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Review

HMGNs, DNA repair and cancer

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ABSTRACT

DNA lesions threaten the integrity of the genome and are a major factor in cancer formation and progression. Eukaryotic DNA is organized in nucleosome-based higher order structures, which form the chromatin fiber. In recent years, considerable knowledge has been gained on the importance of chromatin dynamics for the cellular response to DNA damage and for the ability to repair DNA lesions. High Mobility Group N1 (HMGN1) protein is an emerging factor that is important for chromatin alterations in response to DNA damage originated from both ultra violet light (UV) and ionizing irradiation (IR). HMGN1 is a member in the HMGN family of chromatin architectural proteins. HMGNs bind directly to nucleosomes and modulate the structure of the chromatin fiber in a highly dynamic manner. This review focuses mainly on the roles of HMGN1 in the cellular response pathways to different types of DNA lesions and in transcriptional regulation of cancer-related genes. In addition, emerging roles for HMGN5 in cancer progression and for HMGN2 as a potential tool in cancer therapy will be discussed.

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1. Introduction

High mobility group N (HMGN) family contains five chromatin architectural proteins, which are present in higher vertebrates. Of these proteins, HMGN1, 2 and 4 are expressed ubiquitously [1,2], while HMGN3 and 5 are expressed in specific tissues [3,4]. The HMGNs bind specifically to nucleosome core particles, which consist of 147 bp of DNA, wrapped around an octamer of core histones. The binding of HMGNs to nucleosomes has no sequence specificity and is mediated by their nucleosomal binding domain (NBD), which is the hallmark of this family of proteins. In living cells, HMGNs bind to nucleosomes temporally in a stop-and-go fashion and move continuously between binding sites. However, at any given moment most of the HMGNs are bound to chromatin, since their residence time on nucleosomes is longer than their transit time between nucleosomes. This highly dynamic binding to nucleosomes enables the HMGNs to regulate the chromatin structure both locally and globally [5–8]. HMGNs regulation of the chromatin structure is achieved by their ability to affect the levels of various histone post-translational modifications [9–11], to compete with histone H1 for chromatin binding sites [12,13] and to modulate the activity of chromatin remodeling factors [14]. Through these modes of action the HMGNs can induce de-compaction of the chromatin fiber.

The DNA packaged inside the chromatin fiber is constantly damaged by multiple agents. The insulting agents originate from internal metabolic processes and from external sources such as ultraviolet light (UV) and ionizing irradiation (IR). DNA lesions impose barriers for processes occurring on the DNA fiber, such as transcription and replication. DNA lesions also lead to genetic mutations and chromosomal aberrations, which are among the main causes of cancer development [15–17]. Throughout evolution several systems have evolved to identify the different types of lesions in the DNA, to adjust the cellular physiology to the insult and to repair the damage [17,18]. In humans, approximately 150 genes are dedicated to responding and repairing damage in the DNA [19]. In recent years, additional proteins, which were previously seen only as organizers of chromatin in relation to transcription and replication, were shown to have important roles in the cellular ability to respond to various types of DNA lesions. Among those proteins is HMGN1. This review will describe the roles recently found for HMGN1 in DNA damage response as well as in cancer progression and the potential found for HMGN2 as a therapeutic tool for cancer remission.

2. Role of HMGN1 in the cellular response to UV light

UV light induces several types of DNA lesions of which the cyclobutane pyrimidine dimers (CPD) and (6-4) pyrimidine-pyrimidone photoproducts [(6-4)PPs] are the most abundant [17,18]. These lesions are repaired by the nucleotide excision repair (NER) pathway, which consists of two sub-pathways with different substrate specificity; global genome NER (GG-NER) and transcription-coupled repair (TCR). Both sub-pathways consist of ordered multi-step processes, which differ in the early steps, when the DNA lesions are recognized, but converge in the later steps [20–22].

In GG-NER the whole genome is scanned for lesions by the XPC-RAD23B and the UV-DDB (XPE) complexes, which initiate the repair

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process upon recognition of areas with disrupted base-pairing. On the other hand, in TCR only lesions that interfere with progression of transcription are targeted. DNA lesions that block the progression of RNA polymerase lead to increased binding of Cockayne Syndrome B (CSB) to the polymerase and recruitment of additional factors such as Cockayne Syndrome A (CSA), XAB2 and the histone acetyltransferase (HAT) p300 to initiate the repair process [20–22]. CSB shows homology to the SWI/SNF family of ATP-dependent chromatin remodelers. It has a DNA-dependent ATPase activity a nucleosome remodeling activity and is able to bind both DNA and core histones. These features of CSB are thought to support its ability to alter the topology of the chromosomal DNA [23]. CSA contains WD-40 repeats, a motif that is involved in protein–protein interactions [20], which may be important for recruitment of TCR factors to the damaged site.

Upon initiation of the NER mechanism, in both GG-NER and TCR identical processes take place in order to repair the damage by opening and stabilizing the damaged area, excising the damaged strand, and filling the gap. Genetic impairments in NER are associated with UV-sensitive syndromes such as xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD) [20–22].

The involvement of HMGN1 in the cellular response to UV was first identified by the hyper-UV sensitivity of *Hmgn1*$^{-/-}$ mice in comparison to *Hmgn1*$^{+/+}$ mice. Exposure of *Hmgn1*$^{-/-}$ mice to UV-B led to skin deformations such as acanthosis and localized hyperkeratosis [24], which also occur in the mouse model for XP [25]. In agreement with these results, primary cells prepared from these mice are more sensitive to UV-C; the D_{50} (UV dose which kills 50% of the cells) of *Hmgn1*$^{-/-}$ cells was almost five times lower than the D_{50} of their *Hmgn1*$^{+/+}$ littermates. The hyper-sensitive phenotype of the *Hmgn1*$^{-/-}$ cells was rescued by ectopic expression of wild-type HMGN1. However, ectopic expression of mutated forms of HMGN1, which cannot bind nucleosomes or cannot unfold chromatin, failed to rescue the hyper-sensitive phenotype [24]. Thus, the ability of HMGN1 to support cell survival following exposure to UV is mediated by its ability to interact with chromatin and to unfold it.

Detailed analysis of NER in the *Hmgn1*$^{-/-}$ cells revealed a 3- to 4-fold decrease in the rate of TCR in these cells in comparison to *Hmgn1*$^{+/+}$ cells [24]. TCR is associated with unfolding of the chromatin structure within the lesion area, a process that is thought to be associated with increased acetylation of histones [26,27].

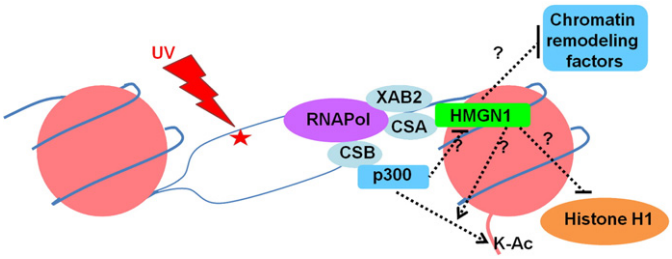

Fig. 1. The involvement of HMGN1 in TCR. UV light induces photolesions (marked in red asterisk) within the genome, which can impose a blockage on transcription. Once elongating RNA polymerase encounters a transcription-blocking photolesion its interaction with CSB is stabilized to initialize the TCR pathway. CSB is required for recruitment of additional factors such as p300, CSA, XAB2 and HMGN1 to initiate the repair of the lesion. p300 may acetylate residues within histone tails in the proximate nucleosomes to the lesion to facilitate chromatin de-compaction and recruitment of repair factors. HMGN1 may be involved in unfolding of the chromatin within the damaged area or in preventing the chromatin from re-folding. HMGN1 can affect the chromatin folding in the following ways: HMGN1 may accelerate the acetylation rate by p300. HMGN1 can prevent binding of histone H1 to the area of the lesion through competition with histone H1 for chromatin binding sites. HMGN1 can inhibit chromatin remodeling factors from re-positioning nucleosomes in the damage area. By these mechanisms HMGN1 may support chromatin de-compaction in the area of the lesion to facilitate repair of the damage. In addition p300 can acetylate HMGN1 directly to reduce the binding of HMGN1 to nucleosomes; a mode of regulation that may serve as a negative feedback loop. The dashed lines indicate hypothesized processes that have not been proven experimentally to occur following induction of DNA damage.

HMGN1 is an essential factor for chromatin unfolding by its ability to increase the activity of HATs such as PCAF [10] and to compete with histone H1 for chromatin binding sites [8,12,13]. Recently, HMGN1 recruitment to sites of TCR was identified. HMGN1 recruitment is dependent on the TCR factor, CSA [28]. It has been hypothesized, but not experimentally proven, that HMGN1 may help to displace nucleosomes that have been re-established behind the stalling RNA polymerase to facilitate regression of the RNA polymerase from the lesion area [21]. Another hypothesis is that HMGN1 may enhance the histone acetylation level in the lesion area [20], a step that may facilitate better accessibility for repair factors after UV exposure (Fig. 1). Recently, HMGN1 was shown to inhibit the function of several chromatin remodeling factors [14]. Thus, it is also possible that HMGN1 could prevent re-positioning of nucleosomes in the damage area by inhibition of chromatin remodeling factors. In summary, HMGN1 activity is important for the repair of DNA lesions following exposure to UV light. The recruitment of HMGN1 to the damage site suggests that it may have a direct role in the repair process, however the exact mechanism how HMGN1 accelerates the repair of the DNA damage is still to be found.

3. Role of HMGN1 in the cellular response to IR

Exposure of living organisms to IR leads to multiple types of DNA lesions including the double-stranded breaks (DSB), which are a dangerous insult for the stability of the genome. Formation of DSB in the genome leads to activation of a tightly regulated cascade of events termed DNA damage response (DDR), which controls the cellular response to the damage. In the DDR, the ternary protein complex MRN is the first recruit to the damage site. It facilitates the recruitment and activation of the major transducer of the damage signal, the ataxia-telangiectasia mutated (ATM) kinase. In parallel MDC1 and 53BP1, which have a role in the activation of ATM, are also recruited to the damage site. Another hallmark of the damage site is phosphorylation of the histone H2A variant H2AX on Ser-139 (in human) to form the γH2AX [29,30]. Phosphorylation of H2AX is mediated by the kinases ATM and DNA-PK following exposure to IR. Activated ATM phosphorylates numerous additional substrates and by that regulates their activity. The substrates of ATM are involved in all cellular aspects relevant to DNA damage response including sensing the damage, repair of DNA lesions, control of cell cycle progression, apoptosis, regulation of gene expression and more [31,32].

The cellular response to IR includes major changes in the organization of the chromatin both locally (at the damage site), as well as globally. Among the local changes are de-condensation of the chromatin [33] possibly by recruitment of chromatin re-modeling complexes such as SWI/SNF [34,35], phosphorylation of histones H2AX and H2B, acetylation of histone H4 on multiple sites [36], ubiquitylation of histone H2A and H2B [37–39], incorporation of Lys-56 acetylated histone H3 to the chromatin [40] and changes in the organization of the heterochromatin localized protein HP1 [41,42]. In addition, global de-condensation of the chromatin was found to occur through phosphorylation of KAP-1 by ATM [43]. At later stages of the repair process, specific chromatin remodeling complexes, such as INO80, are recruited to the damage site to reverse the damage-induced changes [36]. Considering that substantial chromatin re-organization occurs following exposure to IR, it would be expected that ubiquitous chromatin architectural proteins, such as the HMGNs, would be also involved in the cellular response to IR.

The importance of HMGN1 for the cellular response to IR was first identified by the hyper-sensitivity of *Hmgn1*$^{-/-}$ mice to IR; 12 months following exposure to IR the mortality rate of *Hmgn1*$^{-/-}$ mice was more than twice the mortality rate of their *Hmgn1*$^{+/+}$ littermates. The higher death rate of the *Hmgn1*$^{-/-}$ mice was associated with high incidence of lymphomas. Similar hyper-sensitivity to IR was found also in primary cells, which were prepared from these mice. The hyper-

sensitive phenotype of the *Hmgn1*$^{-/-}$ cells was rescued by ectopic expression of wild-type HMGN1. In contrast, ectopic expression of a point-mutated HMGN1, which cannot bind nucleosomes, failed to rescue the hyper-sensitive phenotype [44]. This indicates that the ability of HMGN1 to support cell survival following exposure to IR is mediated by its interaction with chromatin. The defective ability of the *Hmgn1*$^{-/-}$cells to stop at the G2/M checkpoint [44] suggested that HMGN1 may have a role in the activation of the ATM pathway, which controls the G2/M checkpoint response.

Indeed, recently we were able to show that HMGN1 is required for the activation of ATM following IR treatment. Activation of the ATM kinase was measured by the autophosphorylation levels of ATM as well as by the phosphorylation levels of several of its substrates. These two assays revealed a 2- to 3-fold reduction in ATM activation in *Hmgn1*$^{-/-}$ cells in comparison to their *Hmgn1*$^{+/+}$ littermates. The faulty activation of ATM in the *Hmgn1*$^{-/-}$ cells was rescued by ectopic expression of wild-type HMGN1, but not by ectopic expression of a point mutated HMGN1, which cannot bind nucleosomes [45]. Thus, in a similar manner to the IR hypersensitivity phenotype of the *Hmgn1*$^{-/-}$cells [44], the ability of HMGN1 to support activation of ATM is mediated by the interaction of HMGN1 with chromatin. The DSB sensing factors 53BP1, MDC1 and the MRN complex, which are involved in the activation of ATM, were activated correctly in the *Hmgn1*$^{-/-}$cells, suggesting that HMGN1 affects the ATM itself.

More detailed analysis of ATM activation revealed that HMGN1 also modulates the binding of ATM to chromatin [45]. Induction of ATM binding to chromatin after formation of DSB is well established and is associated with its activation [46]. ATM retention to chromatin was compared between *Hmgn1*$^{-/-}$cells and *Hmgn1*$^{+/+}$ cells using biochemical and *in situ* assays, which were developed by Andegeko et al. [46]. With the biochemical assay, the ATM was extracted in a step-wise manner using increasing buffer stringency to distinguish between chromatin unbound protein and chromatin bound protein. In the *in situ* assay, the free ATM was washed away from the nucleus by detergents before the fixation and only then the chromatin-bound ATM, which was left inside of the cells, was immunostained. Significantly, ATM binding to chromatin in *Hmgn1*$^{-/-}$cells was 3-fold higher than in the *Hmgn1*$^{+/+}$ cells both before and after exposure to IR [45]. It seems that in the absence of HMGN1, ATM interacts with chromatin incorrectly prior to any induction of DSB, and to a greater degree following induction of DSB. This faulty interaction of ATM with chromatin prior to the IR-induced damage appears to inhibit the activation of ATM following DSB formation.

In search for the mechanism by which HMGN1 affects the activation of ATM, evidences for direct interaction between the two proteins were not found. HMGN1 is not recruited to the damaged areas and does not co-localize with ATM. However, ATM activation is dependent on HMGN1 ability to bind chromatin. Therefore, we explored a possible indirect mechanism, in which HMGN1 affects the activation of ATM through modulation of chromatin organization [45]. HMGN1 was previously shown to induce acetylation of Lys-14 in histone H3 (H3K14Ac) [9] and to enhance the activity of PCAF, the major HAT that acetylates histone H3 on Lys-14 [10,47]. Therefore, the possibility that HMGN1 affects ATM through modulation of histone acetylation was tested. Indeed, exposure to IR leads to the activation of PCAF [48,49] and to a global increase in the levels of H3K14Ac, in a HMGN1 dependent manner [45]. In order to establish the importance of HMGN1-dependent histone acetylation to the activation of ATM, histone deacetylase (HDAC) inhibitors were used. Addition of HDAC inhibitors to cells reduces the deacetylation rate of histones, and by that leads to an increase in the levels of histone acetylation. Significantly, treatment with HDAC inhibitors reduced the chromatin retention of ATM in *Hmgn1*$^{-/-}$ cells and increased the activation of ATM in these cells to similar level as in the *Hmgn1*$^{+/+}$ cells [45]. Taken together, it appears that HMGN1 modulates the levels of H3K14Ac, both before and following exposure to IR possibly through enhancement of PCAF activity. Through this mechanism, it regulates the nuclear organization of ATM. Moreover, the HMGN1-dependent nuclear organization of ATM prior to induction of DSB is crucial for proper activation of ATM and for the survival of the cells (Fig. 2).

The notion that chromatin organization plays a role in the activation process of ATM was initially based on experiments, in which increased auto-phosphorylation levels of ATM were induced by interference with the structure of the chromatin fiber without breaking it [50]. In these experiments the structure of the chromatin fiber was modified either by salt, by the intercalating agent chloro-

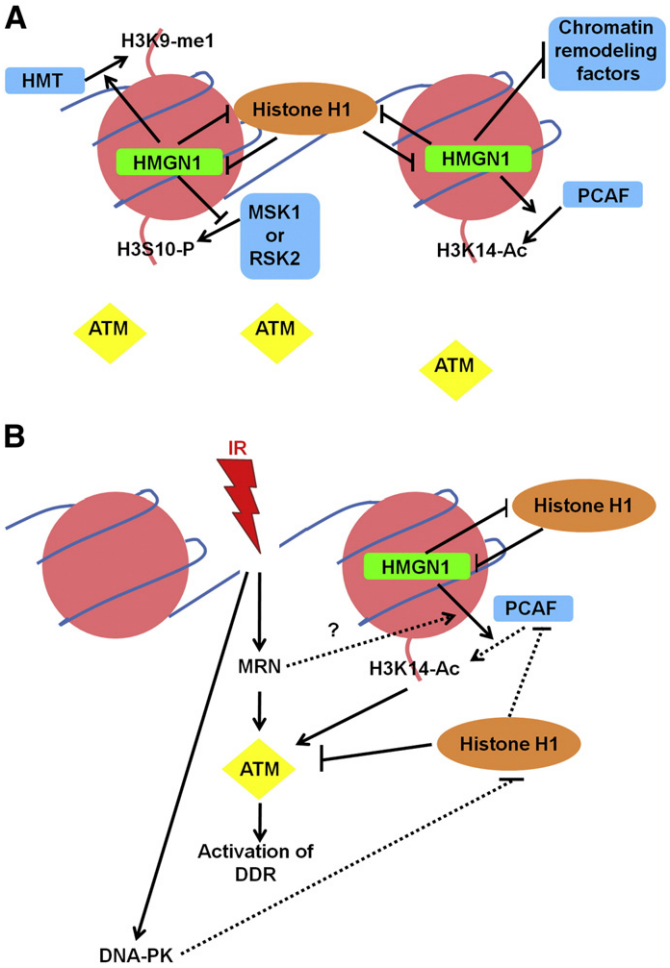

Fig. 2. The involvement of HMGN1 in response to DSB. (A) The importance of HMGN1 to the nuclear organization of ATM under steady state conditions. Prior to any damage, the HMGN1 affects the nuclear organization of the ATM through modulation of the chromatin structure by three possible mechanisms. The first is enhancing the activity of several histone modifiers such as PCAF while inhibiting the activity of other histone modifiers such as MSK1 and RSK2. The second is direct competition with histone H1 for chromatin binding sites. The third is inhibition of chromatin remodeling factors. Through these modes of action HMGN1 may prevent incorrect retention of ATM to the chromatin fiber. Therefore, HMGN1 ensures full potential of activation for the ATM upon formation of DSB. (B) The importance of HMGN1 to the cellular response to DSB. Once DSB are formed the MRN complex is recruited to the damage sites. The MRN complex activates the ATM kinase, which transduces the damage signal and activates the DDR. In addition there is a global increase in acetylation of histone H3 on Lys-14, which is dependent on HMGN1 and probably also on PCAF. Without HMGN1-dependent global chromatin re-organization, the activation of ATM is reduced by 2- to 3-fold. The mechanism of induction of PCAF or other HAT in response to the DSB is not known and may be dependent on the MRN complex. Histone H1 is capable of inhibiting PCAF activation as well as the global DDR. HMGN1 may prevent histone H1 interference with the DDR by direct competition for chromatin binding sites. Histone H1 binding to chromatin may be inhibited by phosphorylation by DSB-activated DNA-PK. The dashed lines indicate hypothesized processes, which have not been proven experimentally to occur following induction of DNA damage. HMT, histone methyl transferase.

quine or by a HDAC inhibitor. Only recently a mechanistic insight into this process has been gained through the HMGN1 knock-out mice [45]. In this mechanism, regulation of histone modifications by HMGN1 along with modifying enzymes dictates the interaction of ATM with the chromatin fiber and the potential of ATM activation following DSB formation.

4. Role of HMGN1 in cancer progression

Cancer progression is thought to be dependent on the accumulation of mutations that change the transcriptional profile of the cell to support its escape from the tight regulation of cell cycle progression [51]. Improper responses to different types of DNA lesions that were detected in *Hmgn1*$^{-/-}$ cells and mice may lead to accumulation of mutations in the genome, which then accelerates the progression of the disease. In addition to the global effects of HMGN1 in the cellular response to DNA lesions, HMGN1 has also local roles in the transcriptional control of proto-oncogenes and tumor suppressor genes.

HMGNs have a role in transcriptional control by affecting the structure of the chromatin fiber. Recruitment of HMGNs to specific promoters may be achieved through their incorporation into various multiprotein complexes [52] or through their direct interaction with transcription factors such as estrogen receptor α (Erα), serum responsive factor (SRF) [53] and PITX2 [54]. Once localized to specific promoter regions, HMGNs can modulate histone modifications [53] and localization of specific nucleosomes [14,55] to shape the chromatin in that region. Under some circumstances, regulation of chromatin remodeling by HMGNs can lead to repression of transcription [53,54], while under other circumstances it can lead to activation of transcription [55]. HMGN1 was shown to bind the promoter of the proto-oncogene *fosB* and to repress its expression by inhibiting histone H3 phosphorylation on Ser-10 (H3S10P) [9]. Other proto-oncogenes and pro-metastatic genes, which are up-regulated in *Hmgn1*$^{-/-}$cells, are *c-fos*, *BCL3* [9] and *N-cadherin* [56]. In agreement with these results the pro-apoptotic gene NGFr is down-regulated in *Hmgn1*$^{-/-}$cells [9]. On the other hand there are proto-oncogenes, which are down-regulated in *Hmgn1*$^{-/-}$cells, such as *JunB* and *c-Jun* [9]. Thus, HMGN1 regulates the transcription of multiple genes involved in tumor progression, mostly, in a way which may suppress the development of cancer (Fig. 3).

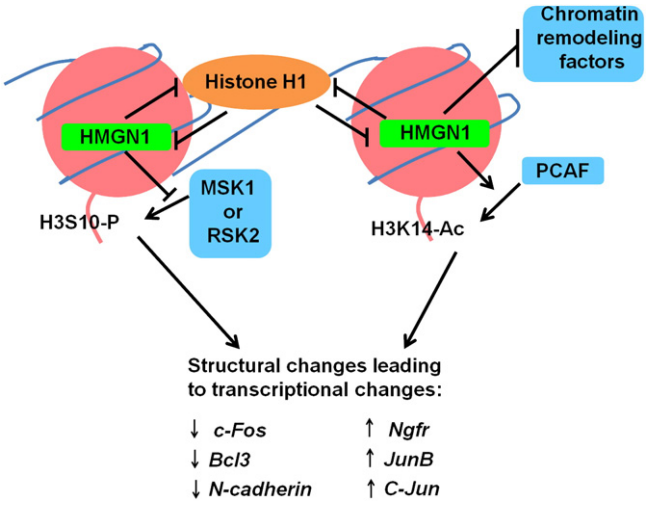

Fig. 3. The involvement of HMGN1 in cancer progression. HMGN1 can modulate the chromatin structure by three mechanisms. The first mechanism is enhancing the activity of several histone modifiers such as PCAF while inhibiting the activity of other histone modifiers such as MSK1 and RSK2. The second mechanism is direct competition with histone H1 for chromatin binding sites. The third mechanism is inhibition of chromatin remodeling factors. Through these modes of action HMGN1 is able to regulate the chromatin structure in promoter regions leading to activation or repression of cancer-related genes.

Taken together the global effects of HMGN1 on DDR and its local effects on the transcriptional control of cancer-related genes it is highly probable that HMGN1 is involved in repression of cell proliferation and tumor progression. Indeed, *Hmgn1*$^{-/-}$mice generates two times more spontaneous tumors than their *Hmgn1*$^{+/+}$ littermates. Primary cells from *Hmgn1*$^{-/-}$mice proliferate faster than cells prepared from their *Hmgn1*$^{+/+}$ littermates and reach senescence after more passages. Moreover, transformed *Hmgn1*$^{-/-}$cells, which were injected into nude mice, induced tumors 5.6 more often than transformed *Hmgn1*$^{+/+}$ cells [44]. In agreement with these results, HMGN1 expression is reduced by 2-fold in highly metastatic breast cancer cells in comparison to low metastatic cells [57]. Overall, through supporting repair of DNA lesions and involvement in transcriptional control of cancer-related genes HMGN1 seems to be important for repression of cancer formation.

5. Perspective

HMGNs are chromatin architectural proteins which until lately were considered to be transcription co-regulators. However, in recent years their role in DNA repair and cancer progression has been established primarily by using HMGN1 knock-out mice [24,44,45]. These studies suggest that the archetype of the HMGNs family, HMGN1, has characteristics of a tumor suppressor gene.

HMGNs act inside the cell in a network of chromatin architectural proteins that compete with histone H1 for nucleosome binding sites [12,13]. Therefore, if HMGN1 supports repair of DNA lesions, it would be expected that histone H1 will have an opposite effect on the repair of DNA lesions. Indeed, recent studies suggest that histone H1 has an inhibitory effect on repair of DNA lesions. Histone H1 was shown to suppress repair of DSB *in vitro* when present in high concentrations. This suppression was released by phosphorylation of histone H1 by DNA-PK, a DSB-activated kinase [58,59]. In mouse embryonic stem cells, reduced amounts of histone H1 led to increased activation of the DDR pathways and to increased rate of homologous recombination [60]. In contrast to these reports, the knock-out of one of the H1 isoforms, H1R, in avian cells increased the cellular sensitivity to the genotoxic agent methyl-methanesulfonate (MMS) [61]. However, the majority of these studies suggest that histone H1 has an inhibitory effect on the DDR pathways.

The above observations suggest that the balance between the relative amounts of HMGNs and histone H1, as well as their chromatin binding abilities, may be important for the regulation of the cellular response to DNA damage. Before occurrence of any damage, sufficient amounts of HMGN1 are important for proper nuclear organization of ATM. Organization which is important for the future activation of ATM following DSB formation. Following exposure to IR, which introduces DSB, binding of HMGN1 to chromatin appears to be critical for induction of epigenetic changes that regulate the proper activation of ATM and the cellular response to the damage [45]. Since histone H1 competes with HMGN1 for chromatin binding sites [12,13], it has the potential to inhibit the activity of HMGN1, which is necessary for the activation of ATM. In addition, histone H1 is also capable of inhibiting histone acetylation by PCAF [62], the same enzyme which is activated by HMGN1 [10,47]. Thus, histone H1 can inhibit HMGN1 activity from at least two different angles, direct chromatin binding and modulation of PCAF activity. In order to overcome the inhibitory effect of histone H1, DNA-PK may phosphorylate histone H1 [58], a modification which is thought to interfere with the ability of histone H1 to bind chromatin. By reducing the chromatin binding capability of histone H1, sustained binding of HMGN1 to chromatin may be achieved and inhibition of PCAF activity may be prevented (Fig. 2).

Following exposure to UV light, recruitment of HMGN1 to the damaged sites by the TCR pathway may help to unfold the chromatin. Unfolding of chromatin within the TCR sites by HMGN1 may be achieved by two mechanisms: removal of histone H1 from the area or

induction of epigenetic changes in the area of the damage. It was suggested that HMGN1 may enhance the levels of histone acetylation in the lesion area by recruited HATs, such as p300 [20]. It should also be noted that p300 can acetylate HMGN1 on several residues, leading to weakening of HMGN1 interaction with nucleosomes [63]. Therefore, p300 may participate in a negative feedback loop to regulate the activity of HMGN1 in the damaged area (Fig. 1). However, it is still to be established whether p300 acetylates HMGN1 or histones *in vivo* in the damaged areas.

In addition to HMGN1, HMGN2 and 5 may be involved in cancer progression. Recently it has been found that in avian cells, disruption of the HMGN2 gene leads to increased cellular sensitivity to UV light. The D_{50} of HMGN2 null cells was 2.5–3.5 times lower than the D_{50} of wild-type cells [64]. Thus, suggesting that HMGN2 may have a role in modulation of the chromatin structure during the process of NER.

Recently, the expression of HMGN5 (previously termed NSBP1) [65] was found to be elevated by 4-fold in highly metastatic breast cancer cells compared to low metastatic cells [57]. In mice, overexpression of HMGN5 in the uterus was associated with the development of uterine adenocarcinoma [66]. Unlike the other HMGNs, HMGN5 contains a long acidic C-terminal portion that facilitates its direct interaction with histone H1 [67]. In addition, the amount of HMGN5 in the cell was estimated to be 10-fold lower than that of HMGN1 or 2 [65]. These differences between HMGN5 and HMGN1 suggest a different mode of action for these two proteins, which may account for the possible positive role of HMGN5 in cancer progression.

Studies on metastasis inhibiting factors and peptides suitable for targeting tumors identified HMGN2 as a potential therapeutic tool for cancer. In the search for tumor invasion-inhibiting factors in bovine liver extracts a peptide of 21 amino acids, which is identical to the C'-terminal part of HMGN2, was identified [68,69]. This peptide, which was termed invasion-inhibiting factor 2 (IIF2) [68], inhibited the migration of several cancer cell lines *in vitro* [69–71]. The inhibitory effect of IIF2 was specifically on the migration rate of the cells rather than on their growth rate or their adhesion properties [69,71]. In experiments in mice, injection of IIF2 inhibited the formation of lung metastasis by 50–60% [69]. The inhibition ability of IIF2 was increased by conjugation it to albumin. This modification stabilized the peptide and resulted in the inhibition of lung metastasis by up to 86% [70,72].

Interestingly, screening of phage-displayed cDNA libraries for peptides that selectively bind tumor cells, identified a peptide identical to the N'-terminal part of HMGN2. This 31 amino acids peptide, which corresponds to the NBD of HMGN2, selectively binds tumor cells both *in vitro* and *in vivo*. Upon binding the tumor cells the peptide is internalized and accumulated in the nucleus of the cells [73]. When the HMGN2 peptide was coupled to a toxin and injected into nude mice bearing tumors from HeLa cells, it led to a significant and selective shrinkage of the tumors [74]. These results raise the possibility of using two different parts of the HMGN2 protein in cancer treatment. The N'-terminal part has the potential to be used as a tool for targeting cytotoxic drugs into tumor cells, and the C'-terminal part might be used as a metastasis inhibitory drug.

In summary, HMGNs appear to have roles in multiple stages of cancer progression from response to and repair of lesions in the DNA through transcriptional regulation of cancer-related genes as well as the potential of HMGN2 to serve as cancer therapeutic molecule. Further research of these aspects should be a promising direction to deepen our understanding of cancer formation and progression.

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