2. **Scope and Objectives**

Cancer results from disturbances of cellular signal transduction and data processing at the genetic and epigenetic level. Among these metabolic reactions becoming dys-regulated in the course of tumorigenesis, eicosanoid biosynthesis from arachidonic acid seems to play a critical role (Marks *et al.*, 2000). A large body of evidence indicates that the metabolism of polyunsaturated fatty acids is critically involved in epithelial cancer development. This holds true, in particular, for the COX and the LOX pathways of arachidonic and linoleic acid metabolism as supported by the accumulation of prostaglandins and related products in human and experimentally induced epithelial tumors. Suppression of these pathways has been found to inhibit tumor formation in animal models such as the initiation-promotion approach of mouse skin carcinogenesis (Muller *et al.*, 2002; Marks *et al.*, 2000; Marks *et al.*, 1999). Considerable evidence suggests that LOXs are involved in epidermal tumor development. Compared with normal epidermis, large quantities of 12(S)-HETE (50–60-fold greater) were found in papillomas and carcinomas induced by 7, 12-dimethylbenzanthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) in a mouse skin tumor model (Krieg *et al.*, 1995). In the same study, 12-LOX enzyme activity was elevated 6-fold in papillomas and 3-fold in carcinomas compared with normal tissue (Chang *et al.*, 1993). Recent studies found that epidermal growth factor (EGF) and TPA up-regulate expression of 12-LOX mRNA in the human A431 cell line (Chang *et al.*, 1993; Chang *et al.*, 1992; Tsai *et al.*, 1989). Also, 12(S)-HETE is the predominant metabolic product of metastatic B16 melanoma cells (Liu *et al.*, 1994). 12(S)-HETE overproduction in papillomas may be a mechanism for progression to malignant carcinoma (Steele *et al.*, 1999). Studies on skin cancer in humans are often limited to epidemiological data for self-evident reasons (Buckman *et al.*, 1998). Squamous
cell carcinomas (SCCs) can be very aggressive and metastatic. Currently the primary form of treatment for these types of skin tumors is excision. However, excision of the initial lesion may not be curative because almost 50% of patients with one non melanoma skin cancer lesion develop another tumor within the next 5 yr at the site or adjacent to the site of excision (Wilgus et al, 2003). Standard chemo therapeutic agents used for the treatment of pre-cancerous skin lesions and non-melanoma skin cancers are not completely effective. Data in a variety of cancer types suggest greater efficacy in treating tumors with combination chemotherapies targeting genes which are overexpressed in the tumors (Wilgus et al, 2004).

It was demonstrated that skin tumor promotion caused by ultraviolet B radiation can be decreased up to 89% by celecoxib, a selective inhibitor of COX-2) in hairless mice (Fischer et al, 1999). A similar study showed that Celecoxib can decrease new tumor formation by 44% in mice that already have tumors (Thompson et al, 2001). In a subsequent study, celecoxib in combination with difluoromethylornithine, caused regression of UV-induced skin tumors to a much greater extent than did each compound alone (Fischer et al, 2003; Chun et al, 2004). In animal models, the p12-LOX expression is also found in xenografts of melanoma (Fischer et al, 2002) and in skin tumors developed by an initiation/promotion protocol (Akunda et al 2007; Fischer et al, 1999).

The foregoing studies on the skin cancers are mainly focused on 12-R-LOX, but no comprehensive study was undertaken on the metabolism of arachidonic acid by various LOX and COX pathways. Hence it would be interesting to identify the specific LOX and COX pathways and understand their role in skin carcinogenesis. With this, the present study was undertaken to study the metabolism of AA in human squamous (epidermoid) cancer cell line A431. Based on these studies the specific inhibitors of these pathways, either alone or in
combination, was undertaken to evaluate their effectiveness in regulating the growth and proliferation of A431 cells. Further, studies were undertaken on the detailed molecular mechanism involved in the regulation of growth of A431 cells by the COX/LOX inhibition.

Thus the specific objectives of the present study were:

- To study the expression pattern of LOX/COX genes in Human epidermoid carcinoma cell line (A431) and compare it with normal skin fibroblast cell line (NIH3T3),
- To study the effect of metabolites and inhibitors of specific LOX/COX enzymes on A431 cells,
- To study the detailed molecular mechanisms involved in the regulation of growth of A431 cells by LOX/COX inhibition,
- To evaluate the effects of COX/LOX inhibition in regulating the growth of A431 xenografts in Swiss mice.