SciMedCentral

Journal of Chronic Diseases and Management

Review Article

Insights into the Precision Medicine of Pancreatic Cancer: Single-Cell Analysis

Negar Jamshidi, Nazanin Jamshidi, Vahid Chaleshi*, and Ehsan Nazemalhosseini

Research Institute for Gastroenterology and Liver Diseases, Shahid Behest University of Medical Sciences, Iran

***Corresponding author**

Vahid Chaleshi, Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Behest University of Medical Sciences, Tehran, Iran, Tel: 98-2122432525

Submitted: 09 January 2024

Accepted: 20 February 2024

Published: 23 February 2024

ISSN: 2573-1300

OPEN ACCESS

Keywords

- Pancreatic Cancer; Single-Cell Analysis; RNA Sequencing

Abstract

The rising incidence of pancreatic cancer (PC) is a global challenge of paramount importance. Understanding the complex nature of this disease is crucial for addressing various issues within the field, including early recurrence, metastasis, and the formidable resistance to chemotherapy and radiotherapy. The complexity of PC arises from the diverse biological phenotypes present in tumor cells, creating a mosaic-like diversity. The lack of comprehensive insights into the functional aspects of cellular and molecular components further complicates treatment, leading to suboptimal outcomes.

To tackle these intricacies, researchers must explore the cellular and molecular foundations of this multifaceted disease. Profiling cellular transcriptomes becomes a powerful tool for understanding the subtle heterogeneity in complex cellular populations within tissues. This approach allows for the deconstruction of molecular processes at the individual cell level, revolutionizing our understanding of cellular identity and function. By carefully examining differences in single-cell genomes, transcriptomes, and epigenetic profiles among tumor cells, both primary and metastatic, collected from patient samples, we gain insights into the diverse landscape of tumor heterogeneity.

This perspective has significant implications for precision oncology in the context of pancreatic cancer. The current study provides a comprehensive assessment of the empirical basis for RNA sequencing technologies, the methodologies for single-cell isolation, and the conceptual framework for integrating these elements into prognostic assessment, genomic accessibility, transcriptional dynamics, and proteomic landscapes. By combining these components, the study aims to establish a coherent framework that facilitates the development of precision medicine approaches tailored to the unique challenges presented by pancreatic cancer.

ABBREVIATIONS

PDAC: Pancreatic Ductal Adenocarcinoma; scRNA-seq: Single-cell RNA-sequencing; LCM: Laser Capture Micro-Dissection; CTC: Circulating Tumor Cells; CSC: Cancer Stem Cells; ITH: Intratumor Heterogeneity; MACS: Magnetic-activated cell Sorting; FACS: Fluorescence-activated cell Sorting; IR: Infrared; UV: Ultraviolet; WGA: Whole Genome Amplification; MALBAC: Multiple Annealing and Looping based Amplification Cycles; MDA: Multiple displacement Amplification; DOP-PCR: Degenerate oligonucleotide primed PCR; MDA: Multiple displacement Amplification; RNA-seq: RNA sequencing; scRNA-seq: Single-cell RNA-sequencing; TME: Tumor Microenvironment; CAFs: Cancer-associated Fibroblasts (CAFs); ECM: Extracellular Matrix; PanINs: Pancreatic Intraepithelial Neoplasia; TAM: Tumor-associated Macrophages; PDFD: Platelet-derived growth factor; HPSC: Human Pancreas Stellate cells; SPARC: Secreted protein acidic and rich in cysteine; HPaStec: Human Pancreatic stellate cells

INTRODUCTION

Pancreatic cancer (PC) stands as a formidable challenge in the realm of malignancies, characterized by its high mortality rates. The formidable challenge arises from delayed diagnosis and limited therapeutic interventions, culminating in its status as one of the most lethal cancers [1,2]. PC is projected to ascend to the second position among leading causes of death before the year 2030 [3,4]. Presently, statistical data from the American Cancer Society underscores the stark reality: a mere 9% five-year survival rate for all PC patients, with a glimmer of hope at 34% for those fortunate enough to undergo tumor resection surgery [5].

Tragically, a significant proportion of PC patients—nearly two-thirds—are diagnosed at a juncture marked by metastasis. The multifaceted challenge of PC stems from its elusive therapeutic solutions owing to the intricacies of primary diagnosis, occurrence, and the ever-looming specter of tumor relapse [6]. The quest for clinical breakthroughs has been

hampered by the intricate biological makeup of tumor cells, compounded by their notable heterogeneity [7,8]. Evidently, the PC stroma's unique attributes, notably the pervasive stromal response characterizing pancreatic ductal adenocarcinoma (PDAC), remain an enigmatic terrain, with activated pancreatic stellate cells (PSCs) taking center stage, yet their fundamental nature remains shrouded in mystery [9].

Unveiling the intricacies of tumor heterogeneity emerges as a formidable imperative, underpinning not only cancer diagnosis but also treatment strategies. Scrutinizing the disparities in RNA transcription and protein expression from cell to cell assumes pivotal importance in the realm of cancer, biology, and personalized medicine [10,11].

The realm of single-cell RNA-sequencing (scRNA-seq) technology emerges as a beacon of hope for deciphering tumor heterogeneity, enabling the delineation of malignant and stromal populations within tumors, and constructing a comprehensive transcriptomic atlas. This microscopic voyage has unveiled distinct pathways guiding tumor progression and distinct transcriptional patterns. Hence, the potency of scRNA-seq shines through as a potent instrument for profiling organs and intricate tumors housing an assortment of cell types orchestrating communication via molecular cues [12,13]. Nevertheless, it's important to underscore that the practical implementation of this methodology encounters ongoing challenges within both research and diagnostic spheres [14-16].

The domain of single-cell technology bifurcates into two pivotal realms: single-cell isolation and subsequent analysis. The bedrock for single-cell analysis rests on precise single-cell isolation techniques, ranging from the stalwart flow cytometry to more advanced methodologies like laser capture micro-dissection (LCM) and microfluidics [10,17,18]. These methodologies unearth the genomic, transcriptomic, and proteomic signatures of individual cancer cells, with single-cell genomic analysis particularly lauded for its multifaceted advantages [19-21].

Central to the single-cell paradigm is its capacity to unearth elusive entities, including circulating tumor cells (CTCs) and cancer stem cells (CSCs), thereby demystifying intratumor heterogeneity (ITH) and illuminating the intricate choreography of tumor metastasis mechanisms. Moreover, it serves as a potent tool for scrutinizing epigenetic modifications, thereby promising diagnostic biomarkers with direct implications for the therapeutic landscape [22-24].

The review explores recent advances in single-cell RNA sequencing in pancreatic cancer, delving into the complexities of single-cell analysis while acknowledging its strengths and limitations. The study highlights the potential of emerging single-cell genomics technologies to provide valuable insights and solutions for understanding this challenging disease.

Single-Cell Isolation Methodologies

Prior to engaging in single-cell analysis, the prerequisite task involves the meticulous isolation and characterization of individual cells. The efficacy of cell isolation technologies is gauged through the prism of three pivotal parameters: throughput, ensuring the capacity to isolate a sizable cohort of cells concurrently; purity, signifying the selectivity in isolating a singular cellular phenotype; and retrieval, entailing a comparative evaluation between the isolated target cell and its counterparts within the specimen [17]. The existing landscape of techniques alludes to distinct advantages tailored to each of these parameters. Drawing from well-established foundations, extant cell isolation methodologies can be systematically categorized into two overarching groups.

Two clusters of cell sorting methods exist: one based on physical attributes like cell size and density, using techniques such as gradient centrifugation and membrane filtration; and another relying on biological traits, employing methods like fluorescence-activated cell sorting and magnet-activated cell sorting. Notably, the first cluster allows label-free single-cell isolation. These techniques pivot on the expression of specific biomolecules on cell surfaces [17,25,26]. This dual-tiered classification is illustrated in Figure 1, with its constituents drawing from contemporary research contributions.

In pursuit of comprehensiveness, Table 1 outlines the foundational underpinnings, merits, and demerits of each technique, providing a valuable resource for researchers and practitioners alike.

A Comparative Analysis of FACS and MACS Techniques

In the pursuit of elucidating disparities and convergences between the Flow Cytometry-based Cell Sorting (FACS) and Magnetic-Activated Cell Sorting (MACS) methodologies, Xu et al. (2014), undertook an investigation targeting the separation of Granulocytic-Myeloid-derived suppressor cells (G-MDSCs) and Monocytic-Myeloid-derived suppressor cells (Mo-MDSCs). The primary aim was to identify efficacious techniques that could bolster the advancement of research concerning these cell types. In response to this inquiry, the present study juxtaposed the FACS and MACS methodologies for the isolation of G-MDSCs and Mo-MDSCs from the spleens of mice afflicted with orthotopic (H22) liver cancer. It was ascertained that both FACS and MACS enabled the sorting of G-MDSCs and Mo-MDSCs with heightened viability and purity, rendering them suitable for both functional and non-functional investigations. Owing to the substantial proportion of G-MDSCs present in the spleens of tumor-bearing mice, both FACS and MACS techniques exhibited the potential to yield G-MDSCs with exceptional purity and yield. However, FACS demonstrated a notable advantage by accomplishing this outcome in a more time-efficient and cost-effective manner than MACS([27].

Both FACS and MACS techniques were compared for CD34 enrichment methods, considering purity, recovery rates, cellular characteristics, and regenerative potential post-myocardial infarction in SCID mice. Tripathi et al. (2020), found similar CD34 cell populations and comparable effects in in vitro and

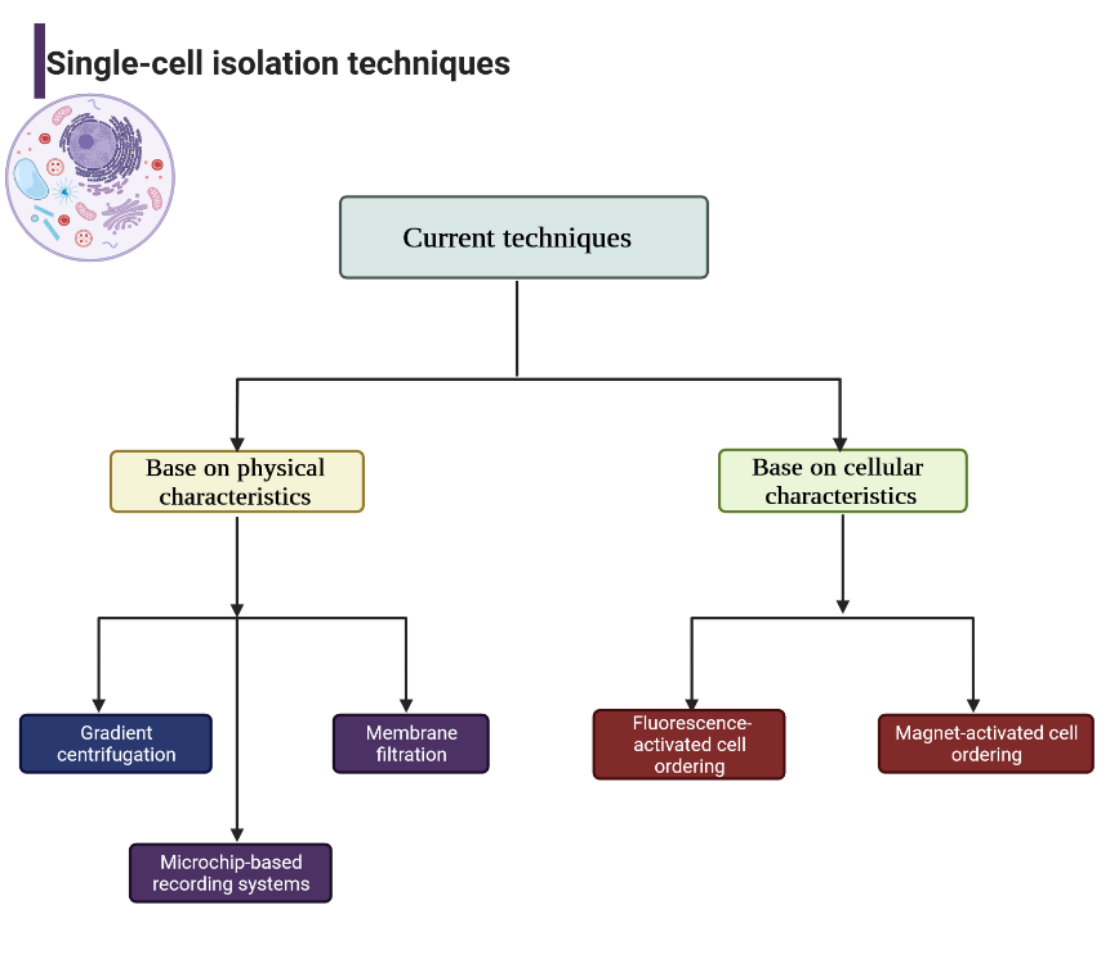


Figure 1 Types of isolation of the single-cell. Physical Isolation: In this method, individual cells are physically separated from each other by using a micropipette or other mechanical device. Chemical Isolation: In this method, individual cells are separated from each other by using a micropipette or other mechanical device. Chemical Isolation: In this method, individual cells are separated from each other by using chemical reagents such as antibodies or enzymes.

Table 1: Advantage and Disadvantages of Mictrofluidics

Advantages	Disadvantages
Microfluidic Systems for Single-Cell Isolation Precision and Detection of Rarity Microfluidic platforms have emerged as robust tools for the precise isolation of individual cells, showcasing remarkable accuracy. Moreover, their utility extends to the isolation of infrequent cell types that might elude detection via alternate methodologies.	Financial Implications of Microfluidics for Single-Cell Isolation Noteworthy, however, is the relatively elevated cost associated with adopting microfluidic systems for single-cell isolation. This financial consideration arises from the requisition of sophisticated equipment and costly reagents.
Efficiency and Economy in Single-Cell Isolation through Microfluidics The implementation of microfluidic systems for single-cell isolation stands out as an economical and efficient approach. Its appeal stems from streamlined sample preparation and minimal reagent consumption, which collectively contribute to its cost-effective nature.	Necessity for Expertise in Microfluidic-Assisted Single-Cell Isolation Proficiency in microfluidic-based single-cell isolation is contingent upon specialized knowledge and skill sets. The requirement for such expertise can pose challenges in acquisition, demanding dedicated and focused training.
Expediency and Reliability in Microfluidics-Based Single-Cell Isolation Microfluidic-enabled single-cell isolation presents a rapid and dependable strategy. Its accelerated ability to segregate cells from substantial samples within brief temporal windows underscores its utility for swift isolation requirements.	Scope Limitations in Sample Size for Microfluidic Single-Cell Isolation A factor to deliberate pertains to the sample size limitations inherent in microfluidic platforms. These constraints render them less viable for handling substantial cell volumes and hence, not conducive to large-scale cell isolation endeavors.
Unintrusive and Secure Single-Cell Isolation via Microfluidics An inherently safe and non-invasive procedure characterizes the microfluidic methodology for single-cell isolation. The absence of harsh chemicals and potentially hazardous materials in the workflow ensures safety while effectively isolating target cells.	Applicability of Microfluidic Single-Cell Isolation to Specific Cell Types It warrants acknowledgment that the utility of microfluidic-based single-cell isolation extends selectively to cell types amenable to facile manipulation and segregation. The technique's efficacy is most pronounced when applied to cells possessing characteristics conducive to its operational parameters.

in vivo characterizations, addressing concerns about MACS purity relative to FACS(28). Evidently, both techniques yielded analogous CD34 cell populations, accompanied by similar advantageous effects during in vitro and in vivo characterizations.

Laser Capture Microdissection (LCM): A Refined Cellular Separation Technique

Laser capture microdissection (LCM) represents a sophisticated approach to isolating individual cells from complex tissues through the precise deployment of laser energy for cell-specific excision. This method offers a remarkable technological advancement in the realm of extracting distinct cell populations or even single cells from solid tissues, all facilitated under the scrutiny of a microscope [29].

The instrumental components intrinsic to the LCM framework encompass several crucial elements: (i) the visual identification of target cells through conventional microscopy, (ii) the ability to channel photoenergy either toward a polymer-cell composite or directly induce tissue photolysis, and (iii) the capability to extract and isolate the intended specimen. The operational configuration necessitates an inverted microscope, an infrared laser, a laser control unit, a microscope stage control mechanism, a digital camera, and a visualization monitor for accurate target assessment [30].

This methodological paradigm revolves around an inverted microscope, wherein a delicate thermoplastic film is strategically positioned over the cell of interest, guided by laser precision. Upon laser-induced melting, the thermoplastic film amalgamates with the underlying cell. Subsequent removal of the film results in the target cells adhering to the substrate while their counterparts remain undisturbed [31]. The subsequent phase entails the gentle transfer of these cells into a microcentrifuge tube containing the requisite buffer solutions tailored to a diverse array of subsequent analyses. Central to this technique's value proposition are its expeditiousness and precision (Figure 1C) [31]. It is noteworthy that while this method bears the potential for contamination, its advantage lies in its minimal disruption to neighboring tissues, which enables molecular comparisons of contrasted cells [32].

Categorically, two distinct variants of LCM systems have emerged: infrared LCM (IR-LCM) and ultraviolet LCM (UV-LCM) [33] [19]. IR-LCM harnesses longer-wavelength light, approximately 810 nm, while UV-LCM employs shorter wavelengths around 355 nm [34]. IR-LCM capitalizes on photoenergy to liquefy polymer caps, thereby isolating tissue samples. In contrast, UV-LCM directly ablates the surrounding tissue, facilitating the isolation of cells of interest [35].

The IR-LCM modality employs brief pulses of infrared lasers at approximately 810 nm, operating through a thin thermoplastic film overlaying the tissue section. These IR beams induce polymer melting overlying the tissue sample, leading to the creation of a tissue-polymer complex that effectively segregates the target cells. Remarkably, the thermofilm concurrently acts as a safeguard against light-induced tissue damage [29]. The precisely focused IR pulse, when applied to the thermofilm, prompts a conformational shift and adhesive interaction with the tissue [36]. Consequently, at the culmination of the dissection process, the cells of interest adhered to the film can be detached and prepared for analysis. Nonetheless, the thin polymer substrate poses a risk of inadvertent attachment and potential contamination, while operator expertise significantly influences the efficacy of the process.

In the context of UV-LCM, utilizing shorter wavelengths of about 355 nm empowers more precise ablation of tissue neighboring the cells of interest [36]. The foundational framework of UV-LCM, conceived by Schutze and Lahr in 1998, involves the direct mounting of tissue onto a membrane [37]. Visual inspection through microscopy facilitates the straightforward isolation of desired cell populations, circumventing the inclusion of undesirable adjacent tissue [29,38]. In contrast to its IR-LCM counterpart, UV-LCM bypasses concerns about unintended cellular adherence due to its capacity for direct photoablation. Additionally, the shorter wavelength enables finer beam precision, particularly beneficial for smaller cells or organelles. The isolated target cells are subsequently propelled into a collector cap via photonic pressure or gravitational forces [36].

Microfluidics in Cell Isolation

Microfluidics technology allows the precise isolation of individual cells from heterogeneous populations through intricate tiny channels. By employing controlled fluid dynamics and pressure differentials, this technique achieves single-cell isolation with high efficiency and speed, surpassing traditional methods. Microfluidics is particularly valuable for isolating rare cells and distinct cell phenotypes. Additionally, it enables real-time analysis of cellular components, expediting detailed and rapid analyses compared to conventional methods.

The prowess of microfluidics in deciphering cellular intricacies stems from its capacity to precisely govern fluidic entities, coupled with its facile and economical manipulability [39]. Leveraging the dissimilarity in dielectric traits among distinct cell types, microfluidic affinity cell chromatography emerges as a notable modality [40]. Fundamentally, this approach hinges on the intricate interplay between antigens, antibodies, ligands, and receptors. Microfluidics utilizes specific antibodies or aptamers to capture cells with particular surface antigens in microchannels, allowing efficient isolation. This technique is highly sensitive, outperforming other methods (Figure 1E). In summary, microfluidics is a powerful and adaptable tool for precise cellular isolation, enabling the exploration of cellular complexities in a controlled fluidic environment.

In the contemporary landscape, the integration of microfluidics with a diverse array of isolation methodologies, encompassing filtration, precipitation, as well as FACS- and MACS-based techniques, has become a prevalent trend. Over the recent years, a multitude of investigations have been conducted within the realm of microfluidic systems, spanning domains such as oncology, microbiology, single-cell analysis, stem cell

research, drug discovery, and high-throughput screening [41]. The versatility of microfluidic technology finds its expression in various applications, particularly in the scrutiny of single-cell genomic alterations, spanning from the intricacies of cancer biology to the domains of environmental microbiology and neurobiology. A notable advantage lies in the adaptability of this approach, which accommodates a broad spectrum of fluids, including bacterial cells, whole blood specimens, protein solutions, antibodies, and buffers [42,43].

Microfluidic technology is utilized for various clinical purposes, including isolating rare cells like circulating tumor cells from blood and bodily fluids. It's also used for isolating and analyzing stem cells and fetal cells from maternal blood. Microfluidics is instrumental in isolating specific cell subtypes, especially cancer cells, aiding advancements in diagnostics and research. Additionally, it's valuable for isolating cells from biopsies for cancer diagnosis and from tissue samples, enhancing possibilities in regenerative medicine.

Yong Zhang et al. (2021), developed the HBEXO-Chip, a specific and rapid microfluidic device for pancreatic cancer exosome separation. This innovation utilizes a 3D herringbone micromixer and the pancreatic cancer exosome-specific antibody, GPC1, improving exosome enrichment efficiency. The study achieved significant outcomes, including enhanced tumor-specific exosome enrichment, successful pancreatic cancer diagnosis by quantifying GPC1+ exosome concentrations, and the identification of a miRNA signature indicative of pancreatic cancer in patient plasma [44].

In a parallel endeavor, María Sancho-Albero et al. contribute a novel microfluidic paradigm and its associated protocol for the isolation of exosomes directly from whole blood [45]. This approach obviates the need for intermediary isolation steps, streamlining the transition of the method to clinical contexts. Notably, the outcomes demonstrate heightened sensitivity in the evaluation of CA19-9 levels within exosomes, as opposed to serum samples, for the cases under examination [45].

A new automated microfluidic system has been developed for cultivating and analyzing 3D organoids, addressing challenges in compatibility with existing technologies. This system allows high-throughput, real-time analysis of organoids, enabling dynamic drug treatments and individual, combinatorial, and sequential drug screens on human pancreatic tumor-derived organoids, thus enhancing preclinical research and personalized therapeutic approaches [46,47].

Technologies for Single-Cell Analysis

There are various techniques for single cell analysis, each of which is briefly described in the Table 2. These techniques are namely: Flow Cytometry, Microfluidics, Mass Spectrometry, Single-Cell PCR, High-Throughput Sequencing, Microscopy and Single-Cell Electrophysiology.

The present techniques bear certain limitations, encompassing cost implications [16]. Singular cell analysis methodologies often entail considerable financial investment owing to the necessity for specialized apparatus. Temporal considerations are also a factor [48]. Such methodologies can demand substantial time investments due to meticulous sample preparation and the scrutiny of individual cells. The precision of these methods is also subject to scrutiny, given the inherent susceptibility to error attributed to minute sample sizes and intricate analyses. Furthermore, the intricacies of these techniques necessitate a profound level of expertise for accurate interpretation [10,49].

This section delves into the realm of single-cell omics, a paradigm shift with profound implications for our comprehension of biology. Single-cell omics bears remarkable significance, serving as a potent instrument for unraveling cellular heterogeneity. The advantages that stem from this approach are manifold: Firstly, it affords an amplified resolution for discerning cellular heterogeneity (a point underscored by (16)). The meticulous scrutiny of single cells bestows a nuanced comprehension of intercellular variations, thus facilitating more precise disease diagnosis and therapeutic interventions. Additionally, it yields an enhanced grasp of gene regulation.

Single-cell analysis provides crucial insights into gene expression, cell communication, development, and the immune system, enhancing our understanding of disease and potential therapies. Emerging tools in genomics, transcription, and proteomics are specifically designed for analyzing individual cells, allowing detailed exploration of cellular mechanisms.

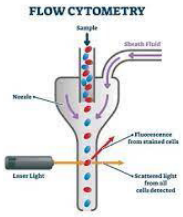
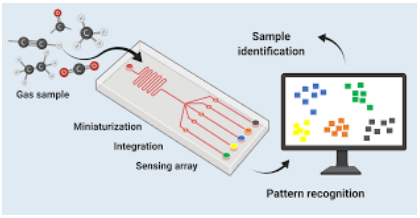
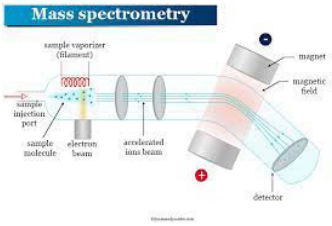
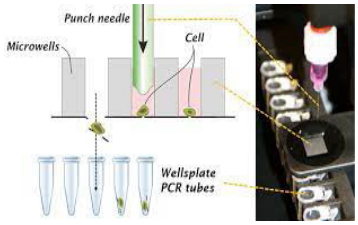
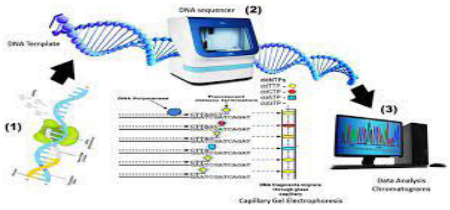
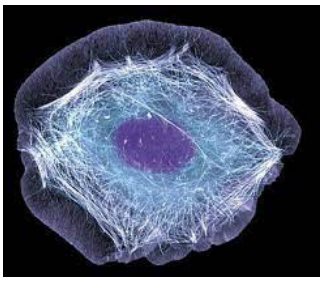
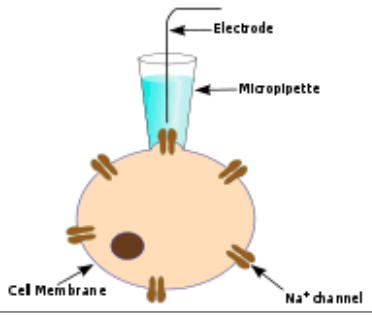
Single-Cell Genomics

The realm of single-cell genomics has prospered into a dynamic field of research, fundamentally devoted to the meticulous scrutiny of individual cell genomes. Pioneering this stride in genomics, researchers have been afforded the unprecedented opportunity to methodically unravel the genetic constituents of singular cells, thereby unraveling the intricate tapestry of genetic variation as expressed at the individual cell level. This transformative approach is uniquely poised to dissect various facets of cellular biology, including but not confined to gene expression dynamics, chromatin architecture, and epigenetic modulations, all while discerning nuances within cell populations. Notably, the ramifications of single-cell genomics resonate emphatically in deciphering the enigmatic underpinnings of intricate maladies and forging innovative therapeutic avenues.

Recent advancements in the landscape of experimental biology have unveiled the profound potential of interrogating the genetic reservoir of individual cells. A groundbreaking discipline, aptly termed single-cell genomics, now enables researchers to meticulously probe the DNA and RNA content of individual cells within the human body, as underscored by the seminal work of [57].

A cornerstone of the single-cell genome sequencing technique lies in its efficacy in unraveling the intricacies of chromosomal

Table 2: Various Techniques for Single Cell Analysis

Technique	Description	Image	Ref
Flow Cytometry	A powerful tool for analyzing the physical and chemical characteristics of single cells.		(50)
Microfluidics	A technology that uses tiny channels and pumps to manipulate and analyze single cells.		(51)
Mass Spectrometry	A technique that can be used to measure the mass of single cells and molecules		(52)
Single-Cell PCR	A technique that can be used to amplify and sequence the genetic material of single cells		(53)
High-Throughput Sequencing	A technology that can be used to sequence the genomes of single cells.		(54)
Microscopy	A technique that can be used to observe the structure and behavior of single cells.		(55)
Single-Cell Electrophysiology	A technique that can be used to measure the electrical activity of single cells		(56)

dynamics, DNA copy number variations, and single-nucleotide polymorphism alterations. Crucially, this method transcends the limitations imposed by a mere pair of gene copies within normal cells, necessitating the replication of cellular DNA. This imperative, referred to as whole-genome amplification (WGA), encompasses three distinct strategies, notably Adapter-Linker PCR [58], multiple annealing and looping-based amplification cycles (MALBAC), and Multiple Displacement Amplification (MDA) [59].

In retrospective synthesis, the nascent techniques of Adapter-Linker PCR engendered the amplification of complete human genomes from solitary cells. Emanating from the integration of PCR amplification and sequences ubiquitously dispersed across the genome, these strategies incorporated sequences ligated to fragmented genomes, or alternatively embraced degenerate or random oligonucleotide priming [60-62]. Nonetheless, these approaches confronted signal attrition across the genome bulk due to disparities in common sequence density and fluctuating PCR efficiency among loci. This predicament exacerbated while commencing amplification with a solitary genome duplicate. Augmenting genome recovery to nearly 10%, Degenerate Oligonucleotide Primed PCR (DOP-PCR), which selectively augmented genome copies and isolated tetraploid nuclei, exhibited promise, albeit accompanied by potential biases linked to the selection for rapidly dividing cells [61].

Significantly, an evolutionary leap in technology has fostered the extension of these methodologies to diploid cells. Distinctive features of these techniques lie in their reliance on thermostable polymerases, inherently prone to higher error rates compared to their thermolabile counterparts, thereby potentiating mutational influx during the amplification process.

Concurrently, a distinct category of WGA techniques embodies isothermal methodologies. Preeminently, Multiple Displacement Amplification (MDA) reigns as the paradigmatic approach, characterized by isothermal random priming and extension orchestrated by φ29 polymerase, distinguished by its robust catalytic activity, minimal error propensity, and strand displacement competence [60,63]. Owing to φ29 polymerase's elevated fidelity, these techniques manifest augmented genome coverage vis-à-vis initial PCR-based methods, accompanied by mitigated error rates. However, the exponential nature of amplification precipitates disproportionate representation of initially amplified loci, an effect exacerbated by elevated fold amplification [64]. The origin of overrepresented loci, whether rooted in stochastic or systematic biases, remains an unresolved enigma. Further exacerbating this, lackluster polymerase activity yields chimeric sequence byproducts, susceptible to amelioration through endonuclease intervention, thus affording debranching of the amplification process to physically segregate amplicons [65,66].

Two hybrid techniques, PicoPLEX and MALBAC, address limitations of PCR and isothermal methods. They combine isothermal amplification with subsequent PCR, using degenerate primers (PicoPLEX) or common sequences and thermal cycling (MALBAC) for efficient and specific DNA amplification. This concerted effort curtails further isothermal amplicon amplification before initiating PCR, culminating in a more even-handed amplification trajectory [58].

In summation, the traverse through the intricate realm of single-cell genomics has unfurled a tapestry of transformative insights. From unraveling the arcane intricacies of cellular genomes to deciphering the enigmatic dance of genomic alterations, this domain not only expands our biological understanding but also heralds the promise of innovative therapeutic frontiers.

Single-Cell Transcriptomics

The realm of single-cell transcript sequencing has emerged as a potent instrument for elucidating the differential gene expression and RNA patterns during primary growth, differentiation, and fetal reprogramming [67]. This technique possesses the capacity to discern myriad transcripts within diverse tissue and cell types, thereby enabling comprehensive transcriptomic profiling [68]. A noteworthy application of this approach lies in the quantitative evaluation of molecular dynamics underlying phenotypic diversity and cellular heterogeneity within a tumor context [69]. The continuous evolution of next-generation sequencing technologies, particularly RNA sequencing (RNA-seq), has facilitated the high-throughput, genome-wide quantification of gene expression levels across various experimental scenarios. Such insights have propelled significant strides in our comprehension of the phenotypic consequences of genetic aberrations in the context of cancer [70]. A repertoire of high-throughput methodologies, platforms, and technologies geared towards single-cell RNA sequencing (scRNA-seq) has been devised and evaluated to enable transcriptome profiling at a single-cell resolution [15,71-76]. It is worth noting that each scRNA-seq approach entails distinct read processing, quality control, and normalization strategies in computational analysis [67].

Single-Cell Proteomics

Proteins, pivotal agents in diverse biological processes, are often generated or secreted in transient bursts and limited quantities (102–104 copies per cell). Given the absence of amplification techniques akin to genomic PCR, the demand for sensitive, multiplexed profiling methodologies in single-cell proteomic analysis has surged. This analytical avenue holds promise in addressing a spectrum of pivotal biological and medical inquiries, spanning from unraveling immune intricacies to deciphering intratumor heterogeneity and intercellular communication [77]. Notably, qualitative insights into protein expression can be gleaned from single-cell DNA and RNA analyses [78].

Conventional protein analysis methodologies, including gel electrophoresis, immunoassays, chromatography, and mass spectrometry, entail substantial cell quantities for scrutiny.

Consequently, the primary obstacles in single-cell protein analysis encompass the scarcity of protein copies and the lack of amplification strategies. Nonetheless, recent strides in flow cytometry, microfluidics, mass spectrometry, mass flow cytometry, and allied techniques have ushered in a new era of single-cell protein investigations, characterized by enhanced sensitivity and specificity [79].

SINGLE-CELL ANALYSIS OF PANCREATIC CANCER HETEROGENEITY

The pancreatic cancer tumor microenvironment is a complex network where cancer cells interact with nonmalignant cells, including fibroblasts, immune cells, endothelial cells, and stem cells, as well as various molecular agents. These interactions support the growth, invasion, and metastasis of pancreatic cancer cells and affect how cancer cells respond to treatments. Stromal cells produce growth factors promoting cancer cell growth, while immune cells can impact the effectiveness of immunotherapies. Hence, acquiring a profound comprehension of the tumor microenvironment in pancreatic cancer stands as an imperative undertaking, crucially informing the formulation of efficacious therapeutic strategies [80].

The tumor microenvironment in pancreatic ductal adenocarcinoma (PDAC) consists of immune cells, fibroblasts, extracellular matrix, and signaling molecules. PDAC exhibits a highly inflammatory and desmoplastic microenvironment, characterized by reactive and dense stroma. This stroma significantly contributes to malignancy and drug resistance in PDAC [81].

Various type of cell in pancreatic cancer and their single-cell analysis

Recent inquiries have probed the impact of therapeutic agents on factors governing stromal regeneration within PDAC. These agents target non-cellular entities, including extracellular proteins and recombinant hyaluronidase, which degrades hyaluronan. Furthermore, their influence extends to the behavior of certain stromal cells, namely cancer-associated fibroblasts (CAFs) and immune cells [82,83]. Accurately quantifying these variables and their continual dynamics remains pivotal in evaluating the progress of interventions and optimizing therapeutic strategies.

Single-cell RNA sequencing (scRNA-seq) reveals profound heterogeneity in cell populations within pancreatic ductal adenocarcinoma (PDAC) tumors, encompassing tumor cells, endothelial cells, cancer-associated fibroblasts (CAFs), and immune cells. Specific markers in these cells correlate significantly with patient survival rates [84,85]. In a 2020 study, researchers identified unique genes like Foxq1 and Onecut2 in pancreatic metaplastic cells, originating from the acinar lineage in genetically modified mouse models. These cells exhibited diverse traits similar to gastric, neuronal, and endocrine cells. Conclusions include the transition of subgroups into ductal structures and PanINs, the presence of multiple metaplastic cell types in premalignant lesions, and communication among metaplastic cells and tissue-resident cells. This knowledge could reveal insights into PDAC progression, malignant cell formation, and new therapeutic targets via mutated Kras comparative analysis in different tissue contexts [86,87].

Cancer-associated fibroblasts (CAFs) and the extracellular matrix (ECM) enhance cancer growth, aggressiveness, and metastasis, while immune suppressor cells (Treg, MDSC, TAM, CD8+ T-cells) counteract tumor progression. PDAC tumors exhibit significant cellular heterogeneity within primary and metastatic sites, influencing tumor categorization and patient prognoses. These findings provide crucial insights into the PDAC microenvironment, offering potential for improved patient management strategies [80].

A: Pancreatic Stellate Cells

Pancreatic stellate cells (PSCs) are crucial fibroblastic constituents within the pancreas, activated by growth factors like TGFβ-1, PDGF, and FGF, leading to collagen and extracellular matrix synthesis. PSCs play a significant role in fibrogenesis in chronic pancreatitis and pancreatic cancer, influencing tumor progression through proteins like Cox-2, PDGF receptors, VEGF, SDF, chemokines, integrins, and SPARC. HPSCs enhance pancreatic cancer cell proliferation, inhibit apoptosis, and promote angiogenesis, making them valuable models for understanding pancreatic tumors and developing innovative strategies against pancreatitis and adenocarcinoma. PSCs exhibit similarities with islet stellate cells (ISCs), and the COL11A gene might regulate their divergence, offering insights into type-II diabetes. Stellate cells are pivotal in metastasis, angiogenesis, and drug resistance, making them potential candidates for early diagnosis and therapeutic interventions in pancreatic cancer [88-92].

B: Cancer-Associated Fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs) are crucial within the tumor microenvironment, influencing cancer cell proliferation and invasion [93,94]. Their presence in the tumor stroma is linked with processes such as angiogenesis, tumor initiation, expansion, metastasis, and drug resistance [95,96]. Unlike normal fibroblasts involved in extracellular matrix (ECM) synthesis, CAFs have unique features including the production of immunomodulatory factors leading to immune suppression [97-99]. They also support tumor progression by providing non-essential amino acids. Single-cell RNA sequencing (sc-RNAseq) on pancreatic ductal adenocarcinoma (PDAC) tissues revealed heterogeneity in cancer cells across patients and associations of specific markers with patient survival rates [84]. The emerging understanding of CAF heterogeneity has implications for therapeutic strategies against PDAC, highlighting the importance of addressing distinct CAF subgroups [47]. CAFs' role in promoting cellular proliferation and metastasis is well-established [100,101]. A murine model of PDAC revealed unique myofibroblastic CAF subpopulations and highlighted diverse roles of CAF subtypes influenced by pathways such as IL1/JAK/STAT3 and TGFβ/SMAD3 [56,102].

Elyada et al. (2019), identified new CAF subtypes that can activate CD4+ T cells, indicating their role as immune modulators [103]. Furthermore, Mao et al. (2021), conducted a transcriptomic analysis on PDAC patients, revealing CAF subtypes' clinical significance and association with varying patient responses and outcomes [104].

C: Circulating Tumor Cells (CTCs)

Metastasis is a primary factor in cancer mortality, necessitating urgent interventions [103,105]. This complex process involves the spread of tumor cells to distant organs [98], with epithelial cancers, making up 80% of global malignancies, accounting for about 90% of cancer deaths due to metastatic progression [97,106].

The detection of metastasis remains challenging. Circulating Tumor Cells (CTCs) migrating into the bloodstream enable cancer spread to other body parts [107]. These cells, though rare among billions of hematopoietic cells, mirror primary tumors in molecular markers and heterogeneity [108,109]. Magnetic-Activated Cell Sorting (MACS) first isolated CTCs in 1998, while the current preferred method is the CellSearch system, approved by the US FDA [110-112]. Improved isolation protocols have expanded CTC research, with studies highlighting their role in disease progression, survival [108,113], monitoring tumor genomes [109,110], and influencing treatment decisions. For instance, the presence of CTCs was noted in colorectal cancer patients as early as 2001, and a high frequency of CTCs is linked with poor prognosis [114,115].

By 2020, CTCs from patient blood became promising biomarkers for pancreatic cancer, aiding in monitoring treatment responses and understanding the disease, especially when showing KRAS mutations [116]. In hepatocellular carcinoma, the relationship between CTCs, Epithelial-Mesenchymal Transition (EMT), and prognosis has been explored [117,118]. Interestingly, only a fraction of CTCs, potentially stemming from Cancer Stem Cells (CSCs), display aggressive metastatic tendencies [119].

Single-cell sequencing has advanced diagnostic capabilities, especially for "fluidic biopsy" of CTCs and understanding molecular heterogeneity in pancreatic ductal adenocarcinoma (PDAC) [116]. Using microfluidics, Yu et al. (2012), identified significant Wnt2 gene expression in murine CTCs, and in vivo studies further revealed its role in promoting metastatic tendencies in pancreatic cancer cells [120]. Moreover, scRNA-seq analysis of murine CTCs and PDAC cell lines showed the importance of specific molecules like SPARC in extracellular matrix (ECM) development. The PDAC CTC mRNA expression profile indicated similarities with CSCs, particularly with markers like CD24, CD44, and ALDH1A1, while SPARC was key in EMT regulation [121].

D: Cancer Stem Cells (CSCs)

Cancer Stem Cells (CSCs) constitute a distinct subpopulation of neoplastic cells characterized by their clonogenicity and self-renewal attributes. Initially recognized in leukemia and subsequently detected across a spectrum of malignancies, such as hematopoietic malignancies [122], breast carcinoma [123], glioblastoma [124], colorectal carcinoma [125], and lung carcinoma [126], these cells are now acknowledged as pivotal entities governing cancer initiation, progression, therapeutic resistance, relapse, and metastasis. Concomitantly, they exhibit the ability for self-renewal, perpetuating their tumorigenic potential [127]. Notably, pancreatic cancer has emerged as a paradigm wherein CSCs orchestrate drug resistance and metastasis [128].

Infrequent in occurrence, CSCs are instrumental in shaping intratumoral heterogeneity [129,130]. Robust evidence underscores the heightened chemoradioresistance of CSC-bearing tumors, attributed to their upregulated anti-apoptotic moieties [129]. Niched within hypoxic/necrotic microenvironments alongside fibroblasts, immune cells, endothelial cells, and extracellular matrix constituents, CSCs find their abode [131]. The saga of pancreatic CSCs commenced in 2007[132], comprising a minute fraction of less than 1% within the cancer cell milieu [133]. In concurrence with prior assertions, their enigmatic origin remains an ongoing inquiry, oscillating between embryonic stem cells, bone marrow, or somatic cells undergoing genetic transformation [134].

The ensemble of PCSC markers encompasses CD133, CD24, CD44, ESA/EpCAM, c-Met, ALDH1, DclK1, CXCR4, and Lgr5 [134], recently implicated in mediating parenchymal interactions pertinent to pancreatic ductal adenocarcinoma (PDAC) infiltration and primary metastasis [82]. Accordingly, an exhaustive molecular portrait of PCSCs assumes paramount importance in the therapeutic landscape of pancreatic cancer.

CONCLUSION AND PROSPECTS

The distressing reality persists that over 90% of patients afflicted by pancreatic ductal adenocarcinoma (PDAC) succumb within a mere five years, despite marked therapeutic strides. The primary drivers of this dire outcome encompass relapse, metastasis, and the vexing specter of drug resistance. In light of these challenges, the imperative of precision medicine becomes manifest, seeking to usher in improved patient management by untangling the intricate interplay of genetic and environmental factors underlying pancreatic disorders. Typically, the identification of pancreatic cancer entails resorting to abdominal imaging modalities such as computed tomography (CT), magnetic resonance imaging (MRI), or a specialized form of ultrasound accessed directly through the oral and esophageal routes, encompassing the gastric and small intestinal domains. A consensus across multiple investigations underscores that the diagnostic precision pivots on the judicious amalgamation of screening modalities, with adjunctive recourse to invasive fine needle biopsy procedures [135,136].

Non-invasive screening methods for pancreatic ductal adenocarcinoma (PDAC) are not yet standardized due to its low prevalence. Genetic analysis-based screening panels show

promise for individuals with familial predispositions and cancer syndromes, enabling personalized precision strategies. However, the impact of early-stage PDAC detection on lethality remains unclear. Investment in non-invasive screening methods may facilitate timely interventions. Genomic models and single-cell RNA sequencing (scRNA-seq) provide insights into cellular heterogeneity and functional roles, aiding the identification of therapeutic targets. Understanding intercellular relationships enhances the search for novel biological markers for therapeutic interventions.

Single-cell RNA sequencing (scRNA-seq) offers a fundamental approach to unravel cellular kinetics, microcosmic subpopulations, and spatial arrangements within PDAC tumors. This technique provides insights into personalized compositions, facilitating the delineation of functional roles for distinct cell states and subpopulations. By employing scRNA-seq and spatial analytics, an intricate molecular atlas of the tumor microenvironment, including cancer stem cells, circulating tumor cells, and stellate cells, is constructed. This diverse cellular landscape holds significant implications for disease relapse and drug resistance, hampering immune surveillance. Single-cell analyses enable the exploration of gene expression signatures in varied cellular contingents, aiding in understanding neoplasm ontogeny and aggression. The ultimate goal is to create biomarker panels tailored for individual cellular units, potentially revolutionizing diagnostic and therapeutic innovations in PDAC research.

ACKNOWLEDGMENTS

We would like to thank the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

REFERENCES

1. Cai J, Chen H, Lu M, Zhang Y, Lu B, You L, et al. Advances in the epidemiology of pancreatic cancer: Trends, risk factors, screening, and prognosis. Cancer Lett. 2021; 520: 1-11.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021; 71: 209-249.
3. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 2014; 74: 2913-2921.
4. Park W, Chawla A, O'Reilly EM. Pancreatic Cancer: A Review. Jama. 2021; 326: 851-862.
5. Sawaya GF, Smith-McCune K, Kuppermann M. Cervical cancer screening: more choices in 2019. Jama. 2019; 321: 2018-2019.
6. Rawla P, Sunkara T, Gaduputi VJWjoo. Epidemiology of pancreatic cancer: global trends, etiology and risk factors. World J Oncol. 2019; 10: 10-27.
7. Mizrahi JD, Surana R, Valle JW, Shroff RT. Pancreatic cancer. The Lancet. 2020; 395: 2008-2020.
8. Sántha P, Lenggenhager D, Finstadsveen A, Dorg L, Tøndel K, Amrutkar M, et al. Morphological Heterogeneity in Pancreatic Cancer Reflects Structural and Functional Divergence. Cancers. 2021; 13: 895.
9. Haeberle L, Steiger K, Schlitter AM, Safi SA, Knoefel WT, Erkan M, Esposito I. Stromal heterogeneity in pancreatic cancer and chronic pancreatitis. Pancreatology. 2018; 18: 536-549.
10. Hu P, Zhang W, Xin H, Deng G. Single cell isolation and analysis. Front Cell Dev Biol. 2016; 4: 116.
11. Zhang Y, Wang D, Peng M, Tang L, Ouyang J, Xiong F, et al. Single-cell RNA sequencing in cancer research. J Exp Clin Cancer Res. 2021; 40: 81.
12. Tirosh I, Izar B, Prakadan SM, Wadsworth MH, Treacy D, Trombetta JJ, et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science. 2016; 352: 189-196.
13. Venteicher AS, Tirosh I, Hebert C, Yizhak K, Neftel C, Filbin MG, et al. Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq. Science. 2017; 355.
14. Linnarsson S, Teichmann SA. Single-cell genomics: coming of age. Springer; 2016.
15. Tanay A, Regev A. Scaling single-cell genomics from phenomenology to mechanism. Nature. 2017; 541: 331-338.
16. Valihrach L, Androvic P, Kubista M. Platforms for single-cell collection and analysis. Int J Mol Sci. 2018; 19: 807.
17. Gross A, Schoendube J, Zimmermann S, Steeb M, Zengerle R, Koltay P. Technologies for single-cell isolation. Int J Mol Sci. 2015; 16: 16897-16919.
18. Xu C, Wang K, Huang P, Liu D, Guan Y. Single-Cell Isolation Microfluidic Chip Based on Thermal Bubble Micropump Technology. Sensors. 2023; 23: 3623.
19. Cherniack AD, Shen H, Walter V, Stewart C, Murray BA, Bowlby R, et al. Integrated molecular characterization of uterine carcinosarcoma. Cancer cell. 2017; 31: 411-423.
20. Kolodziejczyk AA, Kim JK, Svensson V, Marioni JC, Teichmann SA. The technology and biology of single-cell RNA sequencing. Mol cell. 2015; 58: 610-620.
21. Zhang J, Terrones M, Park CR, Mukherjee R, Monthioux M, Koratkar N, et al. Carbon science in 2016: Status, challenges and perspectives. Carbon. 2016; 98: 708-732.
22. Radpour R, Forouharkhou FJ. Single-cell analysis of tumors: Creating new value for molecular biomarker discovery of cancer stem cells and tumor-infiltrating immune cells. World J Stem Cells. 2018; 10: 160.
23. Strimbu K, Tavel JA. AIDS. What are biomarkers? Curr Opin HIV AIDS. 2010; 5: 463.
24. Wang Q, Šabanović B, Awada A, Reina C, Aicher A, Tang J, et al. Single-cell omics: a new perspective for early detection of pancreatic cancer? Eur J Cancer. 2023; 190: 112940.
25. Kashima Y, Sakamoto Y, Kaneko K, Seki M, Suzuki Y, Suzuki A. Single-cell sequencing techniques from individual to multiomics analyses. Exp Mol Med. 2020; 52: 1419-1427.
26. Zeb Q, Wang C, Shafiq S, Liu L. Chapter 6 - An Overview of Single-Cell Isolation Techniques. In: Barh D, Azevedo V, editors. Single-Cell Omics: Academic Press; 2019; 101-135.
27. Xu Y, Zhao W, Wu D, Xu J, Lin S, Tang K, et al. Isolation of myeloid-derived suppressor cells subsets from spleens of orthotopic liver cancer-bearing mice by fluorescent-activated and magnetic-activated cell sorting: similarities and differences. Int J Clin Exp Pathol. 2014; 7: 7545.

28. Tripathi H, Peng H, Donahue R, Chelvarajan L, Gottipati A, Levitan B, et al. Isolation methods for human CD34 subsets using fluorescent and magnetic activated cell sorting: an in vivo comparative study. Stem Cell Rev Rep. 2020; 16: 413-423.

29. Aguilar-Bravo B, Sancho-Bru P. Laser capture microdissection: Techniques and applications in liver diseases. Hepatol Int. 2019; 13: 138-147.

30. Bhamidipati T, Sinha M, Sen CK, Singh KJOM, Longevity C. Laser capture microdissection in the spatial analysis of epigenetic modifications in skin: a comprehensive review. Oxid Med Cell Longev. 2022; 2022.

31. Eells JB, Varela-Stokes A, Guo-Ross SX, Kummari E, Smith HM, Cox E, et al. Chronic Toxoplasma gondii in Nurr1-null heterozygous mice exacerbates elevated open field activity. PLoS One. 2015; 10: e0119280.

32. De Marchi T, Braakman RB, Stingl C, van Duijn MM, Smid M, Foekens JA, et al. The advantage of laser-capture microdissection over whole tissue analysis in proteomic profiling studies. Proteomics. 2016; 16: 1474-1485.

33. Vandewoestyne M, Goossens K, Burvenich C, Van Soom A, Peelman L, Deforce D. Laser capture microdissection: should an ultraviolet or infrared laser be used? Anal Biochem. 2013; 439: 88-98.

34. Gallagher RI, Blakely SR, Liotta LA, Espina V. Laser Capture Microdissection: Arcturus XT Infrared Capture and UV Cutting Methods. Methods Mol Biol. 2012: 157-178.

35. Kummari E, Guo-Ross SX, Eells JB. Laser capture microdissection-a demonstration of the isolation of individual dopamine neurons and the entire ventral tegmental area. J Vis Exp. 2015.

36. Hussain SK, Sundquist J, Hemminki K. Incidence trends of squamous cell and rare skin cancers in the Swedish national cancer registry point to calendar year and age-dependent increases. J Invest Dermatol. 2010; 130: 1323-1328.

37. Schütze K, Pösl H, Lahr G. Laser micromanipulation systems as universal tools in cellular and molecular biology and in medicine. Cell Mol Biol (Noisy-le-grand). 1998; 44: 735-746.

38. Simone NL, Lee JY, Huckabee M, Cole KA, Chuaqui RF, Seshadri C, et al. Molecular analysis of microdissected tissue: laser capture microdissection. PCR applications: Elsevier; 1999.

39. Nguyen NT, Wereley ST, Shaegh SAM. Fundamentals and applications of microfluidics: Artech house; 2019.

40. Sackmann EK, Fulton AL, Beebe DJ. The present and future role of microfluidics in biomedical research. Nature. 2014; 507: 181-189.

41. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature. 2007; 450: 1235-1259.

42. Al-Faqheri W, Thio THG, Qasaimeh MA, Dietzel A, Madou M. Particle/cell separation on microfluidic platforms based on centrifugation effect: a review. Microfluidics Nanofluidics. 2017; 21: 1-23.

43. Lee GH, Kim SH, Ahn K, Lee SH, Park JY. Separation and sorting of cells in microsystems using physical principles. J Micromechan Microengineering. 2015; 26: 013003.

44. Zhang Y, Tong X, Yang L, Yin R, Li Y, Zeng D, et al. A herringbone mixer based microfluidic device HBEXO-chip for purifying tumor-derived exosomes and establishing miRNA signature in pancreatic cancer. 2021; 332: 129511.

45. Sancho-Albero M, Sebastián V, Sesé J, Pazo-Cid R, Mendoza G, Arruebo M, et al. Isolation of exosomes from whole blood by a new microfluidic device: proof of concept application in the diagnosis and monitoring of pancreatic cancer. J Nanobiotechnology. 2020; 18: 1-15.

46. Gómez-Sjöberg R, Leyrat AA, Pirone DM, Chen CS, Quake SR. Versatile, fully automated, microfluidic cell culture system. Anal Chem. 2007; 79: 8557-8563.

47. Schuster B, Junkin M, Kashaf SS, Romero-Calvo I, Kirby K, Matthews J, et al. Automated microfluidic platform for dynamic and combinatorial drug screening of tumor organoids. Nat Commun. 2020; 11: 5271.

48. Navin NE. The first five years of single-cell cancer genomics and beyond. Genome Res. 2015; 25: 1499-1507.

49. Hodne K, Weltzien FA. Single-cell isolation and gene analysis: pitfalls and possibilities. Int J Mol Sci. 2015; 16: 26832-26849.

50. Li M, Liu H, Zhuang S, Goda K. Droplet flow cytometry for single-cell analysis. RSC Adv. 2021; 11: 20944-20960.

51. Reece A, Xia B, Jiang Z, Noren B, McBride R, Oakey J. Microfluidic techniques for high throughput single cell analysis. Curr Opin Biotechnol. 2016; 40: 90-96.

52. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. Mass Spectrom Rev. 2007; 26: 51-78.

53. Hahn* S, Zhong X, Troeger C, Burgemeister R, Gloning K, Holzgreve W. Current applications of single-cell PCR. Cell Mol Life Sci. 2000; 57: 96-105.

54. Grün D, van Oudenaarden AJ. Design and analysis of single-cell sequencing experiments. Cell. 2015; 163: 799-810.

55. Jayan H, Pu H, Sun DW. Recent developments in Raman spectral analysis of microbial single cells: Techniques and applications. 2022; 62: 4294-4308.

56. Le Floch P, Li Q, Lin Z, Zhao S, Liu R, Tasnim K, et al. Stretchable mesh nanoelectronics for 3D single-cell chronic electrophysiology from developing brain organoids. Adv Mater. 2022; 34: 2106829.

57. Trapnell C. Defining cell types and states with single-cell genomics. Genome Res. 2015; 25: 1491-1498.

58. Zong C, Lu S, Chapman AR, Xie XS. Genome-wide detection of single-nucleotide and copy-number variations of a single human cell. Sci. 2012; 338: 1622-1626.

59. Leung ML, Wang Y, Waters J, Navin NE. SNES: single nucleus exome sequencing. Genome Biol. 2015; 16: 1-10.

60. Dean FB, Nelson JR, Giesler TL, Lasken RS. Rapid amplification of plasmid and phage DNA using phi29 DNA polymerase and multiply-primed rolling circle amplification. Genome Res. 2001; 11: 1095-1099.

61. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, et al. Tumour evolution inferred by single-cell sequencing. Nature. 2011; 472: 90-94.

62. Zhang L, Cui X, Schmitt K, Hubert R, Navidi W, Arnheim N. Whole genome amplification from a single cell: implications for genetic analysis. Proc Natl Acad Sci USA. 1992; 89: 5847-5851.

63. Zhang DY, Brandwein M, Hsuih T, Li HB. Ramification amplification: a novel isothermal DNA amplification method. Mol Diagn. 2001; 6: 141-150.

64. De Bourcy CF, De Vlaminck I, Kanbar JN, Wang J, Gawad C, Quake SR. A quantitative comparison of single-cell whole genome amplification methods. PloS one. 2014; 9: e105585.

65. Lasken RS, Stockwell TB. Mechanism of chimera formation during the Multiple Displacement Amplification reaction. BMC Biotechnol. 2007; 7: 1-11.

66. Marcy Y, Ishoey T, Lasken RS, Stockwell TB, Walenz BP, Halpern AL,

et al. Nanoliter reactors improve multiple displacement amplification of genomes from single cells. PLoS Genet. 2007; 3: e155.

67. Stegle O, Teichmann SA, Marioni JC. Computational and analytical challenges in single-cell transcriptomics. Nat Rev Genet. 2015; 16: 133-145.

68. Aldridge S, Teichmann SA. Single cell transcriptomics comes of age. Nat Commun. 2020; 11: 1-4.

69. Zhang Y, Kontos CD, Annex BH, Popel AS. Angiopoietin-Tie Signaling Pathway in Endothelial Cells: A Computational Model. iScience. 2019; 20: 497-511.

70. Cieślik M, Chinnaiyan AM. Cancer transcriptome profiling at the juncture of clinical translation. Nat Rev Genet. 2018; 19: 93-109.

71. Hashimshony T, Wagner F, Sher N, Yanai I. CEL-Seq: single-cell RNA-Seq by multiplexed linear amplification. Cell Rep. 2012; 2: 666-673.

72. Hwang B, Lee JH, Bang D. Single-cell RNA sequencing technologies and bioinformatics pipelines. Exp Mol Med. 2018; 50: 1-14.

73. Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, et al. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. Cell. 2015; 161: 1187-1201.

74. Picelli S, Faridani OR, Björklund ÅK, Winberg G, Sagasser S, Sandberg R. Full-length RNA-seq from single cells using Smart-seq2. Nat Protoc. 2014; 9: 171-181.

75. Ramsköld D, Luo S, Wang YC, Li R, Deng Q, Faridani OR, et al. Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. Nat Biotechnol. 2012; 30: 777-782.

76. Ziegenhain C, Vieth B, Parekh S, Reinius B, Guillaumet-Adkins A, Smets M, et al. Comparative analysis of single-cell RNA sequencing methods. Mol cell. 2017; 65: 631-43. e4.

77. Li L, Yan S, Lin B, Shi Q, Lu Y. Single-cell proteomics for cancer immunotherapy. Adv Cancer Res. 2018; 139: 185-207.

78. Perez OD, Nolan GP. Simultaneous measurement of multiple active kinase states using polychromatic flow cytometry. Nat Biotechnol. 2002; 20: 155-162.

79. Lindström S, Andersson-Svahn H. Overview of single-cell analyses: microdevices and applications. Lab on a Chip. 2010; 10: 3363-3372.

80. Murakami T, Hiroshima Y, Matsuyama R, Homma Y, Hoffman RM, Endo IJ. Role of the tumor microenvironment in pancreatic cancer. Ann Gastroenterol Surg. 2019; 3: 130-137.

81. Pattabiraman DR, Weinberg RA. Tackling the cancer stem cells—what challenges do they pose? Nat Rev Drug Discov. 2014; 13: 497-512.

82. Doherty GJ, Tempero M, Corrie PG. HALO-109–301: a Phase III trial of PEGPH20 (with gemcitabine and nab-paclitaxel) in hyaluronic acid-high stage IV pancreatic cancer. Future Oncol. 2018; 14: 13-22.

83. Hingorani SR, Zheng L, Bullock AJ, Seery TE, Harris WP, Sigal DS, et al. HALO 202: randomized phase II study of PEGPH20 plus nab-paclitaxel/gemcitabine versus nab-paclitaxel/gemcitabine in patients with untreated, metastatic pancreatic ductal adenocarcinoma. J Clin Oncol. 2018; 36: 359-366.

84. Lin W, Noel P, Borazanci EH, Lee J, Amini A, Han IW, et al. Single-cell transcriptome analysis of tumor and stromal compartments of pancreatic ductal adenocarcinoma primary tumors and metastatic lesions. Genome Med. 2020; 12: 1-14.

85. Srinivasan S, Kryza T, Batra J, Clements J. Remodelling of the tumour microenvironment by the kallikrein-related peptidases. Nat Rev Cancer. 2022; 22: 223-238.

86. Parte S, Nimmakayala RK, Batra SK, Ponnusamy MP. Acinar to ductal cell trans-differentiation: A prelude to dysplasia and pancreatic ductal adenocarcinoma. Biochim Biophys Acta Rev Cancer. 2022; 1877: 188669.

87. Schlesinger Y, Yosefov-Levi O, Kolodkin-Gal D, Granit RZ, Peters L, Kalifa R, et al. Single-cell transcriptomes of pancreatic preinvasive lesions and cancer reveal acinar metaplastic cells' heterogeneity. Nature communications. 2020; 11: 1-18.

88. Omary MB, Lugea A, Lowe AW, Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. J Clin Invest. 2007; 117: 50-59.

89. Schnittert J, Bansal R, Prakash J. Targeting pancreatic stellate cells in cancer. Trends Cancer. 2019; 5: 128-142.

90. Ehrlich A, Duche D, Ouedraogo G, Nahmias YJArobe. Challenges and opportunities in the design of liver-on-chip microdevices. Annu Rev Biomed Eng. 2019; 21: 219-239.

91. Amrutkar M, Berg K, Balto A, Skilbrei MG, Finstadsveen AV, Aasrum M, et al. Pancreatic stellate cell-induced gemcitabine resistance in pancreatic cancer is associated with LDHA- and MCT4-mediated enhanced glycolysis. Cancer Cell Int. 2023; 23: 9.

92. Wang X, Li W, Chen J, Zhao S, Qiu S, Yin H, et al. A transcriptional sequencing analysis of islet stellate cell and pancreatic stellate cell. J Diabetes Res. 2018; 2018.

93. Ansems M, Span PN. The tumor microenvironment and radiotherapy response; a central role for cancer-associated fibroblasts. Clin Transl Radiat Oncol. 2020; 22: 90-97.

94. Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H. Cancer-associated fibroblasts: their characteristics and their roles in tumor growth. Cancers. 2015; 7: 2443-2458.

95. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020; 20: 174-186.

96. Dong Y, Skelley AM, Merdek KD, Sprott KM, Jiang C, Pierceall WE, et al. Microfluidics and circulating tumor cells. J Mol Diagn. 2013; 15: 149-157.

97. Ma J, Jemal A, Fedewa SA, Islami F, Lichtenfeld JL, Wender RC, et al. The American Cancer Society 2035 challenge goal on cancer mortality reduction. CA Cancer J Clin. 2019; 69: 351-362.

98. Madar S, Goldstein I, Rotter VJ. 'Cancer associated fibroblasts'–more than meets the eye. Trends Mol Med. 2013; 19: 447-453.

99. Norton J, Foster D, Chinta M, Titan A, Longaker M. Pancreatic cancer associated fibroblasts (CAF): under-explored target for pancreatic cancer treatment. Cancers. 2020; 12: 1347.

100. Öhlund D, Elyada E, Tuveson DJ. Fibroblast heterogeneity in the cancer wound. J Exp Med. 2014; 211: 1503-1523.

101. Zhang J, Chen L, Liu X, Kammertoens T, Blankenstein T, Qin Z. Fibroblast-specific protein 1/S100A4–positive cells prevent carcinoma through collagen production and encapsulation of carcinogens. Cancer Res. 2013; 73: 2770-2781.

102. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, et al. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. Cancer Discov. 2019; 9: 1102-1123.

103. Mao X, Xu J, Wang W, Liang C, Hua J, Liu J, et al. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. Mol Cancer. 2021; 20: 1-30.

104. Plaks V, Koopman CD, Werb Z. Circulating tumor cells. Science. 2013; 341: 1186-1188.

105. Coumans FA, Siesling S, Terstappen LW. Detection of cancer before distant metastasis. BMC cancer. 2013; 13: 1-12.

106. Aier I, Semwal R, Sharma A, Varadwaj PK. A systematic assessment of statistics, risk factors, and underlying features involved in pancreatic cancer. Cancer Epidemiol. 2019; 58: 104-110.

107. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science. 2011; 331: 1559-1564.

108. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004; 351: 781-791.

109. Heitzer E, Auer M, Gasch C, Pichler M, Ulz P, Hoffmann EM, et al. Complex tumor genomes inferred from single circulating tumor cells by array-CGH and next-generation sequencing. Cancer Res. 2013; 73: 2965-2975.

110. Lohr JG, Stojanov P, Carter SL, Cruz-Gordillo P, Lawrence MS, Auclair D, et al. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. Cancer Cell. 2014; 25: 91-101.

111. Lianidou ES, Strati A, Markou AJ. Circulating tumor cells as promising novel biomarkers in solid cancers. Crit Rev Clin Lab Sci. 2014; 51: 160-171.

112. Wang L, Balasubramanian P, Chen AP, Kummar S, Evrard YA, Kinders RJ. Promise and limits of the Cell Search platform for evaluating pharmacodynamics in circulating tumor cells. Semin Oncol. 2016; 43: 464-475.

113. Alix-Panabières C, Pantel K. Circulating tumor cells: liquid biopsy of cancer. Clin Chem. 2013; 59: 110-118.

114. Molnar BE, Buka SL, Kessler RC. Child sexual abuse and subsequent psychopathology: results from the National Comorbidity Survey. Am J Public Health. 2001; 91: 753.

115. Oudin MJ, Jonas O, Kosciuk T, Broye LC, Guido BC, Wyckoff J, et al. Tumor cell–driven extracellular matrix remodeling drives haptotaxis during metastatic progression. Cancer Discov. 2016; 6: 516-531.

116. Chen Y, Li S, Li W, Yang R, Zhang X, Ye Y, et al. Circulating tumor cells undergoing EMT are poorly correlated with clinical stages or predictive of recurrence in hepatocellular carcinoma. Scientific Reports. 2019; 9: 1-9.

117. Rivera-Báez L, Lohse I, Lin E, Raghavan S, Owen S, Harouaka R, et al. Expansion of circulating tumor cells from patients with locally advanced pancreatic cancer enable patient derived xenografts and functional studies for personalized medicine. Cancers. 2020; 12: 1011.

118. Agnoletto C, Corrà F, Minotti L, Baldassari F, Crudele F, Cook WJJ, et al. Heterogeneity in circulating tumor cells: the relevance of the stem-cell subset. Cancers. 2019; 11: 483.

119. Yu M, Ting DT, Stott SL, Wittner BS, Ozsolak F, Paul S, et al. RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis. Nature. 2012; 487: 510-513.

120. Ting DT, Wittner BS, Ligorio M, Jordan NV, Shah AM, Miyamoto DT, et al. Single-cell RNA sequencing identifies extracellular matrix gene expression by pancreatic circulating tumor cells. Cell Rep. 2014; 8: 1905-1918.

121. Trumpp A, Haas SJC. Cancer stem cells: the adventurous journey from hematopoietic to leukemic stem cells. Cell. 2022; 185: 1266-1270.

122. Nguyen M, Osipo C. Targeting breast cancer stem cells using naturally occurring phytoestrogens. Int J Mol Sci. 2022; 23: 6813.

123. Gimple RC, Yang K, Halbert ME, Agnihotri S, Rich JN. Brain cancer stem cells: resilience through adaptive plasticity and hierarchical heterogeneity. Nat Rev Cancer. 2022; 22: 497-514.

124. Ebrahimi N, Afshinpour M, Fakhr SS, Kalkhoran PG, Manesh VS, Adelian S, et al. Cancer stem cells in colorectal cancer: signaling pathways involved in stemness and therapy resistance. Crit Rev Oncol Hematol. 2023; 182: 103920.

125. Lapin M, Tjensvoll K, Oltedal S, Javle M, Smaaland R, Gilje B, Nordgård O. Single-cell mRNA profiling reveals transcriptional heterogeneity among pancreatic circulating tumour cells. BMC Cancer. 2017; 17: 1-10.

126. Lathia J, Liu H, Matei D. The clinical impact of cancer stem cells. The oncologist. 2020; 25: 123.

127. Gzil A, Zarębska I, Bursiewicz W, Antosik P, Grzanka D, Szylberg Ł. Markers of pancreatic cancer stem cells and their clinical and therapeutic implications. Mol Biol Rep. 2019; 46: 6629-6645.

128. Santamaria S, Delgado M, Kremer L, Garcia-Sanz JA. Will a mAb-Based immunotherapy directed against cancer stem cells be feasible? Front Immunol. 2017; 8: 1509.

129. Valle S, Martin-Hijano L, Alcalá S, Alonso-Nocelo M, Sainz Jr B. The ever-evolving concept of the cancer stem cell in pancreatic cancer. Cancers. 2018; 10: 33.

130. Arranz L, Sánchez-Aguilera A, Martín-Pérez D, Isern J, Langa X, Tzankov A, et al. Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms. Nature. 2014; 512: 78-81.

131. Zhou P, Li B, Liu F, Zhang M, Wang Q, Liu Y, et al. The epithelial to mesenchymal transition (EMT) and cancer stem cells: implication for treatment resistance in pancreatic cancer. 2017; 16: 1-11.

132. Dubarry J, Quinton A, Bancons J. Use of colopten in intestinal pathology. Bord Med. 1971; 4: 561-564 passim.

133. Dalla Pozza E, Dando I, Biondani G, Brandi J, Costanzo C, Zoratti E, et al. Pancreatic ductal adenocarcinoma cell lines display a plastic ability to bi-directionally convert into cancer stem cells. Int J Oncol. 2015; 46: 1099-1108.

134. Al-Sukhni W, Borgida A, Rothenmund H, Holter S, Semotiuk K, Grant R, et al. Screening for pancreatic cancer in a high-risk cohort: an eight-year experience. J Gastrointest Surg. 2012; 16: 771-783.

135. Barnes CA, Krzywda E, Lahiff S, McDowell D, Christians KK, Knechtges P, et al. Development of a high risk pancreatic screening clinic using 3.0 T MRI. Fam Cancer. 2018; 17: 101-111.

136. Bartsch D, Slater E, Carrato A, Ibrahim I, Guillen-Ponce C, Vasen H, et al. Refinement of screening for familial pancreatic cancer. Gut. 2016; 65: 1314-1321.

137. Canto MI, Goggins M, Yeo CJ, Griffin C, Axilbund JE, Brune K, et al. Screening for pancreatic neoplasia in high-risk individuals: an EUS-based approach. Clinical Gastroenterol Hepatol. 2004; 2: 606-621.

138. Canto MI, Goggins M, Hruban RH, Petersen GM, Giardiello FM, Yeo C, et al. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. Clin Gastroenterol Hepatol. 2006; 4: 766-781.

139. Canto M, Hruban R, Fishman E. Comparison of CT, MRI, and EUS for detection of prevalent pancreatic lesions (per patient analysis) frequent detection of pancreatic lesions in asymptomatic high-risk individuals screening for early pancreatic neoplasia (CAPS 3 study). Gastroenterology. 2012; 142: 796-804.

140. Canto MI, Almario JA, Schulick RD, Yeo CJ, Klein A, Blackford A, et al. Risk of neoplastic progression in individuals at high

risk for pancreatic cancer undergoing long-term surveillance. Gastroenterology. 2018; 155: 740-751. e2.

141. Gangi A, Malafa M, Klapman J. Endoscopic ultrasound–based pancreatic cancer screening of high-risk individuals: a prospective observational trial. Pancreas. 2018; 47: 586-591.

142. Harinck F, Nagtegaal T, Kluijt I, Aalfs C, Smets E, Poley JW, et al. Feasibility of a pancreatic cancer surveillance program from a psychological point of view. Genet Med. 2011; 13: 1015-1024.

143. Hart SL, Torbit LA, Crangle CJ, Esplen MJ, Holter S, Semotiuk K, et al. Moderators of cancer-related distress and worry after a pancreatic cancer genetic counseling and screening intervention. PsychoOncology. 2012; 21: 1324-1330.

144. Joergensen MT, Gerdes A-M, Sorensen J, de Muckadell OS, Mortensen MB. Is screening for pancreatic cancer in high-risk groups cost-effective?–experience from a Danish national screening program. Pancreatology. 2016; 16: 584-592.

145. Konings IC, Harinck F, Kuenen MA, Sidharta GN, Kieffer JM, Aalfs CM, et al. Factors associated with cancer worries in individuals participating in annual pancreatic cancer surveillance. Fam Cancer. 2017; 16: 143-151.

146. Langer P, Kann PH, Fendrich V, Habbe N, Schneider M, Sina M, et al. Five years of prospective screening of high-risk individuals from families with familial pancreatic cancer. Gut. 2009; 58: 1410-1418.

147. Lathan CS, Cronin A, Tucker-Seeley R, Zafar SY, Ayanian JZ, Schrag D. Association of financial strain with symptom burden and quality of life for patients with lung or colorectal cancer. J Clin Oncol. 2016; 34: 1732.

148. Ludwig E, Olson SH, Bayuga S, Simon J, Schattner MA, Gerdes H, et al. Feasibility and yield of screening in relatives from familial pancreatic cancer families. Am J Gastroenterol. 2011; 106: 946.

149. Maheu C, Vodermaier A, Rothenmund H, Gallinger S, Ardiles P, Semotiuk K, et al. Pancreatic cancer risk counselling and screening: impact on perceived risk and psychological functioning. Familial cancer. 2010; 9: 617-624.

150. Morrow M, Li Y, Alderman AK, Jagsi R, Hamilton AS, Graff JJ, et al. Access to breast reconstruction after mastectomy and patient perspectives on reconstruction decision making. JAMA surgery. 2014; 149: 1015-1021.

151. Poley J-W, Kluijt I, Gouma DJ, Harinck F, Wagner A, Aalfs C, et al. The yield of first-time endoscopic ultrasonography in screening individuals at a high risk of developing pancreatic cancer. Am J Gastroenterol. 2009; 104: 2175-2181.

152. Potjer TP, Schot I, Langer P, Heverhagen JT, Wasser MN, Slater EP, et al. Variation in precursor lesions of pancreatic cancer among high-risk groups. Clin Cancer Res. 2013; 19: 442-449.

153. Schneider R, Slater EP, Sina M, Habbe N, Fendrich V, Matthäi E, et al. German national case collection for familial pancreatic cancer (FaPaCa): ten years experience. Fam Cancer. 2011; 10: 323-330.

154. Shin EJ, Topazian M, Goggins MG, Syngal S, Saltzman JR, Lee JH, et al. Linear-array EUS improves detection of pancreatic lesions in high-risk individuals: a randomized tandem study. Gastrointest Endosc. 2015; 82: 812-818.

155. Verna EC, Hwang C, Stevens PD, Rotterdam H, Stavropoulos SN, Sy CD, et al. Pancreatic cancer screening in a prospective cohort of high-risk patients: a comprehensive strategy of imaging and genetics. Clin Cancer Res. 2010; 16: 5028-5037.