6. Summary

Lipids have long since been recognized as signaling molecules that have the capacity to trigger profound physiological responses. Arachidonic acid (AA) oxygenation via cyclooxygenase (COX) or lipoxygenase (LOX) pathways generates a number of bioactive eicosanoids like prostaglandins, leukotrienes, lipoxins and hydroxy eicosatetraenoic acids (HETEs), which regulate cell growth and proliferation as well as survival and apoptosis. Arachidonic acid released from membrane phospholipids plays a central role in tumor cell proliferation. The overexpression of COX and LOX has been reported in a variety of cancers, including colon, pancreas, skin, prostate, oral, epidermoid and liver, suggesting a role for the eicosanoids in carcinogenesis. Under physiological conditions, however, LOXs are known to regulate normal proliferation and differentiation of epithelial cells and keratinocytes. 12-R-LOX is essential for the maintenance of epidermal barrier function and in the terminal differentiation of the skin. The role of COX is implicated in the maintenance of cell homeostasis and in the regulation of epidermal cell proliferation in vitro.

Non melanoma skin cancers, including basal cell carcinoma and squamous cell carcinomas, are the most common type of cancers and occur more frequently than any other type of malignancy in the human population. Skin irritation and injury lead to a rapid but transient activation of AA metabolism. An understanding of the enzymatic pathways involved in AA metabolism is therefore important. In recent years, it has become clear that there are multiple forms of AA metabolizing enzymes expressed in skin of mice and humans. Elevated levels of PGE$_2$ have been observed in squamous and basal cell carcinomas of the skin and may correlate with an increased propensity for metastatic and invasive behavior. 12-HETE has been shown as one of the main eicosanoids formed by the
epidermis and with the discovery of large quantities of 12-(R)-HETE in human psoriatic lesions, the epidermal 12-LOX has gained considerable interest.

Although the expression of LOX and COX has been reported in various cancer cell lines limited information is available as to whether LOX and COX metabolites are involved in the regulation of epidermoid cancer cell survival. In the present study we elucidate the role of LOX and COX pathways in epidermoid cancer. To evaluate the role of eicosanoids in epidermoid carcinoma, the expression of AA metabolizing enzymes such as lipoxygenases (LOX) and cyclooxygenases (COX) was analysed in human epidermoid carcinoma cell line (A431). These studies revealed the over expression of 12-R-LOX and COX-2 only in A431 cell but not in the normal NIH3T3 cell line suggesting their possible role in promoting the cell proliferation in cancer cells. Incubation of A431 cells with the metabolites of 12-LOX (12-(S)-HETE and 12-(R)-HETE) and COX-2 (PGE$_2$) promoted the growth, suggesting such a possibility. Further studies involving baicalein (a 12-LOX inhibitor) and celecoxib (a COX-2 inhibitor) showed significant reduction in the thymidine incorporation in A431 cells. These studies thus conclusively demonstrate a role for 12-R-LOX and COX-2 in the regulation of A431 cell proliferation. The mode of cell death induced by baicalein and celecoxib appears to be apoptosis, as revealed by reduction in the Bcl-2/Bax ratio, release of cytochrome c, activation of caspase-3 and PARP cleavage.

The signaling pathways that govern cell proliferation, survival and oncogenesis are of prime interest in cancer biology. Most of the signals associated with cell survival trigger growth factor receptors which activated extra cellular signal-regulated kinase (ERK) and PI3/Akt pathways and thus promote cell growth. In the present study baicalein and celecoxib induced apoptosis in parallel with the inactivation of ERK in A431 cells. The metabolites 12-(R)-HETE, 12-(S)-HETE and
PGE$_2$, on the other hand, increased the p-ERK levels, which might be promoting cell proliferation by downregulating apoptosis.

The AKT signaling pathway has been increasingly documented as a prime determinant of tumor promotion and progression in several cell types, including skin. Recent evidences indicate that PI3K/Akt pathway plays a crucial role in tumorigenesis and tumor progression by promoting cell proliferation and inhibiting apoptosis. In the present study the level of phosphorylated Akt was increased in cells treated with the metabolites of 12-LOX and COX-2 and decreased after treatment with celecoxib and baicalein in A431 cells. The results were further validated by the use of ERK specific inhibitor (PD098059) and PI3 Kinase inhibitor (Wortmanin). PD098059 and Wortmanin pretreatment sensitized the cells to baicalein and celecoxib resulting in increase in caspase-3 activity and massive apoptosis suggesting a role for these two survival pathways in the regulation of cell growth in A431 cells. The metabolites of 12-LOX and COX-2, on the other hand increased the phospho ERK and phospho Akt levels, suggesting the involvement of ERK and Akt pathways in the 12-LOX and COX-2 mediated regulation of growth in A431 cells.

Nuclear factor-$\kappa$B (NF-$\kappa$B) and activator protein 1 (AP-1) are the key transcription factors that orchestrate expression of many genes involved in inflammation, embryonic development, lymphoid differentiation, oncogenesis, and apoptosis. The present study clearly demonstrates a regulatory role for COX-2 and 12-LOX in the activation of NF-$\kappa$B as evidenced by its down-regulation with baicalein and celecoxib and up regulation with 12-(R)-HETE, 12-(S)-HETE and PGE$_2$ treatments. As Activator protein-1 (AP-1) is a downstream target of the ERK pathway and also regulates the expression of cell cycle proteins, we examined the effect of baicalein and celecoxib on the DNA binding and transcriptional activities of AP-1. The present study, clearly demonstrates a regulatory role for COX-2 and
12-LOX in the activation of AP-1 as evidenced by its down-regulation with baicalein and celecoxib and up regulation with 12-(R)-HETE, 12-(S)-HETE and PGE$_2$ treatments.

These in vitro studies on epidermoid carcinoma cell line were extended to animal models by monitoring the growth of A431 xenografts in Swiss mice. Both baicalein and celecoxib reduced the tumor weight in A431 xenografts in Swiss mice. The tumor sections of the animals treated with baicalein and celecoxib showed significant apoptosis as compared to tumors from untreated animals. Further studies on nude mice might provide conclusive evidence on the usefulness of COX-2 and 12-LOX inhibition in the control of epidermoid carcinoma. The molecular mechanisms and signaling pathways involved in the regulation of growth in epidermoid carcinoma by 12-LOX and COX-2 pathways are presented in Figure 33.

Figure 33: Schematic representation of the proposed mechanisms involved in the promotion of growth in A431 cells by 12-LOX and COX-2 pathways and the inhibition in the growth by baicalein and celecoxib.
In summary, the present study reveals that 12-LOX and COX-2 have a critical role in the regulation of growth of epidermoid carcinoma and the combined therapy of inhibitors of 12-LOX and COX-2 may be of potential importance.