Pluripotent stem cells (PSCs) are specialized cells, which have remarkable ability to maintain in an undifferentiated state and are capable of undergoing differentiation to three germ-layer lineage cell types, under differentiation-enabling conditions. PSCs include embryonic stem (ES)-cells, embryonal carcinoma (EC)-cells and embryonic germ (EG)-cells. ES-cells are derived from the inner cell mass (ICM) of day 3.5 blastocysts (mouse). On the other hand, EC- and EG-cells have different source of origin and exhibit some differences in terms of their differentiation abilities and culture requirements. These PSCs act as an ideal in-vitro model system to study early mammalian development and cell differentiation and, they could potentially be used for experimental cell-based therapy for a number of diseases. However, one of the problems encountered is the immune rejection of transplanted cells. For this, immune-matched induced pluripotent stem (iPS)-cells have been derived from somatic cells, by forced expression of a few stemness genes. Although, human PSCs lines are being experimented, their cell-therapeutic potential is still far from being thoroughly tested due to lack of our understanding regarding lineage-specific differentiation, homing and structural-functional integration of differentiated cell types in the host environment. To understand these mechanisms, it is desirable to have fluorescently-marked PSCs and their differentiated cell-types, which could facilitate experimental cell transplantation studies.

In this regard, our laboratory has earlier generated enhanced green fluorescent protein (EGFP)-expressing FVB/N transgenic 'green' mouse: GU-3 line (Devgan et al., 2003). This transgenic mouse has been an excellent source of intrinsically green fluorescent cell types. Recently, we have derived a 'GS-2' ES-cell line from the GU-3 mouse line (Singh et al., 2012). Additionally, we envisaged the need for developing an iPS-cell line from the GU-3 mouse and then use them for studying cell differentiation. Thus, aims of the study described in the thesis are to: (1) develop an experimental system to derive EGFP-expressing fluorescently-marked iPS-cell line from a genetically non-permissive FVB/N mouse strain, characterize the established iPS-cell line and achieve differentiation of various cell types from EGFP-expressing iPS-cell line; (2) to study differentiation phenomenon, in particular to cardiac lineage, using select-cardiogenesis modulators and (3) to assess the gene-expression profiles and signaling system associated with cardiomyocyte differentiation of PSCs.

This thesis is divided into four chapters with the 1st chapter being a review of literature followed

by three data chapters. In the chapter I of the thesis, a comprehensive up-todate review of literature is provided pertaining to PSCs, their classification, derivation strategies especially for reprogramming of somatic cells for iPSC generation, their differentiation potential and characterization, particularly to cardiac lineage. Various molecular regulators involved in cardiac differentiation of PSCs with emphasis on epigenetic regulation involving DNA methylation and signaling pathways involved are described in detail. Subsequently, various approaches used for enhanced cardiac differentiation of PSCs and the therapeutic potential of PSC-derived differentiated cell types to treat disease(s) are discussed.

Chapter-II describes the successful establishment of a permanent iPS-cell line (named 'N9' iPS-cell line) from the non-permissive FVB/N EGFP-transgenic GU-3 'green' mouse. This chapter provides results pertaining to detailed derivation strategy and characterization of the 'N9' iPS-cell line which includes colony morphology, expansion (proliferation) efficiency, alkaline phosphatase staining, pluripotent markers' expression analysis by qPCR and immunostaining approaches and karyotyping analysis. Further, in order to thoroughly assess the differentiation competence of the 'N9' iPScell line, assessment of in-vitro and in-vivo differentiation potential of the 'N9' iPS-cell line by embryoid body (EB) formation and teratoma formation in nude mice and its detailed histological analysis showing three germ layer cell types and their derivatives were performed, followed by the generation of chimeric blastocysts by aggregation method. This established N9 iPS-cell line could potentially offer a suitable model system to study cardiac differentiation along with other established PSC lines such as the GS-2 and D3 ES-cell lines and the P19 EC-cell line.

Following the establishment of the system to study cardiac differentiation of PSC lines, efforts were made to understand the biology of cardiac differentiation of PSCs (wildtype and EGFP-transgenic PSC lines and P19 EC-cell line) using small molecules as
modulators. Data pertaining to this is described in Chapter-III. The possible involvement of epigenetic regulation of cardiogenesis for example, DNA methylation changes in cardiogenesis-associated genes is studied using 5-aza cytidine as one of the chromatin modifiers. In order to understand the cardiac differentiation phenomenon, as a consequence of using 5-aza cytidine in cell culture, it was important to investigate its ability to induce/mediate cardiac differentiation. This involved an assessment by quantitating the cardiac beating phenotype and correlating this with enhanced cardiac-gene expression profiles. Further, DNA methylation regulation of

cardiogenesisassociated genes is described using various DNA methylation analysis techniques. Moreover, the possible involvement of other signaling members in mediating the cardiac differentiation is also studied using the P19 EC-cells. Results pertaining to the above findings are described in detail in the Chapter-III.

Chapter-IV is focused on various efforts made towards investigating the ability of ascorbic acid to enhance cardiac differentiation of mouse ES-cells (GS-2 and D3 lines). Ascorbic acid has been implicated to be influencing cardiogenesis and it is reported to enhance differentiation of various cell types under certain culture conditions. Results pertaining to enhancement of cardiac differentiation of PSCs using ascorbic acid are presented in this chapter. This included assessment by quantitating cardiac beating phenotype and its correlation with enhanced cardiogenesis-associated gene expression profiles. Besides, estimation on the sorted cardiomyocyte population, derived from PSCs was also made using mature-cardiac marker. The possible underlying signaling mechanism involved was also studied in detail, using specific inhibitors for pERK (U0126), integrin signaling (pFAK; PP2) and collagen synthesis (DHP), in order to ascertain their involvement in ascorbic acid-mediated cardiac differentiation of mouse ES-cells. Subsequent to the three data chapters (II-IV), separate sections are provided for ‘Summary and Conclusion’ and for ‘Bibliography’, cited in the thesis. The overall scope of the study has been to understand the basic biology of cardiac differentiation from PSCs (EC-cells, iPS-cells and transgenic and wild-type ES-cells) and to assess, by using certain small molecules, whether PSCs could be coaxed to enhance the differentiation to a particular cell type (cardiac). The data contained in this thesis addresses the above theme.