Cancer is a major health issue and leading cause of deaths globally. It can be defined as a complex genetic disease involving uncontrolled cell proliferation with potential to metastasize to other body parts leading to secondary tumor growth. Metastasis is the major cause of deaths among cancer patients. According to WHO report the cancer associated death will rise over 11 million by 2030, if remains uncured. Therefore, it is alarming to explore the cancer progression mechanisms and development of new anti-cancer drugs. In the past decade, the use of Drosophila cancer models has become more popular among the researchers to understand human cancer development and large scale anti-cancer drugs screening. The conserved signaling pathways, lower genetic redundancy, short-life cycle and ease of maintenance make the Drosophila an excellent model organism.

The present thesis is devoted to understand the cancer progression mechanisms and screening of novel Isatin-derivatives in scribble (scrib) knockdown induced cancer in Drosophila wing imaginal discs. Scrib is a tumor suppressor gene and cell-polarity regulator and its loss of function is associated with many forms of human cancers including breast cancer, cervical cancer, pancreatic cancer, colorectal cancer, skin cancer, etc. Drosophila scrib (Dmscrib) gene encodes a protein of relative molecular mass of 195K, consists of 16 LRR (Leucine-Rich Repeats) and 4 PDZ (PSD-95, ZO-1 and Disc large) domains. DmScrib shows overall 37% homology with human Scrib. The LRR and PDZ domains, both are important for Scrib protein localization, protein-protein interactions and regulation of cell proliferation. Also, Scrib has been recognised to interact with major signalling pathways like JNK, Hippo, Wnt and Notch involved in human cancer progression. Hence, to further explore the Scrib loss of function induced cancer progression mechanisms following objective were undertaken:

1. To induce epithelial tissue specific tumor by knocking-down scribble (tumor suppressor gene) and identification of signaling mechanisms leading to cancer progression in the fly.
2. To investigate mitochondrial dysfunction and its role in scribble loss of function (RNAi) driven tumor in epithelial wing imaginal tissue.
3. To screen the anti-tumor activity of Isatin-derivatives on scribble knockdown induced tumor.
Abstract

Objective 1, Objective-2 and Objective-3 have been comprehensively described in chapter-3, Chapter-4 and Chapter-5 respectively of the thesis.

The chapter-1 deals with the introduction and comprehensive review on cancer genetics, cancer associated pathways, role of cell-polarity regulators like Scrib in cancer progression, cell competition as tumor promoting or suppressing factor, role of mitochondrial dysfunction in cancer progression, Isatin derivatives as anti-cancer agents, *Drosophila* cancer models and use of *Drosophila* in anticancer drugs screening etc. Chapter-2 contains detail materials and methods used and followed to carry out the respective experiments. Chapter-3 deals with *scrib* knockdown induced tumor progression in wing imaginal disc. As previously reported, the *scrib* loss of function in clones of small area was conk out and removed by neighbouring wild type cells through cell-competition. While, *scrib* loss in relatively larger area resists their apoptotic removal and grows further and over proliferate. But, how these larger area *scrib* abrogated cells resist apoptotic removal have not been investigated. We have shown that *scrib* knockdown cells in larger area were ‘group protected’ and ‘undead cell’ behaviour of the core cells, away from cell competition, support the growth of *scrib* knockdown cells at the interface of wild type. Further, we have found that the JNK pathway mediated activation of canonical (Wnt/β-catennin) and non-canonical (Wnt/Ca²⁺) signalling leads to over proliferation of the cells. Moreover, the *scrib* knockdown tumor showed highly disrupted actin-cytoskeleton and loss of cell polarity as revealed by phalloidin staining and a cell polarity marker, Dlg, specific, immunostaining. Also, the cytoskeleton disruption and loss of cell polarity were JNK mediated. Moreover, genetically down regulation of JNK pathway restores the actin cytoskeleton arrangements, cell-polarity and wing disc morphology near to wild type. In chapter 3 it has been concluded that, *scrib* loss in major area of wing imaginal discs leads to cancer progression via JNK-Wnt pathway mediated cell-competition.

The chapter 4 is focused on to explore the status of mitochondrial dysfunction/dynamics in *scrib* knockdown tumor. Here, we found that scrib knockdown tumors were highly depolarized along with elevation of total ROS level in comparison to wild type. The mitochondrial membrane potential and total ROS level were measured by Mito Tracker Red and H₂DCFDA staining respectively.
Further, the mitochondrial morphology was analysed by the use of Mito-GFP, a GFP labeled mitochondria expression line. In *scrib* knockdown tumor tissue, mitochondria were highly fragmented, punctate and clustered around the nucleus while, the wild type mitochondria were elongated, well networked and evenly distributed around the nucleus. Moreover, the qRT-PCR analysis also revealed the altered expression of mitochondrial-dynamics regulators in this tumor. The mitochondrial fission promoter, Drp-1, levels were highly elevated while the marf, fusion promoter level was reduced in tumor cells compared to wild type. Further, the reduced expression and mislocalization of Parkin, as confirmed by real time PCR and immunostaining, also suggested the defective mitophagy events responsible for fragmented mitochondrial accumulation around the peri nuclear region. The reduced expression of HtrA-2, an apoptosis initiator in cells with defective mitochondria, might be protecting the apoptotic removal of *scrib* abrogated cells. Further, we have analyzed the mitochondrial fragmentation status upon JNK signaling up and down regulation in *scrib* knockdown cells in comparison wild type. Hence, in chapter 4 it is concluded that loss of *scrib* induces mitochondrial dysfunction, disrupted redox homeostasis and altered expression of mitochondrial dysfunctions collectively co-operating towards tumor progression.

In last, the chapter 5 is focused on the screening of Isatin-derivatives (1d, 3a-e) for antineoplastic and antioxidant activities against *scrib* knockdown tumor of wing imaginal discs using *in-silico* and *in-vivo* approaches. First, the molecular docking studies of these Isatin-derivatives were performed and found that these compounds strongly interact with the Human and *Drosophila* JNKs and follow all required parameters for ADMET and Drug likeness parameters. Hence, the *in-vivo* screening was carried out for all the derivatives against *scrib* knockdown tumor and two lead compounds i.e., Br-Isatin (1d) and BHIO (3d), were identified to rescue the tumor more effectively. The 1d and 3d treatment effectively reduced tumor growth as revealed by the restoration of wing discs morphology to near normal and reduction of GFP bearing tumor cells. Isatin derivatives (1d & 3d) treatment restores actin-cytoskeleton arrangements near to wild type morphology and protects from early pupal deaths about 44% and 68% respectively. These derivatives effectively scavenge elevated ROS and restores mitochondrial membrane potential confirming their anti-oxidant activity. Further, the treatment of Isatin derivatives effectively...
reduces active JNK signalling. The reduction of active JNK signaling confirmed the kinase inhibition activity of these Isatin compounds.

Hence, overall, our study concluded that *scrib* loss of function in large area of wing imaginal discs induce tumor growth by evading cell-competition through group-protected core cells via JNK-Wnt signaling activation, along with mitochondrial dysfunctions. And, the Isatin derivatives with anti-neoplastic and antioxidant activities rescue the *scrib* knockdown associated anomalies of *Drosophila* wing imaginal discs.