## CONTENTS

**Synopsis**  
*i-xxi*  

**List of abbreviations**  
*xxii-xxiii*  

**List of tables**  
*xxiv*  

**List of figures**  
*xxv-xxvi*

### CHAPTER 1  Introduction  1-30

1.1 Definitions of Epigenetics  1  
1.2 Basic Unit of Chromatin Template  2  
1.3 Histone Post-translational Modification  4  
1.4 Histone Variants  7  
1.5 Chromatin Structure: Natural Barrier for DNA-mediated functions  9  
1.6 Chromatin and Genome integrity  10  
1.7 DNA damage response  12  
1.8 Chromatin Structure and Modifications in DNA DSB Repair  13  
1.9 Histone Modifications in DNA DSB Repair  14  
1.9.1 Histone phosphorylation  
1.9.2 Histone acetylation  
1.9.3 Histone methylation  
1.9.4 Histone Ubiquitylation  
1.10 Double Strand Break (DSBs) repair  19  
1.10.1 Chromatin Modifications during Non-Homologous End Joining Repair  
1.10.2 Chromatin Modifications during Homologous Recombination Repair  
1.11 Pre-existing histone modifications and their role in DDR  23  
1.12 Dynamics of histone modifications during cell cycle  23  
1.13 Paradox: Histone Modifications, Cell Cycle and DNA damage  25  
1.14 H3Ser10 phosphorylation: Dual role in interphase and  28
mitosis
1.14.1 H3Ser10 phosphorylation during interphase
1.14.2 H3Ser10 phosphorylation during Mitosis

CHAPTER 2  Aims and objectives

CHAPTER 3  Materials and methods
3.1 Cell Culture
3.2 Trypsinization and sub-culturing
3.3 Synchronization of cells in different phases of cell cycle
3.3.1 Synchronization of cells in G0/G1 phase
3.3.2 Synchronization of cells in S, G2/M and pro-metaphase
3.4 Inhibitor treatment
3.5 Exposure of cells to DNA damaging agents
3.5.1 Radiation
3.5.2 Chemical agents
3.6 Cell viability assay
3.6.1 Trypan Blue Exclusion Assay
3.6.2 MTT assay
3.7 Cell Survival by Colony formation assay
3.8 Cell cycle analysis by FACS
3.9 DNA damage analysis by comet assay
3.10 Localization of histone modifications by Immuno-fluorescence microscopy
3.11 Extraction of histones from cell lines
3.12 Fractionation of total soluble protein and chromatin bound histones
3.13 Protein Estimation by Lowry’s Method
3.14 Resolution of total soluble protein and histones by PAGE
3.14.1 SDS-PAGE of total soluble protein and histones
3.14.2 Coomassie staining
3.14.3 Ammoniacal Silver nitrate staining
3.14.4 Two dimensional SDS-AUT gel electrophoresis of core histones
3.14.5 ‘SDS-silver’ staining of AUT-PAGE
3.14.6 Two-dimensional AUT-SDS PAGE of total histones
3.15 Western hybridization
3.15.1 Electrophoretic transfer of histones from SDS-PAGE
3.15.2 Electrophoretic transfer of histones from AUT-PAGE
3.16 MNase digestion assay
3.17 Mono-nucleosomes prep. and Co-immunoprecipitation
3.17.1 Preparation of nuclei
3.17.2 Purification of mononucleosomes
3.17.3 Mononucleosomal Immunoprecipitation Assay
3.17.3.1 Preparing 50% slurry of protein-G sepharose beads
3.17.3.2 Pre-clearing of lysate
3.17.3.3 Immunoprecipitation
3.18 Chromatin fractionation
3.19 In silico prediction of MSK1 and MKP-1 interaction with native H3 peptide and its PTMs
3.19.1 Homology modeling of MKP-1 and MSK1 structures
3.19.2 Refinement of crystal structure of 14-3-3ζ with native H3 peptide and its posttranslational modifications
3.19.3 Molecular association of MSK1 with histone H3 and its PTM modified structure
3.19.4 Molecular association of native MKP-1 with histone H3 and its PTM modified structure

CHAPTER 4 Results
4.1 Histone Profiling of WRL68 and HepG2 Cell lines
4.2 Cell sensitivity assay at clinically relevant and lethal dose of IR
4.3 Time dependent analysis of γH2AX and cell cycle profile following irradiation of G1-enriched cells
4.4 Decrease in phosphorylation of H3Ser10 and its restoration in response to IR induced damage in G1-enriched cells
4.5 Decrease of H3 Serine 10 phosphorylation is specific to G1 phase of cell cycle
4.6 Decrease of H3 Serine 10 phosphorylation is
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.7</td>
<td>Predominantly from H3.3 variant in G1-enriched cells</td>
</tr>
<tr>
<td>4.8</td>
<td>Reduction of H3Ser10P level is independent of DNA damaging agents and tissue origin in response to DNA damage in G1-enriched cells</td>
</tr>
<tr>
<td>4.9</td>
<td>Decrease in H3Ser10 phosphorylation in G1 cells is associated with deacetylation of histone marks K9, K14 and K56 on H3</td>
</tr>
<tr>
<td>4.10</td>
<td>Phosphorylation of H2AX and dephosphorylation of H3Ser10 are marks on a same mono-nucleosome</td>
</tr>
<tr>
<td>4.11</td>
<td>Reduction of H3Ser10P level is independent of DNA damaging agents and tissue origin in response to DNA damage in G1-enriched cells</td>
</tr>
<tr>
<td>4.12</td>
<td>Decrease in H3Ser10 phosphorylation in G1 cells is associated with deacetylation of histone marks K9, K14 and K56 on H3</td>
</tr>
<tr>
<td>4.13</td>
<td>Phosphorylation status of H3Ser10 in G1 phase cells is correlated with phosphorylation of MAP kinases and their de-phosphorylation by MKP-1 in response to irradiation</td>
</tr>
<tr>
<td>4.14</td>
<td>In silico prediction of MSK1 and MKP-1 interaction with native H3 peptide and its posttranslational modifications (PTM)</td>
</tr>
<tr>
<td>4.15</td>
<td>Reversible phosphorylation of H3Ser10 is mediated through dynamic balance between MKP-1 and MSK1 in G1 phase</td>
</tr>
</tbody>
</table>

**CHAPTER 5** Discussion 95-105

**CHAPTER 6** Summary and Conclusion 106-109

**CHAPTER 7** Bibliography 110-122

**CHAPTER 8** Appendix 123-136
8.1A Cell cycle distribution in G1 enriched population as depicted in Figure (Fig 4.3 and 4.5A)
8.1B Cell cycle distribution in G1 enriched population as depicted in Figure (Fig 4.4 and 4.5B)
8.2 Cell cycle distribution G1, S and Pro-M phase arrested cells as depicted in (Fig 4.7A)
8.3 Cell cycle distribution G1, S and G2/M phase arrested cells as depicted in (Fig 4.7B)
8.4A Cell cycle distribution in G1 enriched population as depicted in Figure (Fig 4.9A)
8.4B Cell cycle distribution in G1 enriched population as depicted in Figure (Fig 4.9B)
8.5 Cell cycle distribution in G1 enriched population of multiple cell lines as depicted in Figure (Fig 4.10)
8.6 Cell cycle distribution in G1 enriched population as depicted in Figure (Fig 4.15 C)
8.7 Line diagram for the secondary structures of MKP-1 and MSK1
8.8 Histone H3 peptide from PDB: 2C1J was modified by phosphorylation of Ser10 and acetylation of Lys9 and Lys14
8.9 Full-length loop structure of histone H3 peptide from PDB
8.10 The Ligplot of the MSK1 and histone H3 docked complexes to analyze hydrophobic interactions
8.11 Ligplot of the MKP-1 and histone H3 docked complexes to analyze hydrophobic interactions
8.12 Cell cycle distribution in G1 enriched population as depicted in Figure (8.21)
8.13 Major List of equipments

CHAPTER 9

Publications

List of Publications
Published manuscripts