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Identification of a pyruvate-to-lactate signature in pancreatic intraductal papillary mucinous neoplasms

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ABSTRACT

Objective: We used transcriptomic profiling and immunohistochemistry (IHC) to search for a functional imaging strategy to resolve common problems with morphological imaging of cystic neoplasms and benign cystic lesions of the pancreas.
Methods: Resected pancreatic cancer (n = 21) and normal pancreas were laser-capture micro-dissected, and transcripts were quantified by RNAseq. Functional imaging targets were validated at the protein level by IHC on a pancreatic adenocarcinoma tissue microarray and a newly created tissue microarray of resected intraductal papillary mucinous neoplasms (IPMNs) and IPMN-associated adenocarcinomas.
Results: Genes encoding proteins responsible for cellular import of pyruvate, export of lactate, and conversion of pyruvate to lactate were highly upregulated in pancreatic adenocarcinoma compared to normal pancreas. Strong expression of MCT4 and LDHA was observed by IHC in >90% of adenocarcinoma specimens. In IPMNs, the pyruvate-to-lactate signature was significantly elevated in high grade dysplasia (HGD) and IPMN-associated adenocarcinoma. Additionally, cores containing HGD and/or adenocarcinoma exhibited a higher number of peri-lesional stromal cells and a significant increase in peri-lesional stromal cell staining of LDHA and MCT4. Interestingly, the pyruvate-to-lactate signature was significantly upregulated in cores containing only low grade dysplasia (LGD) from patients with histologically confirmed IPMN-associated adenocarcinoma versus LGD cores from patients with non-invasive IPMNs.
Conclusion: Our results suggest prospective studies with hyperpolarized [1−^{13}C]-pyruvate magnetic resonance spectroscopic imaging are warranted. If these IHC results translate to functional imaging findings, a positive pyruvate-to-lactate imaging signature might be a risk factor for invasion that would warrant resection of IPMNs in the absence of other worrisome features.

Introduction

For many tumor types, early detection and screening are the main focus of imaging research. Pancreatic cancer, however, is too rare (age-adjusted incidence rate of <13/100,000) [1] to warrant screening of the general population [2]. In the 20% of patients who present with resectable local disease, the median tumor diameter is 3.2 cm, a size which is seldom missed with conventional morphological imaging techniques (computed tomography [CT], ultrasound [US], endoscopic ultrasound (EUS) or magnetic resonance imaging [MRI]) [3].

The majority of pancreatic ductal adenocarcinoma (PDAC) arises from pancreatic intraepithelial neoplasm (PanIN) lesions which are

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Abbreviations

HGD	high grade dysplasia
HP	hyperpolarized
IHC	immunohistochemistry
IPMN	intraductal papillary mucinous neoplasm
LDHA	lactate dehydrogenase A
LCM	laser-capture microdissection
LGD	low grade dysplasia
MCT	monocarboxylate transporter
MGD	moderate grade dysplasia
MRSI	magnetic resonance spectroscopic imaging
TMA	tissue microarray

typically sub-mm in dimension and generally undetectable with current clinical imaging techniques. However, up to 20% of PDAC arises in the context of radiologically detectable cystic neoplasms which are thought to progress along the adenoma-to-adenocarcinoma axis similar to colon polyps [4,5]. The most common of these cystic precursor lesions is the branch-duct (BD)-intraductal papillary mucinous neoplasm (IPMN) [6]. There is a wealth of literature on the diagnostic dilemma of BD-IPMNs and the need to resect high-risk lesions while sparing resection for the majority of patients whose lesions are unlikely to progress [6–9]. While great efforts have been made, including international consensus guidelines and follow-up of these patients with MRI and EUS to capture the small fraction of BD-IPMNs that harbor an invasive cancer, the majority of resections (~75%) for radiologically worrisome BD-IPMNs to date have revealed only low to moderate-grade dysplasia [10–14]. The management of these patients remains a significant challenge, and the fact that these lesions are radiologically detectable suggests that a new functional imaging strategy may be the key to progress.

One of the greatest success stories in functional imaging is sodium iodide symporter- (NIS-) mediated imaging for thyroid cancer. NIS (SLC5A5) is a membrane transporter from the solute carrier (SLC) family and is responsible for the uptake of iodine in thyroid follicular cells as the first step in the synthesis of thyroid hormone. The combined action of NIS and a second trapping (or organification) step allow thyroid cancer cells to accumulate radiolabeled iodine >1000-fold above blood levels at 48 h after administration [15]. This efficient 2-compartment system permits highly sensitive detection of primary and metastatic thyroid cancer deposits with gamma-camera, single-photon emission computed tomography (SPECT), and positron emission tomography (PET) imaging.

Our previous work with NIS [16–18] and the success of SLC transporter-mediated functional imaging (for example FDG-PET) in other tumors led us to investigate the potential of SLC-mediated functional imaging for pancreatic lesions. In an initial microarray screen of 13 pancreatic tumor samples, we discovered SLC16A3 was upregulated in all 13 pancreatic adenocarcinoma samples versus control pancreas samples [19]. SLC16A3 encodes monocarboxylate transporter 4 (MCT4), which is thought to be responsible for cellular export of lactate produced in the glycolytic pathway. While MCT4 activity is not responsible for a direct uptake or trapping process that could be imaged with high resolution SPECT or PET, we reasoned that if this endpoint glycolytic pathway is consistently upregulated versus other competing pathways of pyruvate metabolism in high-risk pancreatic cystic lesions, then it might be worth further exploration as an a potential imaging target for MR spectroscopy following IV injection of hyperpolarized ^{13}C-pyruvate.

In this paper we build on our discovery of MCT4 upregulation in human pancreatic cancer and extend it to the study of endpoint glycolysis in radiologically-detectable but diagnostically-challenging IPMNs, a current and growing clinical problem.

Materials and methods

Transcriptomic studies

Patient tissues were obtained from the Mayo SPORE Pancreatic Cancer Tissue Registry following institutional IRB approval. RNA-Sequencing (methods described previously) was performed on total RNA extracted from a cohort of 21 patient pancreatic adenocarcinoma isolated by laser capture micro-dissection (LCM) [19]. For same-patient control tissue, we laser-captured bulk acinar tissue from 5 patients and normal pancreatic ducts from 16 patients.

Tissue microarray creation and antibody development

A pancreatic adenocarcinoma tissue microarray (TMA) containing samples from 140 patients (3 cores per patient) without prior chemotherapy [19] was screened. A new TMA from IPMN patients was constructed from 140 surgical specimens (3 cores per patient, 8 TMA blocks total) with various grades of IPMN or IPMN-associated invasive adenocarcinoma at surgical pathology. TMAs were stained with H&E or colorimetrically developed with antibodies against LDHA (sc-137243; Santa Cruz Biotechnology, Dallas, TX) or MCT4 (sc-50329; Santa Cruz) at the Pathology Research Core of Mayo Clinic, Rochester, MN. TMA slides were placed in the BOND III (Leica Biosystems, Chicago, IL) stainer for online processing. Slides were treated with Epitope Retrieval 2 solution for 20 min, stained with LDHA antibody at 1:600 dilution or MCT4 antibody at 1:300 dilution (in Bkg Reducing Diluent, Dako, Carpenteria, CA, S3022) for 30 min. Detection was achieved using the Polymer Refine Detection kit as per the manufacturer's instructions (Leica Biosystems). Counter staining was performed for 5 min with hematoxylin. Slides were dehydrated through increasing concentrations of alcohol, cleared in xylene, and coverslipped in xylene-based mounting media.

TMA scoring

Tissue microarrays (TMAs) were scanned at 40X using an Aperio (Leica Biosystems) ScanScope AT Turbo Scanner. Scanned images were reviewed for quality by a research technologist who specializes in digital imaging. Images were de-arrayed and scored using PathXL TMA software (PathXL Ltd.). The pancreatic adenocarcinoma TMA was evaluated for antigen expression by a trained pancreatic pathologist and cores were scored as strong, moderate, weak, or absent; and the percent of cells stained at the highest intensity was recorded. Subcellular localization of the staining was noted for each core. IPMN TMA core morphology was assessed on an H&E stained section by an anatomic pathologist, who recorded presence or absence of adenocarcinoma, presence or absence of IPMN and, if present, IPMN subtype (gastric, intestinal, pancreatobiliary) and grade of dysplasia (low grade, LGD; moderate grade, MGD; high grade, HGD). Adjacent TMA sections stained with antibodies against MCT4 and LDHA were manually assessed by a cytotechnologist under the direction of the same anatomic pathologist. Percentage of negative (0), weak (1), moderate (2), and strong (3) staining was assessed, rounding to the nearest 10%. A histoscore was reported as sum of % of cells stained multiplied by each intensity score (histoscore range of 0–300). Staining on the plasma membrane was assessed for MCT4. Cytoplasmic staining was assessed for LDHA. Peri-neoplastic stromal staining was also

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