The Role of Acinar to Ductal Cell Transdifferentiation in Pancreatic Carcinogenesis

Thesis by

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감사의 글

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이 지 은 올림

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Abstract

Pancreatic cancer is currently the major leading cause of cancer-related deaths in the Western countries with an overall 5-year survival of less than 3%. It is essential step to understand the mechanisms that regulate proliferation and differentiation in the pancreas and to investigate the mechanisms leading to neoplastic transformation for the development of novel therapeutic strategies.

In this report, the observation provides further evidence for the proposal that adult acinar cells can transdifferentiate to duct-like cells. I suggest the spontaneous transdifferentiation of cultured rat pancreatic acinar cells to alter their usual phenotypic expression and attain morphological and functional characteristics of ductal cells. We present *in vitro* system in which the cellular differentiation of pancreatic duct-like cells and their expression of pdx-1, c-kit and SCF appeared to induce stem cell activity potentially. Moreover c-kit might play a critical role in growth and differentiation of normal cell and pancreatic carcinogenesis.

Role of acinar to ductal cell transdifferentiation in pancreatic carcinogenesis

< Directed by professor Si Young Song>

Brain Korea 21 Project for Medical Sciences

The Graduated School of Yonsei University

Ji Eun Lee

I. Introduction

Pancreatic cancer is currently the major leading cause of cancer-related deaths in the Western countries with an overall 5-year survival of less than 3 %. In the Asian countries, the incidence of pancreatic cancer has been increased rapidly during last 2 decades and this change looks like parallel with the gradual Westernized life style including diet and other environmental factors[1]. Understanding the mechanisms that regulate proliferation and differentiation in the pancreas and investigating the mechanisms leading to neoplastic

transformation are essential steps for the development of novel therapeutic strategies.

The protodifferentiated state during early development of pancreas, primitive duct-like structures appear first from which the ductal epithelium, acinar cells and islet cells originate[1]. Morphogenesis of the pancreas during early development is dependent on the expression of insulin-promoter-factor as well as the interaction between ductal cells, islet cells, and acinar cells. After fetal development, however, the ductal cells retain the ability to differentiate into islet cells, but their capacity to differentiate into acinar cells is markedly curtailed[1,2]. Under conditions of subtotal acinar cell necrosis, the proliferation of the remaining acinar cells was shown which leads to complete reconstitution of the pancreas[3].

In adult pancreas, like other organs, replication and differentiation at a minimal level is most likely to be occured to maintain the quantity and quality of the organ. Therefore, even the adult pancreas seems to retain the capacity to regenerate to some extent after injury or in response to some kinds of stimuli. Further, it is also likely that the regeneration process, including replication and differentiation, recapitulates, at least in part, the embryonic

development[4].

Acinar cells are the major tissue compartment of the pancreas and are specialized for synthesizing and secreting a variety of digestive enzymes. It is of interest to note, however, that adenocarcinomas of the duct system make up over 90 % of nonendocrine pancreatic tumors in human, whereas acinar cell carcinomas account for only 1 %[3]. It is generally believed that adenocarcinomas originate from the pancreatic ducts, largely on the basis of morphological similarities.

Recent studies have suggested that acinar cells may also be participated in the histogenesis of adenocarcinomas, during which acinar cells undergo dedifferentiation and give rise to duct-like cells on chemical carcinogenesis[5]. According to the previous reports, pancreatic tissue from MT-TGFá transgenic mice showed uniform pancreatic fibrosis and multiple foci of metastatic ductal epithelium[6]. *In vitro* studies revealed that normal pancreatic acinar cells could change their differentiation commitment pattern and subsequently transform into a ductal phenotype. Also, it is of

interest that the focal regions of regeneration seen in this model resemble the duct-like tubular complexes described in various diseases such as chronic pancreatitis[6], pancreatic adenocarcinoma, and cystic fibrosis and in regeneration of exocrine pancreas after experimentally induced acute pancreatitis. Moreover, several experimental animal models have shown that under certain conditions and in certain stages of development, the differentiation of pancreatic cells can be reactivated. The most important of these models are duct ligation in adult rats[7,8], partial pancreatectomy in rat[9,18], cellopane wrapping of pancreatic head in adult hamsters[10], and streptozotocine-induced beta cell destruction in newborn rats[8,9,33]. The origin of these tubular complexes has been a matter of controversy, with suggestion being made that they result from proliferation or transdifferentiation of acinar tissue leading to duct-like structures[8]. It would therefore be important to understand the growth and differentiation of acinar and ductal cells in the normal pancreas.

The analysis of carcinogenic events by modern techniques of molecular oncology has greatly increased our understanding of

how malignant transformation may happen, but basic questions concerning the cellular origin of cancer still remain. Given the fundamental principle that cancer must arise from cells that have retained the potential to divide, there are two possibilities: the first, tumors arise from dedifferentiation of mature cells that have not terminally differentiation and the second, they arise from maturation arrest of immature progenitor "stem cells".

Transdifferentiaton is essentially different from stem cell-derived neogenesis in that it involves differentiated cells rather than undifferentiated ones. It consists of re-programming of gene expression, which generally occurs via an intermediate stage of de-differentiation[11-14]. Thus, certain terminally differentiated cells retain the capacity to switch off a number of genes and switch on others resulting in a phenotypic switch. In the case of the pancreas, transdifferentiation of acinar exocrine cells to ductal cells is well established[12]. This process has been observed *in vivo* as well as *in vitro* with different mammalian species including human pancreatic cells. *In vivo* it may be involved in the pathogenesis of pancreatitis and pancreatic cancer. In animal models of

pancreatitis, such as induced by TGF-á transgenic mouse[15], duct-ligation[7,8] or chronic inflammation[6], the acino-ductal transdifferentiation[13], or metaplasia, results in the formation of ductal complexes consist of a mixture of trandifferentiated acinar cells and of ductal cells, and eventually these cells can no longer be distinguished on the basis of morphological or immunophenotypic ground[14]. These morphological observations do not completely rule out the possibility that putative multipotential stem cells become activated and proliferate under these conditions. It might be that such a multipotential stem cell could transiently coexpress phenotypic characteristics of two different types of progeny[16,17,18]. Besides looking at the expression of functional characteristics such as amylase and CK19, the "master switch genes," which determine the differentiation state of the cells, remain to be identified[22].

In this paper, I report the question whether ductal cell regeneration or neogenesis in the pancreas depends on stem cells or transdifferentiation of fully differentiated cells. I conclude from the available evidences that there is clue for the existence of

dormant stem cells in the pancreas[19-23]. However, there is some evidence that differentiated exocrine acinar cells retain the capacity to be transdifferentiated into duct-like cells.

II. Methods and Material

1. Animals and Primary culture

The male Sprague Dawley rats weighing between 100–150 g were used to procede primary culture of pancreatic acinar cells. Pancreas were removed, minced and chopped in a spinner flask, and rinsed twice in pancreatic solution A (4 g Glucose, 16.36 g NaCl, 1 M KCl, 1 M $MgCl_2$, 1 M $CaCl_2$) containing 500 mg bovine serum albumin, 500 mg pyruvate, and 60 mg trypsin inhibitors. After rinsing, tissue was digested in a collagenase type IV (Sigma, St. Louis, MO, USA) at 37 °C water bath for 6-8 min and then centrifuged at 1000 rpm for 10 sec. The resulting cell suspension was passed through Nylon mesh filter with 200 ìm pore. After washing these cells, isolated acinar cells were cultured in Waymouth media (Sigma, St. Louis, MO, USA) with 10 % fetal bovine serum (HyClone, Utah, USA) and 10 ìg/ml Dexamethasone (Sigma, St. Louis, MO, USA). During culture, single cell was cloned morphologically by cloning cylinder.

2. Amylase Assay

Amylase activity of cell lysates and supernatant secreted was determined by Caraway methods (Asan Pharm.Co., Korea). One unit of amylase activity is defined as amount of remnant starch that results in the digestion of starch by amylase.

3. Electron microscopy

Ultrastructural studies were performed on cultured cells attached in culture flasks. The cells were washed twice with phosphate-buffered solution (pH 7.4, PBS) and fixed in PBS containing 1 % glutaraldehyde. The cells were postfixed in osmiumtetroxide and the samples routinely processed for transmission electron microscopy.

4. Immunohistochemistry

Immunohistochemical analysis was performed in following manner. Tissues were fixed to 4 % paraformaldehyde and embedded in paraffin. Paraffin sections were fixed to glass slides,

dewaxed, and rehydrated at percentage gradient ethanol sequentially. After quenching of endogeneous peroxidase activity using 3 % H_2O_2 for 20 min, the slides were blocked in 5 % normal donkey serum for 60 min at room temperature. The slides were then treated with primary antibody, rabbit polyclonal anti-c-kit (1:500, Santa Cruze, California), overnight at 4 °C. After washing in PBS, species-specific biotinylated secondary antibody (DAKO, Carpinteria, CA, USA) was applied for 60 min at room temperature and followed by application of Streptavidine (DAKO LSAB Kit, Carpinteria, CA, USA) for 20 min at room temperature. AEC chromogen was applied and the slides were counterstained with hematoxylin (Sigma, St. Louis, MO, USA).

5. BrdU labeling proliferation assay

Cells were cultured in a 96 well plate at 37 °C for 0, 1, 3, 5, 7, 10, 12, and 15 days. Subsequently, BrdU (BrdU labeling kit from Boehringer Mannheim, Germany) was added to the cells and the cells are reincubated for 24 hours. After removing the culture medium the cells were fixed and the DNA was denatured in one

step by adding FixDenat. The anti-BrdU-POD bound to the BrdU incorporated in newly synthesized, cellular DNA. The immune complexes were detected by the subsequent substrate reaction. The reaction product was quantified by measuring the absorbance at 370 nm using a scanning multiwell spectophtometer (ELISA reader, Molecular Devices, USA)

6. RT-PCR

Total cellular RNA was extracted from rat pancreatic acinar cells using RNeasy mini kit (Quiagen, GmbH Germany). Random hexamer-primed reverse transcription of RNA was carried out for 60 min, at 42 °C using Superscript II (Gibco BRL, NY). First strand cDNA served as a template for PCR amplication using Taq polymerase and following degenerate primers. PCR was carried out in a Mastercycler Gradient (Effendorf, Hamberg, Germany) with a denaturation step at 94 °C for 1 min, annealing at each specific following temperature, extension at 72 °C for 1 min, followed by each cycles. Primer sequences are listed in Table 1.

Table 1. RT-PCR primer sequence

Primer	Forward	Reverse	AT(°C)	Cycles	size(bp)
SCF	5' ccg gga tgg atg ttt tgc 3'	5' tgc aac agg ggg taa cat 3'	59	35	794
c-kit	5' tgc tct gcg gtc ctg ttg gtc 3'	5' cct ggc gtt cgt aat tga agt 3'	59	35	391
p48	5' tgc agt cta tca acg acg c 3'	5' gga cag aga tct tcc agt tc 3'	63	40	143
pdx-1	5' ctc gct ggg aac gct gga aca 3'	5' gct ttg gtg gat ttc atc cac gg 3'	63	40	224
β-actin	5' cgt aaa gac ctc tat gcc aa 3'	5' agc cat gcc aaa tgt ctc at 3'	59/63	35	481

7. Real-time quantitative RT-PCR

Primers and probes were chosen with the assistance of the Primer Express software program (PE applied Biosystems, Poster City, CA). Pdx-1 forward primer: 5'-aaaagacccgagcttctgaaaa-3' ; Pdx-1 reverse primer : 5'-gcagacctggcggttcac-3' ; Pdx-1 TaqMan probe : 5'-tttgaggctgcctctcgtgcca-3'. c-kit forward primer : 5'-gcatcagggcgacttcaatt-3' ; c-kit reverse primer : 5'-cactccggaatcgttaactcttg-3' ; c-kit TaqMan probe : 5'-cgaacgccaggagacgctgactatca-3'. The TaqMan probes contain a

fluorophore 5' FAM as reporter and 3' TAMRA as quencher. The concentration of primer, probe, and template giving the highest intensity and specificity of reporter fluorescent signal were based on optimizing preruns. In each assay (96 wells), each sample was run in triplicate for both pdx-1 and c-kit. A PCR master mix, used for pdx-1 and c-kit samples, was aliquoted to each sample tube to a final volume of 25 ul. The PCR reaction mix consisted of; 2× TaqMan Universal PCR Master mix, 900 nM forward primer, 900 nM reverse primer, and 200 nM probe. cDNA was diluted to a stock solution before being aliquoted to each sample tube containing the PCR reaction mix of pdx-1 and c-kit triplicate reactions. In order to compare data under the same conditions, data of the target genes were normalized to an internal housekeeping gene, glyceraldehydes-3-phosphate dehydrogenase (GAPDH), for which data was obtained using TaqMan GAPDH control reagents (PE Applied Biosystems, Poster City, CA, USA). The 96-well sample tray was centrifuged briefly at 2800 rpm for 1 min. The PCR was performed in the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Poster City, CA, USA), which detects

the signal from the fluoregenic probe during PCR. The pre-run thermal cycling conditions were 2 min at 50 °C and 10 min at 95 °C. Thermal cycling conditions were 40 cycles at 95 °C for 15 sec and at 65 °C for 1 min. TaqMan Universal PCR Master mix, Microamp caps and Microamp reation tubes were all from PE Applied Biosystems.

8. Protein extraction and Western Blotting

Proteins were extracted from rat pancreatic acinar cell by solubilizing in lysis buffer (1M beta-glycerophosphate (pH7.2), 50 mM Na vanadate, 0.5 M $MgCl_2$, 0.2 M EGTA, 1 M DTT, 0.5 % Triton ×100, 100 mM PMSF, Protease Inhibitors; Leupeptin, Pepstatin, Aprotinin, and antipain each 5 ì g/ml) on incubation at 4 °C and centrifugation at 13000 rpm for 20 min. Insoluble debris was pelleted, and the protein concentration of the resulting supernatant was determined using the Bradford method. 50 ì g of total protein per lane were loaded and resolved on SDS 10 % polyacrylamide gels, transferred to polyvinylidene difluoride membrane (Immobilon-P, Millipore,

Bedford, MA, USA) and probed with the following primary antibodies: Anti-Human Cytokeratin 19 (1:1000, from DAKO, Carpinteria, CA, USA), Anti-Human Amylase (1:2000, from Sigma, St. Louis, MO, USA), Rabbit polyclonal anti-c-kit (1:1000, from Santa Cruze, California, USA), Rabbit polyclonal anti-SCF (1:1000, from Santa Cruze, California, USA), Rabbit polyclonal anti-p48 (1:1000, from Dr. Francisco X. Real, Pompeu Fabra University, Spain) and Rabbit Anti-Pdx1 (1:1000, from Dr. C. Wright ,Vanderbilt University Medical School, Nashville, TN). The membrane were washed and incubated with horseradish peroxidase-conjugated species-appropriate secondary antibodies (Santa Cruze, California, USA), then developed with enhanced chemiluminescence reagents (Amersham Life Science, UK), and exposed to radiograph film.

III. Results

1. Phenotypic characterization of normal pancreatic acinar cells during transdifferentiation

Isolated pancreatic acinar cells aggregated by 2 to 3 day of culture and start to spread out by 4 to 5 day (Figure 1A-D). The maintenance of this well-differentiated morphology has been limited to the first 6 to 8 days. Acinar cells underwent transdifferentiation during the initial stages of the culture period into duct-like cells. The progressive loss of exocrine differentiation appeared to involve rapid degranulation of zymogen granules by exocytosis and loss of the prominent secretary apparatus (Figure 4A-C). Using BrdU incorporation assay, cell proliferation during the culture period did not appear to account for the increase of ductal cell population which might be possibly included during acinar cell preparation (Figure 3).

At day 0, the exocrine cells within the isolated acini exhibited a high level of cytodifferentiation. The characteristic features of well-differentiated secretory apparatus were apparent

with densely packed zymogen granules in the apical region of the cell associated with the juxtanuclear Golgi apparatus and well-organized rough endoplasmic reticulum (RER) in the basal region (Figure 4). Following 2 days of culture the acini underwent major morphological alterations with the highest density of granules being observed at the periphery of the cell clusters suggesting a reversal of epithelial cell polarity.

From day 2 to 4, the typical ultrastructural appearances of the exocrine acinar cell appeared at day 0 could not be observed any more. Transitional cells lacking differentiated secretary apparatus and containing reduced number of zymogen granules with varying size were the only evidence of an exocrine acinar lineage. These apparent transdifferentiation of exocrine cells were occurred in parallel with an increase in the number of undifferentiated precursor cells with the clusters. Acinar cells were filled with secretory zymogen granules, and acinar cells were partly degranulated; these changes progresseed over the following 3-5 days. Cytoplasmic vacuolation of acinar cells with varing degree was seen in this period (Figure 4D).

Further, the exocrine cells developed primitive microvilli at

the periphery of the cell clusters following the cytoskeletal re-organization (Figure 4E). The overall morphological appearance of the transdifferentiated acinar cells strongly resembled the profile of undifferentiated ductal cells (Figure 4F-G).

2. Transdifferentiation of acinar cells to duct cells

These processes occured in parallel with a significant reduction in the activity and expression of amylase in period from day 0 to day 10 (Figure 2) and simultaneously the epithelial ductal cell markers, cytokeratin 19 were increased (Figure 2B). Cytokeratin 19 was a widely distributed cytokeratin which is expressed in many simple epithelia. It was intermediate filament which was expressed in pancreatic ductal cell and has been used in many studies to identify normal as well as neoplastic pancreatic duct.

3. Characteristics of transdifferentiated pancreatic cells

Expression of pdx-1 was increased gradually from day 3 at both protein level and RNA transcript level (Figure 5A, E, Figure 6A). Both protein and mRNA expression of c-kit were increased, reached a peak around day 5 and then rapidly decreased after day 5 (Figure 5B, F, Figure 6B). The increase of SCF at the level of mRNA and protein were shown and maintained a plateau up to day 10, when large number of epithelial duct-like cell could be observed. SCF expression decreased after day 10 (Figure 5C, G). p48 expression was dramatically diminished and it could not be maintained *in vitro*. However, mRNA level of p48 was not changed until day 10 (Figure 5D, H). The culture of normal rat exocrine pancreas underwent an acinar to ductal phenotypic switch *in vitro* that was also accompanished by the loss of expression of p48.

4. Expression of c-kit in pancreatic carcinogenesis

To clarify whether above *in vitro* results were correlated with *in vivo* during the process of pancreatic ductal metaplasia and pancreatic carcinogenesis, I performed immunohistochemistry for c-kit in the MT-TGFá transgenic mice shown progressive ductal metaplasia development (Figure 7A-D), pancreatic carcinogenesis hamster model treated BOP (Figure 7E-H) and human pancreatic cancer tissue (Figure 7I-N). As expect, c-kit immunoreactivity was expressed only in ductal metaplasia region and islet compartment.

In this study, I have found that c-kit plays a central role in the growth and differentiation of normal and tumor cells as a proto-oncogene. These results indicated that c-kit activity is related to pancreatic tumor development.

IV. Discussion

The plasticity of the differentiated exocrine cell is well documented and involves the re-expression of ductal cell antigens and morphology in response to a variety of stimuli. This differentiation of exocrine tissue has been described in a number of *in vivo* models of pancreatic regeneration. It is generally held that since human pancreatic tumors have a phenotype similar to that of normal ductal epithelium, they must be derived from those ducts. This would imply that carcinogenic events occur in duct cells[5,9,10,18]. However, there are some evidences that the main pancreatic ducts are not the primary sites of origin of pancreatic neoplasia. Acinar cell abnormalities have been described in human pancreatic carcinogenesis[17]. Ultrastructural studies have suggested that the earlist region in the hamster pancreatic cancer model involved the centro-acinar cells or cells of acinar origin. These studies point to the phenotypic plasticity of pancreatic acinar cells but might be explained by the possible leaky expression of acinar promoters in ductal epithelial cells [5,13,14].

Normal human exocrine pancreas was digested using

collagenase and the resulting cellular aggregates were cultured *in vitro*[17]. The phenotype of the digested pancreatic cells was almost exclusively acinar phenotype (amylase-positive, cytokeratin 19-negative, and pdx-1-negative), yet within 4 days of culture the cells had taken on a ductal phenotype (amylase-negative, cytokeratin 19-positive). We could also demonstrate that the rat pancreatic acinar cells could be maintained from primary culture. While the incorporation of BrdU by these cells was not increased with time, the intracellular amylase activity was not preserved, most 90 % of amylase activity being lost after only 5 days of primary culture. In contrast to this gradual decrease of amylase activity, expression of cytokeratin 19 as a ductal marker, was gradually increased with the time course. These results provided direct experimental evidences for the transdifferentiation of pancreatic acinar cells to a ductal phenotype, providing strong support for the hypothesis that acinar cells might represent the target population for carcinogenic events in the pancreas.

I have found that rat pancreatic acinar cells rapidly transdifferentiated and took on a ductal phenotype when cultured *in vitro*. However, I can consider the possibility of presence of

ductal cells at the initial time point which proliferate with the time course. However, this would require a more than 20-fold increase in this cell population within a period of 10 days. Transdifferentiation of acinar cell population into ductal cells rather than expansion of preexisting ductal cells could also be confirmed by the BrdU labeling index *in vitro* and ^{3}[H]-uptake with the time course, which were showing no significant proliferation during the time course. Therefore, I concluded that culture of acinar cells, under these conditions, led to rapid transdifferentiation to a ductal phenotype.

The phenomenon of transforming acinar cell to a duct-like phenotype has been reported previously *in vitro* in the rat, guinea pig, and human[11-13,16]. An acinar unit could be changed a duct-like phenotype by a decrease in cell height and a loss of zymogen granules under certain condition, such as ductal ligation, chronic pancreatitis, and carcinogenesis. On continuous distention of the expression of cytokeratin 19, acinar cells gradually lose their apical cytoplasm and zymogen granules and, eventually, become duct-like cells. The same mechanism may also apply to ductal ligation and chronic pancreatitis. In both cases, ductal obstruction

leads to transformation of acinar cells into cells with duct-like morphology[18].

The duct-like cells that arise from the acinar fragments may contain acinar precursor, which, under appropriate conditions, will be differentiated into acinar cells[24,25]. The understanding of the differentiation and redifferentiation of acinar cells will provide an important implication in the early stage of human carcinogenesis.

PTF1/p48 seems to be another key factor in pancreas ontogeny, as a null mutation of this gene leads to the complete absence of exocrine pancreatic tissue which normally forms the pancreas[26]. In mice with this mutation, hormone-producing cells appear in the mesentery at an earlier embryonic stage and localize into the spleen in late embryonic life[27]. PTF1/p48 is, so far, the only transcription factor that has been found to be selectively expressed in exocrine pancreatic tissue, and it is involved in the expression of pancreatic exocrine enzymes.

The cultures of normal human exocrine pancreas undergo an acinar to ductal phenotypic switch *in vitro* that is also accompanished by the loss of expression of p48. The molecular

mechanisms underlying the down-regulation of p48 gene expression in tumors remain unknown, although at least in human tumors, the p48 gene generally retained. p48 is an excellent marker of the acinar cell differentiation in the pancreas.

The biological effects of the SCF/c-kit system are believed to involve survival, proliferation, differentiation and migration of early stem cell progeny[28-30]. Although SCF and c-kit receptor are widely expressed during normal embryonic development, their expression in the adult is limited. The increase of c-kit mRNA was more gradual than that of SCF and its transcripts maintained a plateau up to day 14, when large number of oval cells could still be observed in fetal liver[28].

The SCF and c-kit mRNA rapidly decreased after day 9 and day 14, respectively in embryonic and adult liver[28,31]. The transcripts for the c-kit receptor are expressed in the early progeny of the hepatic stem cells[28,29,31]. The SCF/c-kit system may be involved in the early activation of the pancreatic stem cells as well as in the transdifferentiation of pancreatic acinar cells, possibly in combination with other growth factor/receptor systems[31].

In this study, I have demonstrated the presence of the SCF/c-

kit systems on the early progeny from the transdifferentiated day 1 cell compartment, indication a role for this ligand-receptor system in the early differentiation of pancreatic exocrine cells. The mRNA level of SCF increased within day 1 after primary culture and reached a peak around day 5. Thus, the expression of SCF preceded the major expansion of the transdifferentiated cell compartment. The level of c-kit transcripts gradually decreased after day 10.

Pdx-1 is pancreatic duodenum homeobox gene. It plays the essential roles in organogenesis of the pancreas and proper differentiation to the islet and acinar cells[32-34]. Of transcription factors, Pdx-1 seems to be a key element, as neonatal mice carrying a null mutation of this gene selectively lack a pancreas, even though the pancreatic buds form in the early stage of ontogeny[33]. Pdx-1 expression is normally seen in all cells of the buds, transiently in the early embryonic stage[35], and then it decreases. Pdx-1 expression increases again in the stage in which islet cell differentiation occurs and becomes localized to â and ä cells in late embryonic stage. It dose not initiate regeneration but may be involved in the differentiation of ductal precursor cells to

mature â cells[15].

This observation provides further evidence for the proposal that adult acinar cells can transdifferentiate to duct-like cells, re-expressing some embryonic protodifferentiated characteristics such as pdx-1. Dedifferentiation of the pancreatic epithelium is not surprising due to the reported high plasticity of this embryonic tissue[32].

In carcinogenesis, the activation of the growth factor, cytokine, and hormone systems is proposed to play significant roles in the development of many types of carcinomas. Among them, it seems that c-kit activation is participated in the development of pancreatic tumor[36-37]. Our previous studies in the animal carcinogenesis model have indicated that pancreatic ductal adenocarcinomas are derived not only from ductal cells but also acinar cells. In the hamster model treated with N-nitrosobis (2-oxopropyl) amine, which mimic human disease in many clinical and biological aspects, we have demonstrated that pancreatic adenocarcinoma is originated not only from ductal cells but also from acinar cells. In fact, as a previous step of carcinogenesis, the

first morphological change that occurs during pancreatic ductal metaplasia has shown at MT-TGFá transgenic mice. In these results, both of these animal models have been expressed strongly for c-kit as well as ductal hyperplasia area in human pancreatic cancer. Therefore, c-kit protein in pancreas appears to be present as a marker for pancreatic ductal adenocarcinoma.

V. Conclusion

In these experiments, I show the spontaneous transdifferentiation of cultured rat pancreatic acinar cells to alter their usual phenotypic expression and attain morphological and functional characteristics of ductal cells. We present *in vitro* system in which the cellular differentiation of pancreatic duct-like cell and their expression of Pdx-1, c-kit and SCF appeared to induce stem cell activity potentially.

VI. Reference

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국문요약

췌장의 암 발생과정에 있어 선방 세포로부터 췌관 세포로의 이행분화의 역할

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췌장은 소도세포, 선방세포, 췌관세포의 세 부분으로 구성되어 있는데 이 중 선방세포(acinar cell)가 90% 이상을 차지하고 있으며 췌관세포(ductal cell)와 소도세포(islet cell)가 10% 정도를 차지하고 있다. 반면, 췌장암은 90% 이상이 췌관선암(ductal adenocarcinoma)으로 진행되며 표현형이 췌관세포로 구성되어 있다. 췌장암의 발생은 췌관세포 자체에서 유래된다는 기존의 이론과 함께, 췌관세포 이외의 세포로부터 분화되어 췌관세포의 표현을 갖게 될 것이라는 가능성이 제시되었다. 이에, 췌장의 분화와 증식을 조절하는 기전에 관한 이해와 종양성 형질전환에 대한 기전 연구는 췌장암의 치료에 필수적인 단계가 될 것으로 생각된다.

본 연구는, 쥐의 췌장의 선방세포를 일차 배양 함으로서 선방세포에서 췌관세포로의 이행분화를 *in vitro* 에서의 동물 모델로 설명하였으며, 췌장의 선방세포는 일차배양의 배지 조건에서 형태적, 기능적인 췌관세포로의 특징들을 얻게 되면서 이행분화하였다. mRNA와 단백질 수준에서의 변화를 확인한 결과, amylase 와 p48 의 발현이 감소하는 동시에 cytokeratin19의 발현이 증가하였고, 이행분화 과정에서 pdx-1, c-kit, SCF 의 발현도 일시적으로 증가하였다. 이러한 사실은, 선방세포로부터 췌관세포로 이행분화되는 공통적인 현상을 보이고 있는 암화과정 모델과 이미 암으로 진행된 환자의 조직에서의 c-kit의 발현은 c-kit 유전자가 췌장의 췌관세포의 이행분화뿐만 아니라, 암화과정에도 관여한다는 사실을 뒷받침한다.

이상의 결과로부터, 췌장세포의 이행분화와 암화과정은 발생학적으로 연관된 세포의 직 간접적인 전환에 의해서 또는 전형성 세포(pluripotent precursor cell)과 같은 중간단계 세포(intermediate cell)의 전환에 의한 것임을 추측할 수 있으며 이에 따른 췌장 내 간세포(stem cell)의 존재 가능성을 제시해 주고 있다.

핵심어: 췌장, 췌관선암, 선방세포, 췌관세포, 암화과정, p48, amylase, c-kit, SCF, pdx-1, cytokeratin 19, 이행분화