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# RNA-Sequencing insights and wide scope approaches in combating Exocrine pancreatic cancer challenges

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**Abstract:** *Transcriptome profiling approaches in RNA-Seq can serve to reveal genes whose differential expression predicts its concurrent association with various cancer pathogenesis and shorter survival rate. By analyzingtranscriptomic data from tumor samples, researchers can identify genes whose expression levels correlate with disease progression. Out of various severe cancer categories exocrine pancreatic cancer remains a devastating malignancy with a poor prognosis. Recent advances with the introduction of powerful next-generation sequencing (NGS) technology RNA sequencing, the identification of genes can lead to the development of drugs or pharmacological agents targeting the pancreatic cancer can be identified more effeciently. This knowledge base can empower the development of non-invasive screening tools for early disease detection and inform the selection of promising therapeutic targets, including the identification of molecular subtypes for personalized medicine approaches. Overall, RNA-Seq-based transcriptome profiling holds immense potential for advancing our understanding and therapeutic management of exocrine pancreatic cancer. This review highlights the current approaches of NGS based transcriptome profiling to expand our understanding of exocrine pancreatic cancer by identifying variational gene expression patterns, molecular subtypes, therapeutic targets and dysregulated pathways, which will add new perspective for future researchers to explore immense potential and understanding of therapeutic management of exocrine pancreatic cancer and drug target application research.*

***Key Words:*** **Transcriptome profiling, next generation sequencing, RNA-Seq, Pancreatic cancer**

## 1. Introduction

Pancreatic cancer is recognized as one of the deadliest illness with comparable rates of mortality and morbidity. Its occurrence is on the rise, yet the five-year relative survival rate is still the lowest among major cancers [1]. This aggressive cancer is marked by a diverse stromal microenvironment, leading to inadequate tumor vascularization and a complicated signaling network that regulates tumor onset, progression, and persistence [2, 3]. Risk factors encompass smoking, alcohol misuse, obesity or metabolic syndrome, aging, and occupational exposure [4, 5]. Pancreatic cancer is also believed to have a familial component. A retrospective analysis involving 175 families with a history of pancreatic cancer, genetic mutations were detected in 28% of family cases [6].The genes frequently linked with pathogenic germline alterations encompass BRCA1, BRCA2, ATM, PALB2, MLH1, MSH2, MSH6, PMS2, CDKN2A, and TP53 [7].

RNA sequencing has revolutionized the study of transcriptomes, offering significant advantages over previous methods such as Sanger sequencing and microarrays. RNA-Seq provides higher coverage by capturing a more comprehensive view of the transcriptome, detecting both

highly abundant and rare transcripts with greater sensitivity, and greater resolution by allowing researchers to quantify gene expression levels more precisely, which is crucial for understanding the dynamic nature of gene expression. Further, the data generated by RNA-Seq facilitates the discovery of novel transcripts, identification of alternatively spliced genes, and detection of allele-specific expressions [8].

The primary objective of transcriptomics are: to compile all types of transcripts, which includes mRNAs, non-coding RNAs and small RNAs, to ascertain the transcriptional organization of genes, including their initiation sites, 5' and 3' ends, splicing patterns, and other post-transcriptional modifications. Lastly, transcriptomics aims to measure variations in the expression levels of individual transcript throughout development and under varying conditions [9]. With the advancement in sequencing technology and "omics" analysis, several novel circulating biomarkers with considerable diagnostic potential have been identified, including metabolites, cell-free DNA (cfDNA), noncoding RNA, and exosomes[10].

Recurrent mutations in genes like KRAS, TP53, CDKN2A, and SMAD4 have been found in pancreatic cancer in previous studies. These discoveries have enhanced our knowledge of how this cancer type develops and advances [11,12]. But pancreatic cancer lacks a molecular taxonomy to guide treatments, unlike other cancers such as breast, prostate, gastric, and colorectal [13]. Tailoring treatment based on molecular targets may enhance outcomes for patients with a grim prognosis. The genetic makeup of pancreatic ductal adenocarcinoma is highly diverse, with most targetable genetic abnormalities being present in less than 10% of cases [14]. Next generation sequencing offers unparalleled possibilities by revealing genetic pathways driving cancer and is thereby advancing personalized medicine [15]. Personalized cancer therapy considers patients' molecular profiles to group them for better treatment outcomes [16, 17].

## 2. Next-generation Sequencing: RNA Seq and its applications in pancreatic cancer

The year 2005 witnessed the emergence of a new generation of sequencing technologies [18, 19].These advanced technologies are referred to as next-generation sequencing (NGS), 'high-throughput' (or even 'ultrahigh-throughput') sequencing, 'ultra-deep' sequencing, or 'massively parallel [20], which can efficiently analyze large number of shorter DNA sequences. Since the completion of the Human Genome Project, there have been significant advancements in genome sequencing technologies, ushering in a new era of "omics" science, fundamentally transforming the investigation of malignant tumors. The advent of NGS has led to enhanced efficiency and decreased expenses, significantly easing the process of cancer genomics exploration and their application in clinical settings [21]. Commercially available commercially available platforms include: 454 (Roche), Genome Analyzer (Illumina/Solexa) and ABI-SOLiD (Applied Biosystems)

A comprehensive examination of various NGS platforms utilizing distinct sequencing technologies is outside the scope of this article. Since this article revolves around transcriptome profiling it will focus on RNA-Seq which allows for a comprehensive assessment of the transcriptome, providing information on the expression levels of thousands of genes simultaneously. Next-generation technologies are increasingly utilized not just for examining stable genomes but also for exploring dynamic transcriptomes through a technique known as RNA-Seq, [22]also called short read massively parallel sequencing. The method of gene discovery has undergone a revolution with the advent of RNA-Seq, a high-throughput assay for sequencing transcribed genes. The potential of RNA-Seq lies in its capacity to provide extensive coverage of the cell's entire transcriptome using a single operation of a high throughput sequencer like the IlluminaHiseq, capable of yielding up to 200 billion bases in a single run [23].

In a standard RNA-Seq experimental workflow, the isolated RNA is converted into complementary DNA (cDNA), proceeding with the preparation of sequencing library followed by sequencing of the library on a next-generation sequencing platform Compared to microarrays, RNA-Seq offers several advantages for studying differential gene expression because it isn't restricted by hybridization issues

such as background noise and saturation, or with problems related to probe sets such as inaccurate annotation and isoform coverage. It's also better at finding genes with low or high expression levels and has a wider range of detection [24]. Advancements in next-generation genome sequencing (NGS) have revitalized the field by aiding the discovery of molecular changes that drive pancreatic cancer progression. Sequencing has shown that pancreatic cancer features highly heterogeneous tumors resistant to conventional chemotherapy and radiation therapy [25].

Most samples within current tumorbiobanks consist of surgical specimens derived from primary tumors but for understanding various aspects of tumor biology, which includes intratumoral heterogeneity, tumor and host interactions, and any changes in disease during therapy, creates the need for biospecimens to be sourced from both the primary tumor and those that represent the patient's condition in specific contexts. Next-generation 'omics' technologies enable comprehensive analysis of tumors[26].The utilization of NGS is on the rise for the detection of diagnostic, prognostic, and predictive mutations within numerous malignant tumors, leading to substantial enhancement in treatment outcomes.

### 2.1 Transcriptome profiling and Expression Analysis

The transcriptome is composed of all the RNAs including mainly the mRNAs, rRNAs, and tRNAs. Moreover, other RNA molecules have also been found which do not encode for proteins, referred to as non-coding RNAs(ncRNAs) and has recently been proven to have regulatory functions that impact both gene expression and protein function [27]. The significance of non-coding RNAs, alternative splicing, and isoform stability in modulating oncogene expression has become increasingly recognized. This focus is crucial because transcriptional addiction is a fundamental dependency of cancer [28]. A recent study outlined the tumor-suppressing function of the non-coding RNA LINC00673 in PDAC [29].The cancer transcriptome comprises of RNA modifications, including alterations in messenger RNA's, which when combined with the genome, offer a holistic understanding of patient's cancer, improving clinical decision-making [30].

To initiate an investigation into the genomic architecture of pancreatic cancer JinpingJia and colleagues, employed high-throughput sequencing techniques to categorize and compare transcribed regions as well as potential regulatory elements, in two human cell lines originating from both cancerous pancreatic tissues and healthy tissues. Through RNA sequencing they detected 2,146 genes exhibiting differential expression in those cell lines, showing enrichment in pathways and biological processes related to cancer, such as cell adhesion, growth factor and receptor activity, signaling, transcription, and differentiation. Paired-end sequencing reads were aligned to the RefSeq database (National Centre for Biotechnology Information (NCBI) Build 37) utilizing the Burrows-Wheeler Aligner (BWA) software [31].

Another study by Mao et al. utilized RNA-Seq to comprehensively characterize the pancreatic ductal adenocarcinoma (PDAC) transcriptome, identifying 2,736 DEGs, including 1,554 upregulated genes, 1,182 downregulated genes, and 6 microRNAs [32]. This further reinforces the value of RNA-Seq for deciphering the pancreatic cancer transcriptome. Furthermore, Kirby et al. demonstrated a correlation between gene expression patterns and survival outcomes in pancreatic cancer patients, highlighting the potential of RNA profiling as a prognostic indicator [33]. Their analysis identified 323 transcripts correlating with survival in the pancreatic patient cohort. Certain survival-associated transcripts were found to confer resistance to gemcitabine treatment in vitro, suggesting that gene expression profiles may influence patient treatment by identifying those who may respond better to non-gemcitabine therapies.

One significant advantage of RNA-Seq is its cost-effectiveness compared to whole-genome sequencing [34]. This has enabled researchers to combine RNA-Seq with innovative bioinformatics methods to detect gene fusions at the transcript level using a paired-end RNA sequencing strategy [34]. Moving beyond mRNA, a comprehensive next-

generation sequencing analysis of the PDAC transcriptome by Muller et al. encompassed both coding and non-coding regions in six tumor tissues and five control tissues [35]. This study revealed novel signatures of long non-coding RNAs (lncRNAs), small nucleolar RNAs (snoRNAs), and mRNAs associated with PDAC [35, 36, 37]. The researchers also identified 13,614 highly expressed coding genes and 432 lncRNAs, with 1,961 mRNAs and 43 lncRNAs exhibiting significant differential expression between cancerous and control tissues [35].

## 3. Identification of molecular subtypes

Pancreatic cancer has been categorized into four primary molecular subtypes according to the expression of transcription factors and their downstream targets, or by analyzing the distribution of structural rearrangements [13]. Transcriptomic profiling allows for the classification of tumors based on gene expression patterns, providing insights into the biology and behavior of different subtypes. Studies on the transcriptional networks, Collisson et al. identified three molecular subtypes: classical, quasi-mesenchymal (QM) and an exocrine-like subtype. The classical subtype is defined by transcription factor GATA6 expression, KRAS dependency, and improved survival. The QM subtype is linked to high tumor grade and worse survival [38].

A significant characteristic of PDAC is its extensive stromal involvement, complicating the acquisition of accurate molecular data specific to the tumor. The analysis of PDAC tumors is hindered due to restricted tumor cellularity and the abundance of stroma intertwined with typical endocrine and exocrine cells. Through digital segregation of tumor, stroma, and normal gene expression, two tumor-specific subtypes have been discovered and confirmed, one of which is a "basal-like" subtype associated with a poorer prognosis and shares molecular similarities with basal tumors found in bladder and breast cancer.

Additionally, distinct "normal" and "activated " stromal subtypes have been characterized, each possessing independent prognostic implications. Their results indicate that RNA subtypes could more accurately depict the molecular characteristics of PDAC and their impact on patient prognosis. They hypothesize that the RNA subtypes identified by them could potentially signify the overall impact of somatic mutations, as well as underscore the significance of tumorstroma. These new findings regarding the molecular makeup of PDAC could potentially be utilized for customizing treatments. Furthermore, an understanding of these subtypes and their prognostic significance might offer guidance in a clinical environment where the selection and timing play a crucial role [39].

Genomic analysis of 456 pancreatic ductal adenocarcinomas performed by Bailey et al, defined molecular subtypes in which RNA expression profiles were used to define the subtypes associated with distinct histopathological characteristics and differential survival. The initial application of unsupervised clustering on RNA-seq data for 96 tumors characterized by a significant epithelial presence (≥40%) to equilibrate stromal gene expression resulted in the identification of four distinct and consistent classes. These were named, squamous, pancreatic progenitor, immunogenic, and aberrantly differentiated endocrine exocrine (ADEX). The squamous subtype is characterized by TP53 and KDM6A mutations, while the pancreatic progenitor subtype expresses genes involved in early pancreatic development. The ADEX subtype displays upregulation of genes related to KRAS activation, exocrine, and endocrine differentiation pathways. Immunogenic tumors contain upregulated immune networks, indicating pathways involved in acquired immune suppression [40].

It’s important to highlight that identifying an altered transcriptome doesn’t necessarily guarantee its phenotypic translation and manifestation. Therefore, it is believed that the integration of various omics technologies could provide a more comprehensive profile of changes for classifying PDAC patients [41].

## 4. Pathway Analysis and Therapeutic Target Identification for Personalized treatments

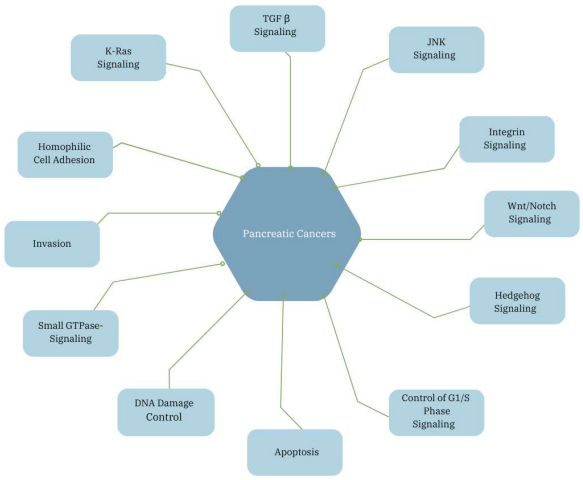


**Figure-1**: The 12 linked core pathways reported with genes which are genetically altered in most pancreatic cancer cases

Genetically modified core pathways and regulatory processes are only noticeable when the genome's coding regions are thoroughly examined. Mutational dysregulation of these core pathways and processes can elucidate the key aspects of pancreatic tumorigenesis. A group of 12 cellular signaling pathways and processes are known to be genetically modified in most pancreatic cancer research [42] as represented in Figure 1.

A study on utilized microfluidics technology, to isolate circulating tumor cells (CTCs) from a genetically engineered mouse model, enabling the generation of an unbiased RNA sequencing profile to identify CTC-specific expression patterns. Their findings indicate that non-canonical Wntsignaling pathways might play a crucial role in promoting metastasis in human pancreatic cancer. Expression of Wnt2 in pancreatic cancer cells inhibitsanoikis, promotes anchorage-independent sphere formation and increases metastatic potential in vivo, indicating its significance in cancer progression. A panel of inhibitors of Wnt-related pathways were tested to find small molecule inhibitors that can suppress the Wnt2 effect on anoikis. 5Z-7-Oxozeaenol, Map3k7 (Tak1) inhibitor eliminated Wnt2-induced tumor spheres without impacting the formation of baseline spheres. Inhibition of Map3k7 (Tak1) kinase, which suppresses the effects of Wnt2, could serve as a potential drug target for metastasis suppression. Tak1 inhibition suppressed Fn1 expression as well and reversed the prosurvival phenotype induced by Wnt2, leading to decreased tumor sphere formation and metastases. The results suggest that Wnt2 plays a role in transmitting survival signals related to metastasis. These signals rely partially on a Tak1-Fn1 signaling pathway. Formation of non-adherent tumour spheres in human pancreatic cancer cells involves upregulation of various Wnt genes. Pancreatic circulating tumour cells (CTCs) in 5 out of 11 cases show enrichment in Wntsignaling. Molecular analysis of CTCs can help in identification of potential therapeutic targets for inhibiting cancer metastasis [43].

The abnormal activation of the Wnt pathway is significantly linked to the formation of various malignant tumors in humans [44]. The DKK4 gene is regulated by the Wnt/β-catenin signaling pathway, and its protein can suppress the pathway activity [45, 46] and the disruption of this feedback mechanism in certain tumors could stimulate tumor development [47, 48]. Ouyang Y and colleagues found that DKK4 is significantly upregulated in pancreatic cancer tissues and explored the correlation between DKK4, and pancreatic cancer progression using high-throughput RNA sequencing and found that DKK4 is linked to tumor formation, organ development, and immune inflammation. The MAPK, Rap1, TNF, and TGF-beta signaling pathways showed notable activation, linked to tumor, immunity, and inflammation. Additionally, construction of pathway interactions reveals how enriched pathways connect to show the gene profile affected by DKK4 in pancreatic cancer cells. And they discovered that the majority of significantly activated signal transduction pathways were connected, directly or indirectly, to the MAPK signalingpathway.Theseinclude the TNF, TGF-beta, Rap1, Ras, Hippo, FoxOsignaling pathways, and others associated with cancer. Nevertheless, the MAPK signaling pathway is the primary signal transduction pathway in DKK4-overexpressing pancreatic cancer cells, indicating that DKK4 could contribute to pancreatic cancer development by activating this pathway abnormally. The outcomes of GO and pathway analyses, alongside differentially expressed gene interaction network, pinpointed genes linked to tumor development such as MAPK3, PIK3R3, VAV3, JAG1, and Notch3 [49].

## 5. Identifying potential therapeutic targets is essential for precision therapy

Certain research studies highlighted kinases as a potential target for precision therapy by analyzing RNA-sequencing data of tumors. Pancreatic cancer cell lines responded to targeting specific kinases like EGFR, PLK2, MET, and AKT2, suggesting them as possible therapeutic targets. The study expanded on nominating outlier kinases specific to pancreatic cancer, known for poor prognosis and resistance to standard treatments. The pancreatic cancer sanctuary, including tumorstroma and immune cells, highlights the demand for the exploration of effective targets. Knockdown inhibition of outlier kinases shows promising results in pancreatic cancer cell lines. Matching actionable targets with suitable therapeutics is crucial.

Targeting MET was more effective in pancreatic cancer cell lines, directed the significance of aligning specific targets from samples with suitable therapeutics. The influential effects of choosing unique outlier kinases along with the oncogenic KRAS mutation, which appears in almost all pancreatic cancer cases and after its knockdown, phospho-ERK levels decreased in KRAS-dependent cells (L3.3 and MIA-PaCa-2) but not in KRAS-independent PANC-1 cells. This suggested that in PANC-1 cells, ERK activity might be maintained through alternative pathways [50].

The PI3K-AKT signaling pathway also plays a crucial role in the development of pancreatic cancer. AKT kinase enhances cell survival, initiates cell division, boosts metabolism, supports growth, stimulates angiogenesis, and aids in DNA repair by phosphorylating various proteins [51]. The PI3K-AKT pathway is influenced by MUC16 [52], FAM83A [53], and NT5E [54], which can serve to be potential therapeutic targets. But MET is presently known to be the most critical gene in this current context of study. The MET proto-oncogene produces the protein MET (c-MET), a membrane tyrosine kinase receptor. MET first binds to its ligand, hepatocyte growth factor (HGF), which is secreted by stromal cells. Upon binding, HGF dimerizes, activating MET, which then can trigger the PI3K/AKTpathway[55].

Researches also reported key oncogene in PDAC, RAS, which significantly influences signaling pathways that regulate cell growth and differentiation, promoting cell proliferation and differentiation while inhibiting apoptosis. Abnormally activated RAS initiates signaling through downstream pathways such as RAF/MEK/ERK, PI3K/PDK1/AKT/mTOR, RALGDS, TIAM1, and RIN1 [56].Activated KRAS reported to boost the natural expression of the upstream protein, epidermal growth factor receptor (EGFR), leading to its excessive activation [57,58]. Elevated RAS levels and heightened EGFR activity significantly amplify MEK/ERK activity, resulting in intraepithelial neoplasia [59]. In a phase II trial, the EGFR inhibitor nimotuzumab extended the overall survival (OS) of patients with advanced or metastatic pancreatic cancer, improving median OS from 6.0 to 8.6 months. Also, patients with KRAS wild-type PDAC resulted in greater benefits, with median OS increasing from 5.6 to 11.6 months compared to those with KRAS mutant PDAC [60].

TP53, a tumor suppressor gene, is the most frequently inactivated in PDAC, with about 70% of patients showing alterations in this gene.[61, 62].Targeting murine double minute 2 (MDM2) is another developing strategy for treating tumors with TP53 mutations. MDM2 counteracts p53 either through direct interaction or by promoting its degradation via the ubiquitin pathway [63]. Thus, inhibiting MDM2 could enhance p53 activity and help control cancers with p53 mutations [64]. Butthere is a current shortage of clinical trials evaluating MDM2 inhibitors in patients with PDAC.

Mutations in tumour suppressors, including TP53, SMAD4, and CDKN2A, contribute to tumorigenesis in PDAC. These molecules are involved in complex molecular networks and have important roles in tumor development and advancement. Various strategies can be used to target these proteins effectively due to their significance. By employing next-generation sequencing (NGS), these abnormal changes can be detected, allowing for the development of strategies and systematic approaches various standard pipelines have now been incorporated that selectively eliminate cancer cells in PDAC patients [65]as shown in Figure 2.

## 6. RNA-Seq Analysis

In order to address wide challenges in exocrine pancratic cancer new approaches for accurate assessment and vast datasets analysis, scientists using bioinformatics principles and constraints involved in the sophisticated process of RNA-Seq analysis [66]. RNA-Seq involves the integration of high-throughput sequencing with computational approaches to capture and measure transcripts within an RNA sample [67]. A typical RNA-Seq workflow often includes the following five sequential steps: 1) initially raw data is preprocessed, 2) followed by aligning reads, 3) with reconstruction of transcriptome, 4) quantifying expression levels, 5) and lastly, conducting differential expression analysis [68].

At each of these stages, it is essential to implement specific measures to assess the quality of the data. Quality assessment for the raw reads includes examining sequence quality, GC content, adaptor presence, overrepresented k-mers, and duplicated reads to identify potential sequencing errors, PCR artifacts or contaminations [69]. The objective of FASTQC is to offer a straightforward approach for conducting quality control assessments on raw sequence data obtained from high-throughput sequencing pipelines. It is a widely utilized tool for conducting these analyses on Illuminareads[70]. For increased mapping efficiency it is necessary for sequences to have undergone appropriate trimming to eliminate adaptor sequences and low-quality tails. Trimmomatic encompasses several processing steps for read trimming and filtering, with its primary algorithmic advancements focusing on the detection of adapter sequences and quality filtering [71].

In RNA-Seq, evaluating expression levels involves aligning relatively short sequencing reads to either a reference genome or a set of transcripts with the help of an alignment tool. The quantification of reads aligned to individual genes is then utilized for estimating expression levels [72]. Alignment in RNA sequencing poses significant complexity due to RNA splicing. Several RNA-Seq algorithms exist, claiming to align sequencing reads with precision and effectiveness, alongside the ability to detect splice junctions [73]. Certain aligners, like HISAT2, STAR [74], and TopHat2 [75], are specifically developed to adeptly identify splice junctions [76]. Unspliced read aligners include Stampy [77] and MAQ [78], which identify matches for short subsequences whereas Bowtie [79] and Burrows wheeler Aligner (BWA) [80] are utilized to find exact matches.

After aligning reads to a particular transcript on a reference sequence, the next step involves allocating them to genes or transcripts, to find out abundance measures. The quantity of reads aligned is then utilized for estimating expression levels associated with that gene.

The second step after read mapping, involves detecting active or expressed genes and isoforms, also called transcriptome reconstruction, and it's a challenging computational task due to three primary factors. Firstly, gene expression levels vary widely, with certain genes being represented by limited number of reads. Secondly, the reads come from both the fully processed mRNA (exons only) and the partially spliced precursor RNA (containing intronic sequences), posing difficulties in identifying mature or fully processed transcripts. Thirdly, short reads and multiple gene isoforms make it complicated to match each read to its originating isoform [81]. Both cufflinks [82] and iReckon [83] utilize established gene structures to aid in transcript assembly, potentially improving the accuracy of exon and transcript boundary determination whereas Oases [84], a de novo assembler, can be used to address partial or low-quality genome builds [85]. Various other transcript assemblers like Scripture [86] and StringTie [87] are also employed in genome-guided reconstruction. In contrast genome-independent algorithms also include Trinity [88] and TransAbyss [89] as de novo assemblers.

After mapping, the reads that align to coding units like exons, transcripts, or genes are counted in a step called counting or quantification, to estimate their expression levels. This is followed by normalization to eliminate sequencing bias. Commonly used quantification tools include HTSeq, which quantifies gene expression by counting mapped reads to exons without transcript assembly. [90]; Cufflinks [91], eXpress [92], MISO [93] and RSEM [94], are used for transcript level quantification.Alignment-free tools like Sailfish [95], Kallisto [96], and Salmon [97] directly associate sequencing reads with

transcripts, eliminating the need for a separate quantification step. They perform well with more abundant transcripts but are less precise in quantifying low-abundance or short transcripts.

For precise gene expression estimation, read counts need to be normalized. The RPKM (reads per kilobase of transcript per million mapped reads) metric normalizes or adjusts a transcript's read count for the gene length and the total number of reads mapped in the sample. For paired-end reads, the FPKM (fragments per kilobase of transcript per million mapped reads) metric further refines this by accounting for the relationship between paired-end reads in the RPKM calculation [84]. One approach employed in the alternative expression analysis by RNA sequencing is Alexa-Seq method, which estimates isoform-level expression by counting reads that map uniquely to one isoform. While effective for some alternatively spliced genes, it is less successful for genes lacking unique exons to gauge isoform expression [98].

Following normalization, when sequence is converted into an expression matrix, the data can be modeled to identify which transcript features likely changed in expression level that is to identify differentially expressed genes. Tools like edgeR [99], DESeq2 [100], and limma+voom [101] are employed for this purpose as shown in Figure 3.

## 7. Challenges and Future Directions

The primary challenge for biologists in this domain is to identify the causes of pancreatic cancer, recognizing potential variations in drivers in distinct cases. Categorizing pancreatic cancers based on drivers has facilitated the evaluation of personalized treatment in animal models and human patients [101]The classification of PDAC into subtypes has clinical potential for precision oncology by identifying patients who might benefit from specific treatments and identifying therapeutic targets [102].

It's important to integrate or combine both transcriptomic and genomic analyses to identify and explore PDAC subtypes, as their mutational and transcriptional profiles differ. Integrating these analyses could provide a more comprehensive view of PDAC's heterogeneity [103,104]. With the progression of sequencing technologies, computational tools must also advance to address emerging technical challenges and support new applications. Further As laboratory techniques improve to allow sequencing of tiny RNA amounts, sophisticated statistical methods will be required to distinguish technical noise from significant biological variation. These advancements will enhance transcriptome analysis in rare cell types and states, helping researchers map biological networks at the cellular level [105].

## 8. Conclusion

Pancreatic adenocarcinoma is a highly lethal disease which is found as a tumor in the exocrine part of the pancreas and is the most common among all pancreatic cancers. Due to its aggressive nature, poor prognosis and limited treatment options, this pancreatic cancer remains a significant clinical challenge with most of them being resistant to standard treatments such as radiation, chemotherapy and surgery. Understanding its molecular landscape on a deeper level is essential for the development of suitable prognostic and therapeutic modalities. Transcriptomic profiling with RNA-Seq revolutionized comprehension of exocrine pancreatic cancer, offering new perspectives on its molecular characteristics, tumor diversity, and treatment prospects. Persistent endeavors to tackle obstacles and utilize RNA-Seq information for precision oncology show potential for enhancing diagnosis, prognosis, and therapy in pancreatic cancer. The emergence of RNA-Seq has transformed transcriptomic profiling by allowing in-depth analysis of gene expression, alternative splicing, and non-coding RNA in exocrine pancreatic cancer. This method provides unparalleled understanding of the molecular mechanisms behind tumorigenesis, progression, and resistance to treatment. Furthermore, with the identification of differentially expressed genes using RNA Seq approaches is crucial for developing new markers for both future detection and treatments will aid future researchers and scientists adding new dimensions of solutions in near future to pancreatic cancer research.

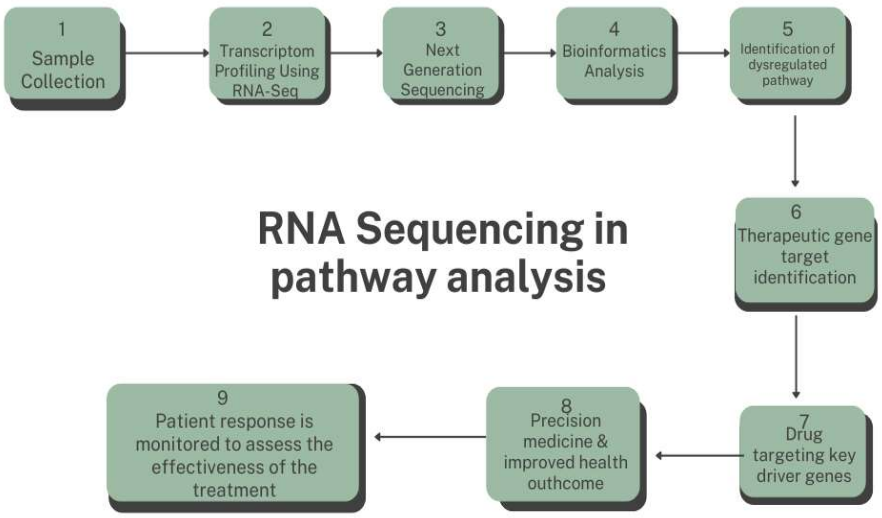


**Figure 2**- RNA Sequencing in pathway analysis and therapeutic target identification for precision medicine

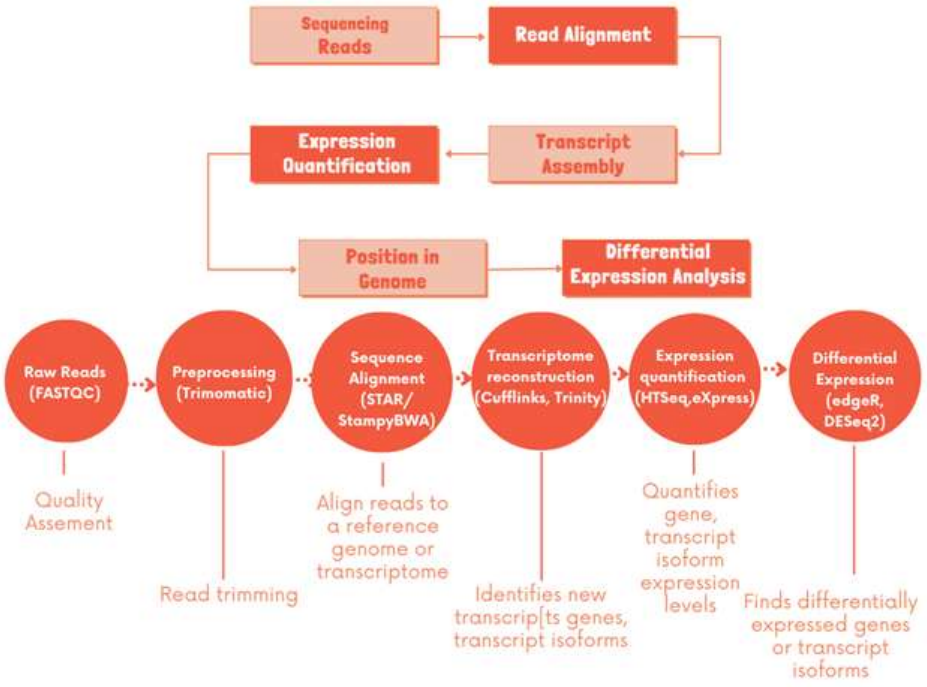


**Figure 3**- RNA-Seq data analysis workflow. This figure illustrates tools and processes involved in RNA-Seq data analysis. These steps are common in almost all RNA-Seq data analysis which includes, quality control, per-processing, sequence alignment, transcriptome reconstruction, expression quantification, and differential expression analysis

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## 10. Authors Contribution

Ariba Anwar, Nesae Akhdash, SabihaZarin Khanam, Aqusa Zaman, Harsimran Kaur Hora, Priyangulta Beckcontributed in literature search and manuscript writing; Priyangulta Beck, HarsimranKaurHora, MukeshNitin manuscript writing, literature searching and proof reading; Ariba Viswaraj Lal, Pinki Raj Sahu, Mukesh Nitin helped with proof reading; MukeshNitin helped with review design, proof reading, guiding.

## 11. Source

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