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Hidden Proteomes: A Systematic Review of Translatable circRNAs in Oncology

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Abstract. Circular RNAs (circRNAs) are covalently closed RNA molecules that were once considered transcriptional noise. Yet, in recent years, a variety of regulatory functions have been reported for circRNAs, and even more surprisingly, translation of circRNAs into microproteins has been demonstrated. This emerging field holds great promise for the field of oncology, since circRNA-encoded proteins have been increasingly implicated in processes such as proliferation, invasion, and metastasis of cancer. This review systematically summarizes the current literature on translatable circRNAs in cancer and outlines the experimental approaches commonly used for circRNA translation identification and validation. The translation of circRNAs is mediated via cap-independent mechanisms, mainly IRES and N6-methyladenosine (m^6A) modifications. The most commonly detected circRNAs and features of their encoded microproteins are being summarized. By synthesizing these findings, this review is intended to serve as a resource for researchers in the field and to encourage further investigation into the hidden proteome and its impact on cancer.

Keywords: Circular RNA, Cancer Proteogenomics, Internal Ribosome Entry Site, m^6A-mediated Translation

1. Introduction

Circular RNAs (circRNAs) are covalently closed RNA molecules generated by back-splicing, which were long considered as non-coding products of splicing noise [1]. Recent research has overturned this perception, revealing that many circRNAs are in fact coding and can be translated into functional microproteins. This translation occurs through cap-independent mechanisms driven by internal ribosome entry sites (IRES) or N6-methyladenosine (m^6A) modifications without requiring a 5’ cap [2, 3].

In the context of cancer, circRNAs have been shown not only to regulate gene expression and act as sponges for microRNAs but also to serve as hidden templates for novel peptides and microproteins. This review highlights the growing importance of circRNAs in the cancer proteome and lays the groundwork for investigating their translational products as potential biomarkers or therapeutic targets.

Based on the existing literature, this review represents one of the first systematic efforts to synthesize experimental evidence on translatable circRNAs in oncology. Specifically, this paper analyzes studies published between 2017 and 2025 that employed rigorous validation techniques such as mass spectrometry (MS) or ribosome profiling (Ribo-seq) to confirm the translation of circRNAs into microproteins in human cancer models. By aggregating these studies, this review aims to provide clarity on the current landscape of validated translatable circRNAs, as well as the methods used and cancer types, and identify common functional themes among their encoded peptides.

2. Methods

A comprehensive literature search was conducted across multiple electronic databases such as PubMed, Scopus, and Web of Science. An appropriate search strategy was used, combining Medical Subject Headings (MeSH) terms and free-text keywords like "circular RNA," "circRNA," "translation," "microprotein," "cancer," and "oncology" to extract all the related articles. The search terms were combined using operators ("AND," "OR") to maximize the sensitivity of the search. The specific search query used was as follows: ("circular RNA" OR "circRNA") AND (translation OR protein OR peptide OR microprotein) AND (cancer OR oncology OR tumor OR neoplasm). The reference lists of all included studies were manually screened to identify any additional studies that may have been missed by electronic search. The search was English language-specific, and the publication year was restricted from 2017 to 2025. To be included, the study should be an original research article with experimental evidence for the translation of at least one circRNA in a cancer scenario. The initial database search yielded 2200 records; 2133 were excluded based on title and abstract, and review articles, editorials, and non-experimental studies were excluded. The full text of the remaining 67 articles was assessed for eligibility, and 52 of these were excluded for various reasons, such as not providing experimental evidence for circRNA translation or not being related to cancer. Ultimately, 15 studies met the inclusion criteria and were included in the qualitative synthesis of this systematic review. After inclusion, data from the selected studies were extracted, including translatable circRNAs, cancer type, validation methods, and the biological functions of the encoded microproteins.

3. Overview of experimental techniques

Like other non-canonical mRNAs, providing definitive proof of circRNA translation is challenging. A usual mRNA has a conserved 5' cap and a 3' polyA tail, which are essential for the initiation of translation. To unambiguously show that a circRNA is translated into a protein, a combination of specialized experimental approaches is needed. This section will present the main experimental approaches used to identify and validate circRNA translation, which includes mass spectrometry-based proteomics, ribosome profiling, and other experimental approaches used as supportive experiments, such as polysome profiling, reporter assays, and Western blot.

Mass spectrometry (MS)-based proteomics is widely regarded as the gold standard for demonstrating protein expression. It detects peptides within complex protein mixtures and infers their amino acid sequences from mass-to-charge and fragmentation patterns, often through database searches [4]. For circRNAs, the power of this technique resides in the ability to detect a peptide that is uniquely spanned across the back-splice junction (BSJ) [5]. Because this sequence is uniquely expressed by the circRNA, detection of this peptide by MS provides strong proof of translation. Yet, proteins derived from circRNAs may be expressed at extremely low levels, making their detection a challenging technical hurdle requiring sensitive instruments and custom protein sequence databases.

On a larger scale, ribosome profiling (Ribo-seq) has been used to discover translatable circRNAs. This high-throughput sequencing method maps the position of all ribosomes translating RNA molecules in a cell. A large number of ribosome footprints on a circRNA suggest that the circRNA is being translated. Ribo-seq can be used genome-wide to screen thousands of potential candidates on a global scale [6]. While ribosome association strongly suggests production of a stable protein, the exact existence cannot be confirmed by this technique.

Apart from MS and Ribo-seq, there are several other experimental approaches that can be used to provide supporting evidence for circRNA translation. These methods, although not as conclusive as MS nor as high-throughput as Ribo-seq, can be used to validate individual circRNAs.

Polysome profiling can be used to separate ribosomes and their mRNAs based on their size. Actively transcribed mRNAs are able to recruit multiple ribosomes and form large complexes known as polysomes. By separating cell lysates on a sucrose density gradient, the polysome fraction can be isolated, and the mRNAs associated with it can be identified. The presence of a specific circRNA in the polysome fraction suggests that it is being actively translated [7]. Polysome profiling is frequently used in conjunction with RT-PCR or Northern blotting to enrich for the circRNA of interest in the polysome fraction.

Reporter assays are frequently used to validate the translation of circRNAs. In a typical reporter assay, the circRNA of interest (or part of it containing the putative ORF) is cloned into a reporter vector such as a luciferase or GFP vector. The construct is then transfected into cells, and the expression of the reporter protein is measured [8]. The presence of a reporter signal provides evidence that the circRNA sequence can be translated.

Western blotting is a commonly used technique for the detection and quantification of specific proteins. If an antibody is available that can specifically recognize the circRNA-encoded protein, a western blot can be performed to provide direct evidence for its existence [3].

Table 1. The overview and advantages of experimental techniques

Technique	Key Advantages
Mass spectrometry	Direct protein evidence; BSJ-spanning peptide detection; high specificity and quantitative capability
Ribosome profiling (Ribo-seq)	Genome-wide detection of ribosome engagement; reveals translation frames and periodicity; high-throughput candidate discovery.
Polysome profiling	Enriches actively translated transcripts; simple biochemical separation of translating mRNAs.
Reporter assays (GFP / luciferase)	Direct functional test of translational capacity; easy manipulation for mutational/mechanistic studies.
Western blot / Antibody detection	Direct protein-level visualization; provides size and abundance information; widely accessible and low-cost.

A combination of these experimental techniques (see Table 1) is typically used to provide supporting evidence for the translation of a circRNA. MS provides the most direct and conclusive evidence, while Ribo-seq provides a global view of the translatome. Other methods like polysome profiling, reporter assays, and western blotting provide supporting evidence.

4. Landscape of validated translatable circRNAs in cancer

The study of the translatable capacity of circRNAs is rapidly extending across several oncology fields. The evidence, gathered from several types of cancer, suggests that there is another layer of proteomic regulation that was previously unknown. This section will present the comprehensive landscape of validated translatable circRNAs in cancer, including identifying the specific types of cancer, commonly reported translatable circRNAs, characterizing the features of microproteins encoded by these circRNAs, and summarizing their diverse biological functions.

4.1. Type of cancers

The study of translatable circRNAs was not limited to any single malignancy but involved a large variety of cancer types, reflecting the broad expression and possible importance of circRNAs in cancer. The most frequently studied cancers were glioblastoma (GBM), hepatocellular carcinoma (HCC), and lung cancer.

Glioblastoma: GBM is the most highly malignant and treatment-resistant brain tumor. Researchers found that translatable circRNAs could provide new insights into the pathogenesis of GBM and offer potential targets for GBM therapy. So far, circ-SHPRH is the best characterized translatable circ-RNA in GBM, which was translated into a 146-amino acid protein (SHPRH-146aa). This microprotein acts as a potent tumor suppressor, and its expression was markedly downregulated in GBM tissues compared with that in normal brain tissues [9]. This study revealed that loss of expression of a circRNA-encoded microprotein could be an important event during tumorigenesis.

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Multiple circRNAs that are translated into functional peptides involved in HCC progression have been reported. For example, circβ-catenin was translated into a novel 370-amino acid isoform of β-catenin, which enhanced the proliferation and invasion of HCC cells by activating the Wnt signaling pathway [10]. This finding is the first report of a functional variant of a well-known cancer protein produced by a circRNA.

Lung cancer includes both Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC). Multiple translatable circRNAs involved in the pathogenesis of lung cancer have been reported. For example, circPTPRA was translated into a 173-amino acid protein (PTPRA-173aa), which promoted NSCLC progression by serving as a decoy for RFN20/40, an E3 ubiquitin ligase, to block the degradation of c-Myc [11].

Across these varied cancer types, the process of circRNA translation is not a rare anomaly but a broadly widespread mechanism that cancer cells can exploit to acquire malignant phenotypes, with the identity of the specific circRNAs and proteins often being highly context-dependent, varying with the tissue of origin and specific molecular subtype of cancer.

4.2. Identified circRNAs

There are hundreds of circRNA predicted to be translatable, and an increasing number have been validated using a combination of techniques such as ribosome profiling, mass spectrometry, and functional assays. A few of these have become recurring players in multiple cancer studies.

The most well-characterized translatable circRNAs so far may be circ-SHPRH. As discussed above, its product, SHPRH-146aa, behaves as a tumor suppressor in glioblastoma. The product protects the full-length SHPRH protein, an E3 ubiquitin ligase, from proteasomal degradation via an interacting ubiquitin-proteasome system protein. This protective interaction, in turn, allows the cell to repair DNA damage and maintain genome stability [9]. The downregulation of circ-SHPRH found in GBM leads to a loss of this protective interaction and likely contributes to the genomic instability observed in this tumor.

Another frequently cited translatable circRNA is circ-FBXW7. circ-FBXW7 is derived from the well-characterized tumor suppressor gene FBXW7. It encodes for a novel 185-amino acid protein referred to as FBXW7-185aa. This microprotein behaves as a potent tumor suppressor by inducing G1 phase cell cycle arrest. It does this by binding and stabilizing the full-length FBXW7 protein, which in turn leads to the ubiquitination and proteasomal degradation of c-Myc, a potent oncoprotein. circ-FBXW7 is often downregulated in various cancers, and loss of circ-FBXW7 is associated with increased tumor growth and proliferation [12].

circ-LINC-PINT is a tumor-suppressive translatable circRNA identified in glioblastoma. It encodes for an 87-amino acid peptide that behaves as a tumor suppressor by binding and inhibiting the transcription of the PAF1 transcription elongation complex. This leads to a suppression in the transcriptional elongation of multiple oncogenes, including c-Myc and genes involved in glycolysis [13].

These examples show a consistent trend where translatable circRNAs, in many cases derived from tumor suppressor genes, give rise to microproteins that augment the function of their linear parent or behave as tumor suppressors through novel mechanisms to inhibit cancer progression.

5. Mechanistic insights

Recent discoveries that circRNAs can be translated into functional microproteins pose several questions about the molecular machinery governing this non-canonical translation event. Unlike the well-conserved, cap-dependent translation of linear mRNAs, circRNA translation requires distinct mechanisms for ribosomes and initiation of protein synthesis. These are not only of interest to merely validate the translation of new circRNA candidates but also to understand how circRNA translation is regulated and dysregulated in cancer. This section discusses current mechanistic insights, with a focus on cap-independent initiation.

CircRNAs lack a 5' cap structure and therefore rely on cap-independent mechanisms to initiate translation. Two widely recognized mechanisms are Internal Ribosome Entry Sites (IRES) and the mediation by N^6-methyladenosine (m^6A) RNA modifications.

IRES are structured RNA sequences that can recruit the 40S ribosomal subunit to an internal position on an RNA, bypassing the need for the 5' cap and the canonical eIF4F scanning complex. The ability of a circRNA to drive translation is strongly dependent on the presence of a functional IRES in its sequence [2]. These elements are defined not by a simple consensus sequence but by their ability to fold into a specific and complex three-dimensional structure that is recognized by the ribosome and a number of trans-acting factors. Importantly, IRES-mediated translation has been reported to contribute to the production of oncogenic proteins in certain cancers. For example, the translation of circ-SHPRH in glioblastoma and circ-FBXW7 in various cancers may be driven by IRES elements. The activity of IRESs can be modulated by cellular stress conditions, which are commonly found in the tumor microenvironment. In general, conditions such as hypoxia or nutrient starvation tend to globally suppress cap-dependent translation efficiency, which may provide a selective advantage for cancer cells that can use IRES-mediated translation to produce several key pro-survival proteins.

m^6A modification is the most abundant internal modification in eukaryotic mRNA and plays a major role in regulating RNA fate, such as translation. In circRNAs, m^6A has been reported to be a major driver of cap-independent translation. The process involves writing m^6A marks on cirRNAs via METTL3-METTL14 complexes and “reading” these marks by specific m^6A-binding proteins (e.g., YTHDF3). Then the subsequent recruitment of translation initiation factors such as eIF4G2/DAP5 and the 40S ribosomal subunit initiates protein synthesis [3]. This m^6A-driven pathway has been found to be responsible for the translation of a large proportion of circRNAs in cancer cells. m^6A and circRNAs are a two-way street: circRNAs not only drive the translation of a subset of circRNAs but can also affect the overall m^6A landscape of a cell, for instance, by sponging m^6A reader proteins. The dysregulation of m^6A writers, erasers, and readers is frequently observed in many types of cancer, which can lead to the aberrant translation of circRNAs and promote the malignant phenotype.

The two mechanisms, IRES and m^6A, are not mutually exclusive and may even cooperate to promote the translation of some circRNAs. The presence of either of these two features is a good indicator that the circRNA is translatable. Increasingly, bioinformatic tools are being developed to predict the presence of these two features in circRNAs.

6. Limitation and challenges

Despite significant progress in the study of translatable circRNAs, several limitations and challenges persist across nearly every stage of the research pipeline, from initial detection and validation of translation to characterization of the resulting microproteins and evaluation of their clinical relevance. Addressing these challenges is essential for the field to mature and for circRNA-based diagnostics and therapeutics to reach their full potential. This section highlights the technical limitations and problems associated with data analysis.

Validation of circRNA translation is technically challenging, and each currently employed approach has inherent limitations that may generate false positives or negatives, complicating the identification of bona fide translated circRNAs.

MS, often considered the gold standard for protein detection, faces specific difficulties in circRNA research. The main challenge is that the novel proteins derived from circRNAs are often of very low abundance relative to the canonical proteome [14]. Thus, the novel proteins are difficult to detect, requiring highly sensitive instrumentation, large sample fractionation ranges, and special enrichment approaches. Peptide identification relies on searching the spectral data against a protein sequence database. For circRNAs, this necessitates the generation of a custom protein database containing predicted sequences of all potential microproteins, especially those spanning the back-splice junction. The correctness of the database is constrained by the quality of circRNA annotation and ORF prediction; inaccurate or incomplete databases may lead to missed identifications. Ribo-seq is a powerful tool for identifying ribosome-associated RNAs on a global scale. However, the presence of ribosome footprints on a circRNA does not guarantee that a full-length, stable, and functional protein will be produced [14]. Translation may be initiated but then stall or terminate prematurely, or the resulting peptide may be rapidly degraded.

These limitations, especially those related to the correctness and size of the databases, are alleviated by the advent of new technologies and methods that will enable the generation of proteomic data much more efficiently. For example, by integrating ribosome profiling (Ribo-seq) evidence with stringent bioinformatic ORF prediction and sample-specific expression thresholds, it can be used to prioritize candidate circRNAs. This substantially reduces the size of custom protein FASTA databases for MS searches and improves sequence accuracy. A further approach can be combining CRISPR/Cas-based functional validation with targeted mass spectrometry or other targeted assays to confirm biological relevance and lower false-positive rates [15].

7. Conclusion

The discovery that a substantial fraction of circRNAs is translated into functional proteins has dramatically changed our view of the coding potential of the human genome. The once-dismissed transcriptomic noise is now regarded as a previously unrecognized source of a vast proteome with enormous implications for cancer biology. This systematic review collates the current knowledge in this dynamic field of research.

The evidence supports the notion that the microproteins encoded by circRNAs are not infrequent exceptions but are potent oncoproteins or tumor suppressors. They play crucial roles in tumorigenesis and act through all the hallmarks of cancer, from cell proliferation and survival to the regulation of the tumor microenvironment and immune response. The non-canonical, cap-independent mechanism of their translation opens new insights into the adaptability of cancer cells and provides new targets that may be therapeutically exploited. The novel sequences generated by the circRNA back-splicing junction not only confer new functions to the encoded proteins but also represent a potential source of tumor-specific neoantigens for the development of next-generation cancer immunotherapies.

Despite the exciting progress made, the field is challenged by a number of technical and conceptual difficulties, including the intrinsic challenges in detection, the need for standardization of annotation, and the inherent biases in the current studies. However, the path forward is clear, with the advent of innovative technologies and ambitious goals. The sophisticated computational tools developed, advances in proteomics and ribosome profiling, and the application of powerful functional genomics technologies, such as CRISPR screening, will certainly continue the trend of accelerated discovery. The ambitious goal of generating a circRNA-Encoded Cancer Microproteome Atlas would certainly provide an indescribable resource for years to come.

In summary, the study of translatable circRNAs has paved the way for a new and exciting era in cancer research. It has called for a more integrative approach to cancer biology, one that integrates genomics, transcriptomics, and proteomics to study the complete complexity of gene expression. A continued and intensified investigation of this hidden proteome is warranted. It would be beneficial if this hidden proteome would reveal new biological mechanisms, a new class of biomarkers, and ultimately, more effective therapies for cancer patients. The age of the hidden proteome has begun, and it is only now starting to reveal itself.

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