INTRODUCTION

In our previous publication we reported a successful in vivo molecular imaging of the pancreas and determine in vivo expression of epidermal growth factor receptor (EGF-R) and survivin using a novel endoscopic ultrasound-guided fine needle imaging (EUS-FNI) technique, which incorporates needle based confocal laser-induced endomicroscopy (nCLE) after intrapancreatic injection of FITC-labeled antibodies. Studies were performed in anesthetized pigs. FITC-labeled specific antibodies against EGF-R and survivin were injected into the tail and neck of the pancreas using a 19 gauge needle introduced under EUS guidance. Thirty minutes later, nCLE was performed using a prototype needle-based confocal laser-induced endomicroscopy probe (Cellvizio AQ-Flex, Mauna Kea Technologies, Paris, France) to determine cellular and tissue localization of EGF-R and survivin in the pancreas. Then pigs were euthanized and specimens of pancreas from areas injected with antibodies were obtained for histologic examination under epifluorescence microscope. Results: EUS-guided nCLE enabled visualization of EGF-R and survivin in pancreatic tissue. Expression of EGF-R and survivin in pancreas was confirmed by histology. EGF-R immunoreactivity was localized to majority of duct-lining cells and to the surface and cytoplasm of many acinar cells. Survivin was localized mainly to the acinar cells. This study demonstrated the feasibility of in vivo, real time visualization of EGF-R and survivin in the pancreas by local injection of FITC-labeled antibodies via EUS-guided fine needle injection, followed by EUS-guided needle based confocal laser-induced endomicroscopy.

Key words: confocal laser-induced endomicroscopy, epidermal growth factor receptor, survivin, pancreas, molecular imaging
induction of experimental pancreatitis and suggested survivin’s role in cell protection and pancreatic regeneration (15). In this regard survivin’s role in pancreas is similar to that in gastric epithelium (16). A recent study demonstrated that EGF (via EGF-R) upregulates survivin expression in pancreatic beta cells during the neonatal period (17), thus indicating a possibility of local autocrine and/or paracrine regulation. Therefore, visualization of EGF-R and survivin expression in vivo in the pancreas is important for a better understanding of their pathophysiological roles.

In the present study we examined the feasibility of in vivo, real time visualization of EGF-R and survivin in the pancreas by local injection of FITC-labeled antibodies via EUS-guided fine needle injection, followed by EUS-guided needle based confocal laser-induced endomicroscopy.

MATERIAL AND METHODS

Experimental design

This study was aimed to establish a new paradigm and to perform in vivo labeling and imaging of EGF-R and survivin in the pancreas. This is more complex than CLE imaging of gastrointestinal mucosa and requires EUS guided administration of FITC-labeled antibodies against EGF-R and survivin using a FNA needle into two different regions of the pancreas. Thirty minutes later an nCLE probe was inserted under EUS guidance to the pancreatic tail and neck areas. Thirty minutes after antibodies injection, a prototype nCLE probe (Cellvizio AQ-Flex, Mauna Kea Technologies, Paris, France) was inserted into pancreas via a 19 gauge needle under EUS guidance and nCLE determination of EGF-R and survivin expression was performed.

At the end of experiments pigs were euthanized using a lethal dose of pentobarbitone and pancreatic sections from the tail and neck areas injected with antibodies were obtained, fixed in 10% buffered formalin and routinely processed for histology. Five µm thin sections were deparaffinized and examined under a Nikon fluorescence microscope.

RESULTS

Needle-based confocal laser-induced endomicroscopy images

Control images were obtained with EUS-guided nCLE within the pancreas without any injection. These images showed no fluorescent structures in the pancreas. After injection of fluorescein labeled anti-EGF-R antibody, EUS-guided nCLE revealed thick and irregular inter-connected bright strands (Fig. 1).

After injection of fluorescein labeled anti-survivin antibody, EUS-guided nCLE revealed a diffuse ground-glass background with thin and ultrathin bright strands with occasional branching (Fig. 2).

Histologic data

EGF-R and survivin were expressed in pancreatic tissue. EGF-R was localized predominantly to the majority of ductal

Fig. 1. nCLE and histologic images of pancreas injected with fluorescence-labeled anti-EGF-R antibody. (A): nCLE image after injection of fluorescein labeled anti-EGF-R antibody, showing thick and irregular inter-connected bright strands. (B) and (C): Micrographs of histologic sections of pancreas showing EGF-R protein localized predominantly to the majority of duct-lining cells and to the surface and cytoplasm of numerous acinar cells.
cells and to the surface and cytoplasm of numerous acinar cells. Survivin was localized mainly to the acinar cells.

DISCUSSION

This study demonstrated feasibility of in vivo, real time visualization of EGF-R and survivin in the pancreas using needle-based CLE in combination with EUS-FNA and injection of FITC-labeled antibodies without necessity of laparotomy. It demonstrated for the first time a successful in vivo visualization of EGF-R and survivin in porcine pancreas using a needle-based CLE probe, EUS-FNA and FITC-labeled specific antibodies. From the technical point of view such study requires an advanced expertise in both CLE imaging and EUS-FNA (18).

This study established a novel method and a paradigm of molecular imaging of pancreas, which has important implications. Needle-based CLE under EUS-FNA guidance allows in vivo visualization of specific regulatory protein and receptors in pancreas, that potentially have important implications for cell growth, proliferation and apoptosis. Moreover, by using antibodies against phosphorylated EGF-R, this procedure will make it possible to determine in vivo, non-invasively the state of receptor phosphorylation/activation and its response to physiological and pharmacological stimuli. Once this method is optimized it will allow quantification of expression of these proteins in a similar way as in our previous ex-vivo study (6).

Previously, Fottner et al. successfully performed in vivo molecular imaging of somatostatin receptors in pancreatic islet cells and neuroendocrine tumors using miniaturized confocal laser-scanning fluorescence microscopy and fluorescein-labeled octreotide in healthy mice and murine models of neuroendocrine tumors (19). However, the visualization and imaging of mice pancreas in that study required laparotomy and the use of handheld probe, which cannot be used in human CLE (19).

Recent pioneering studies using a needle-based CLE probe established a technical feasibility of this method to visualize pancreas in porcine models and in humans (20, 21). However, none of these studies attempted in vivo molecular imaging of important regulatory proteins such as EGF-R and survivin in the pancreas.

In addition to visualization of cellular and tissue structures needle-based CLE enables to study in vivo pathophysiological events in natural tissue environment, and hence functional imaging. In vivo molecular imaging with needle-based CLE can be used in basic science and clinical setting and will enable better understanding of pancreatic pathophysiology.

Conflict of interests: None declared.

REFERENCES


Received: October 29, 2012
Accepted: December 3, 2012

Author’s address: Dr. Kenneth J. Chang, M.D. FACG, FASGE, Professor and Chief, Gastroenterology and Hepatology, Director, Comprehensive Digestive Disease Center, University of California, Irvine Medical Center, 101 The City Drive, Orange, CA, 92868
E-mail: kchang@uci.edu