CHAPTER 1

Introduction: design and rationale of this study
Diabetes mellitus is a chronic disorder that results from a deficiency of insulin. Insulin is produced by the beta-cells of the pancreas and regulates uptake of glucose from the blood into cells. Diabetes mellitus is manifested in two distinct forms: an absolute deficiency of insulin (Type 1) and a relative deficiency of insulin (Type 2) diabetes. Type 1 diabetes mellitus, also known as Juvenile or insulin-dependent diabetes, results from an autoimmune destruction of the beta-cells. Approximately 10% of all diabetics are suffering from Type 1 diabetes. The more common manifestation of diabetes is Type 2 diabetes or non-insulin-dependent diabetes. Type 2 results from insulin resistance, pancreatic beta-cell dysfunction or both. Type 2 diabetic patients are nowadays recommended to change their lifestyle to decrease their symptoms and to avoid too many fluctuations in their glucose levels. There are special lifestyle therapies for these patients. These lifestyle therapies are usually not only successful in decreasing the frequency of episodes of hyperglycaemia but also in decreasing the chance on the associated high blood pressures or high lipid profile. In case the goals are not met, pharmacologic therapy (usually insulin sensitisers) is combined with the lifestyle therapy. In case lifestyle and pharmacologic therapy are not successful insulin therapy is mandatory. A very comprehensive overview of diabetes, its complications and treatment options is given in a supplement of diabetes care (diabetes care. 33, Suppl 1) issued in January 2010. In the following a short review is presented to introduce the experiments given in this dissertation.

Since Type 1 diabetic patients are unable to produce insulin it is necessary to treat these diabetic patients with exogenous insulin. Nowadays most patients are treated with insulin therapy. However, this treatment is not able to regulate the glucose levels on minute-to-minute basis and therefore still exposes the patient to hyperand hypoglycaemia episodes. Long term episodes of hyperglycaemia are associated with various complications such as, cardiovascular diseases (hypertension and/ or dyslipidemia), retinopathy or glaucoma, renal diseases, neuropathy, and diabetic ketoacidosis. The chance on the development of these complications can be decreased by tight glycaemic control by intensive insulin therapy. Unfortunately, however, also intensive insulin therapy is not without risks since it is associated with hypoglycaemic unawareness which is disabling a growing number of patients.
A principally different approach to achieve euglycaemia is to provide the diabetic patient with an endogenous source of insulin by transplantation of endocrine pancreatic tissue. There are two options, i.e. transplantation of the whole pancreas and transplantation of only the islets of Langerhans. The first option, pancreatic organ transplantation, is an established mode of treatment with a world-wide experience of more than 20,000 cases. At present, patient and graft survival rates almost equal those of more conventional grafts such as kidney transplantation. A successful pancreas transplant provides almost normal glucose homeostasis, but it requires major surgery and life-long immunosuppressive medication. The side-effects of life-long immunosuppression in combination with the mandatory major surgery make it doubtful whether this approach will ever be an acceptable alternative for life-long insulin therapy. At present, the majority of research centres restrict themselves to combined pancreas and kidney transplantation in diabetic patients with end-stage renal failure.

Islet transplantation, in contrast to pancreas transplantation, requires no major surgery and there are conceivable approaches that allow for transplantation of islets in the absence of immunosuppression. Approaches currently under investigation are immuno-isolation by encapsulation, immunotolerance, and immunomodulation. The advantage of islet-cell transplantation is that it requires a minimally invasive procedure which can be repeated without major consequences for the recipient. The downside of the method is that it requires 2-4 human donors per recipient. Islets disappear during isolation from the pancreas, but also during and after transplantation. This is not only due to immunological problems, but also because of several viability and metabolic problems due to ischemic periods in the immediate period after transplantation. Recent improvement in this transplantation technology is the administration of non-glucocorticoid immunosuppression (sirolimus, tacrolimus, daclizumab) by the Edmonton-group, which is associated with graft survival in the majority of the transplanted diabetic patients for up to 24 months. This improvement has led to a tremendous growth in the number of research groups involved in human islet transplantation. Nevertheless, due to the limitation of donor shortage islet-transplantation cannot be performed on a world-wide basis.
Therefore, at the moment many research groups are exploring new ways to produce insulin producing cells that can be used for transplantation. One of the technologies that is being investigated is the ability differentiate stem cells into mature beta-cells. Despite the progresses that have been made in the insights and technologies to differentiate stem cells into mature beta-cells a number of challenging questions remain to be answered:

• What are the requirements to manipulate a stem cell to become a fully functional insulin producing beta-cell?
• Which biological factors are involved in the end-stage differentiation of a precursor towards a fully glucose responsive beta-cell?
• How can senescence and genetic disorders like unlimited proliferation in stem cells be avoided?

In order to answer these questions knowledge about the fundamentals of precursor cells is required.

Precursor cells are involved in pancreas development, i.e. during embryogenesis, and during regeneration or growth of the pancreas, i.e. neogenesis. During embryogenesis various phases can be described, i.e. the formation of the primitive ducts, the movement of endocrine and exocrine precursor cells to form islet cell clumps and pre-acini. The final organisation of cells for further differentiation into last stage endocrine and exocrine cells is completed. Precursor cells are not only assumed to be actively involved during embryogenesis, but they are also suggested to be involved during regeneration of the beta-cell mass of the pancreas. During regeneration, new beta-cells can be formed by proliferation of existing beta-cells or by a process called neogenesis, formation of new beta-cells from precursor cells. Moreover, it has been shown that the endocrine pancreas can enlarge the beta-cell mass during increased metabolic demand. This growth is due to proliferation of existing beta-cells and neogenesis. Processes and cells involved in growth of the beta-cell mass can therefore be studied in the pancreas of pregnant animals. The advantage of using pregnancy as a model for pancreatic growth above other models of pancreatic growth, i.e. obesity or beta-cells damage is that the growth of the beta-cell mass during pregnancy is a quick process, which takes place in a well defined time frame. Therefore, in our studies we have used the models of fetal
and neonatal embryogenesis and pregnancy to study the role of precursor cells in development and growth of the pancreas.

**DESIGN AND RATIONALE OF THIS STUDY**

Pancreatic precursor cells have been suggested to have a therapeutic value for the treatment, or even cure, of diabetes mellitus or other pancreas related diseases. Pancreatic precursor cells are responsible for the natural process of pancreas development, but also for regeneration after damage or for natural growth of the pancreas as is taking place for example during pregnancy. Three pancreatic precursor cell populations have been found in the pancreas and several studies showed that these cells play an important role in development of endocrine cells, in particular beta-cells. Although the presence and differentiation potential of these cells have been reported, a detailed description of their localization, phenotypic and genotypic appearance, differentiation capacity, and the potential to contribute to regeneration is still lacking. In the development of the pancreas and of other organ structures the specific proteins nestin, c-met, and c-kit are expressed and some of them had even been proposed to be present on precursor cells.

Therefore, the aim of this thesis was to study the expression of nestin, c-met and c-kit in the embryogenesis and the development and growth of the adult pancreas and the capacity of nestin, c-met and c-kit carrying cell to differentiate toward functional beta-cells.

**Chapter 2** reviews what is known about the different precursor stem cells and the description of the features to fulfill the criteria of being a potential source for the generation of insulin producing cells. At present embryonic stem cells are thought to be the most suitable source for generation of insulin producing cells. Whether pancreatic, thus organ bound precursor cells can play a role in the formation of insulin producing cells via differentiation, remain to be elucidated.

In **chapter 3** we investigated whether cells carrying the c-met and c-kit proteins indeed have some efficacy in stimulating regenerative processes in the adult rat.
pancreas. This was done by infusion of pure, isolated populations of cells in adult rat pancreata previously treated with the beta-cell toxin streptozotocin.

In chapter 4 we performed a histological analysis of nestin, c-met, and c-kit positive cells to investigate the localization of these cells in the endocrine, i.e. the islets, and exocrine, i.e. ductules, exocrine tissue, and blood vessels, compartments in the embryonic, neonatal and physiological growing rat pancreas.

In chapter 5 we investigated whether nestin, c-met, and c-kit positive cells in the neonatal pancreas express molecules that are associated with specific time points in the differentiation process and can therefore be applied as a measure for the differentiation state of the cells. This was done by investigating the expression profile of a number of genes expressed during the early, mid and late phase of differentiation towards endocrine and exocrine cells, and by immunofluorescence detection of their respective proteins.

In chapter 6 we investigated whether isolated c-met and c-kit positive cells from neonatal rats have the profile of pancreatic precursor cells and whether these cells have the potential to differentiate into insulin producing cells after ex vivo manipulation. This was done by positive flow cytometric selection of these cells from neonatal pancreata and subsequent culturing of these cells under beta-cell specific growth factors.

In chapter 7 the results described in this thesis are summarised and discussed, both from a technical point of view as well as from a cell biological point of view.
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