“DECIPHERING HMGB1: ACROSS A SPECTRUM OF DNA AND NUCLEOSOME DYNAMICS”

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by

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Dedicated to my parents, sisters and nephew …..

For their endless love, support and encouragement

CERTIFICATE

This is to certify that the thesis titled "**Deciphering HMGB1: Across a spectrum of DNA and nucleosome dynamics**", submitted by **Mr. Ishu Gupta** to the Indian Institute of Technology Delhi for the award of the degree of "**Doctor of Philosophy**" is a record of the Bonafide research carried out by him, which has been prepared under my supervision and guidance in conformity with the rules and regulations of the Indian Institute of Technology Delhi, India. The results prescribed in it have not been submitted in part or full to any other University for the award of any degree or diploma.

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ABSTRACT

HMGB1 is most abundant nonhistone nuclear protein that has been thoroughly studied for its functions beyond the nucleus, especially in the cytoplasm, where it serves as an autophagy mediator, and in the extracellular matrix, where it operates as an inflammatory molecule. Nonetheless, the complete range of its roles within the nucleus is still not well comprehended. This *in vitro* research sought to address this gap by examining the binding properties of HMGB1 with different types of DNAs, nucleosomes, and chromatin. The research discovered that HMGB1 shows varying binding affinities, displaying a particularly high affinity for modified DNA structures like triplex and bulge DNA. This implies that HMGB1 might be important in detecting and reacting to DNA damage or structural irregularities. Moreover, the study emphasized that HMGB1 binding disrupts nucleosome remodelling by the CHD7 chromatin remodeller, indicating that HMGB1 could impact chromatin accessibility and remodelling activities, potentially influencing transcriptional regulation. The research also showed that HMGB1 attaches to the linker DNA between nucleosomes, possibly affecting chromatin structure and function. These results offer fresh perspectives on the diverse functions of HMGB1, potentially going beyond its established roles in inflammation and autophagy to encompass significant contributions to gene expression, viral replication, and genetic disorders like CHARGE syndrome, where alterations in the CHD7 gene disrupt normal chromatin remodelling. This research consequently paves the way for investigating HMGB1's functions in the nucleus, possibly resulting in innovative treatment strategies for disorders associated with chromatin dysregulation.

सारांश

HMGB1 सबसे प्रचुर मात्रा में पाया जाने वाला नॉनहिस्टोन परमाणु प्रोटीन है जिसका नाभिक से परे, विशेष रूप से साइटोप्लाज्म में, जहां यह एक ऑटोफैगी मध्यस्थ के रूप में कार्य करता है, और बाह्य मैट्रिक्स में, जहां यह एक सूजन अणु के रूप में कार्य करता है, इसके कार्यों के लिए गहन अध्ययन किया गया है। बहरहाल, नाभिक के भीतर इसकी भूमिकाओं की पूरी श्रृंखला अभी भी अच्छी तरह से समझ में नहीं आई है। इस इन विट्रो शोध में विभिन्न प्रकार के डीएनए, न्यूक्लियोसोम और क्रोमैटिन के साथ एचएमजी1 के बंधन गुणों की जांच करके इस अंतर को संबोधित करने की कोशिश की गई। शोध से पता चला कि HMGB1 अलग-अलग बाध्यकारी समानताएं दिखाता है, जो ट्रिपलक्स और उभार डीएनए जैसी संशोधित डीएनए संरचनाओं के लिए विशेष रूप से उच्च समानता प्रदर्शित करता है। इसका तात्पर्य यह है कि HMGB1 डीएनए क्षति या संरचनात्मक अनियमितताओं का पता लगाने और उन पर प्रतिक्रिया करने में महत्वपूर्ण हो सकता है। इसके अलावा, अध्ययन में इस बात पर जोर दिया गया कि HMGB1 बाइंडिंग CHD7 क्रोमैटिन रीमॉडेलर द्वारा न्यूक्लियोसोम रीमॉडलिंग को बाधित करता है, यह दर्शाता है कि HMGB1 क्रोमैटिन पहुंच और रीमॉडलिंग गतिविधियों को प्रभावित कर सकता है, संभावित रूप से ट्रांसक्रिप्शनल विनियमन को प्रभावित कर सकता है। शोध से यह भी पता चला कि HMGB1 न्यूक्लियोसोम के बीच लिंकर डीएनए से जुड़ जाता है, जो संभवतः क्रोमैटिन संरचना और कार्य को प्रभावित करता है। ये परिणाम HMGB1 के विविध कार्यों पर नए दृष्टिकोण पेश करते हैं, संभावित रूप से सूजन और ऑटोफैगी में इसकी स्थापित भूमिकाओं से परे जाकर जीन अभिव्यक्ति, वायरल प्रतिकृति और चार्ज सिंड्रोम जैसे आनुवंशिक विकारों में महत्वपूर्ण योगदान शामिल हैं, जहां CHD7 जीन में परिवर्तन सामान्य क्रोमैटिन रीमॉडलिंग को बाधित करते हैं। . परिणामस्वरूप यह शोध नाभिक में HMGB1 के कार्यों की जांच करने का मार्ग प्रशस्त करता है, जिसके परिणामस्वरूप संभवतः क्रोमैटिन डिसरेगुलेशन से जुड़े विकारों के लिए नवीन उपचार रणनीतियाँ तैयार होती हैं।

HIGHLIGHTS

The principal findings of this study are summarized below:

- HMGB1 preferentially binds to the altered DNA structures
- HMGB1 has high affinity for DNA sequences that are AT-rich as compared to GC-rich
- The binding of HMGB1 increases as the length of the DNA increases
- Triplex DNA is destabilized by the HMGB1 upon its binding and leads to the formation of duplex DNA
- While in case of G-quadruplex structures, HMGB1 acts as a mild destabilizer
- HMGB1 binds to nucleosome with high affinity and this binding impedes the CHD7 mediated nucleosome remodelling
- HMGB1 also have affinity for chromatin, and it binds in the linker region DNA present between two nucleosomes.

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ABBREVIATIONS & SYMBOLS

%	Percent
~	Approximately
°C	Degree Celsius
β-ME	β-mercaptoethanol
µg	Microgram
µL	Microliter
µm	Micrometer
µM	Micromole
APS	Ammonium persulfate
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
$CaCl_2$	Calcium chloride
CD	Circular Dichroism
CHD	Chromodomain helicase DNA binding
DAMP	Damage assisted molecular pattern
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
DTT	1, 4-Dithiothreitol
EtBr	Ethidium Bromide
EDTA	Ethylenediaminetetraacetic acid
EMSA	Electrophoretic mobility shift assay

FAM	Fluorescein amidite
g	gram
GQ	G-quadruplexes
HCl	Hydrochloric Acid
HMGB1	High mobility group box 1
ISWI	Imitation switch
kb	Kilobase pair
KCl	Potassium chloride
kDa	Kilo Dalton
KH_2PO_4	Potassium dihydrogen phosphate
M	Molar
mg	Milligram
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulfate
mM	Millimolar
MNase	Micrococcal nucleas
Na_2HPO_4	Sodium phosphate dibasic
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NCP	Nucleosome core particle
NER	Nucleotide excision repair
$(NH_4)_2SO_4$	Ammonium sulfate
nm	Nanometer

nM	Nanomole
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PMSF	Phenyl methyl sulfonyl fluoride
rpm	Revolutions per minute
RT	Room temperature
SD	Standard deviation
SDS	Sodium dodecyl sulfate
ssDNA	single stranded DNA
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
TBS	Tris-buffered saline
TEM	Transmission Electron Microscopy
TEMED	N, N, N', N'-Tetramethylethylenediamine
TFO	Triplex forming oligonucleotide
TLR	Toll-like receptor