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REVIEW ARTICLE

Laser capture microdissection: techniques and applications in liver diseases

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Abstract
Routine transcriptomic and proteomic analysis are usually performed at a whole organ or tissue level. These approaches provide an average readout of all cell types present within the tissue but do not allow differentiating the profile of specific cell populations. Laser capture microdissection (LCM) constitutes an excellent tool to isolate cell populations or areas of interest within a tissue. By direct visualization, the selected area is excised by a laser and can be further processed for a variety of downstream analyses. This technology has been widely used in the study of liver diseases, from DNA and RNA sequencing to mass spectrometry. However, LCM also has important limitations. To ensure the best integrity of the molecule of interest, optimal tissue preservation, careful tissue sectioning, and optimization of the staining procedure are required. The present review provides a description of the LCM technology, including tips and technical recommendations to perform the procedure, as well as an overview of studies using LCM technology in the field of liver disease.

Keywords Laser capture microdissection · Liver diseases · Tissue preservation · Transcriptome · Proteome · Downstream analysis

Introduction

Molecular profiling of pure cell populations or cells from selected areas of the tissue is essential to better understand healthy and pathogenic conditions [1, 2]. Most of the studies on genomic and proteomic analysis have been performed with whole tissue samples, precluding the analysis of the region or cells of interest. In the last years, several methods have been optimized to isolate pure populations or single cells, including immunofluorescence cell sorting, immunomagnetic cell isolation, affinity column chromatography, among others. Nevertheless, all these methodologies require fresh tissue and previous steps of enzymatic digestion, which may affect gene expression and native protein. To address these drawbacks, LCM (laser capture microdissection) has been developed and is an excellent method for isolating specific cells in their native niche without contamination of the surrounding areas. This technology can identify and isolate pure cell populations, and structures or tissues of interest from fixed tissue or fixed cell cultures by direct visualization of cells under a microscope [3–5]. This microscope is coupled to a laser that excises the selected area, which is thereafter collected in a tube. The dissected areas are then used for further analysis such as RNA and DNA analysis or proteomic studies.

LCM is applicable to a wide range of research areas. It has been used for molecular profiling of tissue, which compares different cell populations, i.e., tumor and non-tumor cells; genomic and proteomic molecular profiling; single cell mutation analysis; and forensic analysis, among others.

Laser capture microdissection technology

The whole process of LCM involves 3 steps (Fig. 1): (1) sample processing, (2) the microdissection procedure, and (3) DNA, RNA or protein extraction followed by downstream analyses.

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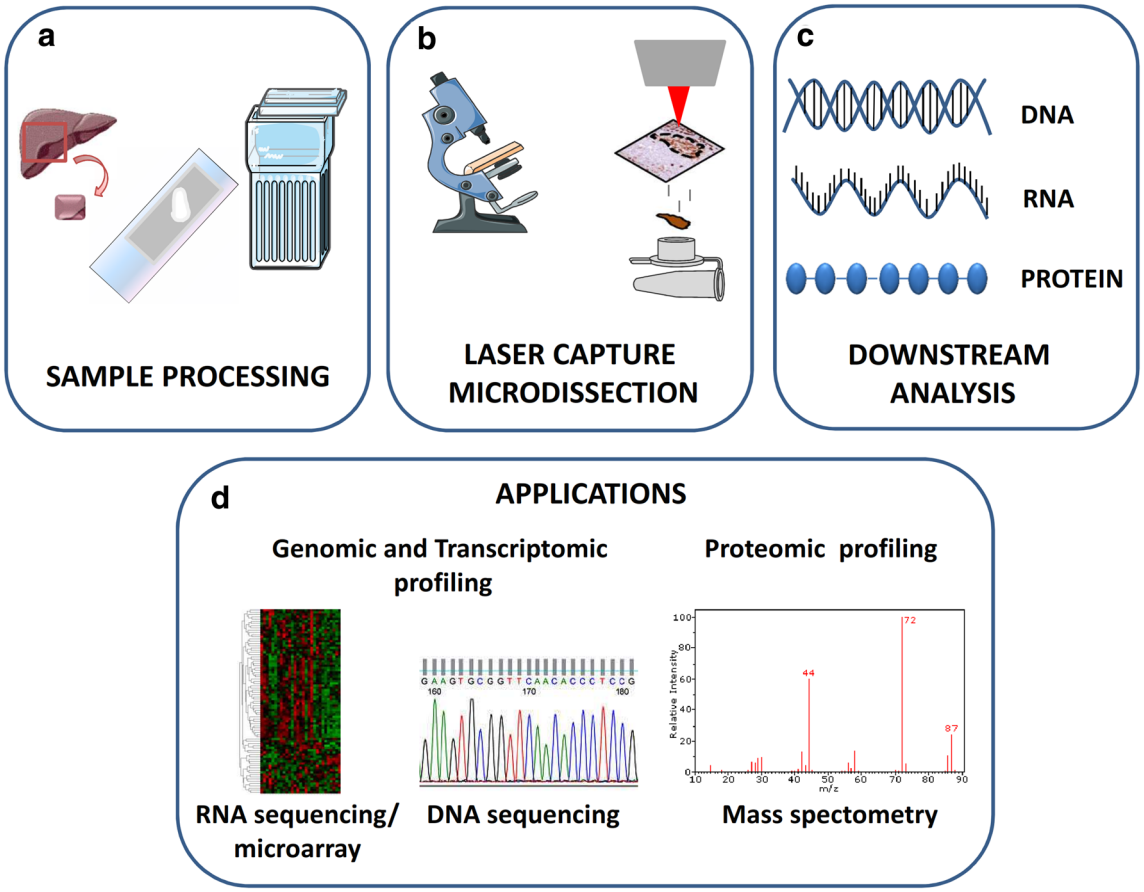

Fig. 1 Laser capture microdissection procedure (LCM). **a** Sample processing; fresh tissue is preserved, sectioned and stained prior to LCM. **b** LCM; selected area is visualized under microscope, excised by laser and collected in the tube's cap. **c** Downstream analysis; DNA, RNA or protein is extracted from the area selected and used for further analysis. **d** Applications; genomic, transcriptomic and proteomic profiling

Sample processing

Prior to performing LCM, sample processing is a critical step to obtain a good yield and quality of DNA, RNA or protein. The main factors that can affect the integrity of the molecules, and therefore, the downstream analysis are the quality of the sample, tissue preservation methods and sample sectioning and staining. For technical recommendations on the LCM procedure see Table 1.

Tissue preservation

Tissue fixation is the most critical step, and it is determined by the duration of the process, the temperature, and the size of the tissue. Indeed, the longer the fixative takes to penetrate the tissue, the greater the chance of RNA or protein degradation due to ubiquitous RNases and proteases. Formalin has been widely used for tissue fixation due to the good morphological preservation obtained. However, the integrity of the molecules can be affected by cross-linking reactions of nucleic acids and proteins. Disruption and denaturation of the cross-links may lead to the recovery of peptides and protein fragments instead of intact proteins. For this reason, frozen or ethanol-fixed tissue offers an alternative to formalin, as they allow obtaining a better yield and integrity of RNA and protein [6]. When performing metabolomics studies, fixation is not recommended as many of the small molecules and metabolites in the chemicals used for fixation are soluble, and they can be delocalized or lost during the procedure [7].

Sample sectioning

When cutting the tissue sample with the microtome [formalin fixed paraffin embedded (FFPE) samples] or cryostat (frozen samples), it is important to carefully design the handling procedure, thickness of the slide, and storage of the sample before LCM, according to the molecular analysis to be performed.

Table 1 Technical recommendations on LCM procedure

	Recommended procedures	Avoiding procedures
Sample processing: tissue preservation, sectioning and staining	Frozen samples allow obtaining better integrity of DNA, RNA and protein	Avoid long fixation times
	FFPE samples provide a good morphological preservation but the integrity of the molecules can be affected by the cross-linking reactions	Avoid formalin based fixation for metabolomic studies
	When performing tissue sections, all the surfaces and instruments should be cleaned with specific reagents to remove DNases and RNases. Bath water should be autoclaved	Tissue sections should not be thinner than 5 µm; less than 5 µM may not contain the whole cell thickness
	Fresh solutions should be prepared for staining protocol. Use DEPC-treated water and nuclease-free reagents. RNase and protease inhibitors can be added to staining reagents	Avoid heat or enzymatic pre-treatments
	If glass recipients are used for staining, they should be baked at 180 °C	
	Complete deparaffinization is essential for DNA and RNA extraction. Yield can be decreased because of an inadequate removal of paraffin	
	Immunostaining should be optimized for each of the antibodies used to minimize the incubation time. It is recommended to use high-affinity antibodies for minimal amount of time	
LCM Procedure	It is recommended to perform LCM immediately after the staining	Do not start microdissection until the sample is completely dried, humidity and bubbles difficult microdissection
	All surfaces should be cleaned with specific RNase and DNase free reagents	LCM procedure should not exceed 1 h–1.5 h. Longer times would lead to a decrease in the yield of the molecule of interest
	When visualization of specific cell populations is not clear, it is recommended to use a consecutive section with standard staining as a guide	
	It is recommended to microdissect as much as possible cells to get a good yield of the molecule of interest	
	When removing the collection tube with the microdissected cells, it is important to do it very carefully because if the cap hits the holder, the samples may be lost	

Sections for LCM are usually thicker than those of conventional immunohistochemical analysis, thereby providing a better cellular yield. The thickness can vary between 5 and 15 µm; less than 5 µm may not contain the whole cell thickness, while a section greater than 15 µm can be difficult to cut and process [8]. To improve tissue adherence to the glass slides, slides without tissue should be placed in a hood under UV rays for 45 min. This step is applied to both cryo- and FFPE-sections.

Before tissue sectioning, all the surfaces and instruments should be cleaned with specific reagents to remove RNases and Dnases, and bath water should be autoclaved. Frozen tissue sections can be stored at − 80º for 1 month or up to 3 months if the molecule to extract is RNA or protein, respectively [6]. On the other hand, FFPE tissue sections can be stored at room temperature, although these sections should be cut as close in time as possible to performing the LCM procedure to preserve epitopes that degrade rapidly [9].

1. Xilol, 20 s
2. Xilol, 20 s
3. Xilol, 20 s
4. Absolute ethanol, 30 s
5. Absolute ethanol, 30 s
6. 95% ethanol, 30 s
7. 95% ethanol, 30 s
8. 70% ethanol, 30 s
9. 70% ethanol, 30 s
10. Destilled water, 30 s
11. Steril PBS 1x, 30 s
12. Primary antibody (Mouse Keratin-7 anti-human, DAKO M7018; 1:50), 10 min
13. Wash with PBS 1x, 1min (x3)
14. Secondary antibody (Dako Envision System HRP Mouse, K4007), 15 min
15. Wash with PBS 1x, 1 min
16. Diaminobenzidine working solution (DAKO, K1011), 2 min
17. Cover the slide with DEPC H_2O and leave it dry for 40 min

*All the steps are performed at room temperature

Box 1 Optimized KRT7 staining for FFPE tissue section

Sample staining

The incubation times in staining for LCM differ from the standard protocols used for routine immunohistochemistry analysis in that they are shorter to reduce degradation and to achieve better integrity of the molecule of interest.

When performing staining (cryo-tissue and formalin/ethanol fixed tissue), it is very important to use nuclease-free water and that all the reagents should be fresh. Moreover, the glass recipients used to perform the staining should be baked at 180 °C. Alternatively, for the processing of a small amount of samples, RNase free sterile 50 ml tubes can be used to perform the staining.

Hematoxylin and eosin constitutes a common staining protocol for LCM. Mayer's hematoxylin can be used without the bluing step, reducing the exposure to water and degradation. There are other staining protocols such as toluidine blue or Nissl that are also suitable for LCM, and they can be performed in one single step, thereby reducing the probability of degradation [9].

Immunohistochemistry and immunofluorescence can also be performed before LCM, although it is important to take into account that the amount and integrity of some biomolecules, especially RNA, can be affected [10]. Box 1 shows a protocol optimized in our laboratory for KRT7 staining in FFPE samples (in press 10.1002/HEP.30472). Immunostaining should be optimized for each of the antibodies used to minimize the incubation time. Moreover, antigen retrieval should be avoided because it is an aggressive process that affects the integrity of the molecules. For this reason, many antibodies may not be useful for microdissection or may require important optimization which may not allow retrieval. It is also important to note that complete deparaffinization is essential to obtain good quality of the molecule of interest, especially DNA and RNA, as protein is usually obtained from frozen samples.

The staining protocols can affect the integrity of the molecule of interest, and therefore, it is recommended to perform LCM immediately after staining [11]. However, if this is not possible, once the samples are dried they can be stored at − 80 °C (based on our experience on FFPE-sections). Fifty ml RNase and DNase free tubes are adequate for storing the samples at − 80 °C. In this way, several samples can be stained at the same time and stored until the LCM procedure.

Tissue microdissection

Two technologies are commonly used for LCM: infrared (IR) and ultraviolet (UV). The IR system is based on the addition of a thermolabile polymer directly on the tissue section placed on the slide. The IR laser pulse melts the polymer resulting in the generation of a polymer-cell composite that is removed from the tissue. To prevent damage to the dissected area, the polymer has a dye that absorbs laser energy, thereby protecting the sample. The UV microdissection technology places the tissue section on a polyethylene terephthalate (PET) membrane slide. The area of interest is cut by UV laser and captured in the cap of the tube placed under the tissue slide. Unlike the IR system, the UV system damages the cells located in the perimeter of the cut area, which may interfere in the final downstream analysis if the amount of cells affected by the laser is higher (> 10%)

than the whole selected area [6]. Despite this limitation, the UV system offers several advantages over the IR system. Avoiding contamination of the microdissected sample is very important for downstream analysis. In the IR system, the area of interest becomes attached to an adhesive film, which may be a source of contamination [12]. Conversely, in the UV system, the area microdissected is directly collected in the tube's cap. Moreover, the laser beam diameter can be thinner (0.5 µm) than the IR [13]. This feature in the UV system is an advantage for analyzing small samples and single cell isolation. As described previously, the optimal thickness of the tissue for LCM can vary from 5 to 15 µm, however, the UV system is able to cut tissue sections of up to 200 µm thick.

As explained above in the Sample Staining section, samples stored at − 80º after staining should be taken out of the freezer to allow slow thawing prior to starting the microdissection procedure. The presence of water bubbles on the slide makes microdissection impossible, and therefore, it is crucial to not open the tube until the tissue is completely thawed and there are no signs of humidity in the slide. Moreover, all the surfaces should be cleaned with specific RNase and DNase free reagents, and the laser should be calibrated to ensure the cut and detachment of the sample.

When performing microdissection, it is important to cut as close as possible to the perimeter of the cells of interest to avoid contamination of other cell types. Once microdissection has been initiated, the procedure should not exceed 1 h and a half, as longer times will affect molecule integrity.

Downstream analysis

After performing the LCM, the microdissected area is placed in a suitable buffer for DNA, RNA or protein extraction. The number of cells or area microdissected should be adjusted according to the amount of RNA, DNA or protein required for the downstream analysis [6]. Currently, there are several extraction kits allowing RNA isolation from small tissue samples from both frozen and FFPE tissues. Based on our experience in microdissection of liver tissue, a minimum area of 1.5 mm^2 from FFPE sections is required for standard RNA sequencing. However, more efficient methodologies for low-input RNA are being developed. Moreover, to obtain enough RNA for further processing, it is also possible to apply amplification protocols.

LCM offers the possibility of performing several downstream analyses. For example, DNA can be used for next-generation sequencing (NSG), pyrosequencing, loss of heterozygosity; RNA can be used for NGS, microarrays, and quantitative polymerase chain reaction (qPCR); and protein can be applied to western blot, reverse-phase protein microarray and mass spectrometry. Moreover, in addition to transcriptomic and proteomic studies, metabolite analysis is becoming an essential procedure to characterize specific tissue, mainly in plants [7, 14, 15].

Mass spectrometry imaging is also being used to record the distribution of several molecules directly from tissue without labeling. It is widely used in clinical research for the identification of biomarkers for diagnosis and prognosis [16]. Imaging of metals is gaining special interest. Several metabolic disorders develop as a consequence of a deficiency or excess of specific trace elements in tissue regions and cellular organelles. For this reason, imaging of the spatial distribution and local concentrations of these elements provides a great advantage in many diseases such as Alzheimer's, Parkinson's or Wilson's disease [17, 18].

LCM in liver diseases

LCM has been widely used in the field of liver disease for a variety of downstream analyses (Table 2). These studies comprise microdissection of liver tissue from patients with hepatitis C virus (HCV) [1, 19–23], hepatitis B virus (HBV) [24–28], alcoholic liver disease (ALD) [29–31], non-alcoholic fatty liver disease (NAFLD) [32], cholestatic liver injury [33–36] and others [37]. LCM allows evaluation of HCV- and HBV-positive hepatocytes in chronic hepatitis [1, 26]. Kandathil et al. [1] observed clusters of HCV infected hepatocytes that were not randomly distributed. They used single cell LCM combined with reverse transcription polymerase chain reaction (RT-PCR) to evaluate infected hepatocytes in patients with chronic HCV and to quantify the viral RNA content per cell. Similarly, Mishiro et al. [26] used LCM coupled with qPCR to analyze the proportion of HBV-DNA in the portal and central areas of hepatic lobules. They observed a positive correlation between the amount of HBV-DNA in hepatocytes from patients with chronic HBV and serum levels of hepatitis B e antigen (HBeAg) [26]. This approach allowed the generation of quantitative data unlike that found in previous studies performing immunohistochemistry and in situ hybridization. Munshaw et al. [23] microdissected hepatocytes from HCV patients with and without fibrosis to find potential genes involved in fibrosis progression within the context of chronic HCV infection. RNA-microarray from both populations of hepatocytes showed a decreased expression of butyrylcholinesterase (BCHE) in hepatocytes from fibrotic tissues [23]. Using the same approach, Honda et al. [19] isolated cells from the liver lobule and portal area of liver biopsies of HCV patients before and after treatment with interferon and ribavirin. They found that patients showing a high expression of interferon and ribavirin-stimulated genes in cells from liver lobules before treatment showed poor response [19].

An important application of LCM in liver disease has been the characterization of cell populations within the

Table 2 Selected laser capture microdissection studies in the field of liver disease

Pathology	Tissue type	Tissue preservation	Staining protocol	Isolated area	Downstream analysis	References
ALD progenitor cells	Human RNA sequencing	FFPE	KRT7	Hepatic		
HVC	Human	Frozen	H	Hepatocytes	RT-PCR	[1]
HVC	Human	Frozen	Toluidine blue	Cells in liver lobules and portal areas	RNA, microarray	[19]
HVC	Human	Frozen	H	Portal tracts and parenchymal segments from pre-cirrhotics and no fibrosis livers	RNA, microarray	[23]
HVB cirrhotic liver	Human	Frozen	HE	Hepatic progenitor cells in cirrhotics and interlobular bile ducts in normal tissue	RNA, microarray	[24]
HVB	Human	FFPE		Peripheral and central hepatocytes	DNA, qPCR	[26]
ASH	Human	Frozen	Cresyl violet	Hepatic progenitor cells	RNA, sequencing	[30]
NAFLD and ALD	Human	FFPE		Hepatocytes and myofibroblasts	DNA, pyrosequencing	[32]
Cholestatic liver injury	Mouse	Frozen	Carbon-labelled[a]	Kupffer cells	qPCR	[34]
Cholestatic liver injury	Human	FFPE	Desmin	HSC	RNA, RT-PCR	[35]
	Mouse	Frozen	Desmin	HSC	RNA, RT-PCR	[35]
PBC	Human	FFPE	HE	Hepatocytes and infiltrating lymphocytes	miRNA, digital PCR	[36]
Acute on chronic liver diseases	Human	Frozen	KRT7	Hepatic progenitor cells	RNA, qPCR and PCR array	[37]
HVB-associated HCC	human	Frozen	HE	Malignant and non- malignant hepatocytes	RNA- microarray	[39]
HCC	Human	FFPE	HE	Normal liver, precancerous lesion and tumour tissue	TaqMan Low Density Array	[40]
HCC	Rat	FFPE	H	Focal tumour lesions	Mass spectrometry	[41]
PSC and CC	Human	FFPE	H	Cholangiocytes and hepatocytes from non-diseased liver, PSC and cholangiocarcinoma	DNA sequencing	[42]
CC	Human	Frozen	H	Normal intrahepatic cholangiocytes and normal hepatocytes	Taqman miRNA (RT-PCR)	[43]
CC	Human	Frozen	cresyl violet	Tumor epithelial and stromal compartments	RNA-microarray	[44]
ICC	Human	Frozen	HE	Stromal cells and non tumor fibrous areas	RNA- microarray	[46]
ICC	Human	FFPE	HE	Tumor and stromal cells	Taqman miRNA (RT-PCR)	[47]
Liver metastasis from CRC	Human	Frozen		Tumour cells from CRC with and without metastases	RNA, microRNA array	[48]
Liver metastasis from CRC	Human	FFPE	cresyl violet	Tumour cells from CRC and their corresponding liver metastasis	RNA, microRNA array	[49]
Liver metastasis from CRC	Human	Frozen	cresyl violet	Tumour cells from CRC with and without metastases	RNA, microRNA array	[50]

ICC intrahepatic cholangiocarcinoma, *CC* cholangiocarcinoma, *PSC* primary sclerosing cholangitis, *HCC* hepatocellular carcinoma, *HBV* hepatitis B virus, *CRC* colorectal carcinoma, *HCV* hepatitis C virus, *PBC* primary biliary cirrhosis, *NAFLD* non-alcoholic fatty liver disease, *ALD* alcoholic liver disease, *ASH* alcoholic steatohepatitis, *FFPE* formalin fixed paraffin embedded, *HE* haematoxylin–eosin, *H* haematoxylin, *KRT7* cytokeratin 7, *HSC* hepatic stellate cells, *RT-PCR* reverse transcription–polymerase chain reaction

[a]Mice were inoculated intravenously with india ink (1:100 in saline) to distinguish kupffer cells

context of liver injury. LCM has been used to analyze the transcriptomic profile of cells without the need for performing the cell isolation procedure and allows the study of the cells in their niche. Mc Daniel et al. used LCM to microdissect hepatic stellate cells from bile duct ligated (BDL) mice that had recovered from cell therapy transplant and

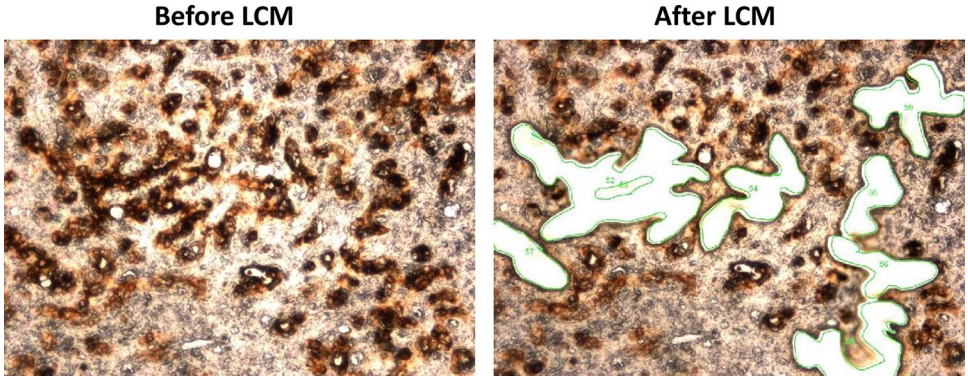

Fig. 2 Laser capture microdissection (LCM) of liver tissue. Microdissection of KRT7$^+$ areas of FFPE tissue sections from patients with alcoholic hepatitis. KRT7$^+$ area before and after microdissection

from patients with primary biliary cholangitis (PBC). qPCR analysis of hepatic stellate cells from the injury-recovered mice showed an increased expression of senescence markers which led to less fibrosis. However, the expression of senescence markers was decreased in stellate cells from PBC patients, indicating that these cells remained active. Gehring et al. isolated Kupffer cells from a BDL mouse model and found that increased expression of IL-6 assessed by qPCR plays an important role in reducing liver damage [34, 35]. Ductular reaction (DR) cells in chronic liver diseases have been a focus of interest in the last years. DR is present in most chronic liver diseases and is characterized by proliferation of hepatic progenitor cells (HPC) [24, 30, 37], (in press 10.1002/HEP.30472). Wang et al. [24] used LCM combined with microarray analysis to evaluate the gene expression profile of DR from HBV-cirrhotic tissue adjacent to hepatocarcinoma (HCC). Similarly, Ceulemans et al. [30] microdissected HPC enriched areas in alcoholic steatohepatitis livers, and RNA sequencing was performed to analyze the transcriptomic profile. Moreover, Spee et al. [37] used LCM to isolate HPC from patients with acute and chronic liver diseases, and a customized PCR array was performed to evaluate the mechanisms involved in HPC proliferation and differentiation. Additionally, HPCs have also been microdissected in our lab from alcoholic hepatitis patients in order to study the gene expression profile by RNA sequencing of ductular reaction in alcoholic liver disease. Figure 2 shows a KRT7 positive area before and after LCM (in press 10.1002/HEP.30472). These studies have provided better understanding of DR proliferation and the biological properties of HPC in chronic liver diseases. LCM has also been applied to epigenetic studies. Katsumi et al. used LCM coupled with digital PCR to identify the cell population expressing miR-139-5p in patients with PBC. By microdissecting hepatocytes and infiltrated lymphocytes they showed an up-regulation of miR-139-5p in lymphocytes [36]. Likewise, Hardy et al. [32] evaluated the source of the hypermethylated PPARγ DNA found in plasma from NAFLD patients. Hepatocytes and myofibroblast enriched areas were microdissected from NAFLD and ALD patients and analyzed by DNA-pyrosequencing, showing a higher degree of methylation in hepatocytes compared to myofibroblasts [32].

- Liver cancer

LCM has been extensively performed in the field of cancer to study hepatocarcinoma (HCC) [38–41], cholangiocarcinoma (CCA) [42–47], and liver metastasis [48–50]. Chronic liver diseases constitute a very important risk factor for the development of HCC. LCM has been used in combination with different downstream analyses in the study of HCC to compare the transcriptome and proteome of malignant and non-malignant cells. Michael et al. [41] used LCM and mass spectrometry to analyze the proteomic profile of focal liver lesions in a rat model of HCC. The proteome characterization gave rise to 11,070 unique peptides which represent 2227 proteins. Known HCC markers were found within these proteins as well as potential new markers for HCC such as aflatoxin B1, aldehyde reductase member 3 and glucose 6-phosphate 1-dehydrogenase [41]. A precancerous liver lesion presents premalignant tissue with a high risk of developing carcinogenesis. Yang et al. [40] combined LCM with TaqMan Low Density Array assay and isolated normal hepatocytes, precancerous tissue and tumor tissue. They identified increased miR-484 in precancerous lesions and demonstrated the potential of this miRNA in cell transformation [40]. Melis et al. [39] compared the transcriptome and HBV expression of whole liver tissue from patients with HBV-associated HCC with microdissected malignant and non-malignant hepatocytes. Microarray analysis from microdissected hepatocytes allowed the identification of two new genes (NUF2 and TTK) not found in whole liver, which were suggested as potential therapeutic targets in HCC [39].

Intrahepatic cholangiocarcinoma (ICC) is characterized by an abundant stroma, and several studies have used LCM to evaluate the microRNA and gene expression profile of this compartment [43, 44, 46, 47]. Asukai et al. [47] used LCM coupled with RT-PCR to analyze miR-130a-3p expression in tumor and stroma cells from patients with ICC. Although

the differences between the two populations were not statistically significant, they were able to stratify patients based on the relative expression of the miRNAs in tumor and stroma (miR-130a-3phigh group and the miR-130a-3plow). Similarly, Chen et al. [43] evaluated the miRNA expression profile of 27 ICC samples, and as controls, cholangiocytes and hepatocytes from normal liver tissues were microdissected. They found 38 miRNAs differentially expressed in tumor samples compared to control samples [43]. Sulpice et al. [46] used LCM combined with microarray analysis to study the tumor stromal compartment to identify biomarkers associated with cancer progression. Functional analysis of up-regulated genes in the stroma showed enrichment in pathways related to the cell cycle, extracellular matrix (ECM) and transforming growth factor beta (TGFB). Moreover, they revealed that the expression of osteopontin, TGFB2 and laminin in the stroma correlated with patient outcome [46]. Similarly, Andersen et al. [44] microdissected the tumor epithelium and stroma, and the microarray analysis showed an overexpression of HER2 in the epithelial compartment, and inflammatory cytokines in the stroma.

Mutations in the coding region have been described in carcinomas of the pancreas, esophagus, biliary tract, and familial melanoma [51]. Taniai et al. [42] used LCM to evaluate the role of p16INK4a gene mutations in the progression of PBC to CCA. Cholangiocytes and hepatocytes were isolated from primary sclerosing cholangitis (PSC) patients with and without CCA, and DNA sequencing was performed. They observed the presence of point mutations in the p16INK4a promoter region in cholangiocytes from PSC patients without cholangiocarcinoma, suggesting the contribution of these mutations in cancer progression [42].

LCM has been used for the study of colorectal cancer (CRC) liver metastasis, mainly to evaluate the transcriptome and microRNA profile of malignant cells isolated from colorectal cancer, the corresponding liver metastasis or both tissues [48, 50]. Iida et al. used LCM combined with miRNA array to analyze tumor stroma from CRC with and without liver metastasis. They observed an up-regulation of miR-221 and miR-222 by RT-qPCR in tumor cells from CRC with liver metastasis, and their expression correlated with mortality [48]. Similarly, Murakami et al. [50] microdissected tumor stroma from CRC with and without liver metastasis and described Tenascin C, a target of miR-198, as a new marker for liver metastasis prognostic. Likewise, Iino et al. [49] evaluated miRNA expression in tumor cells microdissected from primary CRC and their corresponding liver metastasis. The microRNA array showed an overexpression of miR-122 in malignant cells from liver metastasis compared to the primary tumor. Moreover, overexpression of miR-122 led to the suppression of the target cationic amino acid transporter 1 in the CCR tumor, which was suggested as a potential new biomarker to predict postoperative liver metastasis in patients with CCR [49].

Limitations

Although LCM currently constitutes a powerful tool for genomic and proteomic analysis, it has some limitations.

- It is an expensive technology due to the cost of the microdissection equipment.
- Due to inappropriate tissue sample preservation and processing, the yield and quality of DNA, RNA or protein obtained may not be suitable for further analysis.
- The absence of a coverslip on the tissue slide (cryo-tissue and formalin/ethanol fixed tissue) can hinder correct identification of the area to microdissect. A coverslip is important in light microscopy to increase the refractive index of the sample, thereby improving the quality of the image. For samples with poor image quality, standard immunostaining of a consecutive section can be used as a guide to ensure the capture of the correct area.
- Microdissection is a time-consuming technique, mainly due to the optimization of all the steps required to obtain good quality DNA, RNA or protein for the final downstream analysis.
- Cell morphology, tissue structure or histological visualization may preclude single cell or small area microdissection.

Conclusions

LCM has been shown to be an excellent tool for the isolation of tissue areas or specific cell populations within a tissue, without contamination of surrounding cells. Moreover, the areas dissected are available for a variety of downstream analyses in many research fields, including liver diseases. However, LCM technology presents some technical challenges. Specifically, steps concerning tissue preservation, sectioning and staining need to be previously optimized to obtain the best yield and integrity of the molecules of interest.

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Compliance with ethical standards

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Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any study with human participants or animals performed by any of the authors.

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