CHAPTER-1

Introduction
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In maintaining homeostasis, multicellular organisms tightly couple the rate of cell proliferation and cell death. Disruption of this fine balance due to altered regulation of apoptosis (programmed cell death) leads to several life-threatening diseases such as neurodegenerative disorders and cancer. Cancer, which is characterized by a breakdown in the cellular apoptotic machinery, might occur due to alteration of structural and functional properties of critical proteins in the apoptotic pathway and hence interaction between pro- and anti-apoptotic proteins is closely related to the genesis and progression of this deadly disease.

Tremendous progress in apoptotic research has occurred toward understanding the classical caspase-dependent apoptotic pathways with an aim at targeting them for disease intervention. However, complexity of cancer biology draws interest in identifying alternative mechanisms that can promote cell death. Recently, there have been reports of novel extrinsic adaptor-independent and caspase-independent mechanisms of apoptosis. These unique mechanisms are mediated by proapoptotic proteins, human papillomavirus E2 and serine protease HtrA2 respectively [1-3]. Therefore, characterizing the structural and functional properties of these proapoptotic proteins as well as identifying their binding partners will not only delineate their biological functions but will also help understand the apoptotic pathway better and the role of these proteins in cell death and cancer.

**Part-I: Structural and functional characterization of human papillomavirus E2 protein**

Human papillomaviruses (HPVs) are causative agents of cervical cancer which is the second largest cause of death in women worldwide, and is even a bigger challenge in developing countries such as in India due to their economic and social impediments. Out of hundred different human genotypes that have been identified, some such as HPV16 and 18 are associated with different stages of cervical cancer and are termed high risk types. On the other hand, the low risk
viruses, such as HPV6 and 11 cause benign warts and are rarely associated with malignant progression.

Papillomaviruses encode eight major proteins that regulate different viral functions. The early protein E2 acts as a central regulator of viral life-cycle regulating viral gene expression, replication as well as mitotic partitioning of viral genome, and thereby represents a pivotal factor for both the productive cycle and persistent infections by HPVs. Apart from these functions, several groups have demonstrated myriad of other functions that are independent of E2 binding to viral genome such as NFκB activation, induction of apoptosis or regulation of host cell cycle [4]. In particular, proapoptotic activity has been demonstrated for the high risk E2 proteins such as HPV16 and 18 [5, 6]. Although, the exact mechanism of apoptotic induction by E2 is not fully understood, recent literature on HPV18 E2 suggest its involvement in direct interaction with pro-caspase-8 of external apoptotic pathway which might eventually lead to pro-caspase activation and hence initiation of caspase cascade [7, 8].

The extrinsic apoptotic pathway is triggered by ligation of cell-surface “death receptors” followed by formation of multiprotein death-inducing signaling complex (DISC). The DISC comprises oligomerized death receptors such as Fas, the adaptor protein FADD (Fas associated death domain), pro-caspase-8, and cellular FLICE – like inhibitory proteins (cFLIPs). In case of Fas mediated signaling, Fas ligand binds to Fas receptor leading to receptor trimerization followed by binding of FADD to the receptor through its death domain. FADD then interacts with pro-caspase-8 or -10 through homotypic interactions involving death effector domains (DED) of the partners which activate caspase-8 or -10 eventually leading to apoptosis. Interestingly, E2-procaspase-8 interaction has been proposed to bypass the requirement of upstream adaptor proteins which are essentially required for DISC formation, thereby representing a novel adaptor-independent caspase activation pathway. Based on these evidences we hypothesized that E2 influences external cell death pathway by interacting with pro-caspase-8 which might lead to a change in its conformation or stability in turn enhancing its capability to oligomerize and hence
promoting apoptosis. Therefore, our goal was to delineate the mechanism of this novel interaction between high-risk HPV18 papillomavirus E2 and procaspase-8 and how it aids in E2-induced apoptosis using interdisciplinary approach. This information would provide a comprehensive picture of the novel adaptor-independent mechanism of procaspase-8 activation and hence establish a model for E2-induced apoptosis in high risk HPV types. It might also be utilized in future studies to design E2 analogs so as to modulate procaspase-8 activation and hence apoptosis.

**Part-II: Structural and functional characterization of serine protease HtrA2**

HtrA2 (High temperature requirement A2) protein belongs to a unique family of serine proteases that are conserved from prokaryotes to humans. The most elaborately studied protein in this family is *E. coli* periplasmic protein DegP/HtrA that has a dual chaperone and temperature-dependent protease activity [9]. To date four human homologs (HtrA1-HtrA4) of DegP have been identified of which HtrA2 has proapoptotic activity while very little information is available on other human HtrAs [10].

HtrA2 undergoes maturation by autocatalytic N-terminal processing resulting in processed ~36 kDa protein that comprise an N-terminal domain, a serine protease and a PDZ domain (protein-protein interaction domain that primarily binds to the C-terminus of interacting proteins). Moreover, mature HtrA2, has an N-terminal IAP-binding motif (IBM) with which it interacts with the BIR domain of inhibitor of apoptosis proteins (IAPs) such as XIAP, cIAP1 and cIAP2 and relieves the inhibition on active caspases thus promoting apoptosis [11, 12]. Although, HtrA2 has primarily been identified as an IAP-binding proapoptotic protein, its other functions such as caspase-independent induction of apoptosis and serine protease activity are poorly characterized. Recent studies have identified a few antiapoptotic binding partners/substrates of HtrA2 such as PEA-15 suggesting its proapoptotic and proteolytic functions might converge [13]. Moreover, recently it has been observed that the IAPs are also substrates of HtrA2 although the binding
regions for these two proteins are completely different (N-terminal tetrapeptide for IAPs and C-terminal PDZ for PEA-15).

Looking into its complex trimeric three-dimensional structure and trying to account for its low protease activity and narrow substrate selectivity, researchers hypothesized a model which suggests intricate PDZ-protease coordination, rearrangement in their relative orientations and huge conformational changes at PDZ-protease interface are prerequisites for peptide binding and substrate cleavage by HtrA2 [14]. Although PDZ acts as a regulatory domain in all the members of HtrA family, uniqueness of HtrA2 is manifested by its ability to bind subset of proteins, such as IAPs, through its N-terminus and subsequently cleave them [3, 15]. This phenomenon emphasizes multiple modes of HtrA2 activation and regulation, the precise mechanism for which remains to be elucidated. Therefore, our aim was to delineate the structural correlates of HtrA2 activation as well as to develop a universal model for its mechanism of action. A clear understanding of the structural determinants of HtrA2 mediated substrate recognition and cleavage will help define ways of regulating its functions.