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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].

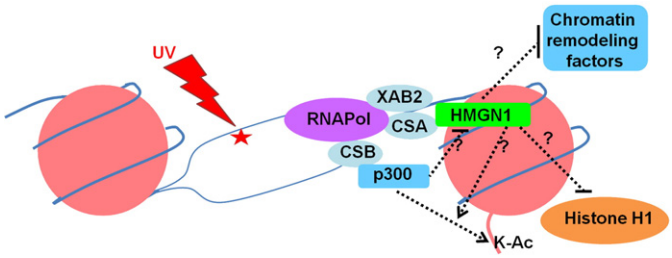

Fig. 1. The involvement of HMGN1 in TCR. UV light induces photolesions (marked in red asterisk) within the genome, which can impose a blockage on transcription. Once elongating RNA polymerase encounters a transcription-blocking photolesion its interaction with CSB is stabilized to initialize the TCR 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-