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Modulation of c-kit expression in pancreatic adenocarcinoma: A novel stem cell marker responsible for the progression of the disease

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers because of late symptoms and resistance to chemotherapy and radiation therapy. We have investigated the appearance of c-kit, a stem cell marker, in both normal adult pancreatic tissue and in cancerous tissue. Apart from some very pale staining of islets of Langerhans, normal pancreas was devoid of staining with antibodies to c-kit. In contrast, in cancerous tissue that still preserves the overall integrity of the pancreatic tissue, there was a clear labeling in islets of Langerhans, which seemed to be co-localized with insulin containing β cells. In other cases, where the pancreatic tissue was completely deteriorated, intensive labeling was clearly evident in remnants of both the exocrine and the endocrine tissues. The duct cells of the adenocarcinoma were moderately but clearly labeled with antibodies to c-kit. In contrast, in metastasis of PDAC, very intensive labeling of c-kit was evident. The location of KRAS, which is strongly associated with PDAC, was also analyzed at the initial stages of the disease, when islets of Langerhans still preserve their integrity to a large extent. KRAS was found exclusively in islets of Langerhans and overlapped in its location with insulin and c-kit expressing cells. It is suggested that the modulation of the expression of c-kit, visualized by antibodies to the oncogene molecule, may play an important role in the formation and progression of PDAC. The absence of c-kit in normal pancreas and its appearance in PDAC is probably due to a mutational event, which probably allows conversion of the β cells into cancer stem cells (CSC). Co-expression of both c-kit and KRAS, typical markers for CSC with overlapping with insulin in islets of Langerhans, strongly support the notion that β-cells play a central role in the development of PDAC. The use of specific drugs that can attenuate the kinase activity of c-kit or target KRAS expressing cancer cells should be tested in order to attenuate the progression of this lethal disease.

Introduction

It has been demonstrated that SCF/c-kit interactions are likely to be involved in mediating differentiation and proliferation of pancreatic islets during fetal pancreatic development and that phosphorylated AKT plays a role downstream in SCF/c-kit signaling (Kaligin et al., 2011). It was also demonstrated that the c-kit receptor is involved in the regulation of glucose metabolism in the fetal rat and human endocrine pancreas through c-kit receptor phosphorylation and in stimulating development and survival of early β-cells (Li et al., 2006, 2007; Wu et al., 2010; Feng et al., 2012). However, there was no conclusive data on the participation of c-kit in the development of pancreatic ductal adenocarcinoma (PDAC) (Tsuura et al., 1994; Pandol et al., 2012). It was suggested that activated KRAS promotes aggressiveness of pancreatic PDAC through the loss of P16 and activation of PDEF-D, but it is not clear whether KRAS is the only active oncogene in this process and whether it is the primer oncogene that initiates the malignant pancreatic process (Bateman et al., 2008). Moreover, it is not clear whether KRAS is expressed in islets of Langerhans at an early stage of PDAC development.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers in humans (Herzel et al., 2006). However, the origin of the tumor growth is still obscure. Theoretically it can originate from the three classes of cells of pancreatic tissues: ductal cells, exocrine cells or endocrine cells of islets of Langerhans, which are comprised mainly of α-cells and β cells (Hennig et al., 2004; Herzel et al., 2006; Balic et al., 2012; Kumar-Shina et al., 2012). Alternatively, it could

Abbreviations: CSC, cancer stem cells; PDAC, pancreatic ductal adenocarcinoma; TSC, tissue stem cells.

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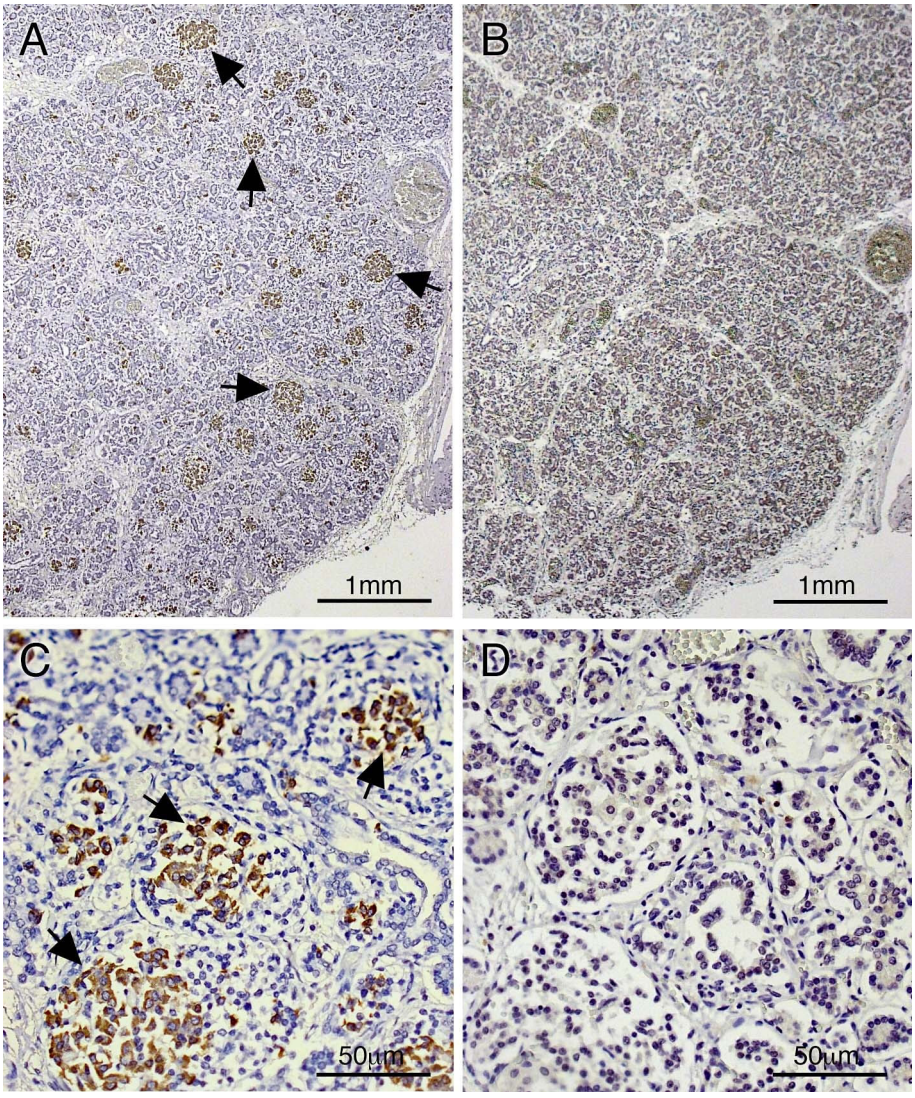

Fig. 1. Immunostaining of normal pancreas with antibodies to insulin and to c-kit. (A) The pancreatic tissue looks compact. Clear staining of islets of Langerhans is visible following staining with antibodies to insulin (arrows). (B) Adjacent section incubated with antibodies to c-kit. No specific staining is evident in the pancreatic tissue (A and B). (C and D) Normal pancreatic tissue incubated with antibodies to insulin and a parallel section stained with antibodies to c-kit (at high magnification). (C) Staining of β cells to insulin is evident in islets of Langerhans (arrows). (D) No staining is evident following incubation with antibodies to c-kit in adjacent section.

also be an imported disease, by the settling of cancer stem cells derived from other tissues on the surface of the pancreas, as was suggested also for ovarian cancer (Curley et al., 2011; Merkwitz et al., 2011; Burgos-Ojeda et al., 2012).

It was recently discovered (Balic et al., 2012) that the markers of stem cells, LGR5 and Nanog, in human pancreas, are uniquely localized in the pancreatic β cells, located in the islets of Langerhans. Moreover, these β cells were clearly involved in the formation of pancreatic PDAC (Amsterdam et al., 2013a,b). However, the question of the molecular events that convert the pancreatic β cells from tissue stem cells (TSC) to cancer stem cells (CSC) remains unresolved. One possibility is that mutation of KRAS is implicated with this process, since in the majority of cases of PDAC, KRAS is mutated (Pandol et al., 2012). However, it is not clear whether this is the only mutation that occurs in PDAC. Moreover, it is not clear whether this is the first mutation that converts TSC to CSC. In view of the various reports that c-kit alters the differentiation and proliferation of α and β-cells of the fetal pancreas (Li et al., 2006, 2007; Wu et al., 2010; Kaligin et al., 2011; Feng et al., 2012), we decided to examine the possibility that the oncogene c-kit is the primary event of the conversion of TSC to CSC. In parallel, we decided to examine the CSC marker KRAS in order to compare its expression in space and time with Nanog, LGR5 and c-kit during the initial stages of PDAC development.

C-kit is a membrane encored protein, which exerts a kinase activity (Lennartsson and Ronnstrand, 2012). It appears in embryonic and fetal life in humans, however, in the adult the expression is silenced (Li et al., 2006, 2007; Wu et al., 2010; Kaligin et al., 2011; Feng et al., 2012). Moreover, it appears in a mutated form in several kinds of cancers, including ovarian cancer (Curley et al., 2011) in gastrointestinal stroma tumors (Miettinen and Lasota, 2006) and in colorectal carcinomas (Sammarco et al., 2004). Since it could serve as a marker of cancer stem cells (CSC), we decided to explore its appearance in the normal and malignant human pancreas, using specific antibodies to this molecule and indirect immunocytochemistry. No labeling with antibodies to c-kit was evident in the normal pancreas. However, in the malignant pancreas, c-kit appeared in the islets of Langerhans in the cancerous pancreatic specimens, with moderate intensity of staining, where only a slight deterioration of the pancreatic tissue was evident. In contrast, in severely deteriorated pancreatic tissue much more intensive labeling of c-kit was evident in the fragmented islets of Langerhans as well as