## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synopsis</td>
<td>1</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>14</td>
</tr>
<tr>
<td>List of Figures</td>
<td>16</td>
</tr>
<tr>
<td>List of Tables</td>
<td>19</td>
</tr>
<tr>
<td><strong>Chapter-1: Introduction</strong></td>
<td>21</td>
</tr>
<tr>
<td><strong>Chapter-2: Review of literature</strong></td>
<td>26</td>
</tr>
<tr>
<td>2.1 Human papillomavirus</td>
<td>27</td>
</tr>
<tr>
<td>2.1.1 Functions of papillomavirus proteins</td>
<td>29</td>
</tr>
<tr>
<td>2.1.2 HPV life cycle and pathogenesis</td>
<td>29</td>
</tr>
<tr>
<td>2.1.3 Current cervical cancer therapies</td>
<td>31</td>
</tr>
<tr>
<td>2.2 Human papillomavirus E2 protein</td>
<td>33</td>
</tr>
<tr>
<td>2.2.1 HPV18 E2-induced apoptosis</td>
<td>34</td>
</tr>
<tr>
<td>2.2.2 Structure and function of typical death-fold domain</td>
<td>37</td>
</tr>
<tr>
<td>2.2.3 Structure and function of HPV E2 protein</td>
<td>43</td>
</tr>
<tr>
<td>2.3 HtrA2 – An overview</td>
<td>46</td>
</tr>
<tr>
<td>2.3.1 Caspase-dependent mechanism</td>
<td>47</td>
</tr>
<tr>
<td>2.3.2 Caspase-independent apoptosis</td>
<td>48</td>
</tr>
<tr>
<td>2.3.3 Alterations in cancer</td>
<td>50</td>
</tr>
<tr>
<td>2.3.4 Structural details</td>
<td>52</td>
</tr>
<tr>
<td>2.3.5 Mechanism of activation</td>
<td>53</td>
</tr>
<tr>
<td><strong>Aims and Objectives</strong></td>
<td>55</td>
</tr>
<tr>
<td><strong>Chapter-3: Methods and Materials</strong></td>
<td>57</td>
</tr>
<tr>
<td>3.1 Cloning, expression and purification of recombinant proteins</td>
<td>58</td>
</tr>
<tr>
<td>3.2 Animal cell culture</td>
<td></td>
</tr>
<tr>
<td>3.2.1 Routine maintenance of cell lines</td>
<td>71</td>
</tr>
<tr>
<td>3.2.2 Transient transfection</td>
<td>73</td>
</tr>
<tr>
<td>3.2.3 Live cell confocal imaging</td>
<td>74</td>
</tr>
<tr>
<td>3.2.4 Preparation of whole cell lysate</td>
<td>74</td>
</tr>
<tr>
<td>3.2.5 Protein estimation using Peterson method</td>
<td>75</td>
</tr>
<tr>
<td>3.2.6 SDS-PAGE</td>
<td>75</td>
</tr>
<tr>
<td>3.2.7 Western blotting</td>
<td>76</td>
</tr>
<tr>
<td>3.2.8 Cell death assays</td>
<td>77</td>
</tr>
<tr>
<td>3.3 Protein-protein interaction analyses</td>
<td></td>
</tr>
<tr>
<td>3.3.1 Pull-down assay</td>
<td>79</td>
</tr>
<tr>
<td>3.3.2 Co-immunoprecipitation</td>
<td>80</td>
</tr>
</tbody>
</table>
3.3.3 Isothermal titration calorimetry 80
3.3.4 Surface plasmon resonance 81

3.4 Biophysical characterization
3.4.1 Circular dichroism spectroscopy 83
3.4.2 Fluorescence spectroscopy 83

3.5 Protein oligomerization and size characterization
3.5.1 Size-exclusion chromatography 90
3.5.2 Glutaraldehyde cross-linking 91
3.5.3 Dynamic light scattering 91

3.6 Serine protease enzymatic assay 92

3.7 Molecular modeling, docking and simulation
3.7.1 Types of interactions 94
3.7.2 General steps for molecular modeling, simulation and docking 96

Chapter-4: Human papillomavirus E2 protein 98

4.1 Understanding protein-protein interactions involved in non-death-fold E2-induced apoptosis 102
4.1.1 Experimental procedures

4.1.2 Results
4.1.2.1 Mapping minimal binding region 106
4.1.2.2 In silico prediction of E2-procaspase-8 binding interface 109
4.1.2.3 Identification of procaspase specific binding site on E2 111
4.1.2.4 Identification of E2 specific binding site on procaspase-8 117
4.1.2.5 Monitoring the effect of interface E2 mutant on cell death 119
4.1.2.6 E2-FADD interaction analysis 120

4.1.3 Discussion 122

4.2 Molecular details of DED assembly and its role in death-fold mediated caspase-8 activation 126
4.2.1 Introduction

4.2.2 Experimental procedures 128

4.2.3 Results
4.2.3.1 Structural basis of DED-DED interaction 134
4.2.3.2 Molecular evidence of procaspase-8 DED assembly 136
4.2.3.3 Molecular basis of FADD DED self-association 144
4.2.3.4 Mechanism of FADD – procaspase-8 assembly 150

4.2.4 Discussion 156
### 4.3 Biochemical and biophysical characterization of E2 transactivation domain

<table>
<thead>
<tr>
<th>4.3.1</th>
<th>Introduction</th>
<th>162</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.2</td>
<td>Experimental procedures</td>
<td>164</td>
</tr>
<tr>
<td>4.3.2 Results and Discussion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.3.2.1 <em>In silico</em> analysis of E2 TAD dimerization</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>4.3.2.2 <em>In vitro</em> characterization of wild-type and mutant E2 TAD</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>4.3.2.3 Unfolding studies</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>4.3.2.4 E2 TAD homo vs. E2 TAD – DED heterodimerization</td>
<td>179</td>
<td></td>
</tr>
</tbody>
</table>

### Chapter-5: Human serine protease HtrA2

| 5.1 | Introduction | 182 |
| 5.2 | Experimental procedures | 184 |
| 5.3 | Results |  | |
| 5.3.1 Elucidating the role of IAPs in activation of HtrA2 |  | |
| 5.3.1.1 Determination of the proteolytic activity of HtrA2 in presence of IAPs | 189 |
| 5.3.1.2 Disruption of the XIAP–HtrA2 interaction prevents protease activation | 190 |
| 5.3.1.3 Interaction analysis | 193 |
| 5.3.2 Determining specificity and catalytic efficiency of HtrA2 |  | |
| 5.3.2.1 Steady-state kinetic parameters for HtrA2 | 196 |
| 5.3.2.3 Comparison of the catalytic efficiency upon N- and C-termini induced activation | 198 |
| 5.3.3 Delineating the global conformational changes following IAP binding |  | |
| 5.3.3.1 Monitoring conformational changes using *in silico* approach | 202 |
| 5.3.3.2 Structural evaluation of peptide bound and unbound HtrA2 | 203 |
| 5.4 Discussion | 206 |

### Chapter-6: Concluding remarks

|  | 211 |

### Chapter-7: Future perspective

|  | 217 |

References

|  | 220 |

Publication Reprints