also in early steps of cancer development. In the first study linking *HMGA1* to MMPs, the *MMP-13* gene was among the list of differentially expressed genes in MCF-7 breast cells overexpressing *HMGA1a* or *HMGA1b* identified from a microarray that included almost 1,200 cDNAs [29]. Differential expression was confirmed using qRT-PCR in these cells. *Integrin-β1* was also identified in this screen and confirmed by qRT-PCR. Using a similar strategy, *MMP-2* was identified as a differentially expressed gene in human prostate cancer

cells engineered to express *HMGA1a* as compared to cells engineered with an empty, control vector [60]. Differential expression of the pre-form of the MMP-2 protein was confirmed by Western analysis. HMGA1 also up-regulates *MMP-2* expression in lung cancer, but only in large cell, undifferentiated lung carcinoma cells [50]. This study showed that HMGA1 occupies the *MMP-2* promoter *in vivo* in undifferentiated, large cell carcinoma cell lines by chromatin immunoprecipitation, but not in cultured cells from other lung cancer subtypes. In addition, both *HMGA1a* and *MMP-2* expression were significantly correlated in cultured, large cell carcinoma cells. Functional studies showed that knock-down of *HMGA1* or *MMP-2* blocked anchorage-independent cell growth. Inhibiting *MMP*-2 also disrupted migration, and invasion, suggesting that this pathway could mediate these tumor progression phenotypes in undifferentiated, large cell lung carcinomas. Another study found that *HMGA1* increases invasiveness and up-regulates *MMP-9* expression in cultured, pancreatic adenocarcinoma cells [70]. Together, these studies suggest that *HMGA1* drives the expression of matrix metalloproteinases in diverse tumor cells. Because many MMP promoters have AT-rich regions and putative HMGA1 binding sites, HMGA1 could orchestrate MMP function in diverse settings, such as cancer and development, and the specific MMP pathway likely depends upon the cellular milieu.

The ability of HMGA1 to activate MMPs could also be relevant in the pathophysiology of Alzheimer's disease. Interestingly, the release of both MMP-2 and MMP-9 proteins is increased in the brain microvessels of patients with Alzheimer's disease and could contribute to angiogenesis [249]. In fact, recent work suggests that aberrant angiogenesis leads to disruption in the blood brain barrier and disease progression [249]. MMP-9 protein was also found to be elevated in postmortem brain tissue of Alzheimer's patients [250]. As discussed earlier, *HMGA1* is increased in the hippocampus of patients with Alzheimer's and *HMGA1* is linked to the production of aberrantly spliced, presenilin-2V (PS2V) [39–42], which appears to play a role in Alzheimer's disease [159–162]. Together, these findings implicate *HMGA1* in the up-regulation of *MMP-2* and *MMP-9* in Alzheimer's, which could lead aberrant angiogenesis along with increases in PSV2.

10.12. Other Protease Genes Regulated by HMGA1

10.12.1. Cathepsin H (CtsH) and Legumain—*CtsH* and *Legumain* were reported to be repressed by Hmga1 in studies comparing wildtype mESCs to those deficient in *Hmga1* [198]. Differential expression of these genes was confirmed by RT-PCR experiments. The CtsH protein functions as both an amino- an endo-petidase that degrades proteins in lysosomes [251]. CtsH also represses apoptosis and induces angiogenesis. *Legumain* encodes another cysteine protease that is localized to the lysosomal/endosomal system and functions in antigen presentation. *Legumain* is also overexpressed in diverse tumors and linked to invasion and migration *in vitro* [252, 253].

10.12.2. Carboxypeptidase E (CpE)—The *carboxypeptidase E (CpE)* gene was reported to be induced by Hmga1 in the same study of mESCs [198]. *CpE* encodes the enzyme carboxypeptidase that cleaves C-terminal amino acid residues to yield biologically active neuropeptides and peptide hormones, such as insulin [254]. Mutations in *CpE* are implicated in type II diabetes; mice lacking *Cpe* have diabetes, infertility, obesity,

osteopenia, and deficits in memory and learning. An alternatively spliced form induces tumor growth and is a biomarker for metastases in a subset of human tumors [254].

10.13. Putative Target Genes Involved in Multiple Transformation Pathways

10.13.1. Inflammation, Proliferation, Angiogenesis

10.13.1.1. *Cyclooxygenase-2* (*COX-2*): *COX-2* was originally identified as a putative HMGA1 target gene in hypoxic vascular endothelial cells [255]. HMGA1's role in regulating this promoter was investigated because the promoter includes an NF-kB site adjacent to an AT-rich region and NF-κB protein or mRNA levels were not induced under hypoxic conditions. In contrast, *HMGA1* mRNA and protein increase in low oxygen tension. Further, HMGA1 binds to an AT-rich region near an NF-κB binding site in the *COX-2* promoter *in vitro* and up-regulates *COX-2* expression in transfection experiments. A subsequent study found that HMGA1 activates *COX-2* expression during uterine tumorigenesis [75, 256]. Uterine sarcomas from *Hmga1a* transgenics have increased *Cox-2*. Treatment of *Hmga1* transgenics with sulindac (a COX-1/COX-2 inhibitor) impairs uterine tumor growth [256]. Both *HMGA1* and *COX-2* are also overexpressed in leiomyosarcomas, a rare and frequently aggressive human uterine sarcoma. Sulindac or celecoxib (a more selective COX-2 inhibitor) disrupt anchorage-independent cell growth *in vitro* and xenograft tumorigenesis *in vivo* in human uterine sarcoma cells, but only in cells with high *HMGA1* levels [256]. This suggests that COX-2 inhibitors could be effective in tumors that dysregulate the *HMGA1-COX-2* pathway. A subsequent study also found that this pathway is important in pancreatic adenocarcinoma [257]. *COX-2* is induced by inflammatory signals, and, like *HMGA1*, could cooperate to elicit a number of cellular pathways involved in neoplastic transformation, including proliferation, angiogenesis, metastasis, and inhibition of apoptosis. Further studies are therefore warranted to elucidate the role of this pathway in other cancers.

10.13.1.2. *Pim-2* and *Pim 1*: The *Pim-2* proto-oncogene was also reported to be induced by Hmga1 in mESCs, based on gene expression microarray and qRT-PCR studies [198]. *Pim-2* is a member of the Pim family of genes, which encode Pim-1 and Pim-2 proto-oncoproteins. Pim-1 and Pim-2 are serine/threonine kinases that function in cell survival and proliferation [175]. Pim-2 phosphorylates cMyc, which enhances its stability and ability to regulate transcription. Pim-2 also phosphorylates BAD, a pro-apoptotic protein, resulting in the release of the anti-apoptotic protein, Bcl-XL. Pim-2 promotes proliferation by phosphorylating cyclin-dependent kinases, such as *Cdkn1a* and *Cdkn1b* [175]. *PIM-1* was also proposed to activate cMYC by phosphorylation and thereby induce *HMGA1* in human prostate cancers associated with trichomonas infection [258]. PIM-1/Pim-1 also promote cell survival and interferes with apoptosis by inhibiting the JNK/p38MAPK signaling pathway, thereby reducing caspase-3 activation [175]. The *HMGA1-PIM* pathways could therefore contribute to oncogenesis through cell survival and proliferation pathways. This pathway also further links *HMGA1* to cMyc function.

10.14. Putative HMGA1 Targets Involved in Ocular Function

10.14.1. Rhodopsin (RHO)—Although *HMGA1* is expressed predominantly in highly proliferative cancer cells or during embryonic development, high levels of *Hmga1* expression were also discovered in terminally differentiated murine photoreceptor cells (rods and cones) of the retina at both the mRNA and protein level [259]. High Hmga1 protein levels could contribute to the ability of the photoreceptor cells to maintain high rates of metabolism and translation. Retinoblastoma cells were also reported to express high levels of *HMGA1* and inhibition of *HMGA1* expression by antisense technology results in repression of the *Rhodopsin (RHO)* gene [259]. Rho protein belongs to a family of G-protein coupled receptors, which are extremely sensitive to light and required for the perception of light. By footprint analysis, HMGA1 binds to an AT-rich region in the *rho* promoter that coincides with the BAT-1 *cis* element, which is also bound by the paired-like homeodomain protein, Crx [259]. Crx is a photoreceptor-specific transcription factor that is thought to be important for photoreceptor cell functioning and differentiation. Mutations in Crx are associated with human diseases such as night blindness characterized by photoreceptor dysfunction, including photoreceptor degeneration, rod-cone dystrophy, retinitis pigmentosa, and Leber congenital amaurosis [175, 259, 260]. In retinoblastoma cells HMGA1 binds to the BAT-1 *cis* element in gel shift assays and transactivates the *rho* promoter in transfection assays. HMGA1 also physically interacts with the Crx protein, shown by GST pulldown assays [259]. Taken together, these findings suggest that HMGA1 is important in regulating the retinal photoreceptor gene *rho* and functions by enhancing transcription through interactions with Crx.

10.14.2. CRYAB—HMGA1 induces expression of the *CRYAB* gene, which encodes the αβ-Crystallin (CRYAB) protein, a heat-shock protein that is highly expressed in the ocular lens of vertebrates [261]. Deficiencies in CRYAB and other crystalline proteins are associated with cataracts. CRYAB also plays a role in cell survival by encoding proteins that function as chaperones for misfolded proteins, thus preventing these abnormal proteins from inducing apoptosis [175]. *In vitro* gel shift assays and *in vivo* chromatin immunoprecipitation demonstrate that HMGA1 binds to a Brahma-Related Gene 1 (BRG1) response element within the *CRYAB* promoter and transfection experiments show that HMGA1 induces *CRYAB* expression. Nucleosome mapping experiments show that this response element is located adjacent to a nucleosome and immediately upstream of the transcription start site. HMGA1 along with the HMGA1 binding sequences are required for maximal *CRYAB* induction, suggesting that HMGA1 helps to orchestrate the optimal chromatin state and assembly of BRG1 to this promoter. Notably, BRG1 is a member of the SWI/SNF chromatin remodeling family. Like other SWI/SNF family members, BRG1 has both ATPase and helicase activity, which facilitate nucleosome sliding for transcription factor assembly at specific regulatory regions of DNA. How *CRYAB* relates to *HMGA1* function will require further study, although emerging evidence indicates that heat shock proteins are important in both cancer and development.

10.15. Genes Involved in Diabetes, and Insulin Resistance

10.15.1. Insulin Receptor—As noted earlier in this review, increasing evidence links *HMGA1* to glucose metabolism and many recent studies underscore the importance of

glucose metabolism in cancer and embryogenesis. HMGA1 regulates expression of several genes that encode proteins involved in glucose homeostasis. In addition, patients with Type 2 diabetes [35–38] were found to have genetic alterations that affect *HMGA1* expression, further implicating *HMGA1* in glucose homeostasis. Type 2 diabetes is characterized by hyperglycemia and reduced sensitivity to insulin. After insulin binds the insulin receptor (IR), a tyrosine kinase receptor, glucose is translocated intracellularly by the glucose transporter type 4 (GLUT4) which mediates the uptake of glucose into adipose tissues and striated muscle cells. HMGA1 was reported to bind to the *IR* promoter at an AT-rich *in vitro*, as shown by gel shift analysis [262]. Transfection experiments showed that the *IR* promoter is repressed when *HMGA1* expression is silenced (in HepG2 hepatoma cells or IM-9 human lymphocytes). Silencing *HMGA1* also decreases radio-labeled insulin binding to HepG2 and IM-9 cells, while overexpression of *HMGA1* increases insulin binding [262]. Subsequent studies found that *HMGA1* up-regulates *IR* expression as a complex with SP1 and C/EBPβ [263]. Co-immunoprecipitation and gel shift experiments show that HMGA1 interacts directly with SP1 and enhances SP1 binding to the *IR* promoters. Similarly, HMGA1 interacts with C/EBPβ and enhances its binding to the *IR* promoter. In addition, co-transfection of plasmids expressing HMGA1 and SP1 activates the *IR* promoter, while transfection of HMGA1 or SP1 alone results in only modest activation of the promoter. Consistent with these findings, *Hmga1*-deficient mice were reported to have a marked reduction in *Ir* expression and phosphorylation in the major downstream targets of the insulin pathway resulting in impaired insulin signaling and decreased insulin secretion. This results in a type 2-like diabetes phenotype with defects in pancreatic beta-cell insulin secretion. As above, four individuals were reported with genetic variants resulting in decreased *HMGA1* expression have a type 2 diabetes phenotype [35, 264]. In addition, alterations in expression of *HMGA1* mediated by expression of the *HMGA1* pseudogene can also lead to post-transcriptional silencing of *HMGA1* and has been implicated in type 2 diabetes in humans [37, 264]. In contrast to type-2 diabetes in humans, however, the *Hmga1*-deficient mice have an increased sensitivity to insulin, requiring higher baseline serum glucose infusions to maintain euglycemia [36].

10.15.2. Retinol Binding Protein 4 (RBP4)—Further studies of *HMGA1* and glucose metabolism reveal that *HMGA1* is required for both the basal and cAMP-induced expression of the gene encoding the retinol-binding protein 4 (RBP4), a protein secreted by adipocytes (thus designated an adipokine) that is involved in glucose metabolism and insulin resistance [36]. In transfection experiments in HepG2 cells, HMGA1 was reported to transactivate the *RPB4* promoter, while inhibiting *HMGA1* expression represses the *RPB4* promoter [36, 37]. HMGA1 binds to this promoter *in vivo,* as shown by chromatin immunoprecipitation experiments. In addition, the *Hmga1*-deficient mice were reported to have reduced expression of *Rpb4* mRNA and lower serum Rpb4 protein levels [36]. In cultured cells, both *HMGA1* and *RBP4* expression are induced by increases in intracellular cAMP, which is known to induce *RBP4* expression. This study also showed that the intraperitoneal injection of glucagon, a hormone that increases intracellular cAMP, results in increased expression of *Hmga1* and *Rbp4* in the liver and fat of wildtype mice. In contrast, the induction of *Rbp4* was attenuated in the *Hmga1*-deficient mice, providing further evidence that HMGA1 induces *RBP4* expression. The *Hmga1*-deficient mice were also reported to have lower

levels of serum free fatty acids, which could contribute to increased sensitivity to insulin. To further assess the role of *HMGA1* and RBP4 in glucose homeostasis, the *Hmga1*-deficient mice were injected with recombinant human RBP4, which attenuated the fall in glucose following insulin administration [36]. Taken together, these findings indicate a potential role for HMGA1 in the regulation of *RBP4* and other genes involved in glucose metabolism. By directly regulating *RBP4* expression, HMGA1 could enhance peripheral insulin sensitivity and ensure adequate glucose uptake in skeletal muscle.

10.15.3. Glut4—As noted previously, the *Hmga1*-deficient mice were reported to have an increased sensitivity to insulin, despite decreased expression of *Ir*. To investigate the mechanisms underlying the increased insulin sensitivity, expression of the glucose transporter, Glut4, was assessed in the skeletal muscle and fat of the *Hmga1*-deficient mice [35]. *Glut4* mRNA and protein were reported to be increased in quantitative RT-PCR and Western assays, respectively. The Akt2 protein kinase is a downstream target of PI3-kinase that is required for insulin-induced translocation of Glut4 to the plasma membrane. Phospospho-Akt protein was reported to be increased in the skeletal muscle and adipose tissues of *Hmga1*-deficient mice compared to wildtype mice and paralleled that of Glut4 protein [263].

10.15.4. GLUT3—HMGA1 also induces expression of the gene encoding the GLUT3 glucose transporter and promotes aerobic glycolysis in colorectal cancer cells [264]. Chromatin immunoprecipitation in human colon cancer (HCT116 and LoVo) showed that HMGA1 binds directly to the *GLUT3* promoter and transient transfection experiments demonstrate that HMGA1 induces expression of this promoter [264]. In primary human colorectal tumors, there was a positive correlation between *HMGA1* and *GLUT3* expression. This study also found that Caveolin-1 induces *HMGA1* expression in colon cancer cells. Interestingly, *CAV-1* and *CAV-2* appear to be repressed by HMGA1 in an ovarian cancer cell line [as discussed earlier; see ref. 222]. It is possible that there is a negative feedback loop whereby HMGA1 inhibits *CAV* expression is some cancer cells, while Caveolin could stimulate *HMGA1* expression in colon cancer and other tumors. Additional work is needed to dissect the role of these pathways in cancer. Nonetheless, these findings further underscore an important role for *HMGA1* in regulating glucose metabolism that could be relevant to abnormal tumor metabolism.

10.15.5. Insulin-like Growth Factor Binding Protein-1 and -3 (IGFBP-1 and IGFBP-3)—IGF binding proteins (IGFBPs) bind to insulin-like growth factors (IGFs) and regulate their bioavailability and distribution. IGF/IGFBPs function, not only in maintaining glucose homeostasis, but recent studies point to a key role for IGFs in cancer [265]. In experimental models, IGFs promote cell proliferation and other oncogenic pathways [265]. In humans, epidemiologic studies suggest that obesity and type 2 diabetes are associated with an increased risk for diverse cancers. *HMGA1* also affects IGF levels by regulating genes encoding IGF binding protein [266]. For example, HMGA1 was reported to bind to the insulin response element (IRE) to induce expression of both the *IGFBP-1* and *IFGBP-3* genes [266, 267]. The IRE is a critical element in hormonal regulation of many genes and HMGA1 binds to this element together with one or more hepatic nuclear factor proteins. Gel

shift and DNase protection assays with recombinant proteins showed that HMGA binds to the IRE in the *IGFBP-1* promoter and suggest that hepatic nuclear factor 3 proteins (HNF3α and HNF3β) form a complex with *HMGA1* [266]. Because the *IGFBP-1* and *IGFBP-3* genes are contiguously arranged on chromosome 7, regulation of both of these genes by HMGA1 was also investigated. Studies using gel shift and chromatin immunoprecipitation analyses in HepG2 cells found that HMGA1 interacts with C/EBP, SP1, and HNF-1α when binding to the *IGFBP-1* and *IGFBP-3* promoters, suggesting that it orchestrates the assembly of these complexes at the IREs [266]. These findings point to yet another pathway regulated by HMGA1 that is involved in glucose metabolism, in this case by inducing expression of proteins that bind to and decrease functional IGF [266, 267].

10.15.6. Mac25, Insulin-Like Growth Factor-Binding Protein-Related Protein-7 or IGFBP-7—The *Mac25* or *IGFBP-7* gene was also reported to be induced by Hmga1 in mESCs compared to mESCs deficient in *Hmga1* [198]. Increased expression of *IGFBP-7* in the *Hmga1*-expressing cells was detected in both microarray gene expression and qRT-PCR experiments [198]. In colorectal cancer cell lines, both IGFBP-3 and IGFBP-7 are linked to proliferation and metastatic progression [268]. Silencing of *IGFBP-7* results in decreased proliferation, colony formation, and metastatic progression to the liver. These results further suggest that *HMGA1* and IGF pathways play an important role in cancer. Because prior studies found that HMGA1 regulates the *IR* [35, 262, 263], *OBESE* [244], *RBP4* [36], *IGFBP-1* [35, 267], *IGFBP-3* [268], *IGFBP-7* [198] and *GLUT4* [263, 269] genes, it is likely that HMGA1 regulates additional genes important in energy utilization, including glucose and fat metabolism. Additional studies are needed to better elucidate its role in metabolism, particularly given recent evidence that alterations in metabolic pathways are critical adaptations in cancer and stem cells that could be targeted in cancer therapy [148–158].

10.16. Other Genes Involved in Metabolism

10.16.1. Cubulin—The *Cubulin* gene encodes the intrinsic factor cobalamin receptor which acts as a receptor for the intrinsic factor-vitamin B12 complex. *Cubulin* was reported to be repressed by *Hmga1* in wildtype mESCs compared to cells deficient in *Hmga1*, as demonstrated by gene expression profile analysis and RT-PCR experiments [198]. Intrinsic factor-vitamin B12 is involved in purine metabolism and DNA synthesis.

10.16.2. Parathyroid Hormone Receptor (Pthr)—The *Pthr* gene encodes a receptor for parathyroid hormone and for parathyroid hormone-related peptide [175]. This receptor is a member of calcium-sensing receptor (CaSR) family of G protein coupled receptors that is activated by elevated levels of calcium and other divalent cations. The CaSR family functions as a multimodal nutrient sensor to maintain calcium homeostasis by sensing extracellular levels of calcium. The receptors can also be activated by other compounds, including polyamines such as spermine, which are increased in some cancers. A prior study in thyroid cancer cells found that PTHR promotes cellular adhesion and migration by coupling to members of the integrin family of extracellular matrix proteins [270]. This gene was reported to be induced by *Hmga1* in wildtype mESCs compared to mESCs deficient in

Hmga1 [198]. Although it is not yet known whether Hmga1 directly regulates *Pthr* expression, this pathway could promote migration and metastatic progression in cancer.

10.17. Development/Cell Fate, Stem Cell & Epithelial-Mesenchymal Transition Genes

10.17.1. Jagged-1 (JAG1)—Notch signaling is an evolutionarily conserved, signaling pathway essential for proper development of diverse organisms, from insects and nematodes to mammals [271–273]. The *JAG1* gene encodes Jagged-1, a ligand for the NOTCH1 receptor. Jagged-1 binding to Notch1 leads to a cascade of proteolytic cleavage events that result in the translocation of the intracellular part of the Notch receptor to the nucleus where it binds to DNA to regulate expression of transcription factors involved in cell fate and morphogenesis [271–273]. In humans, mutations in *JAG1* cause Alagille syndrome, a disorder characterized by development abnormalities affecting which heart, liver, skeleton, and central nervous system [271]. *JAG1* is downstream of canonical WNT signaling and important in normal intestinal stem cell function. *JAG1* is also frequently up-regulated in diverse cancers and thought to contribute to tumor progression and angiogenesis [273]. *JAG1* was discovered in gene expression profile analysis from MCF-7 cells engineered to overexpress *HMGA1a* or *HMGA1b* [29]. Although further studies are needed to determine if HMGA1 directly regulates *JAG1* expression, this pathway could represent a network involved in both development and cancer.

10.17.2. VIMENTIN, E-CADHERIN—When tumor cells metastasize, emerging evidence suggests that they usurp stem cell transcription networks that mediate an epithelial-mesenchymal transition (EMT) and other changes necessary for tumor progression [58, 68, 117]. In poorly differentiated colon cancer cells (HCT116 and SW480), *HMGA1* induces expression of EMT genes, including *VIMENTIN* and *TWIST* (described above under Transcription Factors), while it represses the epithelial gene, *E-CADHERIN. TWIST*, along with *SNAIL* and *SLUG*, were previously shown to be up-regulated by the HMGA2 family member in mouse mammary epithelial cells induced to undergo EMT [274]. Since HMGA1 and HMGA2 induce similar phenotypes when ectopically expressed in cultured cells [28], it is plausible that they share a subset of transcriptional targets. *VIMENTIN* is also up-regulated with the progression from an epithelial to mesenchymal transition [29]. As noted above, silencing *HMGA1* in the high-grade colon cancer cells blocks stem cell properties (three dimensional colonosphere formation and limiting dilution tumorigenesis) as well as oncogenic/tumor progression phenotypes (anchorage-independent cell growth, migration, invasion, and metastatic progression) [68]. These results suggest that *HMGA1* promotes tumor progression through transcriptional networks that facilitate EMT, metastatic progression, and a stem-like state.

10.18. Spindle-Assembly Checkpoint (SAC) Genes

10.18.1. BUB1, Bub1b, Ttk, Mad2/1—The *Bub1b* gene was first discovered to be induced by HMGA1 in an *Hmga1a* transgenic model of leukemia [108]. BUB1 proteins function in activation of mitotic spindle check points. In addition, *BUB1* was identified as a "gene signature" or set of genes associated with poor outcomes and early death in diverse cancers. This 11 gene signature was therefore dubbed the "death from cancer" signature, and was derived by identifying common genes expressed in normal stem cells and metastatic

lesions in a transgenic mouse model of prostate cancer [275, 276]. Interestingly, the expression of this signature correlates with highly malignant tumors, metastatic progression and poor responses to therapy. The *HMGA1-BUB1b* pathway further links *HMGA1* to transcriptional networks in tumors with poor outcomes, stem cells, and possibly to chromosomal integrity given the role of BUB1 in mitosis. More recent studies reported that multiple genes involved in the spindle assembly checkpoint, including *Bub1*, *Bub1b*, *Ttk*, *Mad2/1*, are up-regulated by Hmga1b [277]. This group reported that ectopic expression of *HMGA1* increased expression of all of these genes in colon cancer cell lines and protein levels in mouse fibroblasts and a human colon cancer cell line. They also reported that HMGA1 binding is enriched at the promoters for these genes by chromatin immunoprecipitation in colon cancer cell lines and reporter gene activation downstream of the promoter regions was enhanced by *HMGA1* in transfection experiments. In addition, aberrant mitosis in MEFs deficient in *Hmga1* was reported. In data from The Cancer Genome Atlas, they found that *HMGA1* gene expression correlates with that of spindle-assembly checkpoint genes in colorectal cancer. Together, these data indicate that *HMGA1* may be an important regulator of mitosis in cancer cells through these genes.

10.19. Extracellular Matrix and Cell Structure Genes

10.19.1. Collagen Pro-Alpha1 Type 1 or Col1a1—*Col1a1* was reported to be induced by Hmga1 in mESCs, and increased levels in *Hmga1*-expressing cells were found in microarray gene expression analyses and by qRT-PCR studies [198]. *Col1a1* encodes the major component of type 1 collagen, which is found in most connective tissues, including cartilage. A study of colorectal cancer identified type 1 collagen at the invasive front of colorectal cancer in histopathologic studies [278]. This group also found that culturing colorectal cancer cells in the presence of type 1 collagen induced an epithelial-to-mesenchymal (EMT) transition and stem cell markers. These findings suggest that the *HMGA1-COLIA1* pathway could promote tumor progression, EMT, and a stem like phenotype.

10.19.2. Sparc, Secreted Protein, Acidic, Cysteine-Rich (or Osteonectin)—
Sparc, secreted protein, acidic, cysteine-rich (or *osteonectin*) was reported to be repressed by Hmga1 in wildtype mESCs compared to mESCs deficient in *Hmga1* [198] Repression of *Sparc* was found by microarray gene expression studies and qRT-PCR analysis [198]. The protein encoded by *Sparc* functions in regulating cell growth by mediating interactions with the extracellular matrix and cytokines. *SPARC* has been associated with both tumor suppression and metastasis, the latter of which is thought to relate to its role in changing cell shape which could promote tumor cell invasion [175].

10.19.3. Cyr61, Cysteine-Rich Angiogenic Inducer 61—The same group reported that *Cyr61* (*cysteine-rich angiogenic inducer 61*) is induced by *Hmga1*. This gene encodes a growth-factor inducible protein that promotes endothelial cell adhesion and angiogenesis. It was identified in gene expression profile analysis of mESCs wildtype for *Hmga1* compared to mESCs deficient in *Hmga1*, and confirmed by qRT-PCR [198]. Cyr61 is a secreted protein that promotes proliferation, chemotaxis, angiogenesis, and endothelial cell adhesion

by up-regulating genes involved in inflammation, angiogenesis, matrix remodeling and EMT [279].

11. AREAS FOR FUTURE INVESTIGATION

Although prior studies have uncovered downstream genes regulated by HMGA1, it is likely that these results provide only a glimpse of HMGA1 transcriptional networks in tumorigenesis and normal embryogenesis. Given the central role for HMGA1 in cancer and development, a global investigation of HMGA1 function using state-of-the-art technology with global chromatin immunoprecipitation coupled with sequencing technology in different cellular contexts is needed to further elucidate the molecular pathways induced by HMGA1 in these settings. Of note, many HMGA1 targets reported to date include NF-κB regulatory elements in their promoter regions near the HMGA1 DNA binding sites, suggesting that HMGA1 and NF-κB function together to regulate gene expression [23]. In addition, many NF-κB-HMGA1 gene targets encode important proteins involved in inflammatory pathways associated with cancer. It will be important to determine if NF-κB is required for HMGA1 function in cancer, particularly because NF-κB inhibitors are available and could be exploited in cancer therapy. Both *HMGA1* overexpression and NF-κB activation are prominent in diverse high-grade cancers and further efforts to target these pathways are likely to advance therapy for multiple tumor types. Because HMGA1 is a chromatin remodeling protein that modulates histone and transcription factor binding to chromatin, it is likely that it plays an important role in multiple epigenetic modifications that occur in cancer and development, and this area has not been well-studied.

Studies are also needed to identify, not only transcriptional gene targets, but also noncoding RNAs, regulated by HMGA1. MicroRNAs are non-protein coding small RNA molecules that function as critical regulators of mammalian gene expression [27, 280]. Recent studies indicate that aberrant expression of miRNAs plays a causal role in tumorigenesis. In fact, most high-grade tumors are characterized by global repression of miRNAs and down-regulation of tumor suppressor miRNAs is emerging as a key feature in refractory tumors. Thus, it will be important to identify miRNAs that are regulated by HMGA1 proteins as well as miRNAs that modulate *HMGA1* expression. Such studies should also reveal potential therapeutic targets relevant to diverse tumors. Emerging evidence indicates that pseudogenes play a role in regulating gene expression and *HMGA1* pseudogenes alter *HMGA1* expression and function [37]. Further studies to identify long non-coding RNAs that affect *HMGA1* expression or function should provide important insight on *HMGA1* in cancer and development. Similarly, it is possible that noncoding regions of *HMGA1* could regulate expression of microRNAs, thereby imparting functions independent of the protein coding domains.

Recent studies have uncovered important metabolic adaptations in tumor cells that could be exploited in therapy [148–158]. Indeed, most, if not all, oncogenic proteins and tumor suppressor appear to alter or "reprogram" tumor cell metabolism. As noted, the cMYC oncogenic transcription factor metabolically reprograms cells, forcing them to become "addicted to glucose" *via* aerobic glycolysis (also known as the Warburg effect) and glutaminolysis [154–158]. Interestingly, the dependence upon aerobic glycolysis is also

characteristic of early embryogenesis up to the 16 cell stage, after which both aerobic glycolysis and oxidative phosphorylation occur concurrently. The rapid growth of early embryos could recapitulate growth patterns observed in some cancers [155]. Precisely how oncogenic pathways contribute to metabolic reprogramming is an area of active investigation that is likely to uncover new approaches to target cancer cells. Recent studies also suggest that tumor cells can reprogram the surrounding stroma, and conversely, the tumor stroma can release factors and compounds that help to reprogram the tumor cells themselves. This complexity and how *HMGA1* affects tumor and stromal cell metabolism requires further study.

As outlined here, prior studies found that HMGA1 induces expression of many nuclear genes involved in metabolism, including the *insulin receptor* [263, 264], *RBP4* [36, 37], *GLUT3* [269], *IGFBP-1, IGFBP-3* [266, 267], and *IGFBP-7* [198], and *leptin/obese* [103, 245] genes, indicating that it is involved in both glucose and fat metabolism. Reports of mice deficient in *Hmga1* describe some features present in type 2 diabetes and a small number of patients with type 2 diabetes have genetic lesions that interfere with *HMGA1* expression, further implicating HMGA1 in glucose metabolism [35]. In addition, HMGA1 induces expression of mitochondrial genes [32–34]; how this affects normal and tumor metabolism is still incompletely understood. Thus, studies are needed to dissect the role of *HMGA1* in metabolism in tumors, stroma, and other cells within the tumor microenvironment.

HMGA1 is also enriched in human embryonic stem cells [58, 97, 98], adult stem cells, such as hematopoietic stem cells [96, 97], intestinal stem cells [120], and high-grade or poorly differentiated tumors [27, 281, 282], suggesting that it plays a role in driving the undifferentiated, stem-like state. In addition, its high expression during embryogenesis with absent or markedly decreased levels in differentiated, adult tissues further implicates *HMGA1* in enforcing a stem-like state [98]. Although numerous studies in tumors demonstrate a positive correlation between *HMGA1* and poor differentiation status, there are few studies to elucidate its function in embryonic stem cells. Recent studies highlight a role for HMGA1 as a master regulator in pathways involved in normal stem cells and epithelial-mesenchymal transition, a process important in development and tumor progression [29, 68, 117]. These studies found that HMGA1 orchestrates transcriptional networks and epigenetic pathways involved in maintaining an undifferentiated, pluripotent state in embryonic stem cells and induced pluripotent stem cells [98]. Further work is needed to better understand these networks, not only in normal development, but also in cancer. Such studies are likely to uncover novel roles that could be targeted in cancer and possibly regenerative medicine. Because emerging evidence indicates that stem cells and stem-like cancer cells have epigenetic alterations and changes in chromatin structure, a more detailed understanding of the role of HMGA1 in epigenetic modifications and nuclear chromatin structure should also enhance our understanding of HMGA1 in these settings.

We now know that HMGA1 also has a role in regulating genes involved in DNA repair, such as the *XPA* gene [112]. HMGA1 proteins can bind to 4-way DNA structures *in vitro* and these structures resemble the Holiday junctions formed as intermediate DNA structures during homologous recombination events [61]. HMGA1 proteins also enhance integration of the HIV genome into host DNA [116]. These activities suggest that HMGA1 could

participate in translocation and recombination events. *HMGA1* expression is associated with an increased number of unbalanced translocations in prostate cancer cells, suggesting that HMGA1 promotes such chromosomal aberrations [61]. Given the high levels of HMGA proteins in cancer cells and embryonic stem cells, coupled with its location at the nuclear matrix where recombination events occur, additional studies are needed to better understand the role of HMGA1 in chromosomal integrity and chromosomal structure.

Another largely unanswered question in the field is how *HMGA1* expression is regulated, both during development, postnatally, and in cancer. Although previous studies found that *HMGA1* is up-regulated by hypoxia [256], growth factors stimulation [44] oncogenic factors, such as cMyc [88, 204, 283] AP1 [142, 283, 284] or Wnt/Tcf-4 [285], *HMGA1* expression is up-regulated in cancer cells lacking up-regulation of these pathways, suggesting that there are additional, unknown mechanisms that induce its expression. Preliminary data from our group indicates that *HMGA1* expression is regulated by additional epigenetic mechanisms in stem cells and possibly cancer [97–98] and studies to elucidate these alterations might uncover additional targets for therapeutic interventions in tumors overexpressing *HMGA1*. For example, emerging data also implicates *HMGA1* in microRNA networks and further dissection of miRNA regulation of *HMGA1* should reveal additional pathways relevant to *HMGA1* function. Expression of pseudogenes or noncoding regions of *HMGA1* could also act as a sponge for microRNAs that normally down-regulate *HMGA1* and thereby contribute to overexpression of *HMGA1* [37]. This area requires further research because it could reveal additional pathways that could be exploited in cancer therapy.

A more detailed investigation of the function of each protein domain should provide insight into HMGA1 and novel therapeutic targets. The high degree of conservation of the domains that are required for DNA and protein-protein interactions suggest that these regions are key to its overall function. Indeed, a construct with mutations in the AT-hook DNA binding domains results in dominant-negative function in oncogenic assays [29, 98], which further underscores the importance of DNA binding for HMGA1 activity. While previous research has identified interacting proteins that are important in HMGA1 function [163], the discovery of additional protein-protein interactions should also advance our understanding of HMGA in cancer, development, and other cellular processes.

Substantial evidence also indicates that posttranslational modifications serve as important modulators of HMGA1 function and the specific regulators and sites of modification is an area of active investigation. Since their discovery in 1983 in HeLa cervical cancer cells, HMGA1a and HMGA1b were known to be highly phosphorylated [3, 286–291]. Indeed, HMGA1 proteins, along with histone H1, are among the most highly phosphorylated proteins in the nucleus of cancer cells [286–291]. Subsequent studies found that the AT-hook DNA binding domain sequence matches the consensus sequence for mammalian cyclin dependent kinase 1 or cdk1 (also known as cell division control protein 2 or cdc2 kinase) and phosphorylation of HMGA1 by cdk1 significantly decreases the binding of HMGA1 to DNA [289–291]. This kinase phosphorylates HMGA1b at serine-43 and serine-58, which impairs DNA binding. More detailed analysis showed that phosphorylation at additional sites (threonine-41 and threonine-67) also disrupts the interaction of the amino-terminal

DNA binding domain of HMGA1b to the *IFN-β* promoter at PRDIII-I element [28]. The homeodomain-interacting protein kinase-2 (HIPK2) phosphorylates HMGA1a (at serine-35, threonine-52, and threonine-77) and HMGA1b (at threonine-41 and threonine-66) [291]. Although phosphorylation by HIPK is similar to that observed for cdk1, the kinases exhibit different site preferences for phosphorylation. Another study found that phosphorylation by HIPK reduces the binding affinity of HMGA1 for the promoter of target genes [290]. The effects of cdk1 were more pronounced than those observed for HIPK. Because cdk1 has a greater effect on DNA binding of the second AT-hook, these results suggest that the second AT-hook is a key determinant of DNA binding for HMGA1. Cdk1 also phosphorylates HMGA proteins at the serines near the carboxy-termini, resulting in a more compact protein that may have less oncogenic capabilities [290].

In addition to phosphorylation, acetylation and methylation are important posttranslational modifications that alter HMGA1 function [130, 292–295]. In fact, emerging evidence indicates that HMGA proteins undergo posttranslational modifications (methylation, acetylation and phosphorylation) similar to histones, suggesting that there is an HMGA1 posttranslational modification code (or "PTM code") similar to the modifications that occur on histones. As noted above, HMGA1 proteins in plants and lower organisms are highly homologous to histone H1, suggesting that they evolved from the same protein. Thus, it is not surprising that similar PTMs are found in histones and HMGA1 proteins. As discussed above, acetylation of lysine-65 by PCAF/GCN5 stabilizes the HMGA1 enhanceosome at the *IFN-β* promoter, while acetylation of lysine-71 by CBP disrupts the enhanceosome and halts transcription of *IFN-β* [130]. More recently, both mono- and di-methylation of arginine-25 (in the first AT-hook) has been reported [293, 294]. Indeed, up to 50% of the HMGA1 was found to be monomethylated at arginine-25 in tumor cells. This posttranslational modification was also observed in cells undergoing apoptosis. Protein arginine methyltransferase 6 (PRMT6) was identified as the enzyme responsible for methylation of HMGA1 (at arginine-57 and arginine-59) in cells undergoing apoptosis [293, 294]. These two residues are embedded within the second AT-hook, a key DNA binding and protein-protein interacting domain. A more recent study utilizing innovative technology to monitor posttranslational modifications in intact proteins (called "top-down" proteomics because intact proteins rather than digested proteins are investigated) discovered a striking up-regulation of methylated forms of di- and tri-phosphorylated HMGA1a, but not of its splice variant, HMGA1b, in cancer cells (B16F10 melanoma cells and H1299 lung cancer cells) during stress-induced senescence [295]. Further studies are needed to elucidate the functional consequences of these posttranslational modifications.

CONCLUSION

In summary, numerous studies have established a clear link and functional role for *HMGA1* over-expression and high-grade, aggressive cancers, from its initial discovery in the extraordinarily proliferative HeLa human cervical cancer cells [3] to the more recent identification of HMGA1 as a key transcription factor enriched in poorly differentiated cancers with adverse outcomes [55, 58, 74, 79]. Recent advances in technology to globally assess chromatin binding proteins and epigenetic modifications provides unprecedented opportunities to investigate *HMGA1* in cancer and normal development. In addition, new

approaches to elucidate metabolic adaptations to *HMGA1* should advance our knowledge of tumor metabolism. The discovery of noncoding RNAs as key regulators opens up additional avenues of investigation relevant to *HMGA1*. Moreover, improvements in protein analytical approaches will lead to a better understanding of posttranslational modifications that regulate HMGA1 function as well as proteins that interact with HMGA1. Identification of additional cellular pathways and chromatin modifications involving HMGA1 promises to have a major impact in our ability to understand and treat cancer. These insights may also have relevance to diabetes, cardiac hypertrophy, Alzheimers disease, and other, as yet undiscovered, diseases linked to *HMGA1*.

The ultimate goal of further work in this field is to identify therapeutic strategies to target *HMGA1* in human cancer [reviewed in reference 282]. While many studies cited here underscore the fundamental role of *HMGA1* in tumor progression, there has been relatively little work focused on targeting *HMGA1* in cancer [176]. To date, disrupting the function of oncogenic transcription factors has been challenging given the hurdles of delivery and specificity. Nonetheless, efforts to target critical downstream pathways have shown some promise in preclinical models, such as inhibitors to COX-2 [75, 256, 257] or STAT3 [91, 206, 296]. For this reason, a better understanding of the HMGA1 transcriptome and downstream pathways may reveal novel therapeutic targets. Alternatively, disrupting pathways that induce *HMGA1* in cancer should be an effective strategy and studies to dissect these pathways are needed. In addition, further insight on protein-protein and protein-DNA interactions should reveal new interactions that could be targeted to disrupt HMGA1 chromatin binding and function. The identification of critical posttranslational modifications could also lead to the development of specific inhibitors that impair HMGA1 function. Advances in the stabilization and delivery of short hairpin RNAs or tumor suppressor microarrays that repress *HMGA1* expression may also be effective in cancer therapy. Finally, a better understanding of HMGA1's role in epigenetic alterations and global nuclear structure could also reveal approaches to interfere with its function in malignant transformation. Conversely, studies on *HMGA1* in pluripotent stem cells suggest that enhancing its function will improve stem cell function and may be beneficial in regenerative medicine. Thus, studies to better elucidate the role of *HMGA1* in adult stem cells and approaches to manipulate its levels in this setting are also warranted.

Acknowledgments

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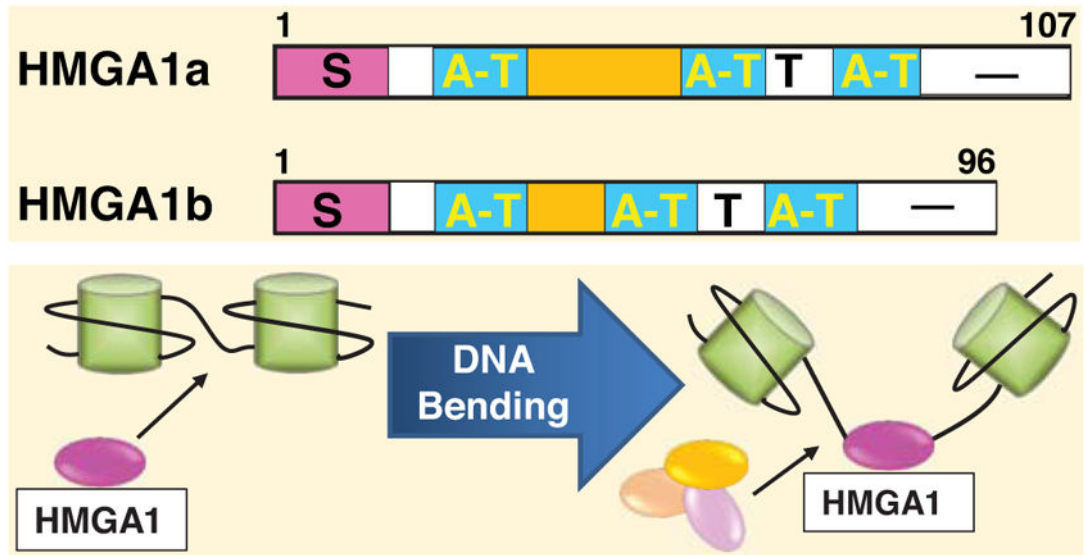
Fig. (1).
HMGA1a and HMGA1b protein isoforms are depicted with the serine (S) and threonine (T)-rich regions, AT-hook DNA binding domains (AT), and the acidic carboxyl terminal (–) region (top). HMGA1 functions as an architectural transcription factor that bends chromatin to enable binding of transcriptional complexes (bottom).
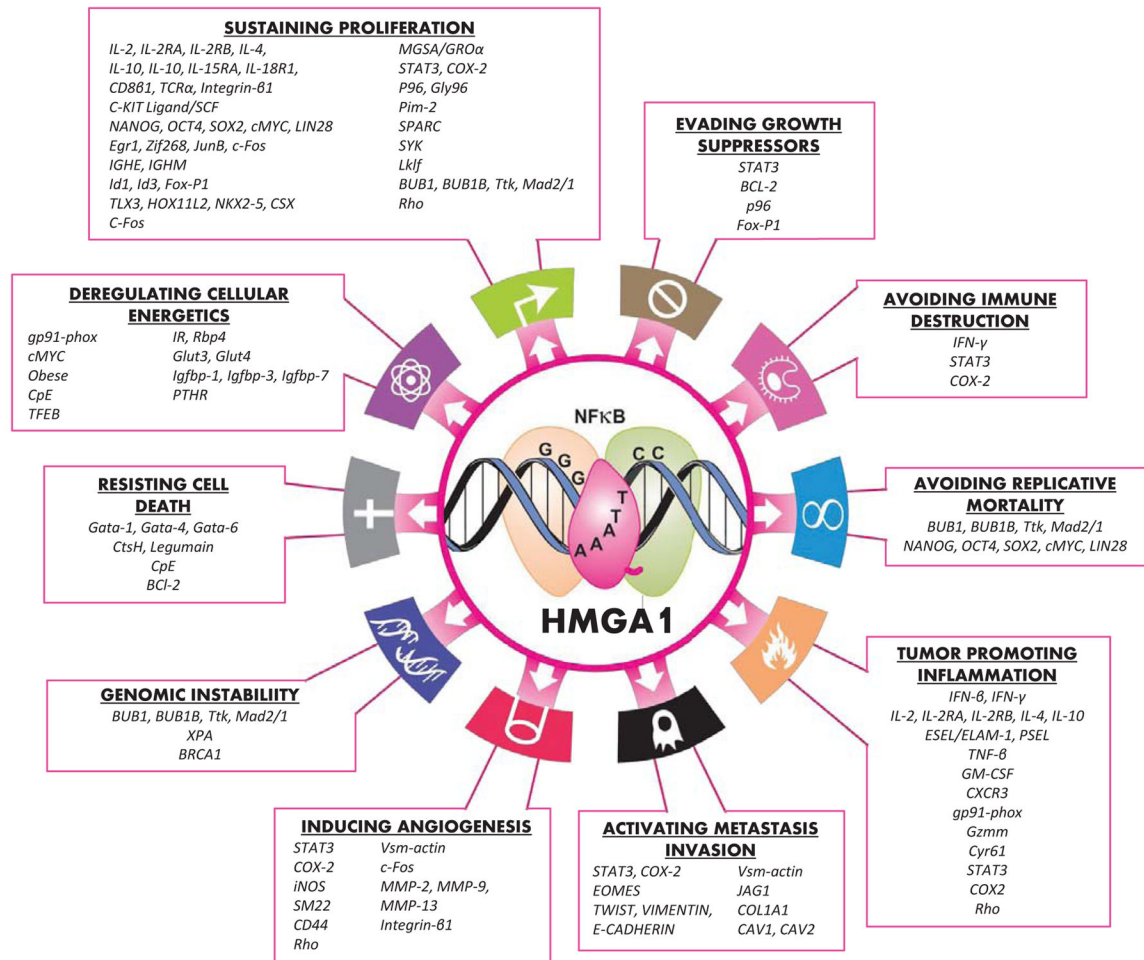
Fig. (2).
HMGA1 transcriptional networks involve all hallmarks of cancer.
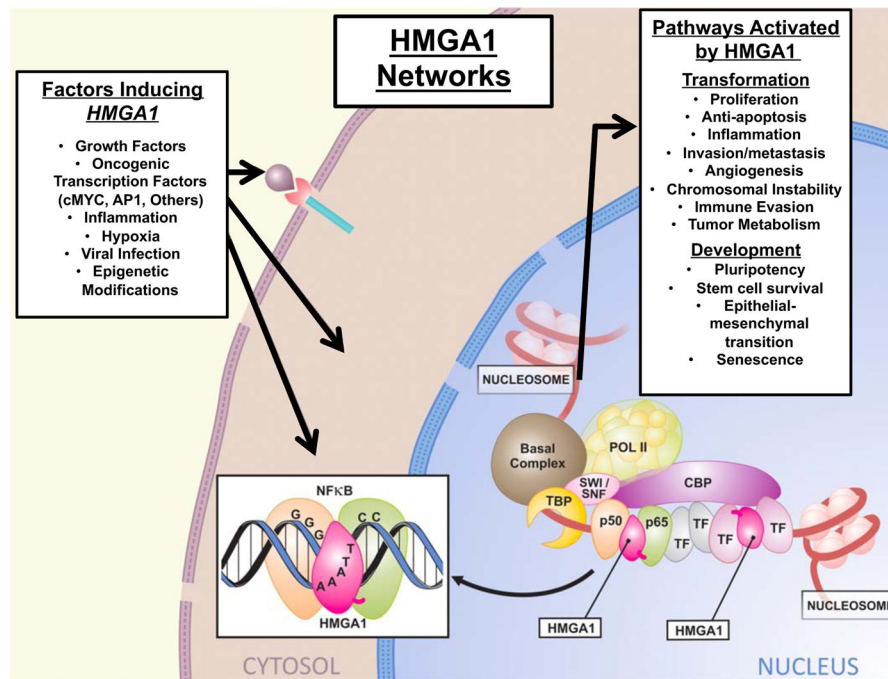
Fig. (3). The HMGA1 Transcriptome

Multiple factors induce *HMGA1* expression, after which the protein isoforms become up-regulated and translocate to the nucleus to induce expression of genes involved in fundamental pathways required for tumor initiation and progression, normal development, and adult stem cell function.

Table 1

Putative gene targets of *HMGA1*.

Cytokine and cytokine receptor genes with promoters with similar (IFN-β- like) enhancer regions

| Gene	Function	Induce/Repress [a]	Organism [b]	Cell Type [c]	Approach/Rationale [d]	Validation Studies [e]	HMGA1-Dependent Expression in Tissues/Cell Lines [f]	Required for HMGA1 Phenotypes [g]	NF-κB Sites [h]	Primary Refs.
Interferon-β (IFN-β)	Antiviral defense, inflammatory response	Induce	H, F, M	HeLa cervical cancer cells, Osteosarcoma, Jurkat T-leukemia cells, MG63 osteosarcoma cells	Expression library screen for IFN-β promoter binding proteins	EMSA, Methylation interference Transfection transcription assay 1, 2, 3	ND	ND	+	Falvo [126], Thanos [127], Du [128]
IFN-γ	Induce T helper cell development	Induce	H, M	Transgenic mouse T cells, Human EL4 T cells	Homology to IL2RA	RNase protection assay, qRT-PCR and ELISA in Transgenic mice, DNase footprinting Assay, EMSA, Transfection transcription assay	Yes	ND	+	Chau [166]
Interleukin-4 (IL-4)	B-cell proliferation/differentiation	Repress	H, M	Mouse E14 T lymphoma cells, human peripheral blood T-cells, Jurkat T-leukemia cells	Promoter analysis	EMSA, Transfection transcription assay DNAase Footprinting	ND	ND	+	Chuvpilo [169]
Interleukin-2 (IL-2)	T-cell proliferation/differentiation, antigen-induced T-cell activation	Induce	H, M	Jurkat T-leukemia cells, human peripheral blood lymphocytes	Promoter analysis	EMSA, Transfection transcription assay DNAase footprinting	+	Y	+	Chuvpilo [169], Hines [189], Fedele [100]
E-Selectin (ELAM-1)	Cell adhesion molecule (CAM), leukocyte rolling and recruitment, inflammatory response	Induce	H, B	Human umbilical vein endothelial cells, bovine aortic endothelial cells (BAEC)	Promoter analysis	EMSA, Methylation interference Transfection transcription assay	ND	ND	+	Whitley [173]
P-Selectin	Cell adhesion molecule (CAM), leukocyte rolling and recruitment, inflammatory response	Induce	M, B	Bovine aortic endothelial cells (BAEC), primary murine lung enothelial cells (MLEC), murine endothelial cells	Promoter analysis	EMSA, Methylation interference ChIP	+	ND	+	Baron [176]
Interleukin-2 Receptor α (IL-2Rα)	T cell proliferation/differentiation, antigen-induced T-cell immune function	Induce	H, M, Mo	Jurkat T-leukemia cells, MT-2 T-lymphocytes, Kit 225 T cells	Promoter analysis	EMSA, Methylation interference Transfection transcription assay DNAase footprinting ChIP Ethylation interference	+	ND	+	John [177, 178], Fedele [100]
Tumor Necrosis Factor-β (Tnf-β)	Inflammation, maintenance of lymphoid tissue & Peyer's patches	Induce	M	PD pre-B murine cells	Promoter analysis	EMSA Transfection transcription assay	ND	ND	+	Fashena [181]
Melanoma Growth Stimulatory Activity/ Growth Regulate α (MGSA/GROα)	Chemokine, inflammation, neutrophil chemo-attractant, induced as melanocytes progress to melanoma	Induce	H	Hs294T melanoma cells, human retinal pigment epithelial cells	Promoter analysis	EMSA, Transfection transcription assay DNAase footprinting	ND	ND	+	Wood [184]

Additional cytokine and chemokine genes

Gene	Function	Induce/Repress [a]	Organism [b]	Cell Type [c]	Approach/Rationale [d]	Validation Studies [e]	HMGA1-Dependent Expression in Tissues/Cell Lines [f]	Required for HMGA1 Phenotypes [g]	NF-κB Sites [h]	Primary Refs.
Interleukin-15 (IL-15)	T-cell proliferation, Generation of cytotoxic and NK T cells, B cell immuneglobulin synthesis	Induce	M	HMGA1/b transgenic mouse splenocytes	Defined as HMGA2 target in HMGA2/T transgenic mouse model	Transfection transcription assay	+	ND	+	Fedele [100]
Interleukin-15 Receptor alpha (IL-15Rα)	T-cell proliferation, Generation of cytotoxic and NK T cells, B cell immuneglobulin synthesis	Induce	M	HMGA1/b transgenic mouse splenocytes	HMGA2 target in HMGA2/T transgenic mouse model	Transfection transcription assay	+	ND	+	Fedele [100]
Interleukin-10 (IL-10)	Promotes B cell proliferation, maintains TH1 cells in an undifferentiated state	Induce	H	Raji Burkitt cells, U937 and K562 acute myeloid leukemia cells, Jurkat T-leukemia cells	Promoter analysis	EMSA	ND	ND	None	Lin [187]
Granulocyte Macrophage (GM-CSF)	Stimulates hematopoietic stem cells to produce granulocytes & monocytes	Induce	H	Jurkat T-leukemia cells	Promoter analysis	EMSA, Transfection transcription assay	+	ND	+	Hines [189]

Gene	Function	Induce/Repress[a]	Organism[b]	Cell Type[c]	Approach/Rationale[d]	Validation Studies[e]	HMGA1-Dependent Expression in Tissues/Cell Lines[f]	Required for HMGA1 Phenotypes[g]	NF-κB Sites[h]	Primary Refs.
Interleukin 2 Receptor β (IL-2Rβ)	IL-2 signaling, T-cell proliferation, inflammatory response	Induce	H, M	HMGA1a transgenic mouse splenocytes, Jurkat T-leukemia cells	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Schuldenfrei [108]
Interleukin 18 receptor (IL-18r1)	Member of IL-1 super-family involved in cell-mediated immunity and inflammation following microbial infection	Induce	H, M	HMGA1a transgenic mouse splenocytes	Microarray gene expression profile analysis	qRT-PCR	+	ND	+	Schuldenfrei [108]
CXCR3	Leukocyte trafficking, inflammatory cell recruitment, inflammation	Induce	H, M	HMGA1a transgenic mouse splenocytes	Microarray gene expression profile analysis	qRT-PCR	+	ND	+	Schuldenfrei [108]
Other genes involved in leukocyte function and inflammation										
T-Cell Receptor α (TCR-α)	Tissue and stage-specific expression and V(DJ) recombination in T-& B-lymphocytes	Repress	H	Jurkat T-leukemia cells HeLa cervical cancer cells	Promoter analysis	*In vitro* transcription	+	ND	+	Bagga [191]
Immunoglobulin heavy chain e enhancer (e enhancer) (Ighe)	V(DJ) recombination in B-lymphocytes; IgE production	Repress	M	BALB/c – derived mouse B lymphoma cells M12.4.1, Abelson virus transformed pre-B cells 18.81, mouse splenocytes	Promoter analysis	EMSA, Transfection transcription assay, DNAase footprinting	+	ND	+	Kim [192]
Immunoglobulin heavy chain mu promoter (μ enhancer) (Ighm)	V(DJ) recombination in B-lymphocytes; IgM production	Induce	M	Ag8.653 murine mature plasmacytoma B cells, 2017 pro-T cells, 38B9 preB cells, BAL-17 mature B cells	Promoter analysis	EMSA, Methylation interference ChIP Pull-down	+	ND	+	Lewis [193]
GP91-PHOX	ROS production in phagocytosis (encodes the heme-binding beta subunit of NADPH oxidase or cytochrome b)	Not known	H	Human endothelial cell library	cDNA expression library screen for CCAAT-box binding proteins in *gp91-phox* promoter	Southwester blot	ND	ND	+	Skalnif [196]
CD8β1	Cytotoxic T-cell receptor, antigen-specific T-cell activation	Induce	H, M	HMGA1a transgenic mouse splenocytes, Jurkat T-leukemia cells	Microarray gene expression profile analysis	qRT-PCR	+	ND	+	Schuldenfrei [108]
Granzyme M (GZMM)	Protein stored in granzymes and involved in apoptosis	Induce	H, M	HMGA1a transgenic mouse splenocytes	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Schuldenfrei [108]
Spleen tyrosine kinase (syk)	Signal transduction in T and B-cells, inflammation, angiogenesis, transformation	Induce	M	Mouse embryonic stem cells	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Martinez-Hoyos [198]
Transcription factors										
Gata-1	Zinc finger transcription factor involved in hematopoiesis	Repress	M	Mouse embryonic stem cells	*Gata1* was tested based on phenotypes found in hematopoietic cells derived from *hmga1*null ES cells	EMSA, Methylation interference ChIP qRT-PCR	+	ND	+	Battista [144]
Gata-4	Zinc finger transcription factor involved in embryogenesis (meso and endoderm-derived tissues), cardiogenesis	Induce	M	Mouse embryonic stem cells and mouse embryonic fibroblasts	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Martinez-Hoyos [198]
Gata-6	Zinc finger transcription factor involved in embryogenesis (meso- and endoderm-derived tissues), cardiogenesis	Induce	M	Mouse embryonic stem cells and mouse embryonic fibroblasts	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Martinez-Hoyos [198]
Lung Krupple-like factor (Lklf)	Zinc finger transcription factor involved in hematopoiesis and an anti-inflammatory response	Induce	M	Mouse embryonic stem cells and mouse embryonic fibroblasts	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Martinez-Hoyos [198]
Zif268	Immediate-early gene involved in mitogenesis and differentiation	Induce	M	Mouse embryonic stem cells, embryonic fibroblasts and liver	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Martinez-Hoyos [198]

Gene	Function	Induce/Repress[a]	Organism[b]	Cell Type[c]	Approach/Rationale[d]	Validation Studies[e]	HMGA1-Dependent Expression in Tissues/Cell Lines[f]	Required for HMGA1 Phenotypes[g]	NF-κB Sites[h]	Primary Refs.
JunB	Immediate-early/proto-oncogene	Induce	M	Mouse embryonic stem cells	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Martinez-Hoyos [198]
c-Fos	Immediate-early/proto-oncogene	Induce	M, F	Murine embryonic stem cells, SL2 droso-phila cells	Microarray gene expression profile analysis, Promoter analysis	RT-PCR, EMSA, Transfection transcription assay	+	ND	+	Martinez-Hoyos [198], Chin [204]
cMYC	Immediate-early/proto-oncogene, pluripotency factor	Induce	M, H	Murine embryonic stem cells, human embryonic stem cells	Microarray gene expression profile analysis; analysis of pluripotency gene expression	RT-PCR, ChIP	+	ND	+	Martinez-Hoyos [198], Shah [98]
NANOG	Pluripotency gene important in stem cells	Induce	H	Human embryonic stem cells, induced pluripotent stem cells	Stem cell gene expression profile analysis	RT-PCR	+	ND	+	Shah [98]
OCT4	Pluripotency gene important in stem cells	Induce	H	Human embryonic stem cells, induced pluripotent stem cells	Stem cell gene expression profile analysis	RT-PCR, ChIP	+	ND	+	Shah [98]
SOX2	Pluripotency gene important in stem cells	Induce	H	Human embryonic stem cells, induced pluripotent stem cells	Stem cell gene expression profile analysis	RT-PCR, ChIP, Protein IF	+	ND	+	Shah [98]
Signal Transducer and Activator of Transcription (STAT-3)	Transcription factor, Intracellular Signaling; oncogene	Induce	H, M, R	HMGA1a transgenic mouse splenocytes and lymphoid tumors, HMGA1a- and 1b Rat1a fibroblast cells, HEL erythroleukemia cells	Microarray gene expression profile analysis	EMSA, Transfection transcription assay, ChIP, qRT-PCR, western blot, IH	+	Y	+	Hillon [91]
Forkhead Box Transcription Factor p1 (FOXP1)	Neuronal development	Repress	H, M	HMGA1a transgenic mouse splenocytes	Microarray gene expression profile analysis	qRT-PCR	ND	ND	None	Schuldenfrei [108]
Eomesodermin (EOMES)	Embryonic development, nueronal and trophoblast development	Induce	H, M	HMGA1a transgenic mouse splenocytes, Jurkat T-leukemia cells	Microarray gene expression profile analysis	qRT-PCR	+	ND	+	Schuldenfrei [108]
TWIST	Embryonic development, epithelial to mesenchymal transition	Induce	H, M	HCT116 & SW480 human colorectal cancer cells	Defined as HMGA2 target	qRT-PCR	+	ND	+	Belton [68]
HAND1	Placental formation, cardiac morphogenesis	Repress	H, M, R	Mouse embryonic stem cells, human thyroid primary and carcinoma cells*, rat thyroid carcinoma cells	Microarray gene expression profile analysis	EMSA, Transfection transcription assay, ChIP, RT-PCR, IH	+	Y	+	Martinez-Hoyos [198]
Tfeb	Lysosomal biogenesis & autophagy	Induce	M	Mouse embryonic stem cells	Microarray gene expression profile analysis	RT-PCR,	+	ND	+	Martinez-Hoyos [198]

DNA damage tolerance, genomic instability

Gene	Function	Induce/Repress[a]	Organism[b]	Cell Type[c]	Approach/Rationale[d]	Validation Studies[e]	HMGA1-Dependent Expression in Tissues/Cell Lines[f]	Required for HMGA1 Phenotypes[g]	NF-κB Sites[h]	Primary Refs.
Xeroderma Pigmentosa A (XPA)	DNA Repair (Nuclear excision repair)	Repress	H	MCF-7 breast adeno-carcinoma cells expressing HMGA1a	Microarray gene expression profile analysis	Methylation interference ChIP RT-PCR Western blot	+	Y	+	Adair [112]
Breast Cancer Type 1 Susceptibility (BRCA1)	Cell cycle regulation, DNA repair (Double-stranded break repair)	Repress	H, M	MCF-7 breast adeno-carcinoma, HMEC human mammary epithelial cells	Promoter analysis	EMSA, Methylation interference Transfection transcription assay, ChIP Western blot Northern blot	+	ND	+	Baldassarre [110]

Genes involved in cell cycle regulation, pro-survival and/or anti-apoptotic pathways

Gene	Function	Induce/Repress[a]	Organism[b]	Cell Type[c]	Approach/Rationale[d]	Validation Studies[e]	HMGA1-Dependent Expression in Tissues/Cell Lines[f]	Required for HMGA1 Phenotypes[g]	NF-κB Sites[h]	Primary Refs.
B-Cell Lymphoma Gene 2 (BCL-2)	Apoptosis Inhibitor	Induce	H, M	ND7 mouse neuroblastoma cells, MCF-7 breast adeno-carcinoma cells, mouse embryo fibroblasts, SAOS-2 osteosarcoma cells	HMGA1-interacting protein array identified p53; p53-regulated genes were therefore studied	EMSA, Transfection transcription assay ChIP Pull-down qRT-PCR Western blot Co-IP	+	ND	+	Esposito [221]

Gene	Function	Induce/Repress[a]	Organism[b]	Cell Type[c]	Approach/Rationale[d]	Validation Studies[e]	HMGA1-Dependent Expression in Tissues/Cell Lines[f]	Required for HMGA1 Phenotypes[g]	NF-κB Sites[h]	Primary Refs.
c-KIT Ligand	Hematopoiesis, hematopoietic stem cell niche, RAS/ERK signaling	Induce	H	MCF-7 breast adeno-carcinoma cells, OCC1 human ovarian cancer cells	Microarray gene expression profile analysis	EMSA, Transfection transcription assay ChIP qRT-PCR	+	ND	+	Treff [222, 223]
Caveolin 1 CAV1	Cellular Signaling	Repress	H	MCF-7 breast adeno-carcinoma cells	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Treff [222]
Caveolin 2 CAV2	Cellular Signaling	Repress	H	MCF-7 breast adeno-carcinoma cells	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Treff [222]
p96 Differentially expressed in ovarian cancer 2 (DOC2)	Negative regulator of mitogenic signaling, induces of c-Fos	Repress	M	Mouse embryonic stem cells & embryonic fibroblasts	Microarray gene expression profile analysis	EMSA, ChIP qRT-PCR	+	ND	+	Martinez-Hoyos [198]
Gly96, immediate-early response gene (IEX-1)	Immediate early gene that regulates proliferation (positively or negatively)	Repress	M	Mouse embryonic stem cells	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Martinez-Hoyos [198]
Vascular smooth muscle proliferation and function										
Inducible Nitric Oxide Synthase (iNOS)	Nitric oxide biosynthesis, blood vessel dilation, angiogenesis	Induce	R, F, H	RASMCs (rat aortic smooth muscle cells), SL2 droso-phila cells, RINm5F insulinoma cells, A549 human alveolar Type II alveolar I epithelial cells	Defined as an NF-κB gene target	EMSA, Transfection transcription assay	ND	ND	+	Perrella [230], Takamiya [231], Darville [233]
Smooth Muscle specific gene 22 (SM22)	Endothelial cell proliferation	Induce	F	SL2 droso-phila cells	Promoter Analysis	EMSA, Transfection transcription assay	ND	ND	+	Chin [237]
Cd44	Cellular adhesion, stem cell marker	Induce	R	RASMCs (rat aortic smooth muscle cells), F9 mouse embryonal carcinoma cells,	Defined as a cytokine-regulated gene & Promoter analysis	EMSA, Transfection transcription assay	ND	ND	+	Foster [239]
Vascular smooth muscle actin (Vsm-actin)	Cytoskeleton	Induce	M	Mouse embryonic stem cells	Promoter Analysis	EMSA, Transfection transcription assay	ND	ND	+	Martinez-Hoyos [198]
Helix-Loop-Helix factors										
DNA-binding protein inhibitor Id-3 (Id3)	Cell growth, differentiation, angiogenesis	Induce	M, R	Mouse embryonic stem cells, mouse splenocytes, FRTL-5 rat epithelial cells	Microarray gene expression profile analysis	EMSA, ChIP, RT-PCR, Western	+	ND	+	Martinez-Hoyos [198]
Adipogenesis										
Obese	Adipogenesis & energy intake	Induce	H, M	Human embryonic kidney HEK) 293 cells3T3-L1 mouse fibroblasts	HMGA interacts with RB & C/EBPβ, C-EBPβ regulated genes studied	Transfection transcription assay	+	ND	+	Melillo [244]
Migration/Invasion/Metastasis										
Matrix Metallo-Proteinase 2 (MMP-2)	Extracellular matrix degradation	Induce	R, H	G-Dunning rat prostate cancer, H1299 lung cancer cells	Microarray gene expression profile analysis in rat prostate cells	Western blot (prostate cells) RT-PCR, ChIP (H1299 lung cancer cells)	+	Y	+	Takaha [60], Hillion [50]
Matrix Metallo-Proteinase 9 (MMP-9)	Extracellular Matrix degradation	Induce	H	MiaPaCa2 and PANC1 huamn pancreatic adenocarcinoma cells	HMGA1 regulates other MMPs and functions in invasion	MMP-9 activity (fluorometric assay), RT-PCR, Transfection transcription assay	+	Y	+	Liau [70]
Matrix Metallo-Proteinase 13 (MMP-13)	Extracellular Matrix degradation	Induce	H	MCF-7 breast cancer cells	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Reeves [29]
Integrin-β1	Cellular Adhesion, migration	Induce	H	MCF-7 breast cancer cells	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Reeves [29]

Gene	Function	Induce/Repress[a]	Organism[b]	Cell Type[c]	Approach/Rationale[d]	Validation Studies[e]	HMGA1-Dependent Expression in Tissues/Cell Lines[f]	Required for HMGA1 Phenotypes[g]	NF-κB Sites[h]	Primary Refs.
Regulators of Multiple Transformation Pathways										
Cyclooxygenase 2 (Prostaglandin G/H Synthase 2)	Lipid Biosynthesis, inflammatory response	Induce	H, M	Vascular endothelium, HMGA1a transgenic mouse uterine neoplasms, MESSA uterine sarcoma cells	Promoter analysis	EMSA, Transfection transcription assay, ChIP, qRT-PCR, western blot	+	Y	+	Ji [255], Tesfaye [75], DiCello [256]
Ocular Function										
Rhodopsin (Rho)	Retinal function, photoreceptor differentiation	Induce	H, M	C57B6 and BALBc mouse ocular tissues, WERI-Rb1 & Y79 retinoblastoma cells	Expression of HMGA1a in photorecep for cells led to exploring a proposed role in gene turnover	EMSA, Transfection transcription assay, DNAse Footprinting, GST-pulldown, qRT-PCR, Western blot, Northern blot, IH	+	ND	+	Chau [258]
Alpha crystalline B chain (CRYAB)	Heat shock protein	Induce	H	Human Lens Epithelial Cells (HLE-B3), SW-13 cells	Differential analysis	EMSA, Transfection transcription assay, ChIP, Nucleosome Mapping	ND	ND	+	Duncan [260]
Diabetes and Insulin Resistance										
Insulin Receptor (IR)	Cell signaling	Repress	H, M, R	IM-9 Lymphocytes, HepG2 and HTC rat Hepatoma cells, 3T3-L1 mouse fibroblasts, pancreatic cells, MCF-7 breast carcinoma	Promoter analysis	EMSA, Transfection transcription assay, ChIP, western, co-IP Southwestern	+	Y	+	Brunetti [261], Foti [262]
RBP4	Insulin Resistance	Induce	H, M	HMGA1-deficient mouse adipocytes, Hepa1 & HepG2 Hepatoma cells, kidney	Defined as gene involved in glucose homeostasis	Transient transfection transcription assays, ChIP, qRT-PCR, western blot, northern blot	+	Y	+	Chiefari [263]
Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1)	Glucose Homeostasis	Induce	H, M	HMGA1-deficient mouse hepatocytes, HepG2 hepatoma cells, MCF-7 breast cancer cells, NIH 3T3 mouse fibroblasts	Promoter analysis	EMSA, Transient transfection transcription Assays, ChIP, qRT-PCR, western, co-IP	+	ND	+	Allander [265], Iiritano [266]
Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3)	Glucose Homeostasis	Induce	H, M	HMGA1-deficient mouse hepatocytes, HepG2 hepatoma cells, MCF-7 breast cancer cells, NIH 3T3 mouse fibroblasts	Promoter Analysis	EMSA, Transient transfection transcription assays, ChIP, RT-PCR, western, co-IP	+	ND	+	Allander [265], Iiritano [266]
Developmental, Stem Cell and EMT										
Jagged-1 (JAG1)	Notch1 ligand involved in cell fate decisions in hematopoiesis & cardiac development	Induce	H	MCF-7 breast cancer cells	Microarray gene expression profile analysis	RT-PCR	+	ND	+	Reeves [29] Reeves
Vimentin (VIM)	Embryonic development, epithelial to mesenchymal transition	Induce	H, M	MCF-7-breast cancer cells, HCT116 & SW480 colon cancer cell lines	Microarray gene expression profile analysis (MCF-7 cells)	RT-PCR IH	+	ND	+	[29], Belton [68]
E-Cadherin	Cellular Adhesion, epithelial to mesenchymal transition	Repress	H	SW480 colon cancer cell line	Expression screen for genes involved in EMT	qRT-PCR	+	ND	+	Belton [68]
Spindle Activating Checkpoint Genes										
Budding Uninhibited by Benzimidazoles 1 Homolog β (Bub1β)	Mitosis, cell cycle regulation	Induce	H, M	HMGA1a transgenic mouse splenocytes (ref 108), Jurkat T-ALL cells, HCT116, NIH3T3, HeLa cells (ref 277)	Microarray gene expression profile analysis	qRT-PCR	+	ND	+	Schuldenfrei [108], Pierantoni [277]

Gene	Function	Induce/Repress[a]	Organism[b]	Cell Type[c]	Approach/Rationale[d]	Validation Studies[e]	HMGA1-Dependent Expression in Tissues/Cell Lines[f]	Required for HMGA1 Phenotypes[g]	NF-κB Sites[h]	Primary Refs.
Budding Uninhibited by Benzimidazoles (Bub1)	Mitosis, cell cycle regulation	Induce	H	HCT116, NIH3T3, HeLa cells	Promoter analysis, Function in phenotypes associated with *HMGA1*	qRT-PCR, Western	+	ND	+	Pierantoni [277]
TTK	Mitosis, cell cycle regulation	Induce	H	HCT116, NIH3T3, HeLa cells	Promoter analysis, Function in phenotypes associated with *HMGA1*	qRT-PCR	+	ND	+	Pierantoni [277]
MAD2/1	Mitosis, cell cycle regulation	Induce	H	HCT116, NIH3T3, HeLa cells	Promoter analysis, Function in phenotypes associated with *HMGA1*	qRT-PCR, Western	+	ND	+	Pierantoni [277]

Legend:

[a] - **Induce or Repress:** Refers to whether the gene expression is induced (up-regulated) or repressed (down-regulated) by *HMGA1/Hmga1*.

[b] - **Organism:** Refers to the organism from which the cell lines or other reagents were used. H=Human, F=Fly, M=Mouse, R=Rat, Mo=Monkey, B=Bovine.

[c] - **Cell Type:** Refers to the specific cell line(s) used for the studies.

[d] - **Approach/Rationale:** Refers to the initial approach or model used to identify the gene target.

[e] - **Validation Studies:** Refers to the **experimental approaches used to validate the original observation, including:** Gel Shift or Electrophoretic Mobility Shift Assay (EMSA); Methylation Interference, Transcription Transfection Assays (an *in vitro* transfection experiments in cells with a reporter construct), DNAse Footprinting, Chromatin immunoprecipitation (ChIP), Ethylation Interference, *in vitro* GST or biotinylated-pull-down; Southwestern blot analysis, qRT-PCR, Western blot, Northern blot, Immunohistochemical analysis, co-immunoprecipitation.

[f] *HMGA1*-**dependent expression in tissues/cell lines:** Refers to whether expression of the target gene depends upon *HMGA1* levels.

[g] - **Required for *HMGA1* Phenotypes:** Refers to whether knock-down or silencing of the target gene also impairs *HMGA1* phenotypes (transformation, proliferation, others).

[h] - **NF-κB binding sites:** Refers to the presence of NF-κB binding sites in the promoters. These were determined either experimentally or using an *in silico* prediction algorithm (MatInspector) [297].

ND = Not done.