INTRODUCTION
Cholesterol homeostasis is one of the significant regulatory events of lipid metabolism in cells. Cholesterol is obtained by the body in two ways; it can be synthesized endogenously within almost all cells from hydroxy methyl glutyryl CoA by the activity of the endoplasmic reticulum bound enzyme HMG CoA reductase. But the prime source of cholesterol is dietary cholesterol. Cholesterol is mainly transported in the blood stream in the form of Low Density Lipoproteins (LDL). LDL consists of an outer phospholipid coat and a protein component mainly ApoB100. The core is almost entirely composed of cholesteryl esters and some free cholesterol. ApoB100 is a 515 kDa protein synthesized in the liver and the intestine. In blood around 70% of the total cholesterol is transported in form of lipoproteins. The total cholesterol content of serum ranges between 150-220 mg/dl in a healthy individual following a 12 hour fast (Goldstein and Brown, 1986). Lipoproteins are classified mainly into four groups based on their densities. Chylomicrons and VLDL have a density between 1.006-1.019. LDL has a density of 1.019-1.063. HDL density ranges between 1.063-1.21.

Cholesterol homeostasis in mammalian cells is controlled by a feedback regulatory system that senses the cholesterol content of membranes and appropriately modulates the transcription of genes required for cholesterol supply. The intracellular biosynthesis & esterification of cholesterol and uptake of extra-cellular LDL are the events of fine tuning process in mammalian cells to maintain intracellular cholesterol homeostasis, which are controlled through complex regulatory circuits. Cellular increase of cholesterol is done by several processes like receptor or scavenger receptor mediated uptake of cholesterol containing lipoproteins, by cholesterol synthesis and hydrolysis of cholesteryl esters by the enzyme cholesteryl ester hydrolase. Decrease is due to efflux of cholesterol from the membrane to HDL via the ABCA -1 (ATP- binding cassette transporter A1) or SR-B1 (class B scavenger receptor B1), esterification of cholesterol by ACAT (acyl – CoA: cholesterol acyl tranferase), and utilization of cholesterol for synthesis of other steroids such as hormones, or bile acids in liver.

LDL that is transported in the circulation enters the cell by way of receptor-mediated endocytosis. LDL binds to a 167 kDa cell surface receptor protein known as
the LDL receptor (LDLR). The LDLR is a type I transmembrane glycoprotein expressed ubiquitously on all cell types except RBCs. Its expression is more pronounced in steroidogenic tissues such as the adrenal cortex and the placenta. The liver also shows a high level of expression of the LDLR due to its role in cholesterol and lipoprotein metabolism. The protein is translated from a 5.3kb polyadenylated mRNA transcribed from the LDLR gene present on chromosome 19p13.3. The gene is comprised of 18 exons and 17 introns and is a mosaic of different domains found in several proteins. These include an EGF precursor homology domain, complement protein C9 homology domain, coated pit localization or clustering domain and a ligand binding extracellular domain (Sudhof et al., 1985).

Schnieder et al. first purified the LDLR from bovine adrenal cortex (Schneider et al., 1980). It has been shown to cluster in restricted regions of the plasma membrane known as coated pits which are responsible for vesicle formation at the time of clathrin coated endocytosis. Mutations in the LDLR result in autosomal dominant familial hypercholesterolaemia (FHC).

Cholesterol homeostasis in cell is largely governed by the cellular level of a cleavable transcription factor called sterol – regulatory element binding protein-2 (SREBP-2). SREBP-2, a resident peptide in the membrane of the endoplasmic reticulum (ER) are tethered to its escort protein called SREBP cleavage activating protein (SCAP). When cholesterol levels are sensed by SCAP to be low, it transports SREBP-2 to the Golgi where SREBP-2 undergoes proteolysis to form an active transcriptional factor. This transcriptional factor then travels to the nucleus where it activates the expression of genes for LDL receptor. Activation by similar mechanism of other transcription factor viz. SREBP1 activates HMG CoA-(3 Hydroxy methyl glutaryl coenzyme CoA) reductase for intracellular cholesterol synthesis. LDL receptor expression is a key determinant of plasma cholesterol level (Brown and Goldstein, 1997, 1999).

The recent studies show that LDLR is highly expressed in the cancer cells (Chen and Hughes-Fullford, 2000). The increased level of cytoplasmic cholesterol, due to more LDL uptake, supports rapid membrane formation by the developing cancer cells (Gal et al., 1982). Some studies also show abundance of Peripheral type Benzodiazepine receptor (PBR) on nuclear membrane of tumor cells (Mukhopadhyay et al., 2006). The subunits of PBR act as cholesterol receptor as
well as cholesterol channel. PBR has been found to be aggressively expressed in human breast cancer cells (Hardwick et al., 1999).

Cholesterol is even found in the nucleus in association with chromatin lipids (Albi and Viola Magni, 2004) but no detailed study has been done so far to elicit the role of this nuclear cholesterol in developing cancers. It is not known whether LDL cholesterol has any role in tumor forming effect by enormous cell proliferation. At least there are reports that in cancer cells the feedback regulation of cholesterol is lost (Chen and Hughes-Fulford, 2001).

Recent studies are immersing with the possibilities of having lipid as one of the energy taking components in the chromatin structure to support cell cycle process or cell proliferation. When the fate of many other lipids in cell nucleus has been well documented; no detailed study, so far, is reported on the role of cholesterol in the cell nucleus towards cell synthesis or cell proliferation. Most of the prevailing studies on cellular cholesterol homeostasis is concerned with atherogenesis and the role of atherogen on intracellular cholesterol homeostasis. This study targets to answer the fate of intracellular cholesterol homeostasis in new cell formation --- a phenomena that causes cell proliferation. Since tumors are rapidly proliferating tissue, normal and/or tumor cell lines are considered to execute the objectives of the study. Transport of cholesterol across the nuclear membrane, intranuclear saturation and any influence of cholesterol on cell cycle proteins towards cell proliferation will be taken into consideration to generate the knowledge whether cholesterol is a partner of cell proliferation process. Various extra cellular inducers and intracellular proteins, which are known to involve in cellular cholesterol homeostasis have been taken into consideration to explore the study. In cancer patients, the LDL level in blood is very low and feedback regulation of cholesterol is lost (Chen and Hughes-Fulford, 2001). This shows two opposite phenomena at the level of LDL receptor functionality. The reason is still unclear and needs to be explored.

Keeping the above facts under consideration, the proposed thesis is planned to compare the feedback regulation of intracellular cholesterol homeostasis and to study its role on cell proliferation in the presence and absence of mitogen as well as carcinogen, with the following objectives:

1. To assess the feedback regulation of LDL receptor by checking the expressions of LDL-receptor, SREBP-2 protein and the rate of LDL uptake by the cells in cell culture system.
2. Evaluation of the cholesterol concentration in cytoplasm and cell nucleus along with the expression of peripheral-type benzodiazepine receptor (a transporter of cholesterol) in the nuclear membrane.

3. To compare the expression of cell cycle proteins e.g. CDK2, cyclin E, with the intracellular cholesterol concentration.

4. To correlate the level of expressions of LDLR / SREBP-2 and LDL uptake profile of cells with the feedback mechanism of LDLR expression, expression profile of cell cycle proteins and extent of cellular proliferation.