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Hypoxia-inducible Factor-1 (HIF-1) in Pancreatic Cancer Cell Aggressiveness and Therapeutic Resistance and the Potential Role for Pancreatic Endocrine Cells in Islet Transplantation

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ABSTRACT

Tissue hypoxia results from an inadequate supply of oxygen (O_2) that compromises biologic functions in various tissues, including both normal and malignant. It is known that a heterodimeric transcription factor hypoxia-inducible factor-1 (HIF-1) mainly mediates this critical adaptation. It regulates the expression of more than 100 genes encoding key factors in cell proliferation and survival, glucose metabolism, invasion, angiogenesis and erythropoiesis. In this thesis I have investigated the roles of HIF-1α in endocrine β-cells of pancreatic islets (paper I) and in exocrine ductal epithelial cells (paper II and III) as well as their surrounding stromal cells (paper IV) of pancreatic ductal adenocarcinoma (PDAC).

Pancreatic islet transplantation is a biological replacement strategy for diabetes mellitus, however the benefits of islet transplantation are only short-term due to the lost grafts over time. Several strategies have been explored to improve the efficacy of islet transplantation. We previously reported a combination of islet preculture and recipient treatment with exendin-4 improved the metabolic outcome of a suboptimal number of rat islets transplanted to diabetic athymic mice. In paper I, we aimed to investigate mechanisms of effects of exendin-4 on islet function and viability in the rodent islet transplantation model with special focus on HIF-1α expression. Our data revealed that short-term preculture with exendin-4 followed by recipient treatment improved the outcome of both free and macroencapsulated islet grafts due to a larger surviving endocrine cell volume. Furthermore, this study has indicated for the first time that the protective effects of the glucagon-like peptide-1 (GLP-1) receptor agonist exendin-4 may be mediated via the HIF-1 pathway.

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignant disease with fatal prognosis. It is characterized by a rapid progression, early metastasis, diagnosis at an advanced stage, and a limited response to chemotherapy and radiotherapy. Since tumour hypoxia is strongly associated with tumour propagation, malignant progression, and resistance to therapy, the study of HIF-1α has emerged a central issue in tumour physiology and cancer therapy. In paper II, we aimed to investigate the relationship of excess glucose, glucose reprogramming and cell migration in human PDAC cells with respect to HIF-1α expression. We found that excess glucose induced HIF-1α expression, increased ATP contents and stimulated migration in MiaPaCa2 pancreatic cancer cells. In addition, non-hypoxic factors contributed to this action in MiaPaCa2 cells as well. The drug-resistant nature of PDAC cells results in a lack of effective chemotherapies, which contributes to the high mortality in patients with pancreatic ductal adenocarcinoma. The microenvironment (such as interactions between cell surface integrins, extracellular matrix components and intra-tumoural hypoxia) is responsible for innate drug resistance. In order to screen new drugs for PDAC treatment, an in vitro model as a more predictive platform is strongly required in this field. In paper III we aimed to develop a 3D model of human PDAC cells, and to further explore mechanisms underlying the transition from 2D to 3D cultures that might be responsible for chemoresistance, including HIF-1 pathway. We successfully established a new high-throughput 3D cell culture drug screening system for pancreatic cancer, which displays increased chemoresistance resulting from enhancement of ECM production, glycolysis and expression of miRNA, hypoxia-inducible genes as well as chemoresistance genes. Our finding is supporting the concept of cell adhesion mediated drug resistance in PDAC. To increase utility and predictive value of our 3D tumour cell model for preclinical drug discovery, in paper IV we generated a hetero-spheroid model with pancreatic stellate cells (PSCs) surrounding a core of cancer epithelial cells, as can be observed in sections from patients with PDAC. Furthermore, gene expression was up-regulated in hetero-spheroids of PDAC cells, including E-cadherin, β-catenin, fibronectin, collagen I, lumican, COX2 and PPP1R1B, compared to mono-spheroids. In addition, we found that HIF-1α expression in hetero-spheroids was associated with the enhanced expression of ECM proteins, cancer stem cell marker (CD24), gene PPP1R1B (DARP-32) and hypoxia-inducible genes, which might alter sensitivity of cancer to chemotherapy.

In conclusion, the present work demonstrates that HIF-1α is an important transcription factor for displaying protective effects of exendin-4 on islet grafts after islet transplantation, understanding the mechanism of Warburg effect in pancreatic cancer cells and describes the development of an organotypic in vitro culture system for PDAC, facilitating the examination of tumor-stroma interactions and improving predictability of drug screening system in pancreatic ductal adenocarcinoma.

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