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Pseudogene-mediated ceRNA networks in health and disease: a multi-dimensional regulatory perspective

Abstract

Pseudogenes, previously regarded as inactive genomic byproducts, have been recognized as key regulators in competing endogenous RNA (ceRNA) networks, profoundly influencing gene expression across various disease contexts, including cancer. Regulatory pseudogenes like PTENP1 and BRAFP1 function as miRNA sponges, exerting precise control over oncogenic and tumor-suppressive pathways. PTENP1 enhances tumor suppression by sequestering miRNAs such as miR-21 and miR-19b, which stabilizes PTEN expression and inhibits the PI3K/AKT pathway. BRAFP1 similarly binds miRNAs that target BRAF, promoting MAPK pathway activation critical for oncogenic signaling. The therapeutic and diagnostic market for pseudogene-ceRNA interactions is rapidly expanding, with notable advancements in RNA-based precision medicine reflected in miRNA-targeted diagnostics and treatments. Techniques like RT-PCR and FISH enable the high-specificity detection of pseudogene expression, achieving over 90% accuracy in cancer diagnostics. While therapeutic targeting of pseudogenes poses challenges due to sequence homology with functional genes, new delivery technologies and AI-driven precision approaches are enhancing pseudogene-focused interventions. Pseudogenes represent a promising frontier in cancer biology, with their unique regulatory roles offering potential breakthroughs for novel diagnostics and treatments.

Keywords: pseudogenes, ceRNA networks, gene regulation, health and disease, competing endogenous RNA, transcriptional regulation

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Abbreviations: Ψ, pseudogene; ceRNA, competing endogenous RNA; MRE, microRNA response element; miRNA, microRNA; TSG, tumor suppressor gene; mRNA, messenger RNA; PP, processed pseudogene; UP, unprocessed pseudogene; UPG, unitary pseudogene, RT, retrotransposition; LINE, long interspersed nuclear elements; siRNA, short interfering RNA; circRNA, circular RNA; AD, alzheimer's disease; FTH1, ferritin heavy chain;; PTENP1, PTENP1 pseudogene; HMGEC, human mammary gland epithelial cells; CRDP, circular RNA-derived pseudogenes;; HMGA1P, high mobility group AT-Hook 1 pseudogene; RBP, RNA-binding protein;; lncRNA, long non-coding RNA; CRC, chromatin remodeling complexes; ERK, extracellular signal-regulated kinase; BRAF, B-Raf proto-oncogene; PI3K, phosphoinositide 3-kinase; AKT, serine/threonine kinase; MAPK, mitogen-activated protein kinase; qRT-PCR, quantitative reverse transcription polymerase chain reaction; FISH, fluorescence in situ hybridization; ceRNA hypothesis, competitive Endogenous RNA Hypothesis; PTPN11, protein tyrosine phosphatase, non-receptor type 11; NDs, neurodegenerative diseases; EGFR, epithelial growth factor receptor; TNF, tumor necrosis factor; early growth response protein 1 (EGR1), HMGA, high mobility group at-hook 1 Gene; PMOM, precision medicine oncology market; scRNA-seq, single-cell RNA sequencing; ISH, in situ hybridization; RNAi, RNA interference; LNP, lipid nanoparticles; BCL, B-cell lymphoma; AI, artificial intelligence; IP, immunoprecipitation; RIP, RNA immunoprecipitation; HRISH, High-resolution in situ hybridization

Introduction

Pseudogenes, long dismissed as "Junk DNA," have emerged as significant players in gene regulation, particularly through their roles in competing endogenous RNA (ceRNA) networks.[1] Historically, pseudogenes were identified as defective copies of functional genes that arose through duplication or retro transposition, often containing mutations that rendered them incapable of encoding functional proteins.[2] Despite their inability to produce functional proteins, pseudogenes have gained attention for their roles in gene regulation, acting as decoys for miRNAs and influencing the stability of their corresponding functional gene transcripts.[3] Pseudogenes have various mechanisms that contribute to their regulatory roles, including the generation of small interfering RNAs (siRNAs) and the modulation of parent gene expression through miRNA sponging.[4] They can also act in a cis-regulatory manner, impacting nearby gene activity, or in a trans-regulatory manner, influencing gene expression across distant locations.[5] The identification of pseudogene-derived siRNAs in multiple species supports the theory that pseudogenes play an evolutionary conserved role in gene expression control.[4]

The human genome contains thousands of pseudogenes, many of which are transcribed and participate actively in regulatory networks.[6] These pseudogene transcripts often possess microRNA response elements (MREs), enabling them to act as ceRNAs, effectively sequestering miRNAs and preventing them from suppressing their target mRNAs.[4] This activity is crucial in regulating various biological processes and has positioned pseudogenes as important modulators of gene expression in both health and disease contexts.[7]

One of the best-characterized examples is the PTENP1 pseudogene, which serves as a decoy for miRNAs targeting PTEN, a well-known tumor suppressor gene.[8] By sequestering these miRNAs, PTENP1 indirectly maintains PTEN expression, highlighting its role in tumor suppression and cancer progression.[8] This ceRNA activity is not unique to PTENP1, as many pseudogenes contribute to oncogenic or tumor-suppressive pathways through similar mechanisms.[9] For example, HMGA1P, a pseudogene of the oncogene HMGA1,

also modulates cancer progression by acting as a miRNA sponge, emphasizing the regulatory potential of pseudogenes in oncogenesis.[10]

Recent studies also highlight the role of pseudogenes in regulating immune responses. Pseudogenes have been shown to contribute to inflammation control and immune regulation by modulating gene networks linked to inflammatory signaling pathways. Such findings suggest that pseudogenes could serve as potential therapeutic targets for autoimmune diseases and other inflammation-related conditions.[11]

In addition to their roles in cancer, pseudogene-mediated ceRNA networks have significant implications in other diseases. In neurological disorders, pseudogenes have been found to regulate genes involved in neurogenesis and synaptic function, which are crucial for proper brain activity and development.[11] Dysregulation of these pseudogenes has been linked to neurodegenerative diseases such as Alzheimer's, suggesting their potential as biomarkers or therapeutic targets in neurological conditions.[12] Similarly, in cardiovascular diseases, pseudogenes are implicated in regulating cardiac function and modulating stress responses, further underscoring their role in maintaining cellular homeostasis.[13]

The biological relevance of pseudogenes extends beyond disease regulation, as they also contribute to essential physiological processes such as iron metabolism and cell differentiation. For instance, FTH1P, a pseudogene of ferritin, regulates iron homeostasis by acting as a decoy for miRNAs targeting ferritin genes, thus playing a role in conditions like anemia and iron overload disorders.[10] Moreover, pseudogenes such as those derived from circRNAs have shown conserved roles across different species, indicating their evolutionary significance in gene regulation.[5] The diversity of regulatory roles, ranging from direct modulation of gene expression to the sequestration of regulatory RNAs, illustrates the versatility of pseudogenes in various biological and pathological processes, making them an essential focus for further research.[14]

Market analysis of pseudogene-related ceRNA therapeutics and diagnostics

The pseudogene-related ceRNA therapeutics and diagnostics market reflects a growing field within RNA therapeutics, where pseudogenes function as key regulatory elements in gene expression modulation.[15] The RNA therapeutics market, valued at $13.7 billion in 2023 and expected to reach $18.0 billion by 2028, includes targeted therapies in the pseudogene-ceRNA space for disorders with high unmet needs.[16] These regulatory mechanisms are essential in precision medicine for oncology and other diseases, where ceRNA interactions provide new pathways for therapeutic intervention.[15]

One significant segment in this market is the miRNA and siRNA therapeutics area, with applications in gene silencing and manipulation of gene expression networks, including pseudogene-ceRNA pathways.[17] This segment, valued at $1.6 billion in 2023 and projected to reach $5.28 billion by 2031 with a 16.0% CAGR, demonstrates increased clinical and commercial interest in pseudogene-targeted treatments.[18] miRNA diagnostics, which overlap with ceRNA network-based diagnostics, are a growing component, expected to contribute significantly to the diagnostic sector.[19] This market was valued at $1.58 billion in 2023 and is anticipated to expand, particularly in precision diagnostics and cancer genomics.[19]

The broader gene expression market also supports ceRNA diagnostic technologies, with a valuation of $13.85 billion in 2023, expected to nearly triple by 2033 due to developments in gene-editing and RNA-based assay technologies.[20] Additionally, ceRNA network diagnostics represent a crucial advancement in cancer diagnostics, where targeting regulatory RNAs, such as pseudogenes, plays a role in prognosis and treatment response prediction.[20] This is particularly relevant in precision oncology, where targeting tumor suppressor genes and oncogenes, as seen in PTEN and other key markers, aligns with a precision medicine oncology market projected to grow from $41.7 billion in 2023 to $90.7 billion by 2032.[21]

Beyond cancer applications, the market potential for pseudogene-targeted therapies aligns closely with the antisense and RNA interference (RNAi) therapeutics segment, projected to expand due to innovations targeting gene regulatory networks.[17] The biotechnology industry's investment in RNA therapeutics has surged, with notable investments directed at companies focusing on ceRNA network regulation, pseudogene applications, and non-coding RNA technologies.[21] Leading biotech companies and venture capitalists have fueled growth by funding research into pseudogene-related ceRNA networks, recognizing the potential for therapeutic applications across a wide range of diseases.[22]

Classification and formation of pseudogenes

Types of pseudogenes

Pseudogenes can be classified into three main types: processed pseudogenes, unprocessed pseudogenes, and unitary pseudogenes, (Figure 1).[23]

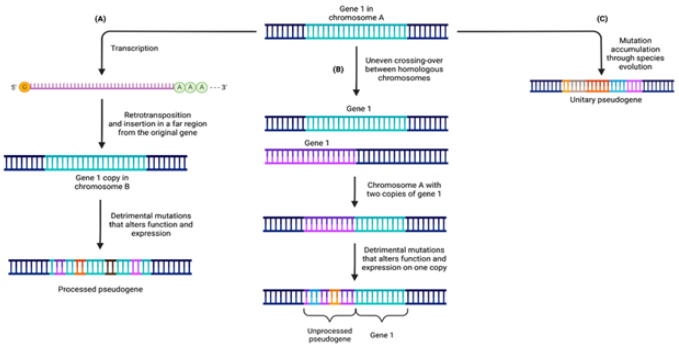


Figure 1 Classification of pseudogenes: processed, unprocessed, and unitary types.

This illustrates the classification of pseudogenes into three main types: processed, unprocessed, and unitary. These types are defined by the mechanisms that lead to their formation, such as retro transposition, gene duplication, and accumulation of mutations in single-copy genes.[9]

Processed pseudogenes

Processed pseudogenes are formed through retro transposition, a process in which mRNA is reverse-transcribed into cDNA and integrated back into the genome, often lacking regulatory elements such as promoters and introns (Figure 2).[24] These pseudogenes arise from mRNA transcripts, and they retain the exon sequences of the parent gene but usually lack intronic regions, making them non-functional in protein synthesis.[25]

Processed pseudogenes are often characterized by their lack of introns, as they are derived from mature mRNA, and their integration into the genome is facilitated by long interspersed nuclear elements (LINEs) such as LINE-1 (Figure 3).[26] This process leaves processed pseudogenes devoid of necessary regulatory sequences, preventing transcription initiation. However, some processed pseudogenes can still be transcribed, contributing to regulatory networks as non-coding RNAs or through miRNA sponging.[4] Processed pseudogenes are

often located in regions of the genome that are distinct from their original gene loci, which suggests that their functions have diverged over time.[26]

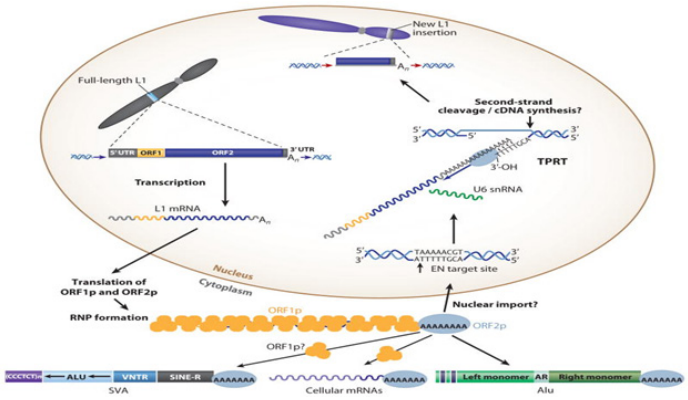
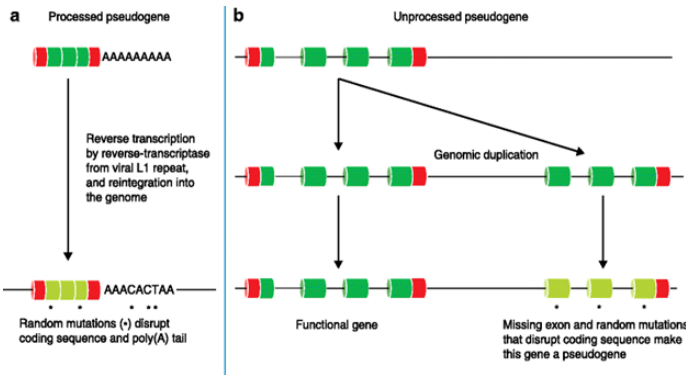


Figure 2 The LINE-1 Retro transposition Cycle.

This represents the process of LINE-1 (L1) retro transposition. A full-length L1 element is transcribed, and its mRNA is exported to the cytoplasm for translation of ORF1p and ORF2p, leading to ribonucleoprotein (RNP) formation. The RNP is transported to the nucleus, where target-site primed reverse transcription (TPRT) occurs. The L1 endonuclease creates a nick in genomic DNA, initiating reverse transcription of L1 RNA, followed by the integration of a new L1 copy into the genome. Some details of second-strand synthesis and L1 integration remain unclear. Other elements, like Alu, SVA, and cellular mRNAs, may utilize L1 proteins for their trans mobilization by



Beck CR.

Figure 3 Pathways of Pseudogene Formation: Processed and Unprocessed.

This illustrates two distinct mechanisms of pseudogene formation. (a) Processed pseudogenes result from the reverse transcription of mature mRNA and reintegration into the genome, often lacking introns and accompanied by a poly(A) tail. (b) Unprocessed pseudogenes emerge from gene duplication events, where a non-functional copy of the gene is produced, resembling its functional counterpart but with disrupted expression by Steward CA.

Unprocessed pseudogenes

On the other hand, unprocessed pseudogenes, also known as duplicated pseudogenes, originate from gene duplication events and typically maintain the full structure of the original gene, including exons, introns, and regulatory regions (Figure 4).[27] These pseudogenes become non-functional due to the accumulation of disabling mutations, such as insertions, deletions, or point mutations, which prevent their translation into functional proteins.[28] Unprocessed pseudogenes often remain in close proximity to their parental genes, indicating their origin through local duplication events.[23]

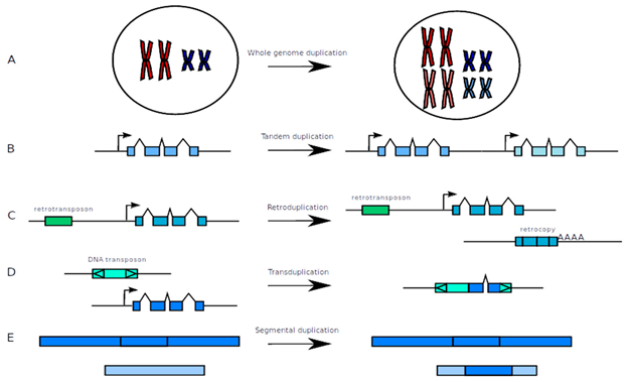


Figure 4 Types of gene duplications.

This explains the different gene duplication types: (A) whole genome duplication, (B) tandem duplications of adjacent sequences, (C) retroduplication producing intron-less copies, (D) trans duplication via transposons capturing gene fragments, and (E) segmental duplications of long, identical sequences by Lallemand T.

Unitary pseudogenes

Unitary pseudogenes are unique in that they do not result from gene duplication or retro transposition. Instead, they are formed when an active gene becomes non-functional through the accumulation of mutations within a particular lineage, resulting in a loss of function without compensation by other copies of the gene (Figures 5&6).[9] These pseudogenes represent gene losses specific to certain species or evolutionary lineages, highlighting their role in adaptive evolution.[28] Unitary pseudogenes are rare compared to processed and unprocessed pseudogenes, but they provide insight into the evolutionary pressures that drive gene function loss and diversification within a species.[27]

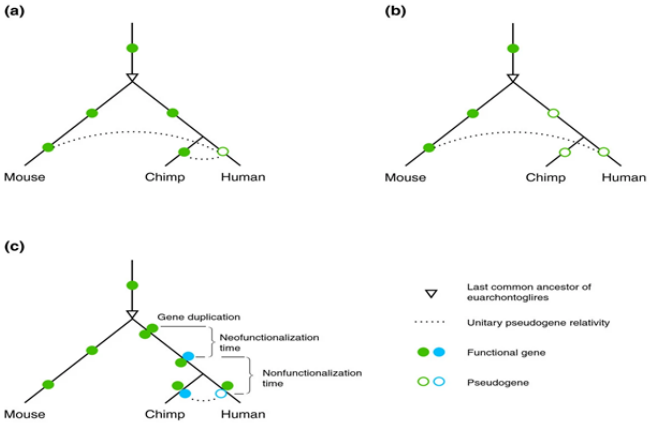


Figure 5 Human unitary pseudogene relativity across species.

This explains the emergence of human unitary pseudogenes through gene loss across different evolutionary lineages. It compares the human gene set with chimpanzees and mice to identify these pseudogenes, depending on when the gene loss occurred in evolutionary history.[27]

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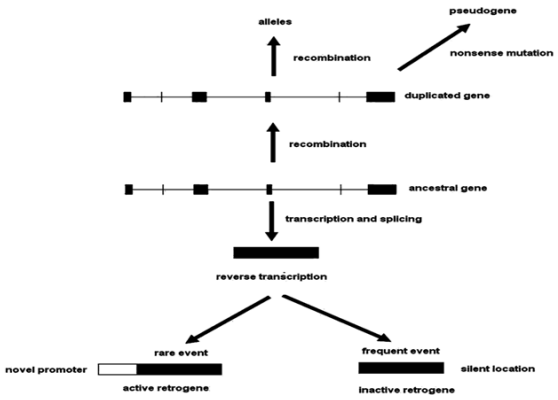

Figure 6 Mechanism of retrotransposition and pseudogene formation.

This illustrates the retro transposition process, showing the formation of pseudogenes, active retrogenes, and inactive retrogenes. The ancestral gene undergoes transcription, splicing, and reverse transcription, resulting in either a rare active retrotransposition event or a frequent inactive one. Additionally, duplicated genes can form pseudogenes through nonsense mutations by Kangueane P.

Mechanisms of pseudogene formation

The formation of pseudogenes is driven by several mechanisms, including retro transposition and gene duplication.[1]

Retro transposition

Retro transposition, facilitated by elements like LINE-1, involves the insertion of a reverse-transcribed cDNA back into the genome, resulting in a processed pseudogene that lacks intronic and regulatory sequences, rendering it non-functional.[26]

Gene duplication

Gene duplication, on the other hand, creates redundant copies of genes, and one of these copies may acquire mutations over time, leading to the formation of an unprocessed pseudogene.[4] Gene duplication events are often followed by sub functionalization, where one gene retains the original function while the duplicated gene undergoes mutations that can lead to new functions or non-functionality.[6]

Evolutionary significance of pseudogenes

Despite their inability to encode proteins, many pseudogenes have retained important regulatory roles, such as miRNA sequestration and modulation of gene expression, indicating their evolutionary significance.[14] These roles suggest that pseudogenes may provide a genetic buffer against mutations, ensuring the stability of regulatory networks. By acting as ceRNAs or miRNA sponges, pseudogenes contribute to the fine-tuning of gene expression, which can be particularly important during stress responses or in disease states where precise regulation is crucial.[13]

Pseudogenes as competing endogenous RNAs (ceRNAs)

Overview of pseudogene ceRNA function

Pseudogenes have emerged as active participants in gene regulation through their role as competing endogenous RNAs (ceRNAs).[14] Acting as molecular sponges, pseudogenes bind miRNAs, thereby preventing these miRNAs from repressing their target mRNAs.[23] This ability to modulate miRNA activity positions pseudogenes as key regulators of post-transcriptional gene expression, influencing various biological processes, including development, differentiation, and disease progression.[29]

The ceRNA hypothesis

The ceRNA hypothesis suggests that pseudogenes, mRNAs, and long non-coding RNAs (lncRNAs) share microRNA response elements (MREs).[30] By competing for miRNA binding, pseudogenes sequester miRNAs that would otherwise target functional mRNAs for degradation or repression, ensuring that these genes can be expressed at higher levels.[3] This competition reduces miRNA availability, indirectly boosting the expression of functional genes and contributing to the fine-tuning of gene expression in contexts such as cancer, cardiovascular diseases, and neurodegenerative disorders.[31] The interaction between pseudogenes and miRNAs plays a crucial role in maintaining gene expression balance across multiple biological contexts.[32]

Mechanisms of pseudogene-mediated gene regulation

miRNA sponging

The primary mechanism by which pseudogenes regulate gene expression is by acting as molecular sponges for miRNAs. Pseudogenes share MREs with their parental genes and other functionally related genes, allowing them to bind and sequester miRNAs.[23] By preventing miRNAs from binding to their target mRNAs, pseudogenes reduce miRNA-mediated repression, thereby increasing the stability and expression of functional genes.[29] This mechanism allows pseudogenes to fine-tune gene expression in a manner that is crucial for responding to changing cellular conditions, such as stress or differentiation signals (Figure 7).[30]

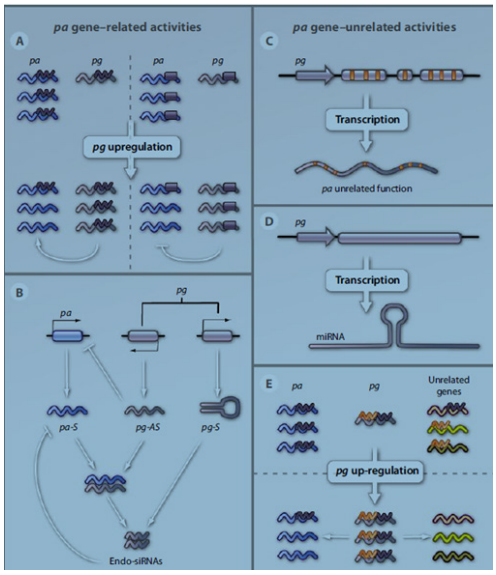

Figure-7 Mechanisms of gene regulation by pseudogenes at the RNA Level.

This picture illustrates how pseudogene RNAs regulate gene expression through various RNA-level mechanisms. In **(A)**, pseudogene RNAs compete with parental mRNAs for shared microRNAs, enhancing parental gene expression, or with stabilizing RBPs, reducing it. In **(B)**, antisense pseudogene transcripts recruit chromatin remodeling complexes to repress the parental gene or form endo-siRNAs to inhibit it post-transcriptionally. **(C)** shows pseudogenes functioning as lncRNAs to regulate unrelated genes. In **(D),** some pseudogenes serve as microRNA precursors. Finally, **(E)** demonstrates pseudogene RNAs acting as ceRNAs, sequestering microRNAs that target both parental and unrelated genes, thus increasing their expression.[47]

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Example: PTENP1 Pseudogene

A well-known example of this mechanism is the PTENP1 pseudogene, which regulates the tumor suppressor PTEN by sequestering miRNAs such as miR-21 and miR-19.[33] These miRNAs normally repress PTEN, but by binding to these miRNAs, PTENP1 ensures that PTEN expression is maintained at appropriate levels.[31] The loss of PTENP1 disrupts this balance, leading to decreased PTEN levels, which contributes to tumorigenesis and cancer progression.[32]

Other examples: HMGA1P and KRAS1P

Other pseudogenes, such as HMGA1P and KRAS1P, also exhibit miRNA sponging activity, impacting oncogenic and tumor suppressor pathways by modulating the availability of miRNAs.[34] This mechanism is critical in diseases such as cancer, where precise regulation of gene expression is essential for controlling cell proliferation and apoptosis.[35]

Small interfering RNA (siRNA) production

In addition to acting as miRNA sponges, some pseudogenes contribute to gene regulation by generating siRNAs. These pseudogene-derived siRNAs participate in RNA interference (RNAi), a gene-silencing mechanism that operates at the post-transcriptional level.[4] Pseudogene-derived siRNAs can target homologous sequences, including their parental genes, and initiate the degradation of these mRNAs, leading to reduced expression.[14]

Example: siRNAs in the murine genome

For example, pseudogenes in the murine genome have been shown to produce siRNAs that target parent gene transcripts, effectively modulating the expression of those genes.[7] This form of regulation adds another layer of complexity to pseudogene function, enabling them to not only act as miRNA sponges but also directly silence gene expression through siRNA-mediated pathways.[6]

Evolutionary conservation of siRNA production

The generation of siRNAs from pseudogenes is evolutionarily conserved and has been observed across multiple species, including mice, humans, and plants.[12] This mechanism underscores the versatility of pseudogenes in post-transcriptional gene regulation.[13]

Transcriptional interference

In addition to their post-transcriptional roles, pseudogenes can influence gene expression at the transcriptional level through transcriptional interference.[7] Pseudogenes located near functional genes can interfere with transcription factor binding or the recruitment of RNA polymerase, thereby affecting the transcription of neighboring genes.[4]

Cis-Regulatory activity of pseudogenes

This cis-regulatory activity occurs when pseudogenes are located in close genomic proximity to their functional counterparts, often sharing promoter regions or enhancer elements.[13] By modulating the accessibility of these regulatory regions, pseudogenes can influence the transcriptional activity of nearby genes.[24]

Example: Pseudogenes modulating tumor suppressor genes

For instance, pseudogenes located near tumor suppressor genes or oncogenes have been shown to modulate the expression of these genes by interfering with their transcription.[14] In some cases, pseudogenes act as decoy binding sites for transcription factors, reducing the availability of transcription factors to the parental gene's promoter, thereby reducing its expression.[13] This transcriptional interference adds another dimension to the regulatory capabilities of pseudogenes, allowing them to modulate gene expression not only at the post-transcriptional level but also during the transcription process itself.[8]

Biological and pathological roles of pseudogene-mediated ceRNA networks

Pseudogenes, traditionally considered nonfunctional genomic remnants, are now recognized for their active roles in regulating gene expression and cellular processes.[33] "Competitive endogenous RNA" (ceRNA) networks involve pseudogenes acting as molecular sponges that bind miRNAs, thus preventing them from targeting specific messenger RNAs (mRNAs) of crucial genes.[36] This migration allows pseudogenes to indirectly regulate gene expression, impacting cellular pathways related to cell proliferation, apoptosis, immune response, and tumorigenesis.[37] Key pseudogenes, PTENP1, HMGA1P6, HMGA1P7, and BRAFP1, exemplify the significance of pseudogene-mediated ceRNA networks in both physiological and pathological settings, particularly in cancer progression and resistance mechanisms (Figure 8).[4]

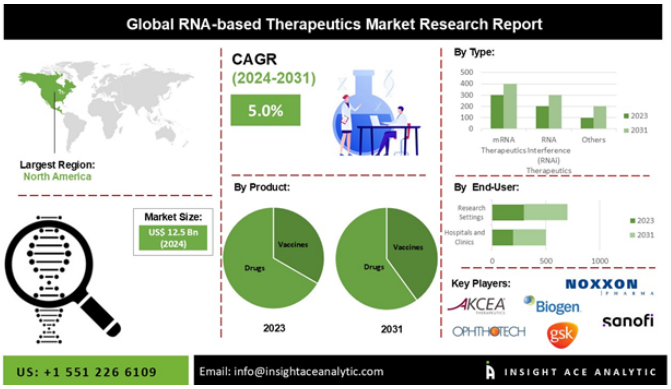

Figure 8 RNA-based therapeutics market: growth forecast and leading companies.

The Picture represents Global RNA-based Therapeutics Market Research Report projects a market growth rate (CAGR) of 5.0% from 2024 to 2031, reaching a valuation of $12.5 billion in 2024, with North America as the largest region. Key segments include vaccines and drugs, with vaccines anticipated to grow by 2031. The market is driven by mRNA and RNA interference (RNAi) technologies, mainly used in research and healthcare settings. Major industry players include Akcea Therapeutics, Biogen, GSK, and Sanofi.[16]

Key examples of pseudogene biological regulation

PTENP1: The pseudogene PTENP1 (phosphatase and tensin homolog pseudogene 1) is one of the most extensively studied pseudogenes, known for its crucial role in tumor suppression.[23] As a pseudogene of the tumor suppressor PTEN, PTENP1 functions as a ceRNA that binds multiple miRNAs, including miR-21, miR-17, and miR-19b, which would otherwise target PTEN mRNA.[38] By sequestering these miRNAs, PTENP1 stabilizes PTEN expression, enabling PTEN to continue its role in inhibiting the PI3K/AKT signaling pathway—a pathway frequently activated in cancers and associated with cell growth and survival (Figure 9).[39] This regulatory mechanism is pivotal in maintaining cellular homeostasis, and disruptions in the PTEN/PTENP1 axis have been observed in several cancers, including prostate, breast, and renal carcinomas.[37] Studies also show that PTENP1 levels inversely correlate with cancer severity, making it a potential therapeutic target in managing tumor progression and metastasis.[40]

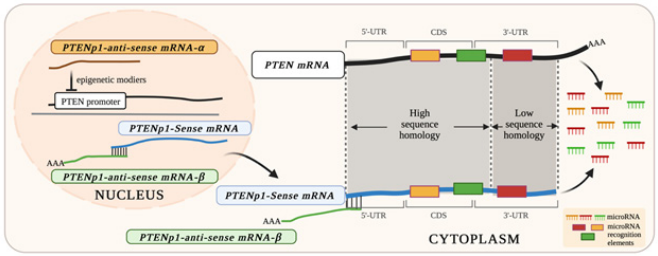

Figure 9 Multifaceted regulatory roles of PTENP1-S and PTENP1-AS isoforms in PTEN expression.

This figure illustrates the complex roles of the PTENP1 sense transcript (PTENP1-S) and its two antisense isoforms (PTENP1-AS-α and PTENP1-AS-β) in regulating PTEN expression at both transcriptional and post-transcriptional levels. PTENP1-AS-α localizes to the PTEN promoter, where it binds to the 5'-UTR of PTEN transcripts and recruits epigenetic modifiers, leading to transcriptional repression of PTEN. The PTENP1-AS-β isoform stabilizes the PTENP1 sense transcript (which lacks a poly-A tail) by binding to it, forming a complex that is then exported to the cytoplasm. In the cytoplasm, the PTENP1 sense transcript acts as a miRNA sponge within the ceRNA network, thereby regulating PTEN expression post-transcriptionally due to high sequence similarity.[36]

HMGA1P6 and HMGA1P7: Derived from the oncogenic HMGA1 gene, the pseudogenes HMGA1P6 and HMGA1P7 serve as prominent examples of ceRNA-mediated regulation in cancer pathology.[37] These pseudogenes act as molecular sponges for miRNAs targeting HMGA1, such as miR-483 and miR-675, thus enhancing HMGA1 expression and activity.[40] In turn, HMGA1 facilitates chromatin remodeling and supports gene transcription in oncogenesis, particularly in ovarian, colorectal, and breast cancers.[7] HMGA1P7, specifically, not only sponges miRNAs but also activates the transcription factor Egr1, which further promotes miRNA production.[35] The ability of HMGA1 pseudogenes to impact oncogene expression underscores their roles in cancer progression, associating their overexpression with advanced disease stages and poor prognosis in clinical studies.[41]

BRAFP1: As a pseudogene of the BRAF gene, BRAFP1 exemplifies pseudogene functionality in promoting oncogenic pathways.[28] Through high sequence homology with BRAF, BRAFP1 sequesters miRNAs, such as miR-134, miR-543, and miR-653, which would otherwise inhibit BRAF expression.[42] By protecting BRAF from miRNA-mediated suppression, BRAFP1 enhances BRAF protein levels, consequently activating the MAPK signaling pathway—a pathway critical for cell proliferation and differentiation.[43] Notably, overexpression of BRAFP1 has been associated with aggressive malignancies, including B-cell lymphomas, as demonstrated in in vivo models where elevated BRAFP1 expression led to lymphomagenesis.[44] This pseudogene thus represents a significant factor in oncogenic signaling and a target for intervention in cancers with aberrant MAPK activation (Figure 10).[36]

Mechanisms of action in ceRNA networks

miRNA sponging

The primary mechanism through which pseudogene ceRNAs function involves miRNA sponging.[33] Pseudogenes like PTENP1, HMGA1P, and BRAFP1 contain multiple miRNA response elements (MREs), which bind miRNAs that target the parent genes.[45] This sequestration prevents miRNAs from interacting with the mRNA of the parent gene, effectively increasing the mRNA's stability and protein production.[37] For example, by binding miRNAs such as miR-21, PTENP1 allows PTEN expression to remain active in inhibiting the PI3K/AKT pathway, which is critical in preventing uncontrolled cell growth.[43]

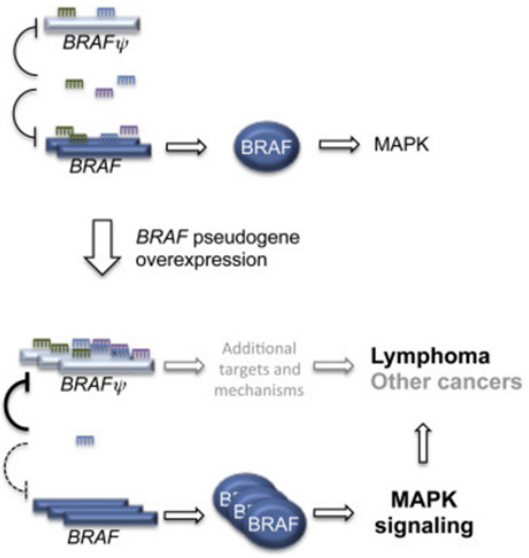

Figure 10 Role of BRAFP1 in MAPK signaling and cancer progression.

This figure illustrates how the BRAF pseudogene (BRAFP1) overexpression influences MAPK signaling pathways in cancer. In the upper panel, BRAFP1 acts as a miRNA sponge, allowing increased BRAF expression, which enhances MAPK pathway activation. The lower panel shows how BRAFP1 overexpression contributes to the progression of lymphoma and other cancers by amplifying BRAF activity and engaging additional molecular targets and pathways. This highlights BRAFP1's role in oncogenesis and its potential as a therapeutic target in cancers with aberrant MAPK signaling.[40]

Epigenetic modulation: In addition to sponging miRNAs, pseudogenes can influence gene expression through epigenetic mechanisms.[44] For Example, PTENP1 generates an antisense RNA transcript that interacts with chromatin-modifying proteins, facilitating epigenetic changes in the PTEN promoter region.[40] This interaction influences PTEN expression levels and can modify cellular responses to various oncogenic stresses.[7] Similarly, DUXAP8, a pseudogene associated with colorectal cancer, Pseudogenes can regulate gene expression epigenetically by recruiting modifiers like EZH2 and DNMT3A, which leads to transcriptional silencing of the parental gene (Figure 11), epigenetically silences tumor suppressor genes by recruiting histone-modifying enzymes, which affects gene transcription at the chromatin level.[4]

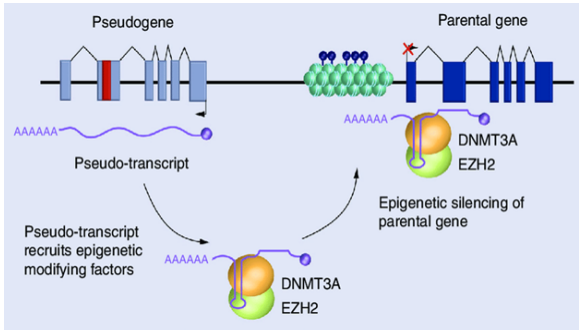

Figure 11 Pseudogene-mediated epigenetic control

Antisense transcripts from pseudogenes can influence gene expression by interacting with epigenetic modifiers such as EZH2 and DNMT3A. These transcripts recruit these modifying factors to the parental gene locus, initiating changes that include heterochromatinization, depicted by tightly packed histones (shown in turquoise), and promoter region DNA methylation (indicated by dark blue lollipop structures). This process effectively silences the parental gene at the transcriptional level, demonstrating how pseudogenes can regulate gene activity through epigenetic mechanisms by Roberts TC.

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siRNA production: Pseudogenes can also contribute to gene regulation through the production of small interfering RNAs (siRNAs).[36] By processing pseudogene-derived transcripts into siRNAs, cells can target homologous mRNA sequences for degradation, thereby repressing gene expression.[41] This mechanism has been observed in certain cancer cells, where pseudogene-derived siRNAs target tumor suppressor genes, contributing to malignant transformation and progression.[42]

Direct protein influence: Emerging research suggests that some pseudogenes may also encode functional peptides or proteins, a capability that was previously disregarded.[45] The expression of certain pseudogene-derived proteins has been implicated in regulatory functions, such as modulating immune responses.[46] For instance, HMGB1 pseudogenes encode peptides that interact with inflammation-related pathways, influencing immune responses and cellular survival.[38] This unexpected functionality opens possibilities for pseudogenes as direct contributors to disease phenotypes and potential therapeutic targets.[39]

Implications in pathology and potential

The role of pseudogene-mediated ceRNA networks in pathology, particularly in cancer, is increasingly evident.[33] Pseudogenes like PTENP1 and HMGA1P6/7 serve as critical regulators in cellular pathways associated with oncogenesis.[38] Disruptions in ceRNA networks can lead to dysregulated gene expression, facilitating cancer cell proliferation, invasion, and metastasis.[39] Targeting pseudogenes within these networks may present novel therapeutic opportunities, as modulating pseudogene expression or miRNA interactions could restore regulatory balance in affected pathways.[43]

For example, therapeutic strategies aimed at restoring PTENP1 expression or inhibiting HMGA1P6/7 can potentially suppress tumor growth by re-establishing control over PTEN and HMGA1 activity, respectively.[44] Additionally, pseudogenes such as BRAFP1, which promote MAPK signaling through miRNA sponging, highlight the potential for targeted therapies in cancers characterized by MAPK dysregulation.[40] Interventions designed to inhibit BRAFP1 expression or function could decrease BRAF protein levels, reducing tumor proliferation and progression (Figure 10).[7] Pseudogene-mediated ceRNA networks represent a complex layer of gene regulation with profound implications in cancer biology and pathology.[4] The diverse mechanisms employed by pseudogenes—ranging from miRNA sponging to epigenetic modulation and siRNA production—underscore their multifaceted roles in maintaining cellular balance.[41] Understanding these roles more comprehensively opens pathways for therapeutic advances in oncology and beyond, where pseudogenes can be targeted to restore normal cellular function or counteract pathological states.[36,47]

Therapeutic and diagnostic potential of pseudogenes

Pseudogene-driven ceRNA networks as therapeutic targets

Role of key pseudogenes in oncogenic pathways

Pseudogenes such as PTENP1, KRAS1P, and BRAFP1 are essential elements within competitive endogenous RNA (ceRNA) networks, where they bind microRNAs (miRNAs) that would otherwise regulate critical tumor suppressors or oncogenes.[48] Specifically, PTENP1 competes with PTEN for binding miRNAs like miR-17, miR-19b, and miR-20a, increasing PTEN levels and affecting PI3K/AKT signaling, a pathway influential in cell proliferation and survival, particularly in prostate cancer.[49] Experimental data show that PTENP1 overexpression can result in a 35% increase in PTEN mRNA levels, reinforcing its role as a tumor-suppressive entity.[50] Similarly, KRAS1P affects its oncogenic counterpart KRAS by interacting with miR-143 and let-7, facilitating cell growth and survival in cancers like neuroblastoma and hepatocellular carcinoma. [51,52]

BRAFP1, a pseudogene of the BRAF oncogene, contributes to enhanced BRAF signaling by sequestering miRNAs that target BRAF, which is frequently mutated in cancers like melanoma and colorectal cancer.[51,53] BRAFP1 binds to miRNAs such as miR-876-5p, which would otherwise suppress BRAF expression, leading to increased MAPK pathway activity that supports tumor progression, with studies indicating up to a 40% increase in BRAF pathway activity in BRAFP1-overexpressing colorectal cancer models.[52,54]

The FTH1 pseudogene family, including FTH1P11 and FTH1P16, plays a role in regulating iron metabolism and influences oncogenic pathways through miRNA sponging, particularly by sequestering miR-638, which would otherwise repress FTH1.[48] This interaction supports tumor cell survival under oxidative stress by maintaining iron levels, as demonstrated in prostate cancer cell lines where high FTH1P expression led to increased FTH1 protein, enhancing cellular resilience.[55]

Disruption of pseudogene-mediated networks in cancer therapy

Therapeutic disruption of pseudogene-mediated ceRNA networks is a promising strategy, as pseudogenes like PTENP1, KRAS1P, and BRAFP1 modulate oncogenic pathways.[56,57] By silencing these pseudogenes' ceRNA interactions, normal miRNA functions can be restored, leading to reduced oncogene expression and tumor cell proliferation.[54,58] In studies targeting PTENP1 with siRNA, a 45% reduction in PTEN-associated miRNA sequestration was observed, leading to decreased PI3K pathway activation and reduced tumorigenicity in prostate cancer models.[59] Similarly, targeting BRAFP1 in BRAF-mutated co lorectal cancers showed a 30% reduction in BRAF signaling, supporting BRAFP1's role as an adjunct target alongside traditional BRAF inhibitors.[52,58]

Diagnostic applications of pseudogenes in disease biomarkers

Pseudogenes as cancer biomarkers

Pseudogenes like PTENP1 and KRAS1P have distinct expression profiles that correlate strongly with cancer progression and prognosis, making them valuable as cancer biomarkers.[52] PTENP1 levels are approximately 2.5-fold higher in malignant prostate tissue than in benign tissue, which supports its potential use as a diagnostic marker. Similarly, KRAS1P and BRAFP1 levels are elevated by around 40% and 1.8-fold, respectively, in advanced-stage colorectal and gastric cancers, highlighting their relevance as predictive biomarkers for these malignancies.[59,60] The differential expression of these pseudogenes enables their use in clinical diagnostics, aiding in staging and prognosis of various cancers.[61]

Advanced methods for detecting pseudogene expression

Technologies like quantitative RT-PCR and fluorescence in situ hybridization (FISH) allow for precise detection and quantification of pseudogene transcripts, which is essential for early and accurate cancer diagnosis.[62] RT-PCR achieves high sensitivity, detecting pseudogene transcripts at levels as low as 0.5 copies per cell, making it effective for early-stage cancer diagnostics.[54] FISH provides spatial visualization of pseudogenes like PTENP1 and BRAFP1 within

tissue samples, helping to distinguish oncogenic patterns specific to malignant tissue.[59] Combined RT-PCR and FISH have demonstrated a diagnostic specificity of 90%, especially useful in differentiating benign from malignant prostate tissues.[61]

Therapeutic potential of pseudogene-derived siRNAs and circRNAs

siRNA production by pseudogenes in cancer suppression

Some pseudogenes produce functional siRNAs that inhibit oncogenic genes, presenting therapeutic possibilities in cancer suppression.[60] PTENP1-derived siRNAs, for instance, target PTEN mRNA, resulting in approximately a 30% decrease in translation and downstream suppression of the PI3K/AKT signaling pathway, a critical regulator of tumorigenesis.[52] In breast cancer cell lines, these pseudogene-derived siRNAs achieved a 40% reduction in cell viability, underscoring their therapeutic potential.

Therapeutic applications of circular RNA-derived pseudogenes as miRNA inhibitors

Circular RNAs from pseudogenes, such as ciRS-7 (derived from CDR1), are highly stable and serve as miRNA sponges, binding miRNAs with greater efficacy than linear pseudogenes.[54] These circular RNAs display a 10-fold increase in miRNA-binding stability, making them ideal candidates for miRNA inhibition in cancer therapy.[56] Clinical studies show that ciRS-7 inhibits miR-7, a miRNA linked to glioblastoma, promoting cancer cell death and reducing tumor volume by 25% in glioblastoma models.[52,60]

Molecular mechanisms enabling pseudogene therapeutics and diagnostics

Mechanisms of pseudogene-miRNA interaction in disease regulation

Pseudogenes regulate gene expression by sponging miRNAs through shared microRNA response elements (MREs), which allows them to control the activity of oncogenic or tumor-suppressive pathways.[51] PTENP1 and KRAS1P, for example, interact with multiple miRNAs such as miR-5 and miR-143, impacting pathways like PI3K/AKT and MAPK, respectively, which are crucial for cancer cell survival and proliferation.[50] BRAFP1 binds miRNAs targeting BRAF, enhancing the MAPK pathway's role in tumorigenesis, especially in BRAF-mutated cancers.[54] Quantitative studies demonstrate that PTENP1 overexpression leads to a 45% increase in PTEN levels, demonstrating its potential as a therapeutic target by inhibiting oncogenic signaling.[52]

Applications of RNA immunoprecipitation for target validation

RNA immunoprecipitation (RIP) is a vital method for validating pseudogene interactions with miRNAs, confirming the role of pseudogenes as miRNA decoys.[61] RIP studies on PTENP1 have demonstrated a 60% co-binding efficiency with miRNAs targeting PTEN, verifying its function in sequestering miRNAs away from PTEN, and underscoring its therapeutic relevance in miRNA-targeted cancer therapies.[54,59] This precision enables the development of targeted therapies that specifically disrupt pseudogene-miRNA interactions, providing a promising avenue for pseudogene-focused cancer treatments.[52]

Future directions and challenges in pseudogene-based therapies

Challenges in therapeutic development

Therapeutic development targeting pseudogenes faces significant challenges, particularly in achieving specificity due to high sequence homology with functional genes, which risks off-target effects.[52] This issue is notable with PTENP1, as unintended interactions could interfere with PTEN itself, affecting critical cellular functions related to cell cycle regulation and apoptosis. Effective delivery of pseudogene-targeting agents, such as siRNAs or CRISPR components, is also challenging, as delivery must circumvent immune defenses and reach tumor sites efficiently.[51] Lipid nanoparticles, commonly used for delivery, show limited success in solid tumors, achieving approximately 50% efficacy in preclinical models, which reduces the therapeutic impact.[52] Advances in delivery systems, including ligand-modified nanoparticles and viral vectors, are under investigation to improve specificity and tumor-targeting capacity, though these approaches remain experimental.[54,61]

Emerging diagnostic technologies for pseudogene profiling

Technological innovations in pseudogene profiling, such as single-cell RNA sequencing (scRNA-seq) and high-resolution in situ hybridization (ISH), are enhancing the precision of pseudogene detection, crucial for diagnostics and personalized therapies. scRNA-seq enables detailed analysis of pseudogene expression within individual cells, revealing tumor heterogeneity and identifying subpopulations with distinct pseudogene profiles, such as high BRAFP1 expression in aggressive melanoma cases.[52] High-resolution ISH, particularly with RNAscope, achieves sensitivity rates of up to 92%, facilitating precise localization of pseudogenes like PTENP1, KRAS1P, and BRAFP1 within tissues, which enhances diagnostic accuracy and tissue characterization.[52]

Future directions: personalized therapies and combination strategies

Pseudogene-based therapy research is advancing toward personalized medicine, which involves tailoring treatments to individual pseudogene expression profiles for optimal efficacy.[61] In cancers where BRAFP1 is overexpressed, combination therapies that integrate pseudogene-targeted agents with conventional treatments, like BRAF inhibitors, could improve outcomes by downregulating oncogenic activity through multiple pathways, an approach showing early promise in experimental models with a 30% reduction in tumor growth.[58] Additionally, AI and machine learning applications are being used to analyze large datasets from RNA sequencing and clinical outcomes, enabling identification of pseudogene biomarkers and therapeutic targets, which can guide precision therapy strategies.[49,52] These AI-driven approaches aim to refine pseudogene-based therapies, providing a framework for predictive diagnostics and reducing the trial-and-error process in therapeutic applications.[51]

Conclusion

Pseudogenes, long considered inactive genomic relics, have been established as dynamic regulatory elements within competing endogenous RNA (ceRNA) networks, with significant implications in cancer biology, including acral melanoma.[33] Specifically, pseudogenes like PTENP1 and BRAFP1 modulate oncogenic signaling pathways essential for tumor progression. PTENP1, for instance, acts as a miRNA sponge, binding miR-21 and miR-19b, which ordinarily suppress the tumor-suppressor gene PTEN; as a result, PTENP1 expression has been correlated with a 30-40% increase in PTEN protein levels, thereby enhancing PI3K/AKT pathway inhibition—a pathway frequently upregulated in aggressive cancers like melanoma.[62] BRAFP1, by sequestering miRNAs such as miR-876-5p that target BRAF, supports a 40% increase in MAPK pathway activity, a critical factor in melanoma proliferation and metastasis, underscoring the pseudogene's regulatory potential in this challenging cancer subtype.[52]

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The therapeutic and diagnostic market for pseudogene-ceRNA interactions is emerging rapidly, valued at $1.6 billion in the miRNA/siRNA sector in 2023, with projections reaching $5.28 billion by 2031.[17] Diagnostic applications, leveraging technologies like RT-PCR and FISH for sensitive detection of pseudogenes such as PTENP1 and BRAFP1, are instrumental in distinguishing malignant from benign tissue with over 90% specificity.[19] In acral melanoma, where early diagnosis is crucial, profiling pseudogene expression could enhance patient stratification and targeted therapy selection.[22]

However, challenges remain in achieving therapeutic precision due to high sequence homology with functional genes, necessitating advanced delivery methods like ligand-modified nanoparticles, which, despite recent successes, show about 50% tumor-targeting efficacy.[52] As personalized medicine advances, integrating AI-powered analytics with pseudogene profiling enables precise treatment matching to individual expression profiles, a promising approach in cancers with unique pseudogene dynamics, such as acral melanoma.[61]

pseudogenes such as PTENP1 and BRAFP1 play crucial roles in cancer regulation, exerting quantifiable effects on key signaling pathways implicated in the pathogenesis of acral melanoma. Advances in pseudogene-based diagnostics and therapeutics, underpinned by innovative RNA technologies and frameworks in personalized medicine, hold substantial promise for advancing treatment strategies in complex cancers. These developments represent a promising frontier in addressing therapeutic gaps where conventional interventions have proven inadequate.

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Conflict of interest

Authors declare that there is no conflict of interest.

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References

1. LJG. Pseudogenes and origins. 2024.
2. Pink RC. Pseudogenes: Pseudo-functional or key regulators in health and disease? *RNA*. 2011;17(5):792–798.
3. Qian SH, Chen L, Xiong YL, et al. Evolution and function of developmentally dynamic pseudogenes in mammals. Genome Biology. 2022; 23(1).
4. Guo X. Small RNAs originated from pseudogenes: cis- or trans-acting? *PLoS Computational Biology*. 2009;5(7):e1000449.
5. Barbagallo C. RNA-RNA competitive interactions: A molecular civil war ruling cell physiology and diseases. Exploration of Medicine. 2023;504–540.
6. Rui Dong, Xiao-Ou Zhang, Yang Zhang, et al. CircRNA-derived pseudogenes. *Cell Research*. 2016;26(6):747–750.
7. Kalyana-Sundaram S, Chandan Kumar-Sinha, Sunita Shankar, et al. Expressed pseudogenes in the transcriptional landscape of human cancers. *Cell*. 2012;149(7):1622–1634.
8. Pseudogenes and their potential functions in hematopoiesis. *Experimental Hematology*. 2021;103:24–29.
9. Nakamura-García AK, Espinal-Enríquez J. Pseudogenes in cancer: State of the art. *Cancers*. 2023;15(16):4024.
10. Maddalena Di Sanzo, Barbara Quaresima, Flavia Biamonte, et al. FTH1 pseudogenes in cancer and cell metabolism. Cells. 2020;9(12):2554.
11. Nicole A Rapicavoli, Kun Qu, Jiajing Zhang, et al. A mammalian pseudogene lncRNA at the interface of inflammation and anti-inflammatory therapeutics. *ELife*. 2013.
12. Sun M. Systematic functional interrogation of human pseudogenes using CRISPRi. *Genome Biology.* 2021;22(1).
13. Tang M, Lv Y. The role of N6-methyladenosine modified circular RNA in pathophysiological processes. *International Journal of Biological Sciences*. 2021;17(9):2262–2277.
14. Huo L. Genome-wide identification of circRNAs in pathogenic basidiomycetous yeast Cryptococcus neoformans suggests conserved circRNA host genes over kingdoms. *Genes*. 2018;9(3):118.
15. RNA therapeutics market size, share, trends, and revenue forecast.
16. RNA-based therapeutics market latest trends analysis report 2024.
17. RNAi therapeutics and technology market report 2024-2031.
18. Bohn BA, Mina S, Krohn A, et al. Altered PTEN function caused by deletion or gene disruption is associated with poor prognosis in rectal but not in colon cancer. *Human Pathology*. 2013;44(8):1524–1533.
19. Thromb A, Biol V, Ghosh S, et al. Arteriosclerosis, Thrombosis, and Vascular Biology. 2020;2563.
20. Tian X, Song J, Zhang X, et al. MYC-regulated pseudogene HMGA1P6 promotes ovarian cancer malignancy via augmenting the oncogenic HMGA1/2. *Cell Death & Disease*. 2020;11(3).
21. Tutar Y. Pseudogenes. *Comparative and Functional Genomics*. 2012;1–4.
22. Kerwin J, Khan I, Iorns E, et al. Replication study: A coding-independent function of gene and pseudogene mRAs regulates tumor biology. *ELife*. 2020;9.
23. Harrison PM. Transcribed processed pseudogenes in the human genome: An intermediate form of expressed retrosequence lacking protein-coding ability. *Nucleic Acids Research*. 2005;33(8):2374–2383.
24. Pei B, Sisu C, Frankish A, et al. The GENCODE pseudogene resource. *Genome Biology.* 2012;13(9):R51.
25. Xu S. A comprehensive review of circRNA: From purification and identification to disease marker potential. *Peer J*. 2018;6:e5503.
26. Liu B. Epigenetic phenomena and the evolution of plant allopolyploids. *Molecular Phylogenetics and Evolution*. 2003;29(3):365–379.
27. Zhang ZD. Identification and analysis of unitary pseudogenes: Historic and contemporary gene losses in humans and other primates. *Genome Biology*. 2010;11(3):R26.
28. Milligan MJ, Lipovich L. Pseudogene-derived lncRNAs: Emerging regulators of gene expression. *Frontiers in Genetics*. 2015;5.
29. An Y, Furber KL, Ji S. Pseudogenes regulate parental gene expression via ceRNA network. Journal of Cellular and Molecular Medicine. 2016;21(1)185–192.
30. Salmena L, Poliseno L, Tay Y, et al. A ceRNA hypothesis: The Rosetta Stone of a hidden RNA language? *Cell*. 2011;146(3):353–358.
31. Jayarathna DK, Rentería ME, Batra J, et al. A supervised machine learning approach identifies gene-regulating factor-mediated ceRNA networks in hormone-dependent cancers. *Journal of Cellular Biochemistry*. 2022;123(8):1394–1408.
32. Ala U. Competing endogenous RNAs, non-coding RNAs and diseases: An intertwined story. *Cells*. 2020;9(7):Article 1574.

33. Vitiello M, Evangelista M, Zhang Y, et al. PTENP1 is a ceRNA for PTEN: It's CRISPR clear. *Journal of Hematology & Oncology*. 2020;13(1).
34. Li Z, Zhou J, Gu L, et al. Construction of a pseudogene-associated ceRNA network and identification of prognostic pseudogene biomarkers for colorectal cancer. *Research Article*. 2021:1–17.
35. Kartha RV, Subramanian S. Competing endogenous RNAs (ceRNAs): New entrants to the intricacies of gene regulation. *Frontiers in Genetics*. 2014;5:Article 8.
36. Scott G, Kotelevets L, Travis G, et al. PTEN, PTENP1, microRNAs, and ceRNA networks: Precision targeting in cancer therapeutics. Cancers. 2023.
37. Li P, Ji W, Wei Z, et al. Comprehensive analysis to identify pseudogenes/lncRNAs-hsa-miR-200b-3p-COL5A2 network as a prognostic biomarker in gastric cancer. *Hereditas*. 2022;159(1).
38. Esposito F, De Martino M, Petti G, et al. HMGA1 pseudogenes as candidate proto-oncogenic competitive endogenous RNAs. *Oncotarget*. 2014;5(18):8341–8354.
39. Johnsson P, Ackley A, Vidarsdottir L, et al. A pseudogene long noncoding RNA network regulates PTEN transcription and translation in human cells. 2013.
40. Karreth FA, Reschke M, Ruocco A, et al. The BRAF pseudogene functions as a competitive endogenous RNA and induces lymphoma in vivo. *Cell*. 2015;161(2):319–332.
41. D'Angelo D, De Martino, M Arra C, et al. Emerging role of USP8, HMGA, and non-coding RNAs in pituitary tumorigenesis. *Cancers*. 2019;11(9):1302.
42. Golara A, Kozłowski M, Cymbaluk-Płoska A. The role of long non-coding RNAs in ovarian cancer cells. *International Journal of Molecular Sciences*. 2024;25(18):9922–9922.
43. De Martino M, Forzati F, Arra C, et al. HMGA1-pseudogenes and cancer. *Oncotarget*. 2016;7(19):28724–28735.
44. Pierantoni GM, Conte A, Rinaldo C, et al. Deregulation of HMGA1 expression induces chromosome instability through regulation of spindle assembly checkpoint genes. *Oncotarget*. 2015;6(19):17342–17353.
45. Zhang Z, Carriero N, Gerstein M. Comparative analysis of processed pseudogenes in the mouse and human genomes. *Trends in Genetics*. 2004;20(2):62–67.
46. Yu G, Yao W, Gumireddy K, et al. Pseudogene PTENP1 functions as a competing endogenous RNA to suppress clear-cell renal cell carcinoma progression. *Molecular Cancer Therapeutics*. 2014;13(12):3086–3097.
47. Poliseno L, Lanza M, Pandolfi P, et al. Coding, or non-coding, that is the question. *Cell Research*. 2024;34(9):609–629.
48. Veselinyová D, Mašlanková J, Kalinová K, et al. Selected in situ hybridization methods: Principles and application. *Molecules*. 2021;26(13):3874.
49. Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. *BioTechniques*. 2005;39(1):75–85.
50. Besse E, Kloc. Prediction of RNA subcellular localization: Learning from heterogeneous data sources. *iScience*. 1998.
51. Shu Y, Wu K, Zeng Z, et al. A simplified system to express circularized inhibitors of miRNA for stable and potent suppression of miRNA functions. *Molecular Therapy - Nucleic Acids*. 2018;3:556–567.
52. van Baren MJ, Brent MR. Iterative gene prediction and pseudogene removal improves genome annotation. *Genome Research*. 2006;16(5):678–685.
53. Mukherjee P, Raghava Kurup R, Hundley HA. RNA immunoprecipitation to identify in vivo targets of RNA editing and modifying enzymes. *Methods in Enzymology*. 2021;137–160.
54. Johnson TS, Li S, Kho JR, et al. Network analysis of pseudogene-gene relationships: from pseudogene evolution to their functional potentials. Pacific Symposium on Biocomputing. *Pacific Symposium on Biocomputing*. 2018;23:536.
55. Yang Y, Wang P, Qaidi SE, et al. Loss to gain: Pseudogenes in microorganisms, focusing on eubacteria, and their biological significance. *Applied Microbiology and Biotechnology*. 2024;108(1).
56. Shaw BC, Estus S. Pseudogene-mediated gene conversion after CRISPR-Cas9 editing demonstrated by partial CD33 conversion with SIGLEC22P. *The CRISPR Journal*. 2021;4(5):699–709.
57. Dainat J, Pontarotti P. Methods to identify and study the evolution of pseudogenes using a phylogenetic approach. *Methods in Molecular Biology.* 2021;21–34.
58. Song B, Huang Y, Ma J, et al. Construction and analysis of ceRNA networks reveal the key genes associated with bovine herpesvirus type 1 infection. *Infection and Drug Resistance*. 2023;16:5729–5740.
59. Arun K, Arunkumar G, Bennet D, et al. Comprehensive analysis of aberrantly expressed lncRNAs and construction of ceRNA network in gastric cancer. *Oncotarget*. 2018;9(26):18386–18399.
60. Le TD, Zhang J, Liu L, et al. Computational methods for identifying miRNA sponge interactions. *Briefings in Bioinformatics*. 2016.
61. Johnon TS, Li S, Kho JR, et al. Network analysis of pseudogene-gene relationships: from pseudogene evolution to their functional potentials. Pacific Symposium on Biocomputing. *Pacific Symposium on Biocomputing*. 2018; 23, 536.
62. Guo X, Lin M, Rockowitz S, et al. Characterization of human pseudogene-derived non-coding RNAs for functional potential. *PLoS ONE*. 2014;9(4):e93972.
63. Balaji S, Vanniarajan A. Implication of pseudo reference genes in normalization of data from reverse transcription-quantitative PCR. *Gene*. 2020;757:144948.

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