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Defining disease with laser precision: laser capture microdissection in gastroenterology

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Abstract

Laser capture microdissection (LCM) is an efficient and precise method for obtaining pure cell populations or specific cells of interest from a given tissue sample. LCM has been applied to animal and human gastroenterology research in analyzing the protein, DNA and RNA from all organs of the gastrointestinal system. There are numerous potential applications for this technology in gastroenterology research including malignancies of the esophagus, stomach, colon, biliary tract and liver. This technology can also be used to study gastrointestinal infections, inflammatory bowel disease, pancreatitis, motility, malabsorption and radiation enteropathy. LCM has multiple advantages when compared to conventional methods of microdissection, and this technology can be exploited to identify precursors to disease, diagnostic biomarkers, and therapeutic interventions.

Keywords

Laser Microdissection; Laser Capture Microdissection

LCM is an exciting technology, developed at the National Institute of Health and National Cancer Institute that has become widespread with applications in analyzing the proteome, genome and transcriptome of human cells. This technology allows the user to isolate pure cell populations of interest rather than taking the average of a heterogeneous tissue sample. This can allow microdissection of key elements of a tissue sample such as tumor cells for protein, DNA or RNA analysis. LCM can be used to analyze frozen, fixed, paraffin embedded tissue, cytological smears, stained cells, living cells, stem cells, and even an individual cell. The speed of this system allows thousands of cells to be captured within several minutes. The unit consists of a microscope, laser unit, control joystick, vacuum system for tissue immobilization, video camera, and a monitor to view the tissue as dissections are in progress. There is a cutting and extracting process that is used to obtain the desired tissue sample. Once the selected tissue is viewed under the microscope, the user selects the cells on the computer screen and uses pulses of an infrared laser to adhere the cells to a thermoplastic film that is in direct contact with the tissue section. This causes the film to remain attached to the selected cells, and the tissue that was not fused by the laser is

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separated when the cap is removed¹. (Fig. 1a, b) Then the cells can be lifted in a microfuge tube containing an extraction solution. The DNA, RNA and proteins are not damaged in this process. Gene chip analysis of LCM crypt base epithelial cells was used to define molecular features shared by adult mouse gut epithelial progenitors².

LCM in Gastrointestinal Cancer

LCM has been applied to the study of many gastrointestinal malignancies including the esophagus, stomach, colon and liver. Streitz et al³ used LCM to investigate the protein expression in patients with high-grade dysplasia of the esophagus and esophageal adenocarcinoma that had undergone esophagectomy. Benign appearing Barrett epithelium (BE) cells, malignant BE and normal epithelium were analyzed and compared for protein expression. Using LCM and mass spectrometry, unique protein peaks (7350, 8446, 10850, 14693) were found in benign appearing BE that were biomarkers for malignant BE. This study demonstrated how LCM can be used to identify individuals who are at increased risk for malignant transformation to esophageal cancer.

Wu et al⁴ used LCM and microarray analysis to study gastric cancer. They were able to distinguish malignant versus benign gastric epithelial cells with an accuracy of 99% using 504 gene profiles including Trefoil 1, 2 and 3. LCM has also been used to study colorectal cancer. Melle et al⁵ used LCM and SDS PAGE to compare protein biomarkers in colorectal cancer cells to adenomas and normal adjacent mucosa. They showed that a biomarker known as heat shock protein 10 (HSP-10) was found to be elevated in colorectal tumors. Similarly, Korekane et al⁶ studied protein expression in the tumor cells of the colonic epithelium. They compared the tumor cells to the normal adjacent epithelial cells in colectomy specimens and found different expression profiles of N-Glycans. Additionally, they noted that using LCM with a BCA protein assay was superior to reverse phase HPLC with mass spectrometry. Using LCM, they identified seven distinct N-Glycans in the tumor cells that would have otherwise been undetected using a conventional method.

Since epidermal growth factor receptor (EGFR) inhibitors have been shown to be effective in inhibiting some solid tumors, many studies have examined EGFR mutations as a target for inhibition. Leone et al⁷ investigated patients with bile duct carcinoma (BDC) and gall bladder (GBC) cancer. Using LCM and gene amplification, they demonstrated that a subgroup of their patients had a mutation in the EGFR gene which is thought to influence tyrosine kinase activity and may be susceptible to targeted therapy with an EGFR inhibitor.

Recently, LCM was used to study Hepatocellular Carcinoma (HCC). Ieta et al⁸ studied Ube2c expression in patients with HCC using LCM, RT-PCR, and microarray analysis. They found that the expression of Ube2c was increased in malignant hepatocytes compared to the normal hepatocytes. Elevated Ube2c expression was also found to be associated with increased tumor invasion into the portal vein and poor survival rates. This study demonstrates how LCM is used to identify biomarkers that can have prognostic value in patients with HCC. In addition, Honda et al⁹ studied hepatocytes from patients with chronic hepatitis B (CHB) and chronic hepatitis C (CHC) who had Hepatocellular Carcinoma (HCC). They utilized LCM and cDNA microarray technology. The investigators found that the CHB specimens had upregulation of genes related to pro-apoptotic activity and cell cycle arrest versus CHC specimens that had upregulation of genes related to anti-apoptosis and cell cycle acceleration. This study demonstrates how LCM can be used to understand the different mechanisms of malignant transformation to HCC.

LCM in Other Gastrointestinal Diseases

LCM has allowed investigators to study inflammatory bowel disease (IBD). Bowel flora has been implicated in the pathogenesis of Crohn's Disease (CD). De Hertogh et al¹⁰ examined cells from the ileum, appendix and colon in patients with CD to study bacterial populations of the bowel flora. They used LCM and PCR amplification to show that Enterobacteriaceae may be the initiating organism in CD related ulcers. Similarly, Ryan et al¹¹ examined the small and large bowel granulomas in patients with CD compared to patients with non-CD related granulomas. Using LCM and PCR amplification to analyze the bacterial DNA of the bowel flora, it was shown that Escherichia Coli DNA is more common in the CD granulomas than non-CD related granulomas. These studies demonstrate that LCM can be used successfully to analyze bowel flora in patients with Crohn's Disease.

Clostridium Difficile is a common cause of antibiotic induced diarrhea in animals and humans. Savidge et al¹² used LCM to study the enterotoxic effects of toxin B compared to toxin A. Fetal human xenografts of the colon were infected with C. Difficile toxin A or B and implanted into the ileum of scid mice. Using LCM to dissect the human colon cells and RT-PCR, this study showed that both toxins were associated with increased levels of interleukin-8 and an increased inflammatory response. Similarly, LCM can be used to study other bacteria such as Helicobacter Pylori. Resnick et al¹³ examined the gastric epithelium from patients with peptic ulcer disease who had gastric biopsies before and after H. pylori eradication. The results showed that 9,680 mRNA transcripts increased and 13,817 decreased after eradication. This study shows how LCM was used to understand the epithelial transcriptome of the gastric antrum to target future research.

LCM can also be used to study the effects of therapeutic radiation on the intestines. Wang et al¹⁴ used LCM to study the pathogenesis of radiation enteropathy. They examined the small bowel of rats after irradiation of their small intestine. They used LCM to microdissect smooth muscle cells from the distal ileum as well as reverse transcription PCR and found that endothelial thrombomodulin expression was decreased and protease activated receptor 1 levels were significantly increased. This demonstrates how LCM can be used to understand the pathogenesis of radiation induced damage of the gastrointestinal tract.

LCM can be used to investigate the pathophysiology of malabsorptive disorders. Gassler et al¹⁵ studied patients with a history of adenocarcinoma of the colon who had undergone resection of their small intestine. They used LCM (Fig. 2) and microarray analysis to show the differential gene expression profiles of the villus and crypt enterocytes in the human small intestine. They demonstrated the differential gene expression of the non absorptive enterocytes in the upper crypt and the absorptive enterocytes in the mid-villus. This application of LCM may lead to targeted therapies for malabsorptive disorders like celiac disease. Similarly, LCM was used by Erwin et al¹⁶ to examine the differential gene expression of crypt enterocytes in a murine model. (Fig. 3) This study demonstrated gut adaptation of the remnant small intestines following small bowel resection and changes in the genes that regulate cell growth. Using LCM, this study demonstrated how the differential gene expression of the crypt enterocyte adapts following small bowel resection. Epithelial barrier function of the small intestine is critical in protecting the interstitium from the harsh luminal environment. Tight junctions are an important component in this barrier function. Clayburgh et al¹⁷ studied the expression of MLCK1 (long myosin light chain kinase splice variant 1) in the crypt-villus axis in the human jejunum. Using LCM they were able to isolate epithelial cells from the villus and crypt and demonstrate that MLCK1 expression was highest in the villus tip and regulated tight junction permeability.

Chronic pain is a predominant feature of chronic pancreatitis. Xu et al¹⁸ have used LCM to explore the pathophysiology of chronic pain. They studied rats with chronic pancreatitis induced by trinitrobenzene sulfonic acid and the mRNA expression of transient receptor potential vanilloid 1 (TRPV1). LCM was used to isolate pancreas specific dorsal root ganglion cells. The mRNA expressed by TRPV1 was found to be increased in rats with chronic pancreatitis. Additionally, they showed that administering TRPV1 antagonists decreased visceral pain associated with chronic pancreatitis. This study demonstrates how LCM can be used to study therapeutic interventions in chronic pancreatitis.

Investigators have used LCM, to isolate cells from the myenteric plexus. Using LCM of the myenteric plexus and the villus epithelium in the jejunum, Guan et al¹⁹ demonstrated that GLP2 stimulated vascular blood flow is mediated by vasoactive neurotransmitters that colocalize with GLP2 in two distinct cell types (neurons and villus epithelium) within the gastrointestinal tract.

Discussion

LCM is a major advance as a microdissection technique compared to previously existing methods, but some disadvantages exist. The equipment may be considered expensive, and a manual dissection may be more cost effective and yield a reliable result. One study showed that LCM may not be necessary to identify tumor cell-specific gene expression profiles, and the macrodissected samples yielded reliable data when compared to microdissected tissue.²⁰ There also may be technical difficulties in preparing the tissue, and adhering the tissue to the film may be challenging. In spite of the disadvantages, LCM has been proven to be an efficient method for obtaining pure populations of cells from a heterogeneous tissue sample. LCM has the potential to help us understand the pathogenesis of many diseases as well as help to target interventions for future drugs. Future applications of LCM may include isolating selected populations of tissue for cultivation, manipulation, and cloning. This would allow a larger population of cells for current and future experiments. It is clear that LCM is a significant advancement in microdissection technology. Combining LCM with common molecular analytic techniques has provided an exciting method to visualize, dissect and analyze cells of the gastrointestinal system with precision and efficiency.

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Abbreviations

LCM	Laser Capture Microdissection
BE	Barrett Epithelium
HPLC	High Performance Liquid Chromatography
GC	Gastric Cancer
SDS PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis

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Figure 1. Laser Capture Microdissection

(A) This diagram demonstrates that when the laser is activated it causes adhesion of the thermoplastic membrane to the cells of interest within the tissue section. (B) Lifting the cap selects the cells that adhered to the thermoplastic membrane.

Figure 2. Enterocytes

(A) Representation of the enterocytes in the crypt-villus axis. (B) H&E staining of small intestine mucosa. (C–F) LCM of the enterocytes from the upper portion of crypts. (G–I) LCM of the enterocytes from the middle portion of the villi. This figure is from Gassler et al.¹⁵

Figure 3. Mouse Enterocyte Crypt

(A) The morphology of the extracted crypts and adjacent cells is maintained during capture, showing that the correct population of cells is obtained. (B) RT-PCR of cell compartment specific genes for cDNA made from total RNA isolated from LCM-extracted crypt cells, showing the specificity of the LCM procedure for crypts. This figure is from Erwin et al.¹⁶

Table 1

LCM applied to Gastroenterology

Pathology	Tissue Type	Substrate Analyzed	LCM Isolated cells	Additional Techniques used	Results	Source
Barrett's Esophagus	Human	protein	Epithelial Cells of the distal esophagus and GE junction	MALDI mass spectrometry	Protein peaks found in benign appearing Barrett's epithelium can be an early biomarker of adenocarcinoma.	Streitz et al ³
Gastric Cancer	Human	cDNA	Normal and malignant gastric epithelial cells	cDNA microarray	The differences between the genetic expression of malignant gastric cells and benign gastric cells are demonstrated.	Wu et al ⁴
Colon Cancer	Human	protein	Normal and malignant colonic cells	BCA, mass spectrometry, HPLC	LCM can be used in colon cancer research to detect N-Glycans otherwise undetected by conventional methods.	Korekane et al ⁶
Colon Cancer	Human	protein	Colonic epithelial cells	protein chip array, SDS PAGE	HSP-10 expression is increased in colorectal cancer cells.	Melle et al ⁵
Biliary Carcinoma	Human	DNA	Cholangiocyte tumor cells	PCR, DNA amplification	EGFR somatic mutations are found in a subgroup of patients with biliary malignancies.	Leone et al ⁷
Hepatocellular Carcinoma	Human	RNA	Hepatocytes	reverse transcription PCR, microarray	Elevated Ube2c expression is associated with tumor invasion.	Ieta et al ⁸
Hepatocellular Carcinoma	Human	cDNA	Hepatocytes, infiltrated lymphocytes	cDNA, microarray	Malignant transformation of hepatitis B involves upregulation of pro-apoptotic and cell cycle arrest genes versus hepatitis C which had upregulation in anti-apoptotic and cell cycle acceleration genes.	Honda et al ⁹
Crohn's Disease	Human	DNA	Mucosal cells of the small and large bowel	RT-PCR	CD related granulomas have more E. Coli DNA than non-CD related granulomas.	Ryan et al ¹¹
Clostridium Difficile	Human	RNA	Colon epithelial cells from the fetal human intestine	RT-PCR	IL-8 and inflammatory markers were increased with both C. difficile toxin A and B.	Savidge et al ¹²
H. Pylori	Human	mRNA	Gastric epithelial cells	RT-PCR	The transcriptome of antral epithelial cells becomes altered after treatment for H. Pylori.	Resnick et al ¹³
Radiation Enteropathy	Rat	mRNA	Small intestine smooth muscle cells	in-situ hybridization, RT-PCR	Thrombomodulin was decreased and protease activated receptor 1 levels were increased in chronic pancreatitis.	Wang et al ¹⁴

Pathology	Tissue Type	Substrate Analyzed	LCM Isolated cells	Additional Techniques used	Results	Source
Malabsorption	Human	RNA	Enterocytes of the villus and crypt of the Small Intestine	microarray, in-situ hybridization	Genetic profiling of microdissected enterocytes shows the differential gene expression profiles of the villus and crypt enterocytes.	Gassler et al ¹⁵
Malabsorption	Mouse	cDNA	Crypt cells of the small intestine	RT-PCR	Crypt enterocyte adaptation occurs following small bowel resection.	Erwin et al ¹⁶
Malabsorption	Human	mRNA	Jejunal epithelium from the villus and crypt	Immunofluorescent Microscopy	MLCK1 expression is related to epithelial barrier regulation in the small intestine	Clayburgh et al ¹⁷
Pancreatitis	Rat	mRNA	Pancreas specific neurons in the dorsal root ganglion	RT-PCR	TRV1 antagonist causes a decrease in abdominal pain in chronic pancreatitis.	Xu et al ¹⁸
Motility	Pig, Human	mRNA	Villus Epithelium, Myenteric Plexus of the jejunum	RT-PCR	GLP-2 is localized with VIP, eNOS, 5HT in enteric nerves or enteroendocrine cells	Guan et al ¹⁹