High-mobility-group chromosomal proteins, HMGA1 as potential tumour markers

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The high-mobility-group, HMGA1 (formerly HMG I/Y) family of non-histone, chromosomal proteins consisting of HMGA1a, HMGA1b and HMGA2 are known as 'architectural transcription factors' because of their specific protein–protein and protein–DNA interactions. In the recent past, the HMGA1 family has got special attention and has been given several names like 'oncoproteins', 'enhancers', 'multifunctional proteins', 'architectural elements', 'tumour markers', etc. The increased interest in the last few years in these small molecular weight proteins is due to their high expression in neoplastic transformation of cells and metastatic tumour progression. Because of their elevated levels found in a wide variety of human cancers, they are suggested as novel diagnostic tumour markers. These proteins have three conserved binding domains called 'AT-hooks' which bind to the narrow minor groove of the DNA in the AT-rich sequences and bring conformational changes in DNA and chromatin. They are also known to participate in protein–protein interaction in the organization of transcriptional complex and assembly of 'enhanceosome'. Since HMGA1 proteins are the only oncoproteins known so far, which bind to the DNA minor groove, they are also used as potential targets for anti-tumour drugs.

INTERACTIONS of proteins with DNA play a very significant role in regulating various cellular processes like replication, transcription, recombination and repair. Although most of the studies on chromatin are devoted to the interactions of DNA with histones[1], the importance of the non-histone chromosomal proteins in the structural and functional complexity has only recently got the deserved attention[2–6]. Among the non-histone chromosomal proteins, the 'high-mobility group proteins', popularly known as HMG proteins are the best studied. The name HMG was coined because of their rapid movement in the gel electrophoresis[7]. The 'canonical HMG proteins' are grouped into three families with characteristic functional motifs: HMG-B with 'HMG-box', HMG-N with 'nucleosomal binding domain' and HMG-A family with 'AT-hook'. The proteins containing any of the above three motifs are classified as 'HMG motif proteins'. Although the structural information about these proteins is well documented, very little is known about their cellular function. Most of the data suggest that these proteins serve as 'architectural elements' in chromatin[5].

HMG-B consisting of HMGB1 and HMGB2, which have more than 82% amino acid sequence identity, are the largest (~ 25 kDa), most abundant and highly conserved family[7]. They bind to DNA with little sequence specificity and induce unwinding, bending and supercoiling; they are also suggested to participate in the regulation of chromatin structure[8]. The HMGN family consists of HMGN1 and HMGN2 present in cells of higher eukaryotes and is supposed to modulate the effect of chromatin on transcription[5]. The third family, HMGA will be discussed in detail in this review.

With the phenomenal rate at which the data on HMG proteins is increasing in the literature, it is impossible to include all the three groups of HMG proteins in the present review and therefore we restrict ourselves to the discussion on HMGA family only. The interest in HMGA1 proteins is mainly two-fold. Firstly, the HMGA1 family stands out from the rest of the non-histone proteins, because of its emerging links to cancer and suggested diagnostic marker[9–13]. Secondly, the HMGA1 proteins are very flexible, multifunctional and play a complex role in the transcription and cellular functions.

Nomenclature

The HMG proteins and the related HMG motif proteins are reported in several mammalian species (human, mouse, calf, etc.), non-mammalian species (*Drosophila*, Xenopus, chicken, etc.), even in plants (maize, *Arabidopsis*, tobacco, etc.)[14] and the nomenclature in the literature has been quite arbitrary. However, last year all the HMG genes, proteins and protein products were given systematic nomenclature in the website maintained by the Mouse Gene Nomenclature Committee. Table 1 gives the old and new names of mouse and human HMGA family. (For a complete list for all the three families consult http: www.informatics.jax.org/mgihome/nomen/genefamilies/hmgfamily.shtm).

Structure of HMGA proteins

HMGA family proteins consisting of HMGA1a, A1b and A2 were originally identified as low molecular

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Table 1. Old and new nomenclature of HMGA family alongwith the accession number for the literature search in Pubmed

Protein		Gene		
Old name	New name	Old name	New name	Accession no.
HMG-I; HMGI; HMG I; HMG-I/Y a-protein	HMGA1a	HMGA1	Hmga1	L17131(H); AF286367(M)
HMG-Y; HMGY; HMG Y	HMGA1b	HMGA1	Hmga1	M23618(H); J04179(M)
HMG-I/R	HMGA1c	HMGA1	Hmga1	AF176039(H)
HMGI-C	HMGA2	HMGA2	Hmga2	L46353(H); L41617(M)

weight, basic, non-histone, chromosomal binding proteins[5]. HMGA1a (11.9 kDa) (ref. 15) and HMGA1b (10.6 kDa) (ref. 16) are identical in sequence, except for an 11-amino acid internal deletion in the latter, and are produced by alternate splicing of transcript from a single gene[17,18]. HMGA2 is encoded by a different gene[19], but shares some structural and amino acid sequence homology with HMGA1. Surprisingly, in higher plants also, proteins containing AT-hook motifs and a globular domain of histone H1 are reported, suggesting an interplay of linker histone H1 and HMGA1 and A2 proteins in chromatin and gene regulation.

The amino acid sequence comparison of human HMGA1a, A1b and A2 is given in Figure 1. The important structural feature of the HMGA family is the presence of three DNA-binding domains BD I, BD II and BD III called 'AT-hooks', which enable them to bind to the narrow minor groove of AT rich sequence of 15–18 base pairs of contiguous AT residues in DNA helix[20] (Figure 1). The three separate BD motifs which randomly appear in the sequence are shown in Figure 1. The palindromic pentapeptide sequence of the AT-hook motif, Pro–Arg–Gly–Arg–Pro (PRGRP), is the most highly conserved sequence in the HMGA family of proteins and is also identified in a wide variety of DNA-binding proteins[21]. The main region of the BD motif is actually the central RGR, which binds strongly to DNA. The central BD II plays an important role in binding to DNA, while BD I and BD III seem to have less involvement in making protein–DNA contacts.

Figure 2 shows the structure of PRGRP region of BD motif in HMGA and its DNA complex with AT-hook[22]. From the NMR and CD studies on pure HMGA proteins *in vitro*, it is reported that they exist almost 75% as random coil[23,24]. Recognition of the minor groove 'A-T' sequences by the HMGA proteins is confirmed by the NMR structural report on the co-complex of the HMGA–DNA[22]. PRGRP acquires a specific, planar, crescent-shaped structure only after binding to DNA[24] and adopts β-turn conformation (shown in Figure 2). Therefore the HMGA proteins, due to their property of reversible transition from 'random coil' to 'ordered structure' (with crescent form of BD), are also known as 'flexible proteins'. Being chromosomal proteins, it is not surprising to see about 25% of positively charged amino acid residues in HMGA proteins; however, presence of a fair number

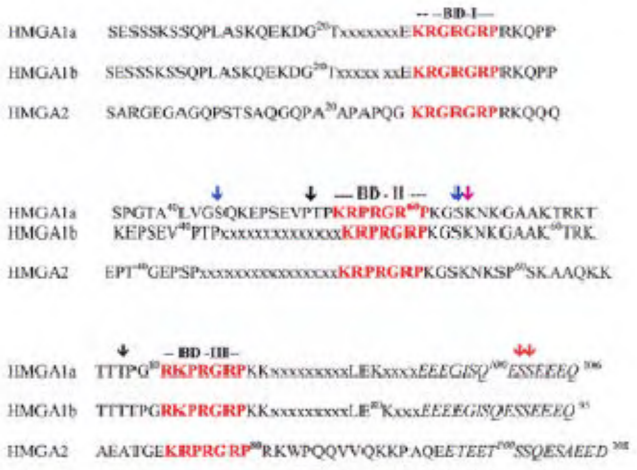

Figure 1. Alignment of the amino acid sequences of human HMGA1a[15], HMGA1b[16] and HMGA2 (ref. 19). HMGA1b, A2 are short by 11–12 amino acid stretch, which is internally deleted. The binding domain sequence KRPRGRP[68] in the three DNA-binding domains BD-I, II and III is shown in red colour. Note that the C-terminals shown in italics, are rich in glutamic acid and the deletions are represented by 'x'. Residues phosphorylated and shown as arrows by cdc2 kinase[32,33] are Thr 52, 77 (black); by CK2[34] are Ser 101, 102 (red) and by protein kinase C-α[45] are Ser 43 and 63 (blue). Lysine 64 acetylated by CBP[39] acetyl transferase is shown as a pink arrow.

of prolines (Figure 1) is rather unique. By using site-directed mutagenesis it has been shown that the conserved proline residues in the PRGRP are critical in maintaining the structure. The prolines of the PRGRP in the free (unbound) protein exist in *trans* configuration, while the central glycine is quite flexible. Therefore, the peptide backbone of the PRGRP is forced to assume a narrow concave structure that can fit well in the deep narrow minor groove of DNA with AT sequences. The two arginines on either side of the glycine residue also help in stabilizing the complex with DNA by (a) making electrostatic contacts with the phosphates on the surface of the groove, and (b) hydrophobic interactions (of the aliphatic methylenes of the side chain) with adenine bases of DNA which determine the orientation of the AT-hook binding in the minor groove. The net affinity of binding of these BD motifs actually depends on DNA conformation and species of the HMGA that it belongs to. Depending on the sequence, organization and length of the DNA substrate, the HMGA protein binding can cause changes in linear conformation of DNA[25]; super-

helicity of closed circular DNA and unwinding of the intrinsically bent DNA[26]. It is interesting to note that HMGA1 proteins bind strongly to DNA with a large number of short AT-hooks (4–6 base pairs of AT) which are well spaced, rather than to a DNA sequence with a large continuous AT stretch[27]. Simultaneous binding of two or more AT-hooks of HMGA with DNA results in asymmetric neutralization of DNA, which increases the strength of HMG–DNA complex. Binding of HMGA1 to poly d(A-T) which exists in the classical B-DNA conformation is shown to be non-specific, although no binding data with HMG protein are reported on poly (dA)·(dT) which shows A-type of DNA conformation. It has also been reported earlier that peptide binding to poly d(A-T) is significantly different from that of poly d(A).d(T)[28]. The HMGA proteins, as well as the BD motif itself are shown to bind preferentially to non-B forms of DNA, such as four-way junctions[29], supercoiled plasmids[30] and distorted regions of DNA found on isolated nucleosome core particles[31], but the mechanism of this binding is still unknown. In addition to the number and spacing of AT stretches in DNA, the correct helical phasing of HMGA-binding site on the DNA is also very important, as seen in the β-interferon gene promoter. Therefore, the reversibility of the unordered-to-order structure of HMGA proteins is quite amazing, and explains its role in the biological activity.

Phosphorylation and acetylation of HMGA proteins

Since HMGs are basic proteins like histones, it is not surprising that they are highly phosphorylated and the extent of phosphorylation, particularly at residues Thr 52 and Thr 77 from N-terminal residues of BD II and BD III, respectively by Cdc2 kinase is cell cycle-dependent[32,33]. The two–four consecutive serine residues near the C-terminal are predominantly phosphorylated by Casein kinase II[34]. Although the effect of post-translational modification (phosphorylation) by protein kinase C, MAP kinase, of HMGA family *in vivo* is not yet known, however, the binding affinity to A.T DNA *in vitro* is greatly reduced after phosphorylation. Phosphorylation by Cdc2 inhibits the binding of BD-I in HMGA1 to the protein-binding domain, PRD-III-1 element on DNA in IFN-β promoter. While in HMGA2, phosphorylation of BD II makes it derail from the minor groove and binds to

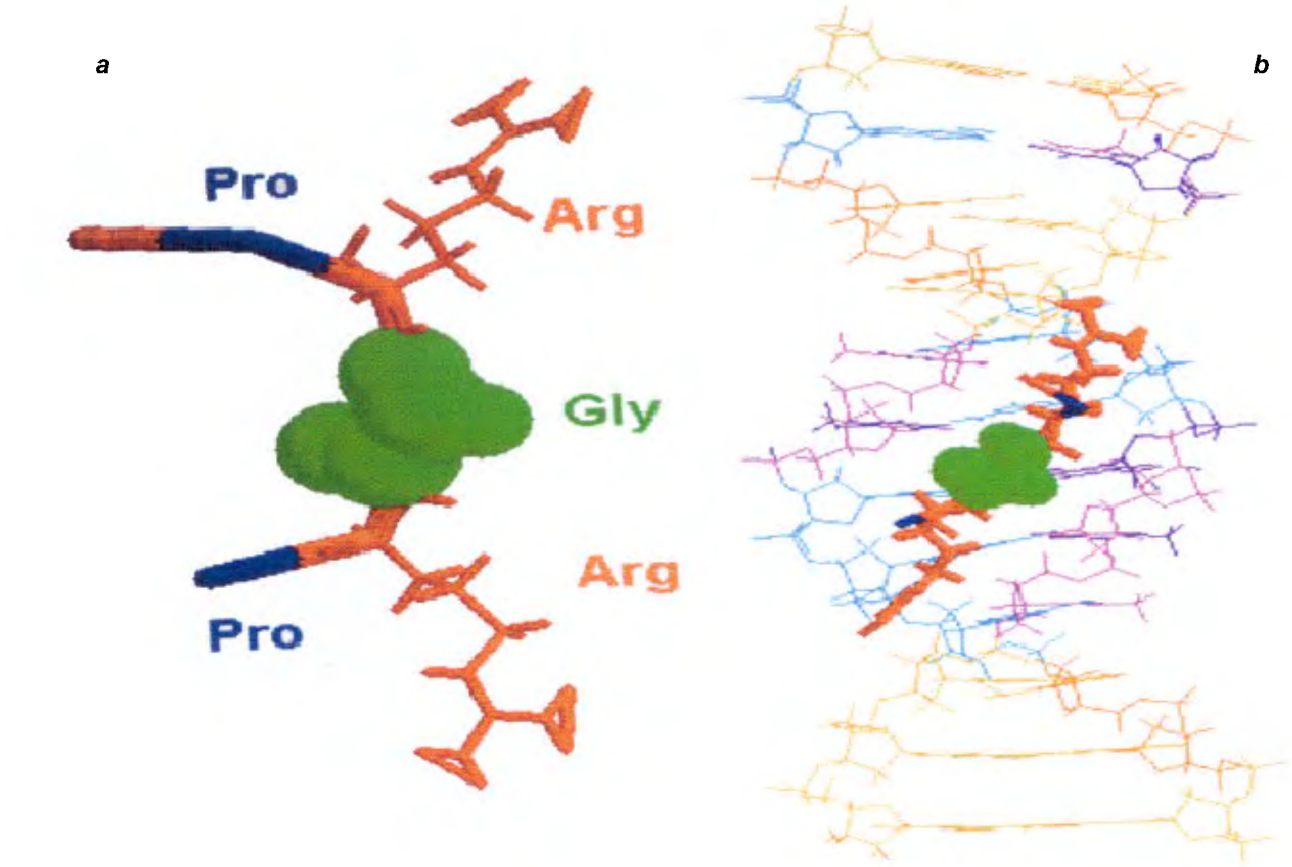

Figure 2. ***a***, AT-hook motif (PRGRP) which takes a crescent shape on binding to DNA; ***b***, Binding of PRGRP in HMGA1 to AT-hook motif in the minor groove of DNA[22].

DNA via sugar-phosphate backbone[35]. The regulation of phosphorylation/dephosphorylation of the HMG1 proteins seems to be important during eukaryotic development, although functional significance needs to be established by further studies.

Interactions with DNA and chromatin

In humans, *Hmga* gene is located on the short arm of chromosome 6p21, a region that is involved in rearrangements, translocations and other abnormalities correlated with a number of human cancers[18]. In mouse, the *Hmga1a* gene is located in the *t*-complex region of chromosome 17; a number of genes of this region are known to cause embryonic lethal mutations[36]. *Hmga2* location on chromosomal locus 12q14-15 (ref. 37) G/Q and C-band in humans and mouse chromosomes, respectively, suggests that the HMGA proteins are immunolocalized to the A-T-rich region during metaphase, HMGA1a proteins are specially associated with particular regions of chromosomes. It adds support to the idea that they may be actively involved in dynamic changes in chromatin structure occurring during the cell cycle and chromosome condensation[17]. It is suggested that HMG proteins are involved in the regulation of genes that are activated by growth factors like IGF-I, PDGF and FGF.

Interaction of HMGA family of proteins with DNA and chromatin has been well characterized[5]. By combining a variety of experimental techniques like methylation-interference, minor-groove ligand-binding complexation studies, DNA foot-printing studies, etc. HMGA proteins have been shown to bind to stretches of A.T-rich B-form DNA[20,38]. Foot-printing experiments with purified proteins indicate that *in vitro*, HMGA family proteins do not bind to all stretches of A.T-rich DNA with equal affinity, indicating that they recognize structure rather than the sequence[39,40]. HMGA1a proteins interact with homeodomain-binding sequences and block the transcriptional factors binding to 5′-TAAT-3′ sequences of DNA[38,41]. It has been suggested that HMGA family recognizes base unpairing regions (BUR)[42], the key structural element of matrix-associated regions (MARs). This interaction is linked directly to metastatic breast cancer phenotype. It has been demonstrated that mutations within the A.T-hook domains of HMGA1a, homologue of HMGA family, affect binding to gene promoters but not to four-way junction DNA[43]. The *in vivo* observations confirm that HMGA proteins preferably bind to isolated scaffold-associated regions (SAR) and in fact they compete with histone H1 for such A.T sequences.

Cellular function of HMGA as 'molecular glue' in the enhanceosome

In addition to their more general function in chromatin as architectural proteins, HMGA proteins have been reported to regulate transcription in specific genes. The first example of *in vivo* transcriptional regulation by HMGA was reported by Fashena *et al.*[44] from studies on the promoter region of TNF-β that was constitutively expressed in transformed B-cell line. Activation of eukaryotic gene expression relies on the formation of a multi-protein enhanceosome complex on promoters and enhancers adjacent to the transcription site[44]. Among the cellular functions of the HMGA family, one that is best understood is their *in vivo* role in regulating the expression of a variety of genes lying in close proximity to A.T-rich promoter sequences in either a positive or negative manner[30]. However, they seem to have no transcriptional activity[45] and perhaps function as architectural transcription factors[35–37]. They facilitate interaction of sequence-specific DNA-binding proteins to their target DNA sites and act as a bridge between two DNA-binding proteins bound to nearby *cis*-elements[38]; therefore, they are called 'molecular glue' in enhanceosomes[41,42]. HMGA family proteins have been shown to be an essential component of enhanceosomes, which are higher order transcription enhancer complexes formed when several distinct transcription factors assemble on the DNA in a stereo-specific manner[42]. Figure 3 is a schematic representation of the 'enhanceosome' complex of IFN-β gene where two molecules of HMGA1a protein are involved (based on ref. 46). Thus they function *in vivo* as both structural components of chromatin and auxiliary gene transcription factors. The *in vitro* transcription and electron microscopy studies have been reported to regulate long-range enhancer-dependent transcription on DNA and chromatin by changes in DNA topology[39] by HMGA proteins. HMGA family is also an activator of interferon and IL-2 receptor genes, and a suppressor of IL-4 and immunoglobulin genes. In these cases, localized binding of DNA is pro-

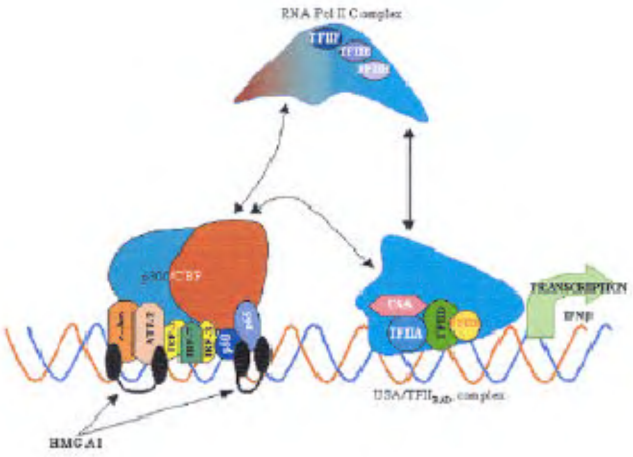

Figure 3. Schematic representation of the 'enhanceosome complex' based on Maniatis *et al.*[46] HMGA1 proteins act as 'enhancers' to form the nucleosome in the transcriptional initiation complex of human IFN-β gene[46]. Two molecules of HMGA1 (shown in black), one binding to c-Jun, ATF-2 and the other p50, p65 complex act as 'molecular glue'.

posed to facilitate assembly of multicomponent enhancer-binding complexes, which control gene activity. HMGA family has been shown to interact directly with DNA-bound transcription factor, including NF-Kβ, ATF-2/c-jun and SRF, to enhance their binding affinity and transactivation potential[21,22]. Table 2 provides a list of some of the important genes regulated for either activation or repression by HMGA1. It may be noted that the genes that are modulated by HMGA2 are not yet identified.

In a similar manner, HMGA family modulates binding of transcription factors to the U5 region of HIV type-1 proviral promoter. HMGA1 family has also been implicated in retroviral cDNA integration[29]. Down-regulation of nitric oxide synthase-2 by transforming growth factor beta-1 (TGF-β1) is reported to be associated with the down-regulation of HMGA family of protein synthesis. On the other hand, binding of HMGA family to a positioned nucleosome helps in transcription of human interleukin-2 receptor alpha gene.

High expression of HMGA proteins and cancer: Diagnostic marker

Considerable attention in recent years has been focused on the HMGA family proteins due to their enhanced expression during neoplastic cellular transformation and increased metastatic potential of several human cancers. Data available have well-demonstrated an increased expression of these proteins during embryogenesis, lymphocyte activation, tumourigenesis, tumour progression and malignant transformation. HMGA1 expression is maximal during embryonic development[47] and in rapidly proliferating cells[48,49]. In contrast, these proteins are undetectable or expressed at very low levels in normal adult tissues[50], indicating their critical role in cell proliferation, differentiation, embryonic growth and mesenchymal cell function. It is being suggested that these proteins are a characteristic and diagnostic feature of the transformed cellular phenotype[51,52].

Human *HMGa1* gene located on 6p21 has eight transcribed exons and four promoter/enhancer regions and human *Hmga2* located on 12q14-15 has five exons. Deletion within the 3′ exons of the *Hmga2* and fusion with other genes are characteristic for a variety of tumours. *Hmga2* truncations and their fusion with other domains interfere with the native properties of the protein and its regulatory function. Mutations in *Hmga2* gene have resulted in pygmy-type of mice[53]. The chromosomal rearrangements corresponding to the AT-hook motifs of HMGA proteins are seen in non-malignant tumours[54,55]. There is enough evidence at present from the experiments on nude mice[56] and transgenic mice[57] where HMGA protein expressions were concomitant with malignancy and metastatic tumours. It has been observed that alterations in the *Hmga* gene family play an important role in the generation of benign and malignant tumours. Rearrangements of the *Hmga* genes associated with AT-hook-binding domains have been found frequently in benign tumours of mesenchymal origin in humans. The chromosomal translocations in *Hmga2* are reported in lipomas, for example, myeloid leukaemia, breast hamartoma. In the reported cases, gene rearrangements were caused by chromosomal translocations involving regions 12q13-14 and 6p21, where *Hmga2* and *Hmga1* genes, respectively are located. Alterations in the expression levels of the HMGA1 family proteins are associated with a large variety of tissues, including thyroid[9], prostate[58], uterus[59] and colorectum[60], skin[61,62], breast[42], lung[63], neuroblastoma[64] and elevated levels of these proteins are highly correlated with cancer where over-expression of HMGA1 is untruncated and complete.

What are the factors or agents that regulate the *Hmga1* gene expression? A variety of factors/agents activate *Hmga1* gene; some important factors involved in the regulation of this gene are listed in Table 3. This may not be a complete list, but certainly gives an idea about the complexity involved.

Immunohistochemical studies with specific HMGA family antibody showed high levels of these proteins in pancreatic duct cell carcinomas[65]. In a recent study, Wood *et al.*[11] have found HMGA family to be a new *c*-Myc target gene, thus suggesting *HMGA* to be a potential oncogene. Nonetheless, the elevated expression of mRNA and proteins of HMGA has been useful as a diagnostic tool for differential diagnosis. Although numerous studies have demonstrated that over-expression of the

Table 2. Some important genes that are regulated for activation or repression by HMGA1*

Positive regulation	Negative regulation
Murine TNF-beta[44]	Human IL-4 (ref. 75)
Human IFN-beta[69]	Murine E immunology[76]
Human IL-2 receptor alpha[70]	γ-globilin[77]
Human E-selectin[71]	T-cell receptor-alpha[78]
Inducible nitric oxide synthase (iNOS)[72]	Alpha-2 collagen[77]
Jun B and fra-1 (ref. 73)	
C. fos[74]	

*Genes that are regulated by HMGA2 are not yet known.

Table 3. Some key factors that control *Hmga1* gene expression

Growth factor	Transcription factor
Transforming growth factor-α (TGF-α)[79]	AP-1 (ref. 81)
Epidermal growth factor (EGF)[80]	c-Myc[11]
Platelet-derived growth factor (PDGF)[51]	Human papillomavirus E6 protein[83]
Fibroblast growth factor (PGF)[51]	
Calcium inophore[81]	
Phorbol ester[81]	
IFN-β1 (ref. 82)	

HMGA family of architectural transcription factors is frequently associated with both neoplastic transformation and metastatic tumour progression, little is known about their molecular roles in these events. One logical question that comes up frequently is whether we can block tumour progression by inhibiting the HMGA protein synthesis. Indeed, suppression of HMGA protein synthesis mediated via adenoviruses has been shown as potential therapy of human malignant neoplasias. Consistent with these findings, inhibition of HMGA family has been shown to prevent transformation. It has been speculated that binding of HMGA family to the A.T-rich sequence of cDNA brings about a conformational change, promoting pre-initiation complex formation or activity in HIV.

HMGA1 oncoproteins: Target for anti-tumour drugs

HMGA1 family of oncoproteins which bind to the DNA in the minor groove have become an attractive target for anti-tumour chemotherapy[86]. By using immuno-precipitation procedure, fragments of DNA *in vivo* were isolated, which were covalently cross-linked to the anti-tumour drug FR 900482 and AT-hook-binding domain of the HMGA1a. The drugs FR 900482 (ref. 66) and FR66979 (ref. 67) are structurally similar to the well-known minor groove DNA-binding drug, mitomycin C. Since HMGA1 is the only minor groove-binding oncoprotein presently known, it is possible that these non-histone chromosomal proteins are amongst the important *in vivo* targets of this family of anti-cancer drugs.

Conclusions: The road ahead

Many questions regarding the role of HMGA family proteins in organizing chromatin architecture, regulating gene expression and tumourogenesis are yet to be answered. Much of the ambiguity is due to the fact that the relationship between chromatin structure and gene regulation involves a complicated interplay of DNA, transcription factors, histones and nuclear scaffold proteins. It is, however, clear that the expression of HMGA family proteins is very high in tumour progression and malignant transformation, but nothing concrete is known about why and how these non-specific proteins selectively regulate expression of specific groups of genes.

Studies on HMGA family suggest that this specificity partly involves protein–protein interactions with specific transcriptional factors, although interactions with other architectural components are also possible. There is increasing evidence to support that HMGA proteins facilitate the organization of enhanceosome complexes in specific genes. The ability of HMG proteins to recognize the structure in the enhancer/promoter regions of different genes explains why they are called multifunctional proteins. Another characteristic feature of HMGA proteins is the AT-hook-binding domains, which are not specific to the AT-containing sequences, but are specific to the structure or the structural region in DNA. The fact that they prefer binding to multiple domains of short AT stretches compared to a single large AT stretch, actually helps them bind strongly to DNA. The induced, ordered structural elements in the HMGA protein in the presence of DNA also are chosen carefully during evolution.

Clinically, differential diagnosis between pancreatic carcinoma and benign pancreatic lesions such as adenoma, hyperplasia, etc. remains a major problem. Determination of the expression levels of HMGA family gene/proteins could contribute to the detection of even a small number of cancer cells. Although, in the future, several questions regarding the mechanism of HMGA proteins have to be answered, these proteins still have the potential to be a diagnostic marker in a variety of cancers. Future in-depth research in this area will be able to answer the key questions regarding the function of HMGA proteins in cancer and these oncoproteins can be potential target for designing anti-tumour drugs.

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