Summary and Discussion
Hyaluronan or hyaluronic acid (HA) is a high molecular weight, negatively charged, non-sulfated, multifunctional linear glycosaminoglycan present in the extracellular matrix of many tissues in the human body as well as in many other higher organisms. HA was originally regarded as a ubiquitous space filling molecule and a principal structural component of the pericellular matrix. Its immense role in diverse cellular process particularly in cell proliferation, cell motility, migration and cancer-biology is well established. In the last two decades, diverse localization of HA and its enormous regulatory influence on many vital physiological processes make it indispensable for normal development in almost all higher eukaryotes. Though, initially not much emphasis had been given on the nuclear localization of HA, now it seems a physiologically relevant phenomenon in association with nuclear function. A significant increase in HA synthesis occurs just prior to mitosis, enabling cells to become dissociated from surrounding cells and lose adhesion from the surrounding ECM as a preparation for cell-division. The mitotic events in proliferating cells are also associated with increased and rapid metabolism of HA which actually precedes the large cyclin D3 response that controls the formation of the monocyte adhesive ECM.

It is also postulated that HA mediates almost all of its cellular functions via a number of hyaladherins, such as RHAMM, Cdc37, CD44 and others. One of the most important and interesting hyaladherins is our protein HABP1, which is identical to p32/ gC1qR. HABP1/p32/gC1qR is a negatively charged, ubiquitous, multifunctional chaperon protein which has been evolutionarily conserved, synthesized as a 282 amino-acid long proprotein, which possesses a distinct mitochondrial localization signal that gets cleaved to generate 209 amino acid mature HABP1 following its mitochondrial translocation. HABP1 binds strongly with intracellular, cytoplasmic and cell-surface HA. HABP1 has a direct or indirect role in vastly different cellular responses and physiological processes such as cell-
adhesion and proliferation, signal transduction, tumorogenesis, inflammatory reactions, blood coagulation, apoptosis and others. The multifunctional nature of HABP1 may be attributed to its interaction with several proteins of different cellular compartments and thus mediates its diverse cellular responses. It has been reported to interact with a vast array of proteins, critically involved in regulation of cell proliferation and polarity.

To identify the functional characterization of HABP1 in simple eukaryotes, HABP1 was overexpressed in fibroblast and HeLa cells where we observed growth inhibition and apoptosis induction. In continuation, we designed to downregulate the expression of HABP1 using vector based on RNAi technique and examined the phenotypic changes, along with morphological appearance and growth related analysis. Finally, we used the synchronized HeLa cells as a model as it provides a useful tool to study cell cycle dependent events and its regulation. We used several cell cycle blockers and correlated the level of HABP1 and its localization pattern in different stages of cell divisions. The synchronized HeLa cells were treated further with the ligand of HABP1, HA, a known mitogen and examined the level and localization of HBP1, along with the expression of regulatory protein Cdc25 and ERK1.

As presented in Chapter 1, initially we confirmed a 50% reduction in HABP1 level with gene disruption and a significant reduction in growth rate was recorded. HABP1 level is reduced in the cytosol of HeLa cell line, but no signal was detected in the nucleus, suggesting its nuclear exclusion, corroborated by the reduced nucleo-cytoplasmic ratio. An inhibition in G2/M phase and an increase in subdiploid population by was reported using FACS analysis. Apoptosis induction with downregulation of HABP1 was finally confirmed with Bax expression, upregulation of p53, tumor suppressor gene and p21, cell cycle inhibitory protein along with the nuclear translocation of p53. Mdm2, the p53 regulatory protein was visibly downregulated suggesting that apoptosis may be mediated through p53.
dependent pathway. Not only upregulation of the cell cycle inhibitory proteins was observed, but also the expression of AKT and ERK1 activation were reduced, suggesting a blockage of cell survival pathway. However with mitotracker staining, no disruption of mitochondria and no excess generation of ROS were detected.

The previous reports of upregulation of HABP1 in cisplatin induced apoptotic cells and stable fibroblast transfectant expressing HABP1 demonstrate the accumulation of HABP1 in the mitochondria creating mitochondrial dysfunction and apoptosis. The present report confirms that even the downregulation of HABP1 results in apoptotic induction in a p53 dependant pathway suggesting that a balance in HABP1 level is critical in normal cellular processes.

In the synchronized HeLa cells, the level of HABP1 expression was upregulated in G1 phase and reduced in S and G2 phases. Subsequently, the synchronized cells were blocked at different stages of cell cycle using specific blockers, anticancer drugs and thereafter the cellular morphology and HABP1 level and localization profile were studied. HeLa cells blocked at G1 stage showed visibly smaller nucleus whereas nucleo-cytoplasmic ratio was higher in the cells on blocking of S and G2 phase of cell cycle. In association with the morphological change, a significant increase in expression of HABP1 was noted in G1 phase whereas reduction in HABP1 level in S and G2 phases were observed. This has been substantiated by the differential distribution pattern of HABP1 during different stages of cell cycle in HeLa cells treated with different cell cycle blockers. HABP1 was shown to be uniformly expressed and distributed in the cytosol and nucleus in the cells blocked at G1 while excluded from nucleus when the cells were blocked at S and G2 stages. Subsequently a HABP1 coat surrounds the cell during premitotic stage during which the cytoplasm had completely rounded up to divide. Along with HABP1 level, the cell cycle regulatory protein Cdc25B is also shown to be varied in different stages. The treatment of HA,
a mitogen, on synchronized HeLa cells enhanced the cell growth at the level of 50μg and 100 μg. Enhanced expression of HABP1 level was observed at G1 stage and an increased accumulation in Cdc25B levels in G2 phases.

In conclusion, the present work suggests a direct role of HABP1 in regulation of cell cycle progression, as downregulation of HABP1 inhibits cell growth, induces morphological aberration, induction of apoptosis as reflected in the increase in subdiploid population with the expression of Bax, upregulation of p53 and p21 and lowered level of Mdm2 along with the reduced expression of AKT, the cell survival and impaired ERK activation, the indicator of reduced mitogenic activity. Its role in cell cycle progression is apparent as its level is varied during different stages even in synchronized cells. Most interestingly, though the nuclear localization of HA was not appreciated by the scientific community initially and the reliability of extramitochondrial localization of a mitochondrial protein like HABP1 was questioned, it continues to be verified and accepted as with the present observation. Nuclear localization of HABP1 and its profile is similar to its ligand HA, allowing us to speculate that its interaction with other nuclear protein may be responsible to carry out its diverse activity in the nucleus.