Exp Dermatol. 2015 Mar;24(3):187-93. doi: 10.1111/exd.12604.

Laser capture microdissection followed by next-generation sequencing identifies disease-related microRNAs in psoriatic skin that reflect systemic microRNA changes in psoriasis.

Løvendorf MB[1], Mitsui H, Zibert JR, Røpke MA, Hafner M, Dyring-Andersen B, Bonefeld CM, Krueger JG, Skov L.
Author information

Abstract

Psoriasis is a systemic disease with cutaneous manifestations. MicroRNAs (miRNAs) are small non-coding RNA molecules that are differentially expressed in psoriatic skin; however, only few cell- and region-specific miRNAs have been identified in psoriatic lesions. We used laser capture microdissection (LCM) and next-generation sequencing (NGS) to study the specific miRNA expression profiles in the epidermis (Epi) and dermal inflammatory infiltrates (RD) of psoriatic skin (N = 6). We identified 24 deregulated miRNAs in the Epi and 37 deregulated miRNAs in the RD of psoriatic plaque compared with normal psoriatic skin (FCH > 2, FDR < 0.05). Interestingly, 9 of the 37 miRNAs in RD, including miR-193b and miR-223, were recently described as deregulated in circulating peripheral blood mononuclear cells (PBMCs) from patients with psoriasis. Using flow cytometry and qRT-PCR, we found that miR-193b and miR-223 were expressed in Th17 cells. In conclusion, we demonstrate that LCM combined with NGS provides a robust approach to explore the global miRNA expression in the epidermal and dermal compartments of psoriatic skin. Furthermore, our results indicate that the altered local miRNA changes seen in the RD are reflected in the circulating immune cells, suggesting that miRNAs may contribute to the pathogenesis of psoriasis.

PLoS One. 2016 Mar 21;11(3):e0151775. doi: 10.1371/journal.pone.0151775. eCollection 2016.

High-Throughput Microdissection for Next-Generation Sequencing

Avi Z. Rosenberg,[1,‡] Michael D. Armani,[2,‡] Patricia A. Fetsch,[3,‡] Liqiang Xi,[4] Tina Thu Pham,[4] Mark Raffeld,[4] Yun Chen,[2,5] Neil O'Flaherty,[2] Rebecca Stussman,[2] Adele R. Blackler,[2] Qiang Du,[2] Jeffrey C. Hanson,[1] Mark J. Roth,[3] Armando C. Filie,[3] Michael H. Roh,[6] Michael R. Emmert-Buck,[2,7,*] Jason D. Hipp,[1] and Michael A. Tangrea[2,8,*]

Serena M. Bagnasco, Editor

Associated Data

Supplementary Materials

Data Availability Statement

Abstract

Histol Histopathol. 2015 Nov;30(11):1255-69. doi: 10.14670/HH-11-622. Epub 2015 Apr 20.

Laser capture microdissection: Big data from small samples.

Datta S[1], Malhotra L[1], Dickerson R[1], Chaffee S[1], Sen CK[1], Roy S[2].
Author information

Abstract

Any tissue is made up of a heterogeneous mix of spatially distributed cell types. In response to any (patho) physiological cue, responses of each cell type in any given tissue may be unique and cannot be homogenized across cell-types and spatial co-ordinates. For example, in response to myocardial infarction, on one hand myocytes and fibroblasts of the heart tissue respond differently. On the other hand, myocytes in the infarct core respond differently compared to those in the peri-infarct zone. Therefore, isolation of pure targeted cells is an important and essential step for the molecular analysis of cells involved in the progression of disease. Laser capture microdissection (LCM) is powerful to obtain a pure targeted cell subgroup, or even a single cell, quickly and precisely under the microscope, successfully tackling the problem of tissue heterogeneity in molecular analysis. This review presents an overview of LCM technology, the principles, advantages and limitations and its down-stream applications in the fields of proteomics, genomics and transcriptomics. With powerful technologies and appropriate applications, this technique provides unprecedented insights into cell biology from cells grown in their natural tissue habitat as opposed to those cultured in artificial petri dish conditions.

Sci Rep. 2018 May 8;8(1):7189. doi: 10.1038/s41598-018-25320-z.

Cell-type specific expression of oncogenic and tumor suppressive microRNAs in the human prostate and prostate cancer.

Kumar B[1], Rosenberg AZ[2], Choi SM[1], Fox-Talbot K[3], De Marzo AM[1,3], Nonn L[4], Brennen WN[3], Marchionni L[3], Halushka MK[2,3], Lupold SE[5,6].

Author information

Abstract

MiR-1 and miR-143 are frequently reduced in human prostate cancer (PCa), while miR-141 and miR-21 are frequently elevated. Consequently, these miRNAs have been studied as cell-

autonomous tumor suppressors and oncogenes. However, the cell-type specificity of these miRNAs is not well defined in prostate tissue. Through two different microdissection techniques, and droplet digital RT-PCR, we quantified these miRNAs in the stroma and epithelium of radical prostatectomy specimens. In contrast to their purported roles as cell-autonomous tumor suppressors, we found miR-1 and miR-143 expression to be predominantly stromal. Conversely, miR-141 was predominantly epithelial. miR-21 was detected in both stroma and epithelium. Strikingly, the levels of miR-1 and miR-143 were significantly reduced in tumor-associated stroma, but not tumor epithelium. Gene expression analyses in human cell lines, tissues, and prostate-derived stromal cultures support the cell-type selective expression of miR-1, miR-141, and miR-143. Analyses of the PCa Genome Atlas (TCGA-PRAD) showed a strong positive correlation between stromal markers and miR-1 and miR-143, and a strong negative correlation between stromal markers and miR-141. In these tumors, loss of miR-1 and gain of miR-21 was highly associated with biochemical recurrence. These data shed new light on stromal and epithelial miRNA expression in the PCa tumor microenvironment.

PMID: 29739972 PMCID: PMC5940660 DOI:10.1038/s41598-018-25320-z

Gut. 2019 Jan 18. pii: gutjnl-2018-317706. doi: 10.1136/gutjnl-2018-317706. [Epub ahead of print]

Experimental microdissection enables functional harmo nisation of pancreatic cancer subtypes.

Maurer C[#1,2,3], Holmstrom SR[#1,2,3], He J[#1,4,5], Laise P[1,4,5], Su T[1,3], Ahmed A[1,3], Hibshoosh H[1,5], Chabot JA[1,6], Oberstein PE[7], Sepulveda AR[1,5], Genkinger JM[1,8], Zhang J[9], Iuga AC[1,5], Bansal M[10], Califano A[1,4,5], Olive KP[1,2,3].

Author information

Abstract

OBJECTIVE:

Pancreatic ductal adenocarcinoma (PDA) has among the highest stromal fractions of any cancer and this has *complicated*attempts at expression-based molecular classification. The goal of this work is to profile purified samples of human PDA epithelium and stroma and examine their respective contributions to gene expression in bulk PDA samples.

DESIGN:

We used laser capture microdissection (LCM) and RNA sequencing to profile the expression of 60 matched pairs of human PDA malignant epithelium and stroma samples. We then used these data to train a computational model that allowed us to infer tissue composition and generate virtual compartment-specific expression profiles from bulk gene expression cohorts.

RESULTS:

Our analysis found significant variation in the tissue composition of pancreatic tumours from different public cohorts. Computational removal of stromal gene expression resulted in the reclassification of some tumours, reconciling functional differences between different cohorts. Furthermore, we established a novel classification signature from a total of 110 purified human PDA stroma samples, finding two groups that differ in the extracellular matrix-associated and immune-associated processes. Lastly, a systematic evaluation of cross-compartment subtypes spanning four patient cohorts indicated partial dependence between epithelial and stromal molecular subtypes.

CONCLUSION:

Our findings add clarity to the nature and number of molecular subtypes in PDA, expand our understanding of global transcriptional programmes in the stroma and harmonise the results of molecular subtyping efforts across independent cohorts.

KEYWORDS:

pancreatic cancer

PMID: 30658994 DOI:10.1136/gutjnl-2018-317706

J Vis Exp. 2018 Jun 14;(136). doi: 10.3791/57762.

Optimization of Laser-Capture Microdissection for the Isolation of Enteric Ganglia from Fresh-Frozen Human Tissue.

May-Zhang AA[1], Deal KK[1], Southard-Smith EM[2].

Author information

Abstract

The purpose of this method is to obtain high-integrity RNA samples from enteric ganglia collected from unfixed, freshly-resected human intestinal tissue using laser capture microdissection (LCM). We have identified five steps in the workflow that are crucial for obtaining RNA isolates from enteric ganglia with sufficiently high quality and quantity for RNA-seq. First, when preparing intestinal tissue, each sample must have all excess liquid removed by blotting prior to flattening the serosa as much as possible across the bottom of large base molds. Samples are then quickly frozen atop a slurry of dry ice and 2-methylbutane. Second, when sectioning the tissue, it is important to position cryomolds so that intestinal sections parallel the full plane of the myenteric plexus, thereby yielding the greatest surface area of enteric ganglia per slide. Third, during LCM, polyethylene napthalate (PEN)-membrane slides offer the greatest speed and flexibility in outlining the non-uniform shapes of enteric ganglia when collecting enteric ganglia. Fourth, for distinct visualization of enteric ganglia within sections, ethanol-compatible dyes, like Cresyl Violet,

offer excellent preservation of RNA integrity relative to aqueous dyes. Finally, for the extraction of RNA from captured ganglia, we observed differences between commercial RNA extraction kits that yielded superior RNA quantity and quality, while eliminating DNA contamination. Optimization of these factors in the current protocol greatly accelerates the workflow and yields enteric ganglia samples with exceptional RNA quality and quantity.

PMID: 29985370 PMCID: PMC6042974 [Available on 2019-06-14] DOI: 10.3791/57762

BMC Mol Biol. 2018 Jun 19;19(1):7. doi: 10.1186/s12867-018-0108-5.

Laser capture microdissection for transcriptomic profiles in human skin biopsies.

Santoro S[1], Lopez ID[2], Lombardi R[3], Zauli A[1], Osiceanu AM[1], Sorosina M[1], Clarelli F[1], Peroni S[1], Cazzato D[3], Marchi M[3], Quattrini A[2], Comi G[1,4], Calogero RA[5], Lauria G[6,7], Martinelli Boneschi F[8,9].

Author information

Abstract

BACKGROUND:

The acquisition of reliable tissue-specific RNA sequencing data from human skin biopsy represents a major advance in research. However, the complexity of the process of isolation of specific layers from fresh-frozen human specimen by laser capture microdissection, the abundant presence of skin nucleases and RNA instability remain relevant methodological challenges. We developed and optimized a protocol to extract RNA from layers of human skin biopsies and to provide satisfactory quality and amount of mRNA sequencing data.

RESULTS:

The protocol includes steps of collection, embedding, freezing, histological coloration and relative optimization to preserve RNA extracted from specific components of fresh-frozen human skin biopsy of 14 subjects. Optimization of the protocol includes a preservation step in RNALater® Solution, the control of specimen temperature, the use of RNase Inhibitors and the time reduction of the staining procedure. The quality of extracted RNA was measured using the percentage of fragments longer than 200 nucleotides (DV_{200}), a more suitable measurement for successful library preparation than the RNA Integrity Number (RIN). RNA was then enriched using the TruSeq®RNA Access Library Prep Kit (Illumina®) and sequenced on HiSeq® 2500 platform (Illumina®). Quality control on RNA sequencing data was adequate to get reliable data for downstream analysis.

CONCLUSIONS:

The described implemented and optimized protocol can be used for generating transcriptomics data on skin tissues, and it is potentially applicable to other tissues. It can be extended to multicenter studies, due to the introduction of an initial step of preservation of the specimen that allowed the shipment of biological samples.

KEYWORDS:

Idiopathic neuropathy; Laser capture microdissection; RNA sequencing; Skin biopsy; Transcriptomics

PMID: 29921228 PMCID: PMC6009967 DOI: 10.1186/s12867-018-0108-5