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The Plasticity of Pancreatic Stellate Cells Could Be Involved in the Control of the Mechanisms that Govern the Neogenesis Process in the Pancreas Gland

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1. Introduction

Mammalian pancreas is a gland that plays an important role in the regulation of energy balance and nutrition. Through the synthesis and release of protein digestive enzymes and hormones, which are involved in the absorption, it uses and stores the digested nutrients. This gland divided into two compartments with exocrine and endocrine functions, together with the stroma surrounding the pancreatic parenchyma, plays important roles in the homeostasis of the body. Moreover, they are involved in the maintenance of the function of the organ, including the regenerative process observed after injury of the pancreatic tissue. However, to understand this relationship, it is necessary to understand the embryological mechanisms that control the development of the pancreatic tissue. This embryological pathway begins from the precursor cells located in the endoderm, which is able to promote the pancreatic morphogenesis after responding to specific external and internal signals. Therefore, knowledge of the different networks created by neighbouring embryonic tissues will be essential for understanding the complexity of this morphogenetic process.

The organogenesis process of the pancreas gland is originated from stem cells located in the endoderm, which have the capacity to promote the development of the exocrine and endocrine compartments, identified in the adult gland from mammals. This phenomenon follows a specific gene network activity which is regulated by specific transcription factors (Jensen J, 2004). This complex process can be summarized into three steps identified by different investigators. The first step is accomplished through the action of specific signals that are originated from the mesoderm (Sander M and German MS, 1997). In the second step, the primitive endocrine cells, which are scattered throughout the undifferentiated

epithelium, proliferate and promote the primitive islets cells located in the surrounding mesenchyma. Moreover, the mesenchymals signals are important to promote the development of islet cells and increase the number of beta cells at the end of the process. All these signals also promote vascularization (Kim SK and Hebrok M, 2001; Scharfmann R 2000; Reusens B and Remacle C; 2006). In the last step, the gland is remodeled into two functional compartments (Habener JF et al. 2005). In the adult pancreas, these two compartments exhibit different physiological roles. In addition, they have an important relationship and cellular interaction.

The pancreas like other tissues is considered like a dynamic organ, able to adapt to different physiological situations, such as diabetes, obesity or in gestation. This dynamic adaptation is based on the regulation of the beta cell mass in order to maintain glucose homeostasis. There are different mechanisms that control this process, which include: apoptosis, necrosis, hypertrophy, hyperplasia and neogenesis. However, little is known about some of these processes, and in particular, the cells which are involved. In the case of the neogenesis process, many studies supported the idea that it occurs via cells which are located in, or which are associated with, the ductal epithelium of the exocrine compartment of the pancreas. One of the approaches used for investigating this hypothesis is the application of the immunocytochemical and immunohistochemical techniques. These techniques are important because they help to identify the cell population involved in the process without losing the architecture of the tissue. Moreover, they are important tools for the phenotyping of the cell population when isolated from the tissue and checked while maintained *in vitro*.

2. Historical perspective of stellate cells

In 1876 Karl von Kupffer described for the first time a new population of cells in the liver called "sternzellen " or stellate cells, due to their stellate appearance. These cells located in the space of Disse had cytoplasmatic inclusion bodies indicating to have a phagocytic function. Initially, Kupffer classified them into the "Waldeyer's perivasculare Bindgewbszellen" or reticulo-endothelial system. However, this author changed opinion and the cells were considered phagocytes and were referred to as "special endothelial cells of the sinusoids" (Kupffer C 1876). However, it was not until the beginning of the 20th century when Zimmerman described them as dendritic perisinusoidal cells surrounded by reticular fibers and named them hepatic pericytes. Later, the Japanese Anatomist Dr. Ito described a new cell population in the liver, which were located in the perisinusoidal space and contained abundant amounts of fat droplets in their cytoplasm. These cells, known as "Ito-cells" are able to store and deliver vitamin A and other liposoluble vitamins. Moreover, they are involved in the regulation of sinusoidal tone, local blood supply, and tissue repair and fibrosis. The cell presents several thick cytoplasmatic processes which are protuded directly from the perikaryon (primary process) and extended onto the outer surface of the sinusoidal entohelial cells (Ito T et al. 1951). In summary, these cells have received other names, such as: fat storing cells, pericytes, parasinusoidal, and lipocytes. Several studies demonstrated that all these cell populations shared most of their cellular and physiological characteristics and seemed to correspond to the same population. For that reason, and in order to avoid confusion, in 1996 the international community of investigators unified the nomenclature and defined

these cells as a "Stellate cells" (no authors listed, 1996). Soon after, Kent and Popper demonstrated that the stellate cells were linked to the pathogenesis of hepatic fibrosis (Hirosawa K and Yamada E, 1973). This important finding promoted the identification of this cell type in extrahepatic organs (pancreas, spleen, adrenal, ductus efferent and uterus) in rodent and humans (Geerts et al., 2001).

In addition, the presence of these cells in a wide variety of species, ranging from lampreys (primitive fish) to humans and in all major tissues, indicated their importance in the development of the different organs (Wake K 1987).

2.1 Stellate cells in pancreatic tissue: historical perspective

Vitamin A storing cells were first described in the pancreas by Watari, *et al.,* in 1982, using fluorescence and electron microscopy. In 1990, Ikejiri, et al., confirmed the previous results and also showed the presence of vitamin A as a autofluorescence stained in normal pancreatic sections from rats and humans. In 1997, Saotome, et al., described the presence of the myofibroblast-like cells in human pancreas, and their involvement in the extracellular matrix remodeling during the fibrosis process. However, these independent observations had not been realized to be related until 1998, when Bachem, et al., and Apte, et al., defined these two populations of cells as pancreatic stellate cells, in two different stages of activation (Quiescent and Active).

2.1.1 The embryological origin

The embryological origin of the stellate cells is unclear. Importantly, there are few studies conducted to resolve this dilemma. Most of them have been described in the liver. For that reason, different observations of these cells in liver have been extrapolated to other organs including the pancreas. However, numerous theories on the linage of these cells have been presented. The hepatic stellate cells (HSC) are proposed to be derived from mesenchymal cells that separate the pericardial and peritoneal cavities of the embryo (Morita M et al. 1998; Naito N and Wisse E 1977). However, the specific microfilaments identified in their cytoplasm and morphology, resembling the astrocyte cells from astroglia in the Central Nervous System, could also be indicating a neural-ectodermal origin (Niki T et al. 1999; Friedman SL 2000). This last observation was difficult to reconcile with the mesenchymal origin described before. Recently, the identification in bone marrow of fibroblast /myofibroblast cells, which share some HSC characteristics, suggests that stellate cells could be derived from hematopoietic stem cells (Susking DL and Muench MO, 2004; Baba S et al. 2004; Ogawa M et al 2006). In conclusion, new experimental designs are required in order to understand the embryological origin of these cells. Moreover, the possibility to use the lineage–specific promoters to drive the transgene expression could contribute to the clarification of this problem and enable the understanding of the biology of these cells.

2.1.2 Biology of pancreatic stellate cells

Pancreatic stellate cells (PSC) are located in different spaces: periacinar, perivascular and periductal of the exocrine compartment of the pancreas. They represent approximately 4% of the total cells of the gland. The cells are closely in contact with acinar, endothelial and

ductal cells and establish a strict cellular communication between them through long processes containing numerous filaments and microtubules. These cells play an important role in the pancreatic pathology of the exocrine compartment of the pancreas, such as chronic pancreatitis and pancreatic cancer. In all these injury processes, PSC and HSC have shown an important phenotype transformation to a so-called activated form. In this state, the cells are able to produce large amounts of extracellular matrix proteins (EMC), fibronectin and laminin resulting in the extensive fibrosis. In this stage, the cells showed: a typical characteristic spindle –shaped, absence of the retinol in the cytoplas, the increment of the myofilaments, as in the GFAP and vimentin, as well as the presence of the new myofilament (α-SMA). Moreover, the production of multiple factors with a paracrine, autocrine and chemoattractant actions can be detected (Jasper, R 2004, Morini S et al. 2005; Omary MB et al. 2007; Kordes C et al 2009) (Fig.1 A,B). In contrast, when the cell are in the quiescent form, they present: abundant droplets of vitamin A in the cytoplasm, are less positive for desmin, vimentin, nestin and GFAP intermediate filaments, and the cytoplasmatic processes are not observed. In addition, a non-proliferative state is observed in the cells (Pinzani, M. 1995; Apte MV et al. 2003). The transitional stage of the cells was observed and the cells share some of the ultrastructural and functional characteristic for these two differentiated stages described previously.

The mechanism implicated in this transformation process is not determined yet. *In vivo*, different signal transduction pathways have been described and all, including infiltrating leucocytes and damaged acinar cells, are able to initiate and maintain the activated phenotype. However, most of the information about the activation mechanism has derived from *in vitro* studies of rodent PSC maintained in culture. These cultures, initially express the molecular markers of the quiescent cells and it is easy to observe the presence of the cytoplasmic lipid droplets by oil red stain (Apte MVet al., 1988, Mato E et al 2009). However, in a short amount of time, most of the cells in the culture showed a proliferative phenotype with α-SMA and ECM protein expression. These molecules are associated with the activated phenotype (Haber PS et al. 1999). Several authors have associated this phenomenon to *in vitro* changes of Rho-ROCK pathways regulated by the actin cytoskeleton (Masamune A et al. 2003). PI 3-kinase activity is required for PDGF-stimulated PSC migration, but not cell proliferation (McCarroll JA et al. 2004). Moreover, the role of the enzymes involved in the mitogen-activated protein kinase (MAPK) family have been described : Jun N-terminal kinase JNK and p38, which are involved in the transcriptional control and PSC activation, and are mediators of signals induced by pro-inflammatory cytokines and cellular stressors (Masamune A et al. 2003). On the other hand, ligands of the nuclear receptor PPARγ (peroxisome proliferator-activated receptor γ) such as 15-deoxy-Δ12,14-prostaglandin J_2 and troglitazone (an antidiabetic drug of the thiazolidinedione group) stimulate maintenance of a quiescent PSC phenotype *in vitro* have been described (Masamune A et al. 2002). In summary, despite that several intracellular mediators involved in the control of the PSC activation and desactivation have been identified, most of them are unknown.

Furthermore, some authors have documented a significant increment of the PSC in the regenerative areas of the pancreas after suffering an acute pancreatitis, induced in rodent. These observations, plus the identification of the PSC positive for nestin marker, support the idea that this population could be involved in the pancreatic regeneration process (Zimmermann A et al. 2002, Ishiwata T et al 2006).

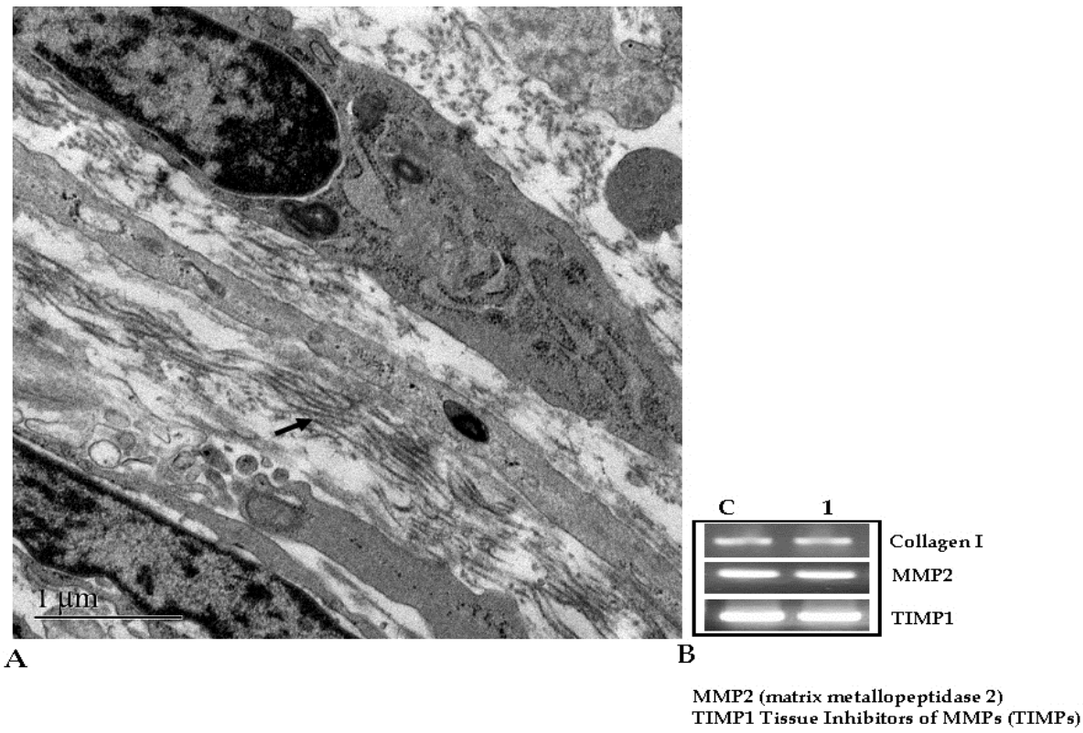

Fig. 1. A. Transmission electron micrographs of activated pancreatic stellate cells *in culture*. The arrow show abundant collagenous fibers compatible with collagenous type I. B. RT-PCR expression involved in the EMC remodeling (Mato E. et al., unpublished data)

3. Pancreatic progenitor cell: historical perspective

One of the important reasons to find progenitor cells in the pancreas is to cure Diabetes Mellitus. This metabolic disorder is a common and serious disease in our society and is the most rapidly growing chronic disease of our time. It has become an epidemic that affects millions of people around the world. For that reason, there has been an increasing in interest scientific community to identify the cell populations with stem or progenitor properties in the pancreatic tissue. This finding could represent a significant therapeutic advance in this disease.

The first description of stem and progenitors cells in adult tissue was in bone marrow and the nervous system (Weissman IL 2000; Fuchs E and Segre JA 2000). Although it is accepted that similar cells can exist in the other adult tissues and organs, they are not always easy to find. One of the reasons for limited number of studies on these cells relates to the fact that they do not have specific biological markers. Thus, finding of progenitor cells in the pancreas is a challenge. There is some evidence in the pancreas that progenitor cells exist in the neogenesis process, which can be induced by cellophane wrapping of the pancreas (Rosenberg L et al. 1998), partial pancreatectomy (Bonner-Weir S et al. 1993), streptozotocin-induced diabetes (Fernandes A et al. 1997), and also during pregnancy (Bonner-Weir S 2000). Some authors, Rosenberg in 1998 and Rafaeloff in 1997, have only associated this phenomenon with gene (*Reg*) and proteins (islet neogenesis, INGAP) which are expressed during the process, but not with progenitors cells. However, cell participation is possible.

Research has been launched to investigate the process of neogenesis and the cells that may be involved in this mechanism. Understanding this process will be the key since it will allow us to restore the function of the gland lost during the illness.

3.1 Progenitor cells in the pancreas tissue

3.1.1 Ductal cells

Most of the studies favor the pancreatic duct as a potential source of progenitor cells in adult pancreas (Rosenberg L 1998; Bonner-Weir S 2000). These studies are based on the information about the important role the primitive ductal epithelium has during the pancreas embryogenesis of the pancreas as a source for the islet development (Madsen OD et al. 1996, Sander M and German MS, 1997). Moreover, Gu and coworkers described the presence of endocrine cells within the adult ductal system (Gu D and Sarvetnick N 1993) and also identified beta cells associated with the human ductals (Bouwens and Pipeleers, 1998). Finally, the ability of ductal cells to expand *in vitro* and to form insulin-producing islet-like structures has also been demonstrated (Bonner-Weir S et al. 2000; Ramiya VK et al. 2000).

3.1.2 Pancreatic islet as a cellular source

Another interesting hypothesis was to propose the pancreatic islet as a progenitor cell source, based on the analysis of islet regeneration in mouse pancreas models after the administration of streptozotocin. The results showed the presence of the insulin-producing cells following the injury into the adult islets. This study suggested the existence of the two types of progenitor cells, one of them expressed Glut-2 and the other coexpressed insulin and somatostatin (Guz Y et al. 2001).

Nestin-positive cells, neurogenin-3 positive cells and hormone-negative immature cells, with proliferative capacity *in vitro* has been found in rats and human islets. This supports the idea of the existence of the multipotential cells in the islet (Kodama S et al 2005). However, their participation in islet regeneration and neogenesis *in vivo* has not yet been demonstrated (Zulewski H et al. 2001). Despite the explosion in the number of *in vitro* studies that describe different types of cells with progenitor capacity within the island, there is also some critical work demonstrating that the reactivation of genes required for endocrine cell development, such as neurogenin 3, are not implicated directly in the regeneration of pancreatic tissue after pancreatectomy (Lee CS et al. 2006).

Cells with the capacity to be differentiated not only in the lineage of endocrine cells, but also in other cellular lineages, such as exocrine and glials cells, have been identified (Seaberg RM et al 2004). These progenitors could be of different origins (ductal cells or cell located inside the islets). These cells showed different molecular markers, such as "the hepatocyte growth factor receptor", c-Met. This receptor tyrosine kinase plays an important role in tumour growth by activating mitogenic signaling pathways (Seaberg RM et al 2004; Suzuki A et al., 2004).

Other authors identified cells presenting a differentiated morphology and named them "small cells". Although these cells are positive for several pancreatic markers (PDX-1, sinaptoficin, insulin, glucagon, somatostatin, pancreatic polypeptide), they also expressed

markers of undifferentiated cells, such as: alfa-fetoprotein and Bcl-2. Surprisingly, these cells were negative for nestin and cytokeratin 19, indicators of pluripotency and ductal origin. Functional analysis showed that they have the capacity to present a glucose response, but they did not respond to secretatgogues, such as IBMX (Petropavlovkaia M and RosenbergL , 2002).

3.1.3 Hematoipoietic stem cells as a progenitor cells in pancreas

Hematoipoietic stem cells have been proposed, as a new progenitor source in pancreas. In 2002, this hypothesis was formulated by Lerner, et al., who identified a population defined as Side Population, or SP, from a bone marrow origin. This SP cell population, described for the first time by Goodel MA, et al., corresponded to a small subpopulation of cells with an enriched stem cell activity and showed a "low" Hoechst 33342 dye staining pattern. Subsequent studies attributed this SP phenotype to the expression of stem cell markers sucha as MDR1 and Nestin, and also co-expressed ABCG2, an ATP-binding cassette (ABC) transporter (Zhou S, 2001). ABCG2 gene is expressed in several rodent tissues, such as in the intestine, kidney and testes (Tanaka, Y 2005). The precise physiological function of these transporters in progenitor and differentiated cells is unknown and it has been postulated that they confer protection against a number of xenobiotics, thus maintaining the regenerative capacity of the tissue (Leslie, E.M, 2005). The identification and isolation of ABCG2 positive cells in pancreatic tissue may be a new potential source of adult multipotential stem/progenitor cells, useful for the production of islet tissue for transplantation into diabetic subjects (Fetsch, PA, 2006). The presence of these cells in pancreas tissue is controversial.

3.1.4 Epithelia Mesenchyma Transition (EMT)

Finally the concept of Epithelia-Mesenchymal transition or EMT has been described during the regeneration endocrine pancreas and in the cancer development. The EMT could permit that adult cells can be differentiated into the fibroblastic-like cells as a step of transition to other cellular lineage. Recently this process has been linked with the maintenance of stem cell phenotype. However, the molecular mechanism to control the EMT process remained to be demonstrated (Gershengorn MC et al. 2004; Bonner-Weir S et al. 2004).

An explosion of publications in the last decade tried to discover what type and where the progenitors cells are localised in the pancreatic tissue. We can conclude that the number of progenitor cell types in the pancreas may not be too limited to the cells already described. It is possible that the pancreas may contain an unidentified cell population at rest, as described in oval cells in the liver, capable of initiating their proliferation during the process of neogenesis. This opens the opportunity to explore new cell populations that form the pancreatic parenchyma.

4. Immunocytochemical investigation of the role of pancreatic stellate cell as progenitor cell

The plasticity of the stellate cells phenotype during tissue injury is a proven fact and may indicate that these cells can be presented in progenitor cell features. These findings suggested a novel aspect of the stellate cell biology must be necessary investigated.

The first marker identified in HSC was nestin. Nestin, a marker for neural stem cells, was identified in HSC during the transition from the quiescent to the activated phenotype in cells maintained in culture, but no association with a progenitor role was suggested by the authors (Niki T et al. 1999). Later, other markers were identified in the HSC: CD133 (prominin-1), a glycoprotein also known in humans and rodents as a Prominin 1 (PROM1), and expressed in the adult and embryonic stem cell and Oct4 (octamer-binding transcription factor 4), also known as POU5F1 (POU domain, class 5, transcription factor 1), protein involved in the self-renewal of undifferentiated embryonic stem cells (Mizrak D et al. 2008; Niwa H et al. 2000). These two markers were able to maintain an undifferentiated phenotype without losing the ability participates in liver regeneration (Kordes C et al 2009). Finally, the HSC were able to be differentiated into endothelial or hepatocyte-like cells (Kordes eC t al. 2007; Kubota H et al 2007). Following these findings an increasing number of papers about this topic were published.

The existence and lineage of progenitor cells in the pancreas, as well as their origin and location, is a topic of debate and, although several hypotheses had been proposed, it is not yet proven. Moreover, the possibility that the PSC can act as a progenitor cell is not clear.

Nevertheless, it is also important to remark that PSCand hepatic stellate cells are identical, have a common origin and both share transcriptional level, exhibiting organ-specific variations of the common transcriptional phenotype and (Bucholz M et al 2005; Omary MB et al 2007). This scenario suggests that the progenitor role for PSC could be a reality. In 2002, nestin-positive cells were identified in normal adult rat pancreas and during its regeneration. Interestingly, most of these cells presented the morphology characteristic of stellate cells. Nestin, in pancreas as in liver, was confirmed as a main marker of stellate cell activation. Other roles, including the marker of progenitor cells, were not confirmed (Lardon J 2002).

The question that needs to be addressed is whether PSC, after overexpressing some specific pancreatic transcription factors, such as Pdx1 or NeuroD1, have the ability to present the transdifferentiation process, which permits conversion into insulin-producing beta cells.

One approach to conduct these studies and broaden the possibility of unraveling the mechanisms that control self-renewal, is to explore the cell roles after their isolation and establishment of the cell culture. The first description of the stellate isolation from tissue was in 1977 by Galamos JT. The study was characterized by growth mesenchymal cells derived from liver tissue, which have probably been derived from stellate cell (Galamos JT et al. 1977). Later, density gradient centrifugation was used after *in situ* digestion of the tissue, based on their buoyancy attributable to intracellular vitamin A. The density gradient separation method remains the most widely used approach for stellate cell isolation, but criticism of this method favours the isolation of quiescent cells, which are rich in vitamin A (Friedman SK, 2008). Later, transgenic and knockout mouse models have been developed for the isolation following the standard method of murine stellate cells or for performing *in situ* analysis with specific stellate markers. However, one limitation of the technique is the large number of animals needed to obtain an adequate cell yield (Henderson NC et al. 2006; Kalinichenko Vet al. 2003). To solve this problem, stable cell lines obtained from human and mouse model would be an important advantage for many investigators in order to study stellate cell biology. Several methods have been described to establish from HSC cultures and pancreas cell lines, such as: long-term culture, transfection with simian virus 40 (SV40) T antigen, or ectopic expression of telomerase (Vogel S et al. 200; Murakami K et al 1995;

Apte MV et al. 1998; Kruse ML 2001; Sparmann G 2004; Masamune A et al. 2003; Satoh M et al 2002; Jesnowski R et al 1999; Löhr M et al 2001). The disadvantage of the cell lines is that they differ somewhat in their state of activation or in transcription expression and the results obtained must be validated in the *in vivo* model. Finally, the description of the cryoperservation technique for freezing primary stellate cell lines is an important advance for sharing the cells between different laboratories (Neyzen S et al. 2006).

In this context our group initiated a new research field, focusing in the identification of progenitor cell in pancreas tissue through ABCG2 transporter as a progenitor cell marker. This marker was identified as a molecular determinant of the Side-Population (SP) phenotype. However, there is no information about its expression on the pancreatic cells. Recently, overexpression of the breast cancer-resistance half-transporter protein (BCRP1) was found to be responsible for the occurrence of mitoxantrone resistance in a number of cell lines (Doyle LA et al, 1998; Miyake K et al, 1999; Litman T et al, 2000). Based on in these findings, we isolated a mitoxantrone-resistant cells population from pancreata of lactating rats by mitoxantrone selection through the ABCG2 transpoter (Fig. 2 A, B, C).

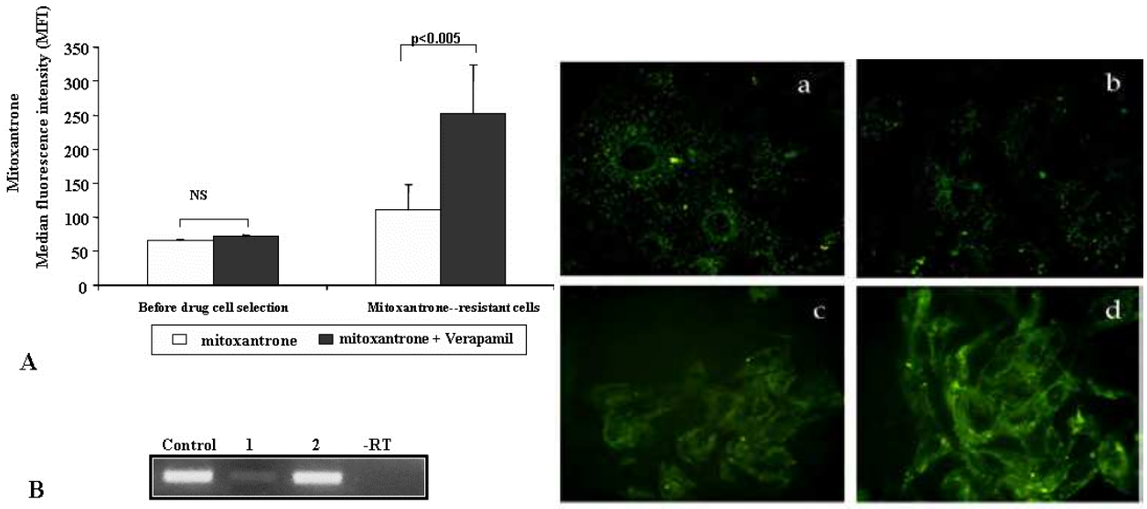

Fig. 2. ABCG2 expression, and drug uptake and retention assays in primary cell cultures (mitoxantrone-resistant cells and unselected cells). (A) One-hour drug accumulation assay with and without verapamil. The cells were preincubated with 5μM verapamil for 15 min. Subsequently, cells were treated with 8 μM mitoxantrone and assayed for drug accumulation. Each condition is the mean of three experiments ± SD. Verapamil increased the intracellular concentration of mitoxantrone in the mitoxantrone-selected drug-resistant cells. The experiment was performed in triplicate, and a representative histogram was shown. (B) The ABCG2 expression in the cells from cultures: unselected cells (line 1) and mitoxantrone-resistant cells at Stage 2 (line 2) was determined by RT-PCR. The ARIP cell line was used as a positive control of the reaction (Control), – RT corresponds to amplification in which reverse transcriptase was excluded from the reaction (negative control). (C) cells treated with mitoxantrone for 2 ' (a) and 10' (b) or treated with mitoxantrone plus verapamil (ABCG2 inhibitor) for 2′ (c) and 10′ (d) (Reproduced with permission, from Mato E. et al. Identification of a pancreatic stellate cell population with properties of progenitor cells: new role for stellate cells in the pancreas . Biochem. J. 421; 181–191© the Biochemical Society)

Next, cells were expanded, checking that the cells present in culture a fibroblast features (Fig 3 A)

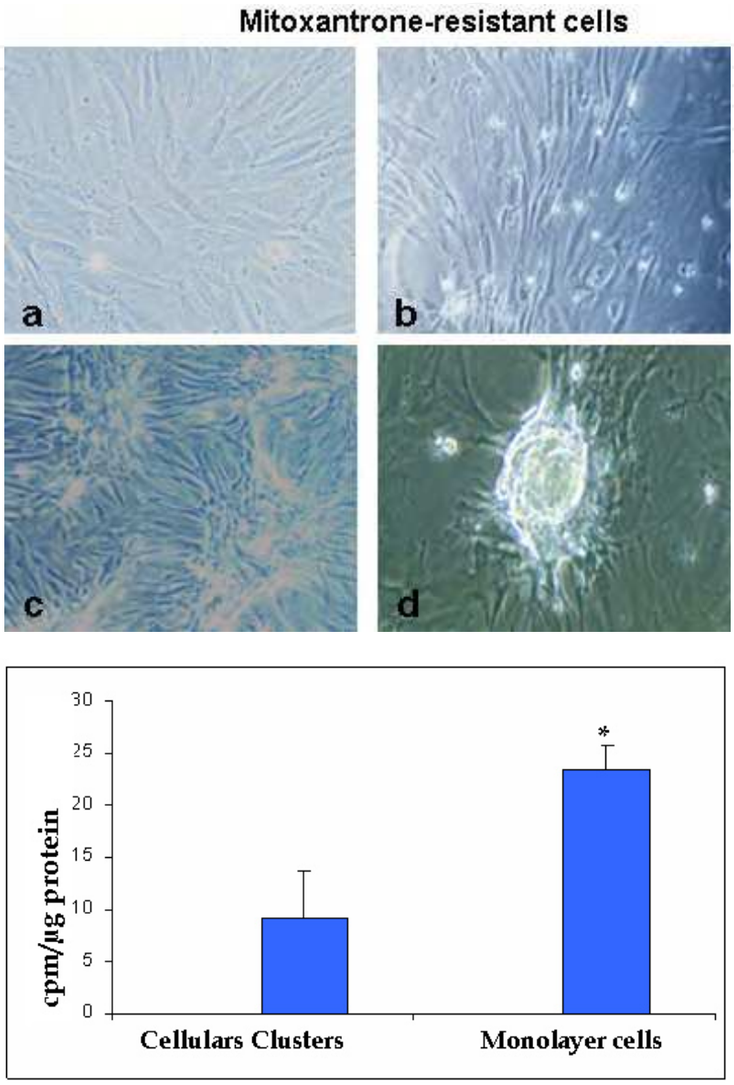

Fig. 3. Phenotype of Cell Line from mitoxantrone-resistant cell population. A The mitoxantrone-resistant cells became overgrown by cells with a fibroblastoid morphology (a,b). Spontaneously, some cells began to form three-dimensional cell clusters (c,d,e). B. Representative Histogram of the tritiated thymidine incorporation in cellular cluster and monolayer cells * $p<0.05$. (Reproduced with permission, from Mato E. et al. Identification of a pancreatic stellate cell population with properties of progenitor cells: new role for stellate cells in the pancreas . Biochem. J. 421 ;181–191© the Biochemical Society)

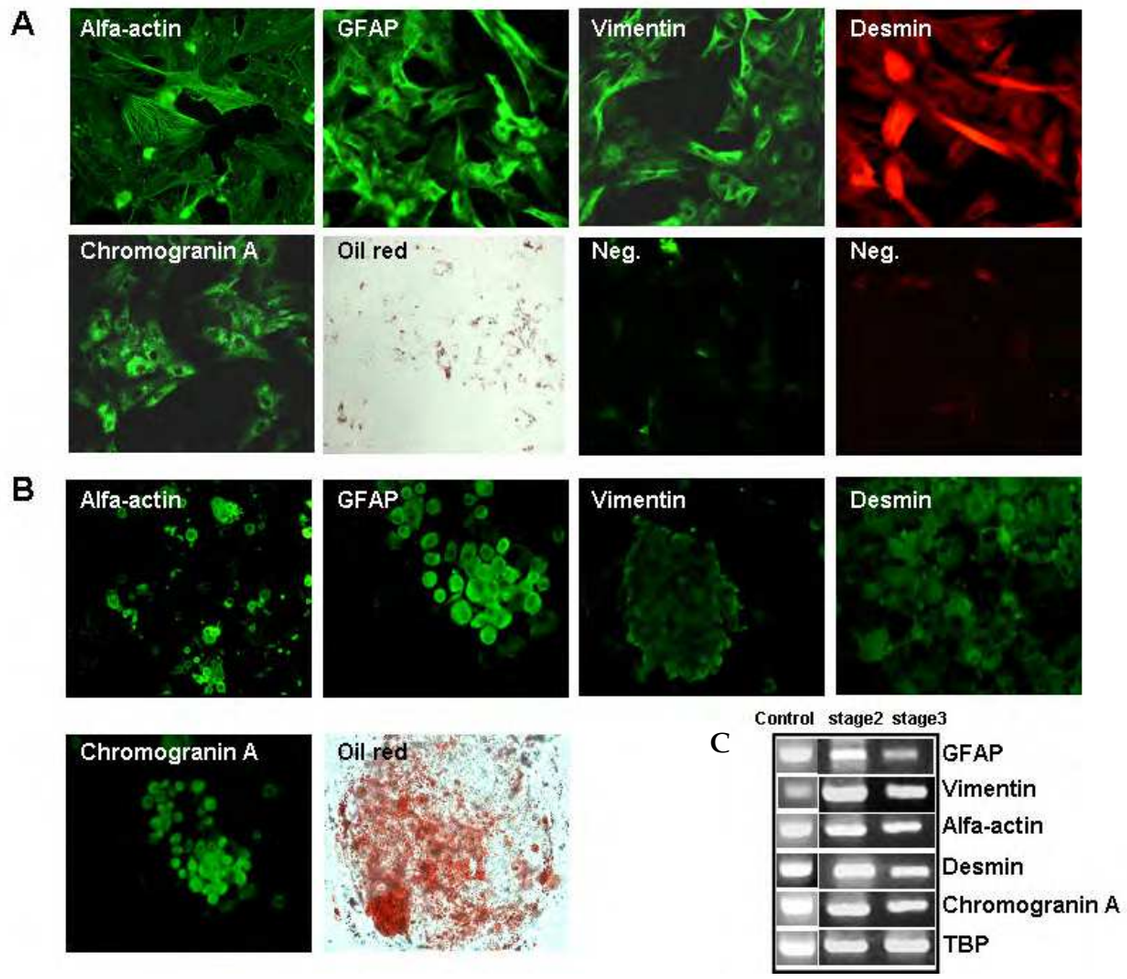

Fig. 4. Mitoxantrone-resistant cells were phenotyped by immunofluorescence and RT-PCR using pancreatic stellate markers. (A) Mitoxantrone-resistant cells at Stage 2 express the markers: alfa-Actin, GFAP, vimentin, desmin, and chromogranin A. To confirm the presence of the vitamin A stored in the fat droplets, oil red staining was performed. (B) Disaggregated from mitoxantrone-resistant cells at stage 3 were immunophenotyped for the same markers, including the oil red staining. Negative controls (Neg) were used. (X20 original magnification). (C) These results were confirmed by RT-PCR using one µg of total RNA of the mitoxantrone-resistant cells in both stages (stage 2 (monolayer cultere) and stage 3 (cellular cluster)). Control cell lines were used as a control reaction. (Reproduced with permission, from Mato E. et al. Identification of a pancreatic stellate cell population with properties of progenitor cells: new role for stellate cells in the pancreas . Biochem. J. 421 ;181–191© the Biochemical Society)

The existence of a fine balance between proliferation and differentiation process is accepted by the research community. This balance promotes the differentiation from adult stem cell to postmitotic cells through decreasing or increasing the ratio of proliferation, permitting the maintenance of the stem cell population in adult tissues (Soria B, 2001). The observation of the behavior of mitoxantrone resistant cells in culture was interesting. The results indicated

that, while the cells with fibrobastoide appearance have showed a rapid and constant growth after clustering formation, they modified their behavior showing a significant reduction in their growth, without stopping completely (Fig.3, B). The results suggested the ability of the cell to be reprogrammed.

Finally the immunocharacterization of these cell cultures in monolayer and cellular cluster showed a stellate phenotype, characterised by vitamin A uptake (oil red staining) and steallate markers presence (Fig. 4 A, B).

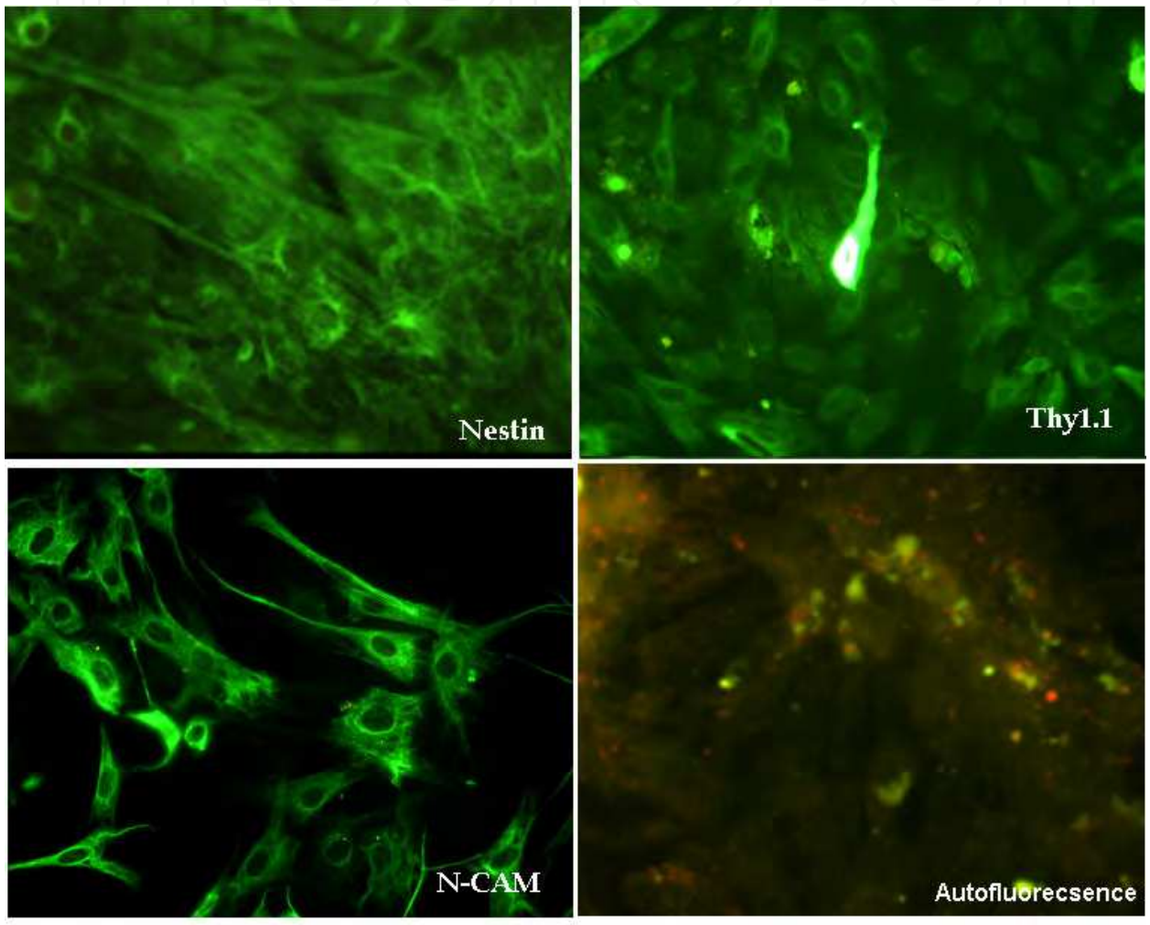

Fig. 5. Characterization of progenitor markers in mitoxantrone-resistant cell population. Nestin, Thy1.1 and N-CAM protein expression was detected by immunostaining in culture from mitoxantrone-selected drug-resistant cells (Modified with permission, from Mato E. et al. Identification of a pancreatic stellate cell population with properties of progenitor cells: new role for stellate cells in the pancreas . Biochem. J. 421 ;181–191© the Biochemical Society)

Moreover, they share markers of the adult stem cells, such as: ABCG2, Nestin, Thy1.1, and N-CAM. The latter marker participates in signal transduction and in cell type segregation as a mediator of cellular junctions during organogenesis (Esni F et al. 1999) (Fig. 5).

Little it is known about the role of Fibroblast growth factor and their receptor in stellate cells. FGF belongs to a large family of molecules that retain a high homology at the genetic level. These growth factors induce pleiotropic responses, causing effects in both embryonic development and in adult tissue (Steiling H and Werner S, 2003). Their actions are mediated by four receptors of the tyrosine kinase membrane and present different isoforms (b and c) by splicing (Itoh N and Ornitz DM, 2004). Fibroblast growth factors receptors (FGFR) have been detected over time during the development of the pancreas. In addition, their ligands, such as Fibroblast growth factor: 1, 7, 9, 10, 11, 18 (Dichmann DS et al., 2003), and the subtype of the FGFR 2, called FGFR2b, seem to have a key role in the exocrine development (Miralles F, et al. 1999). Recently, FGF7 and FGF10 have been involved in maintaining the cells in an undifferentiated stage and controlling the self-renewal of the pancreatic precursors (Elghari L et al. 2002; Norgaard GA et al, 2003). The positive gene expression for FGFR2IIIb, FGFRIII2c, FGFR1, and their specific ligands (FGF 1,7,and 10), were showed for the first time in our cell cultures (Fig. 6, Mato et al. unpublished data).

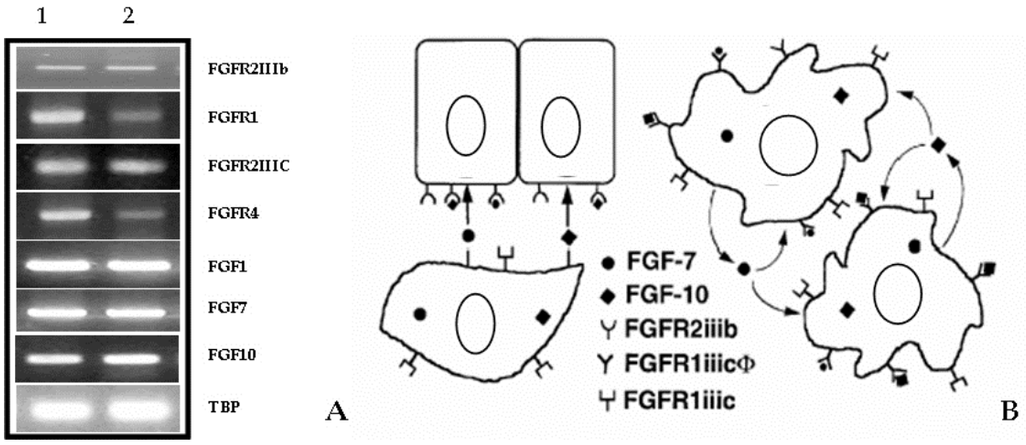

Fig. 6. Expression of the Fibroblast growth receptor and Fibroblast growth factors in the cells from cultures. Expression of FGFRIII2b, FGFR1, FGFR4, FGFR2IIIC, FGF1, FGF7, FGF10 in the cells from cultures: monolayer cells (line 1) and at clusters cells (line 2) was determined by RT-PCR. B Proposed autocrine (A) and paracrine (B) model through FGFR and their ligands of the PSC in: ductal cell, exocrine cells or themselves. (Mato E. , unpublished data)

This finding may suggest that FGFR and their ligand are involved in epithelial-mesenchymal communication of PSC and, in addition, the autocrine effect allows the maintenance of its cell population in the pancreatic tissue. On the other hand, pancreatic stellate cell do not express endocrine genes. However, during cell expansion, a spontaneous cell differentiation occurs and these cells showed a weak expression of PDX-1 in to the nucleus and the cytoplasm of the cells (Fig. 7 A, B). This gene, also known as (insulin promoter factor-1, islet/duodenum homeobox-1, somatostatin transactivating factor-1, or insulin upstream factor-1 and glucose-sensitive factor), plays a key transcription factor in the endocrine differentiation pathway and is also essential for differentiation of endocrine cells in the gastric antrum. The results suggest a transdifferentiation process. However, the molecular mechanisms of this process are unknown. In additon, few studies are investigating the effect of culture medium and additional protein components on the viability and maturation of the cells (Royer PJ et al 2006). Our results underscore the

importance of defining culture medium composition in experimental procedures, in order to identify new soluble factors involved in the processes of cellular transdifferentiation.

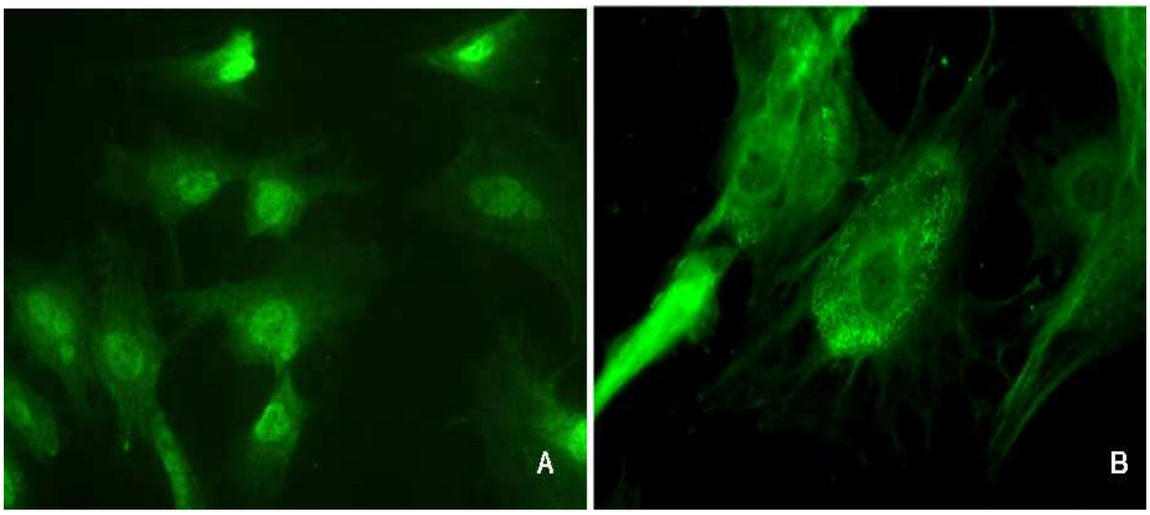

Fig. 7. Expression of Pdx-1 transcription factor in the cells from cultures (Monolayer stages). Pdx-1 protein expression was detected by immunostaining fluorescent in culture from mitoxantrone-selected drug-resistant cells. A.- Nuclear staining (X40 original magnification) B.- Cytoplasmatioc staining (X60 original magnification) (Mato E. unpublished data).

Identifying instructive signals that induce differentiation during organogenesis will be important to determine how such signalling networks are established and how they elicit multiple signalling responses in endodermal cells to activate appropriate genetic programs (Ratineau C et al 2003). Several signalling molecules have been implicated in induction of specific endodermal cell types. However, few of these factors have been examined in adult pancreatic tissue (Sttaford D et al 2006). One of these factors is GLP-1, secreted from the L-cells of the distal ileum and colon. This substance has been suggested to play an important role in increasing beta cell mass by inducing the neogenesis or transdifferentiation through the expression of Pdx-1 in ductal or islets cells (Yue F et al. 2006; Abraham EJ et al 2002; Hui H et al 2001).

Also, matrigel secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells, is a gelatinous protein mixture that provides a semisolid medium that resembles the complex extracellular environment found in many tissues and is used as a substrate for three-dimensional cell culture. The addition of exendin-4 (analog to GLP-1) and matrigel to our cellular model was needed to proceed to the differentiated stages and permit detection of insulin, IAPP, glucagon, GLUT2 and the convertases PC1/3 and PC2 expression (Fig. 8 A, B). In contrast, expression of the transcription factor p48 and other exocrine genes, such as amylase, were not detected. Interestingly enough was the observation of the cytokeratin 19 (CK19) expression. These intermediary filaments present in cells of the epithelial origin, such as ductal cells, indicate that the cell could be involved in the mechanism to control the mesenchymal-epithelial transition (MET). This phenomenon consists of a promising source of cells for replacement therapies, but can also be involved in the carcinogenesis process (Mato E et al. 2009).

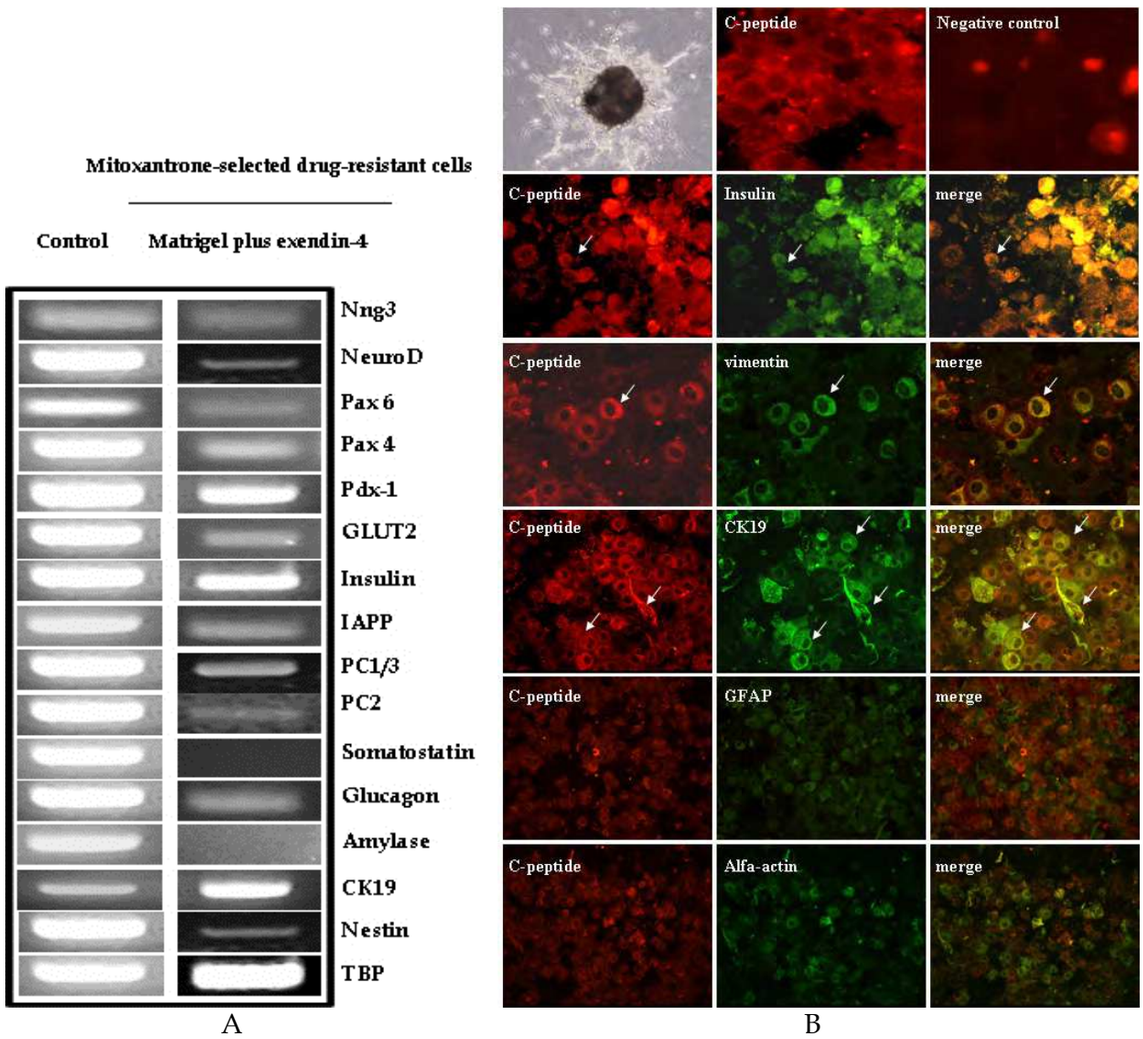

Fig. 8. Pancreatic gene expression profiles and co-immunolocalization of different markers by cytospin-prepared cells obtained from disagregated cellular clusters after exedin-4 treatment. A.- Gene expressions profile after matrigel plus exendin-4 treatment in mitoxantrone-resistant cell cultures. B.- Representative cellular cluster after treatment with matrigel plus exendin-4. The markers were visualized in red: c-peptide, green: insulin, vimentin, CK19, GFAP, alfa-actin, and yellow as the merges. The MIN-6 cells were used for the immunohistochemistry control. (Reproduced with permission, from Mato E. et al. Identification of a pancreatic stellate cell population with properties of progenitor cells: new role for stellate cells in the pancreas . Biochem. J. 421 ;181–191© the Biochemical Society).

The molecular mechanisms and the receptors involved in EMT process are not indentified yet. Most of the evidence suggests that integrin could play an important role. On the other hand, the basement Membrane Matrix is an effective culture medium for the attachment and differentiation of both normal and transformed anchorage dependent on epithelioid and other cell types. The use of these three-dimensional culture systems may be particularly relevant to such efforts by recapitulating a more physiological microenvironment (Han YP et al. 2004; Phillips PA et al. 2003; George PC 2005). During the matrigel growth, substantial

ultrastructural changes in the cells were observed. The cells presented a smaller and more homogenous cell size with round nuclei and electron-dense homogenous chromatin, a significant increase in the number of mitochondria, lipid droplets in the cytoplasm and abundant electron-dense granules were also observed. In contrast to the cellular cluster growth in a normal condition medium, the quiescent stellate cells had a high presence of fibers compatible with collagen fibers (Fig. 9 A).

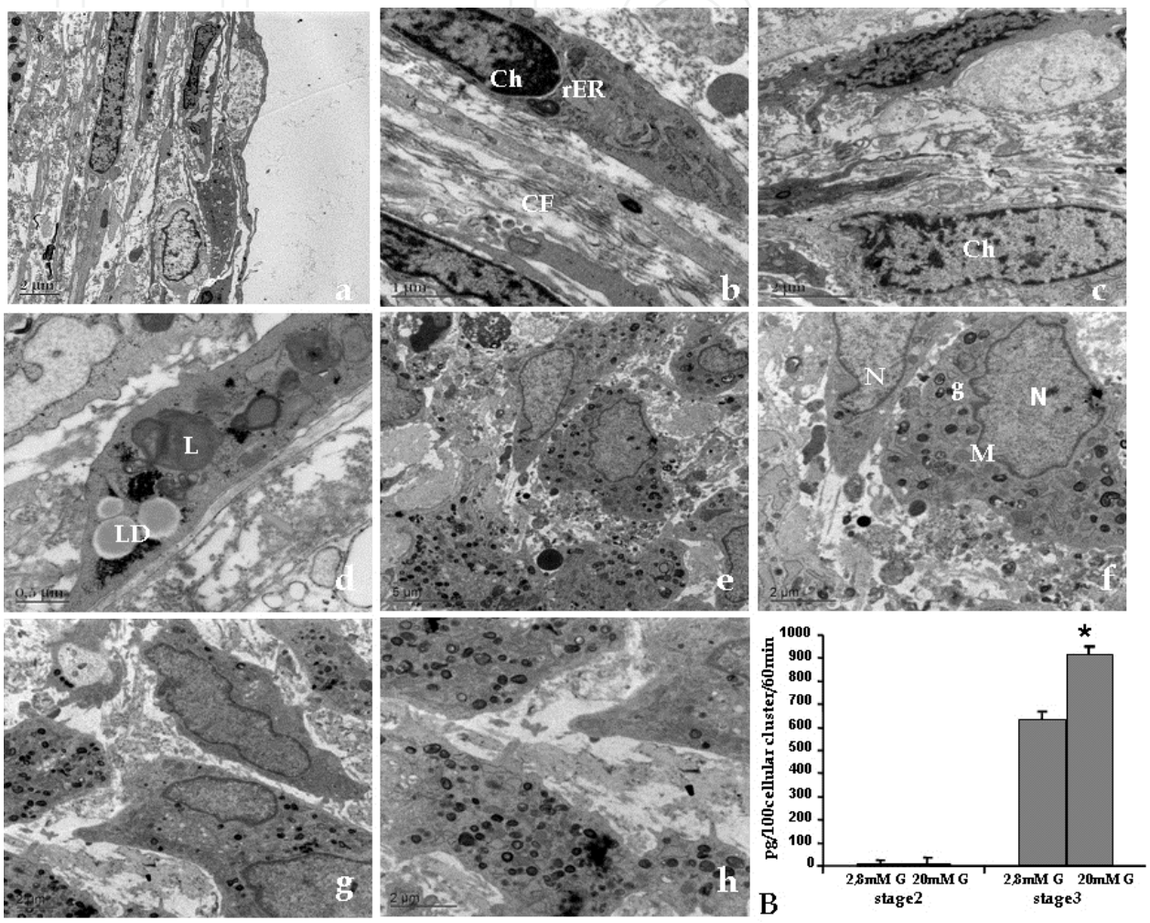

Fig. 9. Ultrastructural changes and insulin release in the Mitoxantrone-resistant cells at stage 3 after differentiation treatment with medium 3. (A) Transmission electron micrographs of undifferentiated cells (a-d) show high hypertrophy in the rough endoplasmic reticulum (rER), lipid droplets (LD), lysosomes (L) and collagenous fibers (CF). Two types of electron-dense chromatin structure were observed (Ch). However, the differentiated cells (e-h) presented a homogenous size with a round nucleus (N), at times indented, abundant mitochondria (M), and electron-dense granules in the cytoplasm were observed (g). (B) Insulin secretion after 1 hour of glucose stimulation at 20 mM *vs*. 2.8 mM. The results were normalized to 100 cell clusters (n=3) * $p < 0.05$ (employing Student's t-test) (Reproduced with permission, from Mato E. et al. Identification of a pancreatic stellate cell population with properties of progenitor cells: new role for stellate cells in the pancreas . Biochem. J. 421 ;181–191© the Biochemical Society).

Gene expression and ultrastrucutral changes detected in the cell culture growth support the idea of the ability of cells to release insulin into the medium. In this scenario, insulin secretions of several sets of cell clusters were measured by static incubation at low (2.8mM) and high (20mM) levels of glucose. Eventhough, insulin levels detected in the cell clusters were lower compared to mouse islets, an increase of 44% was detected after stimulating cellular clusters with high level of glucose. (Fig. 9 B). However, future experiments will have to demonstrate that the secretion of insulin is not only constitutive (Kuliawat R et al. 1994). Furthermore, the expression of specific markers of stellate cells remained after maintaining the cell in matrigel condition. These results may indicate the differentiation process has not been fully completed and the cells still maintained characteristics of stellate cells (Fig. 8).

An interesting strategy in order to investigate the biology of these cells is the use of proteomic approaches, since it is a useful tool for displaying protein expression patterns in the cell. For that reason, this approach has been used in active as well as quiescent stellate cells. (Kawada N et al. 2001; Pauki JA et al. 2011 (a); Paulo JA et al. 2001 (b); Wehr AYet al. 2011). In this context, the proteomic study of our cellular culture secretome was preformed. The results showed that some of these proteins have potentially great influence on the physiology of the stellate cells themselves and/or on neighbouring cells, indicating a paracrina and /or autocrine action. Moreover, we have identified some novel factors that were clustered in the differentiation/development-related proteins, such as AHNAK, Gap43, and DIXDC1 (unpublished data from Mato E et al). However, further experiments are required to investigate the interaction within these different genes.

In summary: The pancreatic stellate cells is a fascinating nonendocrine cellular model that could represent a new source of cells involved in regenerative medicine of the pancreas in the future. However, more studies are needed to understand the molecular mechanisms that control their cellular plasticity. Certainly, the use of imunocytochemical and immunohistochemical techniques, complemented with cell -tracking methods, will be important tools to unravel the role of these cells during the tissular regeneration process both in the pancreas and in the liver.

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6. References

Abraham E.J., Leech, C.A., Lin, J.C., et al. (2002). Insulinotropic hormone glucagons-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. Endocrinology 143,3152-3161

Apte M.V. et al. (1998). Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. Cancer Res.43:128–133

Apte M.V., Haber PS, Applegate TL, et al. (1998). Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. Gut 43:128–133

Apte M.V., Haber PS, Darby SJ, et al. (1999). Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. Gut 44:534–541

Bachem M.G. et al.(1998). Identification, culture, and characterization of pancreatic stellate cells in rats and humans. *Gastroenterology* 115:421–432

Baba S., Fuji H, Hirose T, et al. (2004). Commitment of bone marrow cells to hepatic stellate cells in mouse. J. Hepatol 40, 255-260

Bonner-Weir S. (2000). Perspective: postnatal pancreatic cell growth. Endocrinology 141:1926-192

Bonner-Weir S., Baxter L.A., Schuppin G.T., et al. (1993). A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. Diabetes 42:1715-1720

Bonner-Weir S., Inada A., Yatoh S., et al. (2008). Transdifferentiation of pancreatic ductal cells to endocrine β-cells. Biochem Soc Trans 36: 353–356

Bouwens L. and Pipeleers D.G. (1998). Extra-insular beta cells associated with ductules are frequent in adult hunman pancreas. Diabetologia 41, 629-633

Bunting K.D. (2002). ABC Trransporters as phenotypic markers and functional regulators of stem cells. Stem Cells 20, 11-20

Buchholz M., Kestler H.A., Holzmann K., et al. (2005). Transcriptome analysis of human hepatic and pancreatic stellate cells: organ-specific variations of a common transcriptional phenotype. J Mol Med 83,795-805

Deichmann D.S., Miller C.P. Jensen J. et al.(2003). Expresión and misexpression of members of the FGF and TGFbeta familias of growth factors in the developing mouse pancreas. Dev Dyn 226,663-674

Doyle L.A., Yang W, Abruzzo LV, et al. (1998). A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci USA 95: 15665–15670

Esni, F., Taljedal, I.B., Perkl, A.K., et al. (1999). Neural cell adhesion molecule (N-CAM) is required for cell type segregation and normal ultrastructure in pancreatic islets. J. Cell. Biol. 144,325-337

Elghari L., Cras-Meneur C., Czernichow, P et al. (2002). Role for FGFR2IIIb-medianted signals in controlling panceatic endocrine progenitor cell proliferation. Proc Nat Sci USA 99, 3884-3889.

Fernandes A., King L.C., Guz Y., et al. (1997). Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. Endocrinology 138:1750-1762

Fetsch, P.A., Abati, A., Litman, T., et al. (2006). Localization of the ABCG2 mitoxantrone resistance-associated protein in normal tissues. Cancer Lett. 235, 84-92

Friedman S.L. (2000). Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J.Biol.Chem 275:2247-2250

Friedman S.L. (2008). Hepatic Stellate Cells: Protean, Multifunctional, and Enigmatic Cells of the Liver. Physiol Rev January 88; 1 125-172

Fuchs E., Segre J.A .(2000). Stem cells: a new lease on life. Cell 100:143-155

Galambos J.T., Hollingsworth MA, et al. (1977). The rate of synthesis of glycosaminoglycans and collagen by fibroblasts cultured from adult human liver biopsies. J Clin Invest 60: 107–114

Geerts, A. (2001). History, heterogeneity, development biology, and functions of quiescent hepatic stellagte cells. Semin Liver Dis 21, 311-255

Gershengorn M.C., Hardikar A. A. , Wei Ch ., et al. (2004). Epithelial-to-Mesenchymal Transition Generates Proliferative Human Islet Precursor Cells. Science 24: Vol. 306 no. 5705 pp. 2261-2264

Georges P.C. and Janmey P.A. (2005). Cell type-specific response to growth on soft materials. Journal of Applied Physiology; 98: 41547-1553

Gu D., Sarvetnick N. (1993). Epithelial cell proliferation and islet neogenesis in IFN-transgenic mice. Development 118:33-46

Guz Y., Nasir I., Teitelman G. (2001). Regeneration of pancreatic cells from intra-islet precursor cells in an experimental model of diabetes. Endocrinology 142:4956-4968

Goodell M.A., Brose K., Paradis G., et al. (1996). Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 1996; 183: 1797–806

Habener J.F., Kemp D.M. and Thomas M.K. (2005). Minireview: transcriptional regulation in pancreatic development. Endocrinology 146, 1025-1034

Haber PS, Keogh G.W., Apte M.V., et al. (1999). Activation of pancreatic stellate cells in human and experimental pancreatic fibrosis. Am J Pathol 155:1087–1095

Han Y.P., Zhou L., Wang J., et al. (2004). Essential role of matrix metalloproteinases in interleukin-1-induced myofibroblastic activation of hepatic stellate cell in collagen. J Biol Chem 279: 4820–4828

Henderson N.C., Mackinnon A.C., Farnworth S.L., et al. (2006). Galectin-3 regulates myofibroblast activation and hepatic fibrosis. Proc Natl Acad Sci USA 103: 5060–5065

Hirosawa K. and Yamada E., (1973). The localization of vitamin A in the mouse liver as revealed by electrón microscopy radioautography. J Electron Microsc (Tokyo) 22, 337-346

Hui, H., Wright, C., Perfetti, R. (2001). Glucagon-like peptide 1 induces differentiation of islets duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells. Diabetes 50,785 –796

Ito T. (1951). Cytological studies on stellate cells of kupffer and fat storing cells in the capillary wall of human liver (abstract). Acta Abat Jpn; 26:42

Ito T. (1973). Recent advances in the study on the fine structure of the hepatic sinusoidal wall: A review. Gunma Rep Med Sci, 6:119-163

Itoh N. and Ornitz D.M. (2004). Evolution of the Fgf and Fgfr gene families. Trends Genet 20,563-569

Ishiwata T., Kudo M, Onda M et al. (2006). Defined localization of nestin-expressing cells in L-arginine-induced acute pancreatitis. Pancreas 32,360-368

Ikejiri N. (1990). The vitamin-A storing cells in the human and rat pancreas. Kurume Med J 37:67–81.

Jasper, R. (2004). Molecular regulation of pancreatic stellate cell functions. Ml Cancer 3, 26

Jensen J. (2004). Gene regulatory factors in pancreatic development. Dev Dyn 229, 176-200

Jesnowski R., Müller P., Schareck W., et al. (1999). Immortalized pancreatic duct cells *in vitro* and *in vivo*. Ann NY Acad Sci; 880:50–65.

Kalinichenko V.V., Bhattacharyya D, Zhou Y, et al. (2003). Foxf1 +/– mice exhibit defective stellate cell activation and abnormal liver regeneration following CCl4 injury. Hepatology 37: 107–117

Kawada N. , Kristensen D.B. , Asahina K., et al. (2001). Characterization of a Stellate Cell Activation-associated Protein (STAP) with Peroxidase Activity Found in Rat Hepatic Stellate Cells. The Journal of Biological Chemistry, 276, 25318-25323

Kim S.K. and Hebrok M. (2001). Intercellular signals regulating pancreas development and function. Gene Dev 15, 111-127

Kordes C., Sawitza I., Haussinger D. (2009). Hepatic and pancreatic stellate cells in focus. Biol Chem 390:1003–1012.

Kodama S., Toyonaga T. Kondo T. et al. (2005). Enhaced expresión of PDX-1 and Nng3 by exendin-4 durin beta cell regeneration in STZ-rtrated mice. Biochem Biophys Res Commun 327, 1170-1178

Kordes C., Sawitza I., Häussinger D. (2009). Hepatic and pancreatic stellate cells in focus. Biol Chem. Oct;390(10):1003-12.

KordesC., Sawitza I., Muller-Marbach A. et al. (2007). CD133+ hepatic stellate cells are progenitor cells. Biochem Biophys Res Commun 352,410-417

Kubota H., Yao H.L. and Reid L.M .(2007). Identifiaction and characterization of vitamin A-storing cells in fetal liver: implications dor functional importance of hepatic stellate cells in liver development and hematopoiesis. Stem Cells 25,2339-2349

Kruse M.L., Hildebrand P.B., Timke C., et al. (2001). Isolation, long-term culture, and characterization of rat pancreatic fibroblastoid/stellate cells. Pancreas;23:49–54

Kuliawat R. and Arvan P. (1994). Distinct molecular mechanism formprotein sorting within immature secretory granules of pancreatic beta-cells. J Cell Bio 126,77-86.

Lardon J., Rooman I., Bouwens L. (2002). Nestin expression in pancreatic stellate cells and angiogenic endothelial cells. Histochem Cell Biol. 2002 Jun;117(6):535-40. Epub 2002 May 14

Lee C.S., De Leon D.D., Kaestner K.H. et al (2006). Regeneration of pancreatic islets after partial pancreatectomy in mice does not involve the reactivaion of neurogenin-3. Diabetes 55,269-272

Lechner A., Leech C.A., Abraham E., et al. (2002). Nestin-positive progenitor cells derived from adult human pancreatic islets of Langerhans contain side population (SP) cells defined by expression of the ABCG2 (BCRP1) ATP-binding cassette transporter. Biochem. Biophys. Res. Commun. 293,670-674

Leslie E. M., Deeley, R.G., Cole, S. P. (2005). Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. Toxicol. Appl. Pharmacol. 204, 216-237

Litman T., Brangi M, Hudson E, et al. (2000) The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter MXR (ABCG2). J Cell Sci 113(Part 11): 2011–2021

Löhr M., Müller P., Zauner I., et al. (2001). Immortalized bovine pancreatic duct cells become tumorigenic after transfection with mutant k-ras. Virchows Arch 438: 581–590

Madsen O.D., Jensen J., Blume N., et al. (1996). Pancreatic development and maturation of the islet cell studies of pluripotent islet cultures. Eur J Biochem 242:435-445

McCarroll J.A., Phillips P.A, Kumar R.K., et al. (2004). Pancreatic stellate cell migration: role of the phosphatidylinositol 3-kinase(PI3-kinase) pathway. Biochem Pharmacol 67:1215-25

Masamune A., Kikuta K, Satoh M, Satoh K, Shimosegawa T (2003). Rho kinase inhibitors block activation of pancreatic stellate cells. Br J Pharmacol 140:1292-1302

Masamune A., Satoh M., Kikuta K., et al. (2003). Establishment and characterization of a rat pancreatic stellate cell line by spontaneous immortalization. World J Gastroenterol 9:2751–2758

Masamune A., Satoh M., Kikuta K., et al. (2003). Inhibition of p38 mitogen-activated protein kinase blocks activation of rat pancreatic stellate cells. J Pharmacol Exp Ther 304:8-14

Masamune A., Kikuta K., Satoh M,. et al. (2002). Ligands of peroxisome proliferator-activated receptor-γ block activation of pancreatic stellate cells. J Biol Chem 277:141-147

Mato E., Lucas M., Petriz J. et al. (2009). Identification of a pancreatic stellate cell population with properties of progenitor cells: new role for stellate cells in the pancreas . Biochem. J. 421 ;181–191

Mizrak D., Brittan M., Alison M.R. (2008). "CD133: Molecule of the moment". J Pathol 214 (1): 3–9

Miralles F., Czernichow P., Ozaki K. et al. (1999). Signaling through fobroblast growth factor receptor 2b plays a key role 96,6267-6272

Miyake K., Mickley L, Litman T, et al. (1999). Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res 59: 8–13

Morita M. et al. (1998). Analysis of the sinusoidal endothelial of the feta rat liver a sinusoidal enthelial cell specific antibodyt, SE-1. Cell Struct Funct 23:341-348

Morini S., Carotti S, Carpino G, et al.(2005). GFAP expression in the liver as an early marker of stellate cells activation. J Anat Embryol. 110(4):193-207

Murakami K., Abe T., Miyazawa M., et al. (1995). Establishment of a new human cell line, LI90, exhibiting characteristics of hepatic Ito (fat-storing) cells. Lab Invest 72: 731–739

Naito N. and Wisse E. (1977). Observation on the fine structure and cytochemistry of sinusoidal cells in fetal and neonatal rat liver, In: Wisse E., Knook D., ed. Kupffer Cells and Other Lever Sinusoidal Cells. Amstenrdam: Elvesier/North Holland Biochemicak Press; 497-505

Neyzen S., Van de Leur E., Borkham-Kamphorst E., et al. (2006). Cryopreservation of hepatic stellate cells. J Hepatol 44: 910–917

Niki T., Pekny M., Hellemans K., et al. (1999). Class VI intermediate filament protein nestin is induced during activation of rat hepatic stellate cells. Hepatology. 29(2):520-7

Niwa H., Miyazaki J., Smith A.G. (April 2000). "Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells". Nat. Genet. 24 (4): 372–6

No authors listed (1996). Hepatic stellate cell nomenclature. Hepatology 23(1):193

Norgaard G.A., Jensen J.N., Jensen J. (2003). FGF10 signaling maintauns the pancreatic progenitor cell state revealing a novel role of Notch in organ development. Dev Bio 264,323-338.

Ogawa M., La Rue A.C., Drake C.J. et al (2006). Hematopoietic origin of fibroblasts/myofibroblasts: Its patholophysiologic implications. Blood 108, 2893-2896

Omary M.B., Lugea A., Lowe A.W., et al. (2007). The pancreatic stellate cell: a star on the rise in pancreatic diseases. J Clin Invest 117: 50–59

Paulo J.A., Urrutia R., Banks PA, et al. (2011). Proteomic analysis of a rat pancreatic stellate cell line using liquid chromatography tandem mass spectrometry (LC-MS/MS). Proteomics. Sep 25

Paulo J.A., Urrutia R., Banks P.A., et al. (2011). Proteomic Analysis of an Immortalized Mouse Pancreatic Stellate Cell Line Identifies Differentially-Expressed Proteins in Activated vs Nonproliferating Cell States. J Proteome Res. 2011 Oct 7;10(10):4835-44

Petropavlovkaia M. and Rosenberg .L (2002). Identification and characterization of small cells in the adult pancreas: potential progenitor cells? Cell Tissue Res 310, 51-58.

Phillips PA, Wu M J , Kumar RK , et al. (2003). Cell migration: a novel aspect of pancreatic stellate cell biology Gut 52:677-68.2

Pinzani, M. (1995). Novel insights into the biology and pahysiology of the Ito cell. Pharmacol Ther 66, 387- 412

Ramiya V.K., Maraist M., Arfors K.E., et al. (2000). Reversal of insulin-dependent diabetes using islets generated *in vitro* from pancreatic stem cells. Nature Med 6:278-282

Rafaeloff R., Pittenger G.L., Barlow S.W., et al. (1997). Cloning and sequencing of the pancreatic islet neogenesis associated protein (INGAP) gene and its expression in islet neogenesis in hamsters. J Clin Invest 99:2100-2109

Ratineau, C., Duluc, I., Pourreyron, C., et al. (2003). Endoderm- and mesenchyme-dependent commitment of the differentiated epithelial cell types in the developing intestine of rat. Differentiation 71,163-169

Reusens B. and Remacle C. (2006). Programming of the endocrine pancreas by the early nutricional environment. Int J Biochem Cell Bio 38, 913-922

Royer, P.J., Tanguy-Royer, S., Ebstein, F., et al. (2006). Culture medium and protein supplementation in the generation and maturation of dendritic cells. Scandinavian Journal of Immunology 63,401-409

Rosenberg L. (1998) Induction of islet cell neogenesis in the adult pancreas: the partial duct obstruction model. Microsc Res Tech 43:337-346

Sander M. and German MS (1997). The beta cell transcription factors and development of the pancreas J Mol Med 75,327-340

Satoh M., Masamune A, Sakai Y, et al. (2002). Establishment and characterization of a simian virus 40-immortalized rat pancreatic stellate cell line. Tohoku J Exp Med 198:55–69

Sparmann G, Hohenadl C, Tornoe J, et al. (2004). Generation and characterization of immortalized rat pancreatic stellate cells. Am J Physiol Gastrointest Liver Physiol;287:G211–G219

Scharfmann R. (2000). Control of early development of the pancreas in rodent and humans: implications of signals from the mesenchyme. Diabetologia 43, 1083-1092

Seaberg R.M., Smukler SR, Kieffer TJ et al (2004). Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreaticv lineages.Nat Biotechnol 22 1115-1124

Soria B., Skoudy A., Martin F. (2001). From stem cells to beta cells: new strategies in cell therapy of diabetes mellitus. Diabetologia 44:407-415

Steiling H. and Werner S. (2003) Fibroblast growth factors: key players in epithelial morphogenesis, repair and cytoprotection. Curr Opin Biotechnol 14, 533-537

Susking D.L. and Muench M.O., (2004). Searching for common sgtem cells of the hepatic and hematopoietic systems in the human fetal liber: CD34+ cytokeratin 7/8+ cells express markers for stallte cells. J Hepatol 40, 261-268.

Suzuki A., Nakauchi H., Taniguchi H. (2004). Prospective isolation of multipotent pancreatic progenitors using flow-cytometric cell sorting. Diabetes.53(8):2143-52

Sttaford, D., White, R.J., Kinkel, M.D., et al. (2006). Retinoids signal directly to zebrafish endoderm to specify insulin-expressing beta-cells. Development 133,949-956

Tanaka Y., Slitt A.L.,Leazeer T.M., et al. (2005). Tissue distribution and hormonal regulation of the breast cancer resistence protein (Bcrp/Abcg2) in rats and mice. Biocehm.Biophys.Res.Commun. 326, 181-187

Von Kupffer C. Ueber Sternzellen der leber. In: Abdruck aus Verhandlungen der Anatomischem Gesellschaft auf der 12 Versammlung in Kiel vom 17-20 April 1898, ed von Bardeleben K., pp80-86 (Gustav Fischer, Jena)

Vogel S., Piantedosi R., Frank J., et al. (2000). An immortalized rat liver stellate cell line (HSC-T6): a new cell model for the study of retinoid metabolism in vitro. J Lipid Res 41: 882–893

Watari N., Hotta Y., Mabuchi Y. (1982). Morphological studies on a vitamin A-storing cell and its complex with macrophage observed in mouse pancreatic tissues following excess vitamin A administration. Okajimas Folia Anat. Jpn.58:837–858

Wehr A.Y., Furth E.E., Sangar V., et al. (2011). Analysis of the human pancreatic stellate cell secreted proteome. Pancreas. 2011 May;40(4):557-66

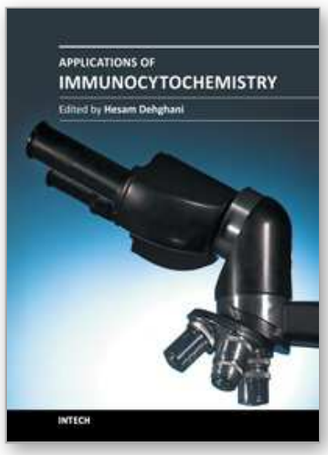
Weissman I.L. (2000) Stem cells: units of development, units of regeneration, and units in evolution. Cell 100:157-168.

Yue F., Cui, L., Johkura, K., et al. (2006). Glucagon-like peptide-1 differentiation of primate embryonic stem cells into insulin-producing cells.Tissue Engineering 12,2105-2115

Zimmermann A., Gloor B., Kappeler A. et al. (2002). Pancreatic stellate cells contribute to regeneration early after acute necrotising pancreatitis in humans. Gut 51,574-578

Zulewski H., Abraham E.J., Gerlach M.J., et al. (2001). Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. Diabetes 50:523-533

Zhou S., Schuetz J.D., Bunting K.D., et al. (2001). The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 7: 1028–34



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Immunocytochemistry is classically defined as a procedure to detect antigens in cellular contexts using antibodies. However, over the years many aspects of this procedure have evolved within a plethora of experimental setups. There are different ways to prepare a given specimen, different kinds of antibodies to apply, different techniques for imaging, and different methods of analyzing the data. In this book, various ways of performing each individual step of immunocytochemistry in different cellular contexts are exemplified and discussed. Applications of Immunocytochemistry offers technical and background information on different steps of immunocytochemistry and presents the application of this technique and its adaptations in cell lines, neural tissue, pancreatic tissue, sputum cells, sperm cells, preimplantation embryo, arabidopsis, fish gonads, and Leishmania.

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