Aims and Objectives
The ubiquitous glycosaminoglycan hyaluronan (HA), which is a major constituent of extracellular matrix, has diverse biological roles in vertebrates. These include its role as a vital structural component of connective tissues, formation of loosely hydrated matrices that allow cells to divide and migrate (e.g. during development and metastasis), cell adhesion, inflammation, regulation of multi-drug resistance and intracellular signaling (Tammi et al, 2002). The diverse range of activities of HA results from the large number of HA binding proteins (hyaladherins) that exhibit significant differences in their tissue expression, cellular localization, specificity, affinity and regulation.

The 34 kDa **Hyaluronan Binding Protein (HABP1)** is a member of hyaladherin family that binds with a high specificity to HA. It was originally isolated from rat kidney using HA-Sepharose affinity chromatography and was identified as a glycoprotein containing sialic acid (D'souza and Datta 1985; Gupta *et al*, 1991). HABP1 has been shown to be identical to p32, a protein co-purified with human pre-mRNA splicing factor SF2 (Krainer *et al*, 1991) and the receptor of globular head of complement protein C1q (gC1qR) (Ghebrehiwet *et al*, 1994). Other homologues of HABP1 are Mam33p (Seytter *et al*, 1998), a mitochondrial matrix protein associated with oxidative phosphorylation in *S. cerevisiae* and YL2, an HIV-rev binding murine homologue (Tange *et al*, 1996). Although the exact physiological function of HABP1 remains to be defined, *in vitro* and *in vivo* experiments have proposed it to have a role in macrophage cell adhesion, signal transduction, mammalian reproduction and infection.

HABP1 appears to be a multifunctional protein that interacts with a wide range of biomolecules residing at different subcellular locations. Considerable controversy has surrounded the localization of HABP1 since its cDNA sequence does not predict a traditional
membrane-anchoring domain, and has a probable mitochondrial targeting sequence. The open reading frame of HABP1 encodes a proprotein of 282 amino acids, which is post-translationally processed by the removal of the first 73 amino acids through cleavage between serine$^{73}$ and leucine$^{74}$ resulting in generation of the mature HABP1 of 209 amino acid residues (Honore et al, 1993). The initial 73 amino acids are presumed to contain the mitochondrial targeting sequence. Interestingly, HABP1 has been found to be present in a number of cellular compartments including the mitochondria, nucleus, cell surface and cytoplasm where it interacts with diverse range of other proteins. HABP1 is reported to interact with nuclear proteins including pre-mRNA splicing factor (SF2/ASF), TFIIB, lamin B receptor, CCAAT-binding factor (CBF) and cytoplasmic proteins like ERK of MAP kinase pathway, protein kinase C, cytoplasmic domain of alpha$^{1B}$-adrenergic receptor. HABP1 has been identified as a putative receptor protein for extracellular plasma proteins like high molecular weight kininogen, Factor XII, and C1 component protein C1q. HABP1 also interacts with the components of extracellular matrix like vitronectin and HA indicating its possible role in cell adhesion and motility. Several studies have shown HABP1 to be exclusively mitochondrial. Yeast homologue of HABP1, p30 is reported to play an important role in mitochondrial oxidative phosphorylation (Muta et al, 1997). HABP1 interacts with BH3-only protein Hrk in mitochondria suggesting its possible role in apoptosis (Sunayama et al, 2004). In addition to cellular proteins, HABP1 also interacts with numerous pathogenic proteins like adenovirus core protein V, Epstein Barr virus nuclear antigen-1, Hepatitis C Virus core protein, HIV-1 tat, HIV-1 rev, Rubella virus capsid protein, Listeria monocytogenes surface protein In1B etc., indicative of its possible role under conditions of pathogenic stress (Leeuwen et al, 2001). An extensive study based on immuno-gold electron microscopy of cultured cells
(Raji, CHO, HeLa, human fibroblasts and B-SC-1) as well as rat tissues (adrenal glands, cerebellum, cerebral cortex, heart, kidney, liver, pituitary, pancreas, skeletal muscle, spleen, testis and thyroid) showed HABP1/p32/gC1qR to be primarily located in mitochondria. However, strong reactivity of the protein was also shown to be present in zymogen granules, condensing vacuoles, endoplasmic reticulum and on the cell surface of pancreatic acinar cells, on the cell surface of microvascular endothelial cells in pancreas and kidney, on the cell surface and nuclei of splenic lymphocytes, and acrosomes of developing spermatids in testes (Soltys et al, 2000). These results indicate that HABP1 is primarily a mitochondrial protein that may also localize outside mitochondria in certain cells and tissues. The diverse subcellular localization of HABP1, coupled to its various interacting proteins suggest that it could be a component of the trafficking pathway connecting the nucleus, mitochondria and cytoplasm and the export pathway to the cell surface (Leeuwen et al, 2001). Moreover, HABP1 exhibits structural flexibility which is influenced by the ionic environment under in vitro conditions near physiological pH which may play an important role in its binding towards different ligands (Jha et al, 2003). The crystal structure of HABP1 shows it to be a trimer with a doughnut shaped quaternary structure and a non-crystallographic 3-fold symmetry, without any distinct domains. The trimer has an asymmetric charge distribution along its surface; one side has highly negatively charged residues, while the other side possesses positive polarity. This polarity in charge distribution may have functional implications for the protein (Jiang et al, 1999).

Studies from our laboratory have shown that HABP1 is an endogenous substrate for MAP kinase and phosphorylation of endogenous HABP1 was observed following treatment of J774 cells with PMA. HABP1 is reported to be highly phosphorylated in
transformed fibroblasts and phosphorylation of HABP1 may be involved in cellular transformation and cellular signaling (Majumdar et al, 2002). Ectopic expression of HABP1 in simple eukaryote Schizosaccharomyces pombe leads to morphological aberrations and inhibition in cell growth with the interaction of cell cycle regulatory protein cdc25 (Mallick and Datta, 2005). HABP1 upregulation is shown to be important for cisplatin induced apoptosis. The number of apoptotic cells was shown to reduce significantly if the expression of HABP1 was knocked down using short interfering RNA specific to the HABP1 transcript (Kamal and Datta, 2005). Stable transfectants in cervical cancer cell line HeLa, overexpressing mature and proprotein forms of HABP1 exhibit growth inhibition, morphological changes, and apoptosis induction as shown by increase in percentage of subdiploid population, Bax induction and defective actin polymerization. A reduction in the anchorage independent growth of the stable clones overexpressing HABP1 was observed indicating a rescue from tumorigenesis by HABP1 overexpression (Anupama, 2005).

Constitutive expression of HABP1 in normal fibroblast cell line has shown to inhibit growth, formation of autophagic vacuoles, and induction of apoptosis at 60 hours without media change (Meenakshi et al, 2003). It has been reported from our lab that upon constitutive overexpression of mature HABP1 in fibroblast cell line F111, it accumulates in the mitochondria which leads to generation of reactive oxygen species (ROS), mitochondrial dysfunction and ultimately apoptosis (RoyChowdhury et al, 2008). The above observations indicate an important role of HABP1 in excessive generation of ROS which results in perturbation of cell growth, proliferation and induction of apoptosis.

Liver and intestine provide first and second line of defense against oxidative xenobiotics degradation pathway. A wide range of cytochrome P450 family of oxidative metabolizing enzymes,
conjugating enzymes and several membrane transporters are known to co-ordinate for protecting the body against diverse chemical insults. Human liver cancer line, HepG2 is known to possess many functional xenobiotic-metabolizing enzymes. HepG2 cells have high levels of important protective enzymes such as Mn-superoxide-dismutase and Cu/Zn-superoxide-dismutase, as well as catalase, glutathione peroxidase, glutathione reductase and thioredoxin reductase (Murakami et al, 2002). Glutathione (GSH) is the main non-protein thiol in the mammalian cells which participates in many critical cellular functions, including antioxidant defense storage of cysteine, maintenance of intracellular redox state, and modulation of cell growth. Many studies involving lymphocytes and fibroblasts have established that increased GSH level was associated with an early proliferative response and was essential for cell to enter S phase (Shaw and Chou, 1986; Hamilos et al, 1989). Increased glutathione levels have been reported during proliferation of rat hepatocytes, which stimulates hepatocytes to shift from Go to G1 phase of the cell cycle (Lu and Ge, 1992). The increased GSH levels have been attributed to increased availability of cysteine and induction of y-glutamylcysteine synthetase heavy subunit (GCS-HS). It is known that glutathione (GSH) levels increase in hepatocytes during active proliferation (Huang et al, 2000). It has been shown that GSH levels is increased in human liver cancers as a result of increased expression of GCS-HS and GSH synthetase at the transcriptional level (Huang et al, 2000). Moreover, in liver, HA is primarily metabolized by sinusoidal endothelial cells but the role of liver epithelial cells in this process is elusive.

It is known that overexpression of mature form of HABP1 in F111 cells, leads to generation of oxidative stress and ultimately apoptosis, therefore, it would be interesting to study the effect of HABP1 overexpression in a system where endogenous levels of antioxidants
like GSH are high. Overexpression of mature form of protein may lead to aggregation of protein, improper localization because of absence of localization signals and misfolding which can lead to generation of cellular stress. So, we will be using a full-length HABP1 construct for generation of stable cell line as it is expected to undergo the processing in the similar way as the endogenous protein.

Therefore, in this study, we have chosen an alternative human liver epithelial cancer cell line (HepG2), as a model system to examine the function of HABP1 under conditions of high anti-oxidant levels and to study whether the phenotypical cellular effects (like apoptosis, vacuolation) are specific to cell types of the same organ.

To answer these queries we initiated this project with the following aims and objectives:

1. To generate and characterize a stable human liver cell line overexpressing full-length HABP1 and study the effect of HABP1 overexpression on cell growth and morphology.
2. To study the effects of stable HABP1 overexpression on various cellular parameters like cell adhesion, cell migration, ROS formation, response to anti-cancer drugs and HA distribution.
3. To investigate the cellular pathways that may be modulated as a consequence of stable HABP1 overexpression. The upstream and downstream effectors of the pathways will be explored by microarray analysis.