4.2 Conclusions

Fas associated death domain (FADD) is a pivotal signaling component of programmed cell death. FADD plays an important role in embryonic development, cell cycle regulation, inflammation, immunity, necroptosis and autophagy apart from apoptosis. Activation of death receptor signaling allows interaction of FADD with procaspase-8/10 to form DISC and further execution of downstream signaling of apoptosis. The death receptor apoptosis negatively regulated by anti-apoptotic protein cFLIP. Interestingly, FADD provides a common platform for binding of procspase-8 and cFLIP to direct cell death or survival signals. In the present study we unravelled the cellular dynamics of FADD in regulation of cell death and survival signaling.

The following salient finds were achieved from this study:

- Expression of FADD was found low as compared to cFLIP in different origin of cancer and transformed cells. The results indicate that low expression of FADD and elevated expression of $cFLIP_L$ in cancer and transformed cells restrict apoptosis. In contrast, the overexpression of FADD activates cascade of apoptotic signaling with simultaneous downregulation of $cFLIP_L$.
- Moreover, the over expression FADD and knockdown of $cFLIP_L$ challenge the mitochondrial integrity and pulverize the membrane potential by altering the expression of Bcl-2 and cytochrome c to facilitate extrinsic and intrinsic signaling of apoptosis.
- Tumor Necrosis Factor-α (TNF-α) canonically induces the activation of NF-κB and associated gene product cellular FLICE-like inhibitory protein ($cFLIP_L$) to promote cell survival. The present study delineates a novel molecular mechanism of FADD in regulation of NF-κB activation. The results suggest that, induced expression of FADD abrogates the endogenous expression of anti-apoptotic protein cIAP2 and stabilizes RIP1 protein to hinder the activation of NF-κB. Moreover, FADD promotes JNK1 dependent ubiquitination of $cFLIP_L$ to induce apoptosis, independent of TNF-α response. Thus, FADD shows enormous potential to act on the core pro-survival machinery of NF-κB and serves as an important functional component in apoptosis signaling.
- Autophagy plays a cytoprotective role against cell death during cell proliferation in cancer and provides microenvironment for cell survival by availing them nutrition. In

this study it was found that the induced expression of FADD suppresses the autophagic stress and directs the cells for apoptotic death. Moreover, the selective knockdown of $cFLIP_L$ accumulates autophagic stress, but induced expression of $cFLIP_L$ mask this effect. Thus, a balanced expression of FADD and $cFLIP_L$ determines fate of cell survival or survival.

- The activation of inflammatory cytokines provides an additional line of defense to cancer cells against apoptosis. It was noticed that induced expression of FADD suppresses LPS induced activation of NF-κB and the expression of pro-inflammatory cytokines IL-1β. Moreover, the induced expression of FADD regulates the canonical inflammasome activation to restrict the processing and maturation of IL-1β.
- The apoptotic and anti-inflammatory competency of FADD was evaluated with targeted delivery of FADD protein within cancer cells. The human FADD was cloned and expressed to conjugate with cell permeable peptide (CP). It was noticed that the conjugated CP-FADD successfully translocates across the membrane and initiates apoptotic cell death in cancer cells. Moreover, CP-FADD effectively suppresses the expression of inflammatory cytokine IL-1β. In addition, the apoptotic efficacy of CP-FADD conjugate was comparable to the conventional apoptosis inducers.

This study delineates a novel molecular mechanism of FADD mediated programmed cell death signaling in cancer cells, which could offer a great potential for cancer therapeutics in the future.