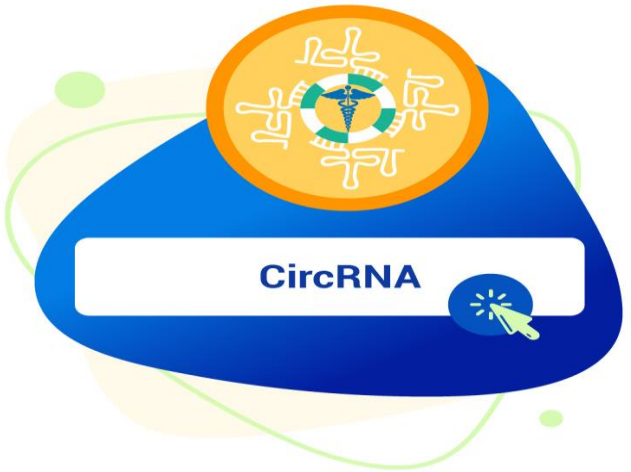
Presentation Title

Applications of Circular RNAs in Disease Diagnosis and Therapy via Signaling Pathways



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Introduction to RNAs

What is RNA?

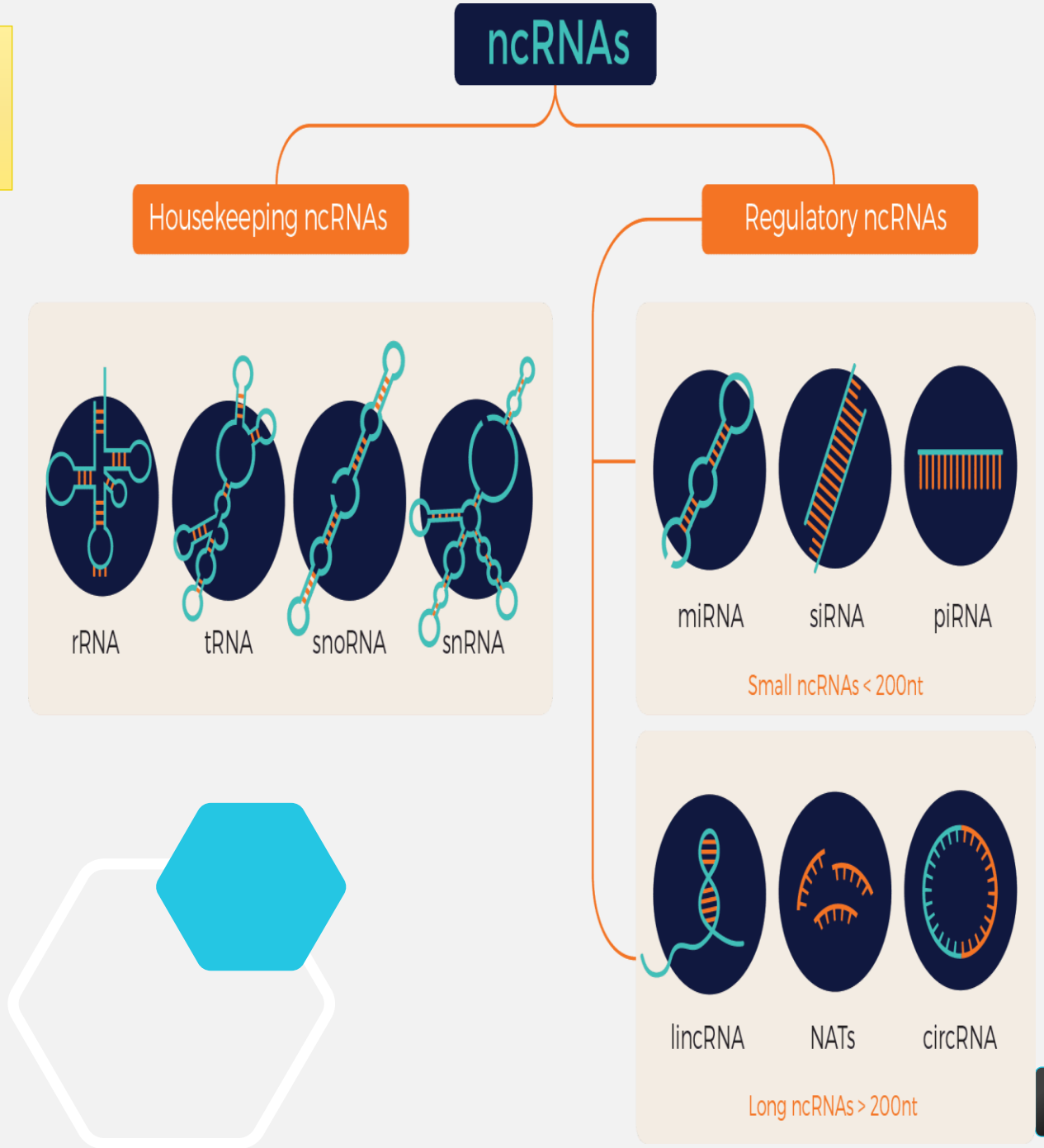
- RNA (Ribonucleic Acid) is a fundamental biomolecule essential for life, involved in protein synthesis, gene regulation, and cellular signaling.
- It is typically a single-stranded molecule composed of nucleotides that include a ribose sugar, phosphate group, and nitrogenous bases (Adenine, Uracil, Cytosine, Guanine).

Structural Features of RNA

- ✓ Unlike DNA, RNA contains the sugar ribose instead of deoxyribose, which has one more oxygen atom making RNA more reactive.
- ✓ RNA uses Uracil (U) instead of Thymine (T) found in DNA.
- ✓ Its single-stranded nature allows RNA to fold into complex three-dimensional shapes necessary for diverse biological functions.

Types of RNA and Functions

1. Messenger RNA (mRNA): Carries genetic code from DNA to ribosomes for protein synthesis.
2. Transfer RNA (tRNA): Delivers specific amino acids during protein synthesis.
3. Ribosomal RNA (rRNA): Structural and catalytic component of ribosomes.
4. Non-coding RNAs (ncRNAs): Includes microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) that regulate gene expression and cellular processes.



Introduction to Circular RNAs (circRNAs)

Definition of circRNAs:

- Circular RNAs (circRNAs) are a type of non-coding RNA characterized by a covalently closed continuous loop structure.
- Unlike linear RNAs, circRNAs have no free 5′ cap or 3′ poly-A tail, which makes them resistant to exonuclease degradation, granting them enhanced stability in the cell.
- They are generated mainly from back-splicing events of precursor mRNA (pre-mRNA), where a downstream splice donor site is joined to an upstream splice acceptor.

Structural Features:

Circular structure: circRNAs form a closed loop through covalent bonding between their 3′ and 5′ ends.

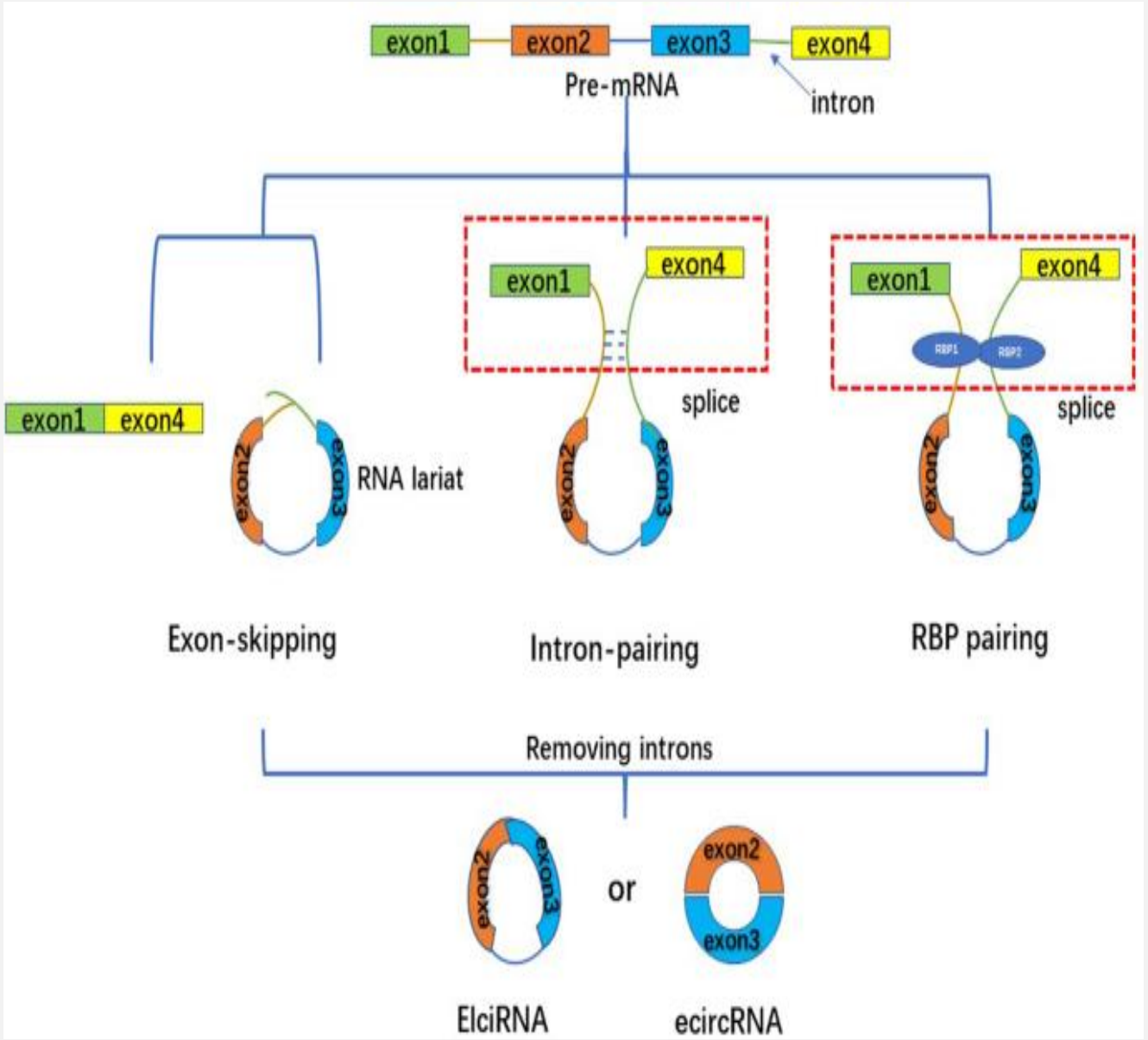
No free ends: This unique structure prevents them from typical degradation pathways that affect linear RNAs.

Size: They vary in length, mostly originating from exons but can also derive from introns or both.

Table

Differences Between circRNAs and Linear RNAs

Feature	Linear RNA	Circular RNA (circRNA)
Structure	Linear strand with 5′ cap and 3′ poly-A tail	Covalently closed circular molecule, no ends
Stability	Less stable, prone to exonuclease degradation	Highly stable, resistant to exonucleases
Biogenesis	Canonical splicing	Back-splicing of pre-mRNA
Functional Roles	Protein coding (mRNA), regulatory (miRNA, lncRNA)	Mainly gene regulation and miRNA sponging



Types of circRNAs

I. Exonic circRNAs (EcircRNAs): Consist solely of exons and are the most common type. They mainly localize in the cytoplasm and regulate gene expression post-transcriptionally.

II. Intronic circRNAs (ciRNAs): Derived from introns; are usually located in the nucleus and may regulate transcription.

III. Exon-intron circRNAs (EIciRNAs): Contain both exon and intron sequences, often involved in regulating gene expression at the transcriptional level.

Biosynthesis of Circular RNAs (circRNAs)

Physical and Biological Features of circRNAs

High Stability

circRNAs have a unique covalently closed loop structure without free 5′ or 3′ ends, making them highly stable compared to linear RNAs.

This structure protects them from degradation by exonucleases, enzymes that typically degrade linear RNAs.

Resistance to Exonucleases

Due to the lack of free ends, circRNAs are resistant to exonucleolytic degradation, resulting in a longer half-life inside cells.

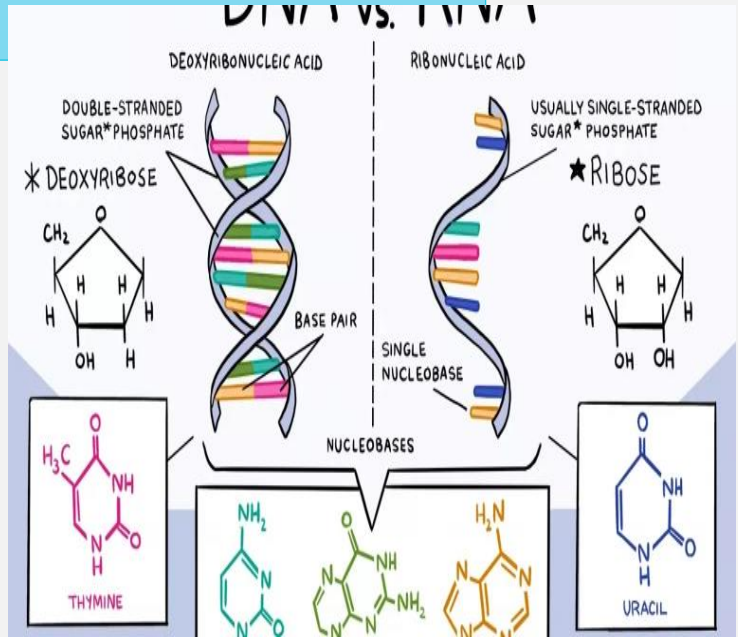
This resistance makes circRNAs reliable molecules for sustained regulatory roles in the cell.

Tissue and Cellular Distribution

circRNAs are expressed in a tissue-specific and cell-type-specific manner.

They are found in various tissues and often accumulate in the cytoplasm but some types reside in the nucleus.

Their specific patterns suggest specialized roles in cellular processes and gene regulation.



Biological Functions of Circular RNAs (circRNAs)

1. Role as miRNA Sponges

- circRNAs can act as "miRNA sponges" by binding and sequestering microRNAs (miRNAs), preventing miRNAs from interacting with their target mRNAs.
- This reduces miRNA-mediated repression, leading to increased expression of miRNA target genes.
- Example: ciRS-7 (CDR1as) contains over 70 binding sites for miR-7 and regulates its availability, influencing brain development and disease.
- The sponge function makes circRNAs crucial regulators in diverse biological and pathological processes like cancer progression and neurological diseases.

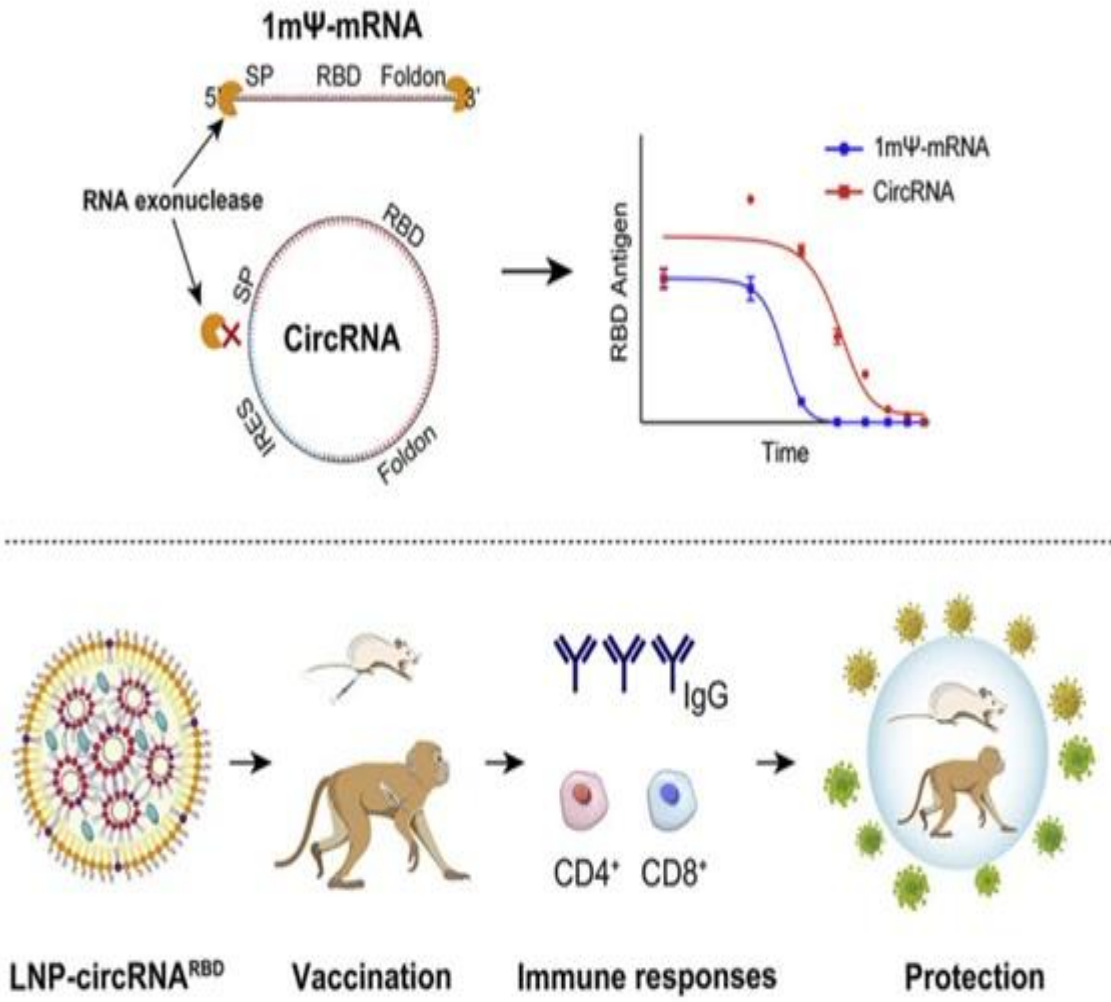
2. Interaction with RNA-Binding Proteins (RBPs)

- Diagram illustrating circRNA binding miRNAs (sponge mechanism), preventing miRNA repression of target mRNA.
- Schematic showing circRNA interaction with RBPs influencing gene regulation.
- Flowchart connecting circRNA functions to overall gene expression regulation.

Biological Functions of Circular RNAs (circRNAs)

3. Regulation of Translation and Gene Expression

- circRNAs can compete with linear mRNA for splicing machinery, indirectly affecting gene expression.
- Some circRNAs may also interact with ribosomes or translation factors, modulating protein synthesis.
- They play roles in fine-tuning cellular functions by impacting RNA stability and translational control.



Signaling Pathways Related to circRNAs

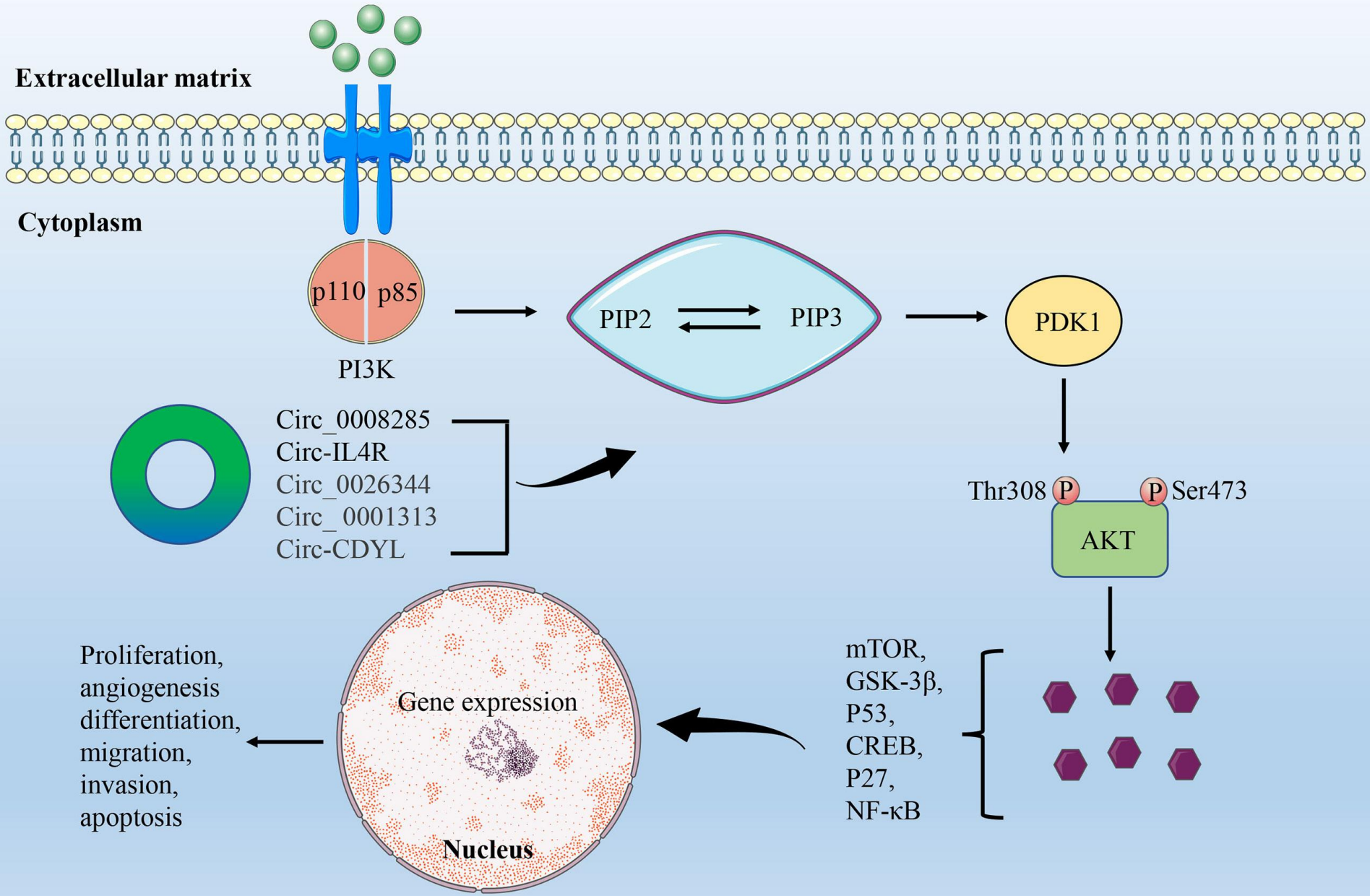
Signaling pathways related to circular RNAs (circRNAs) include their regulatory roles in critical cell signaling mechanisms such as PI3K/Akt, MAPK/ERK, Wnt/β-catenin, and Notch pathways. These pathways are involved in cancer progression, cell proliferation, apoptosis, inflammation, and other cellular processes.

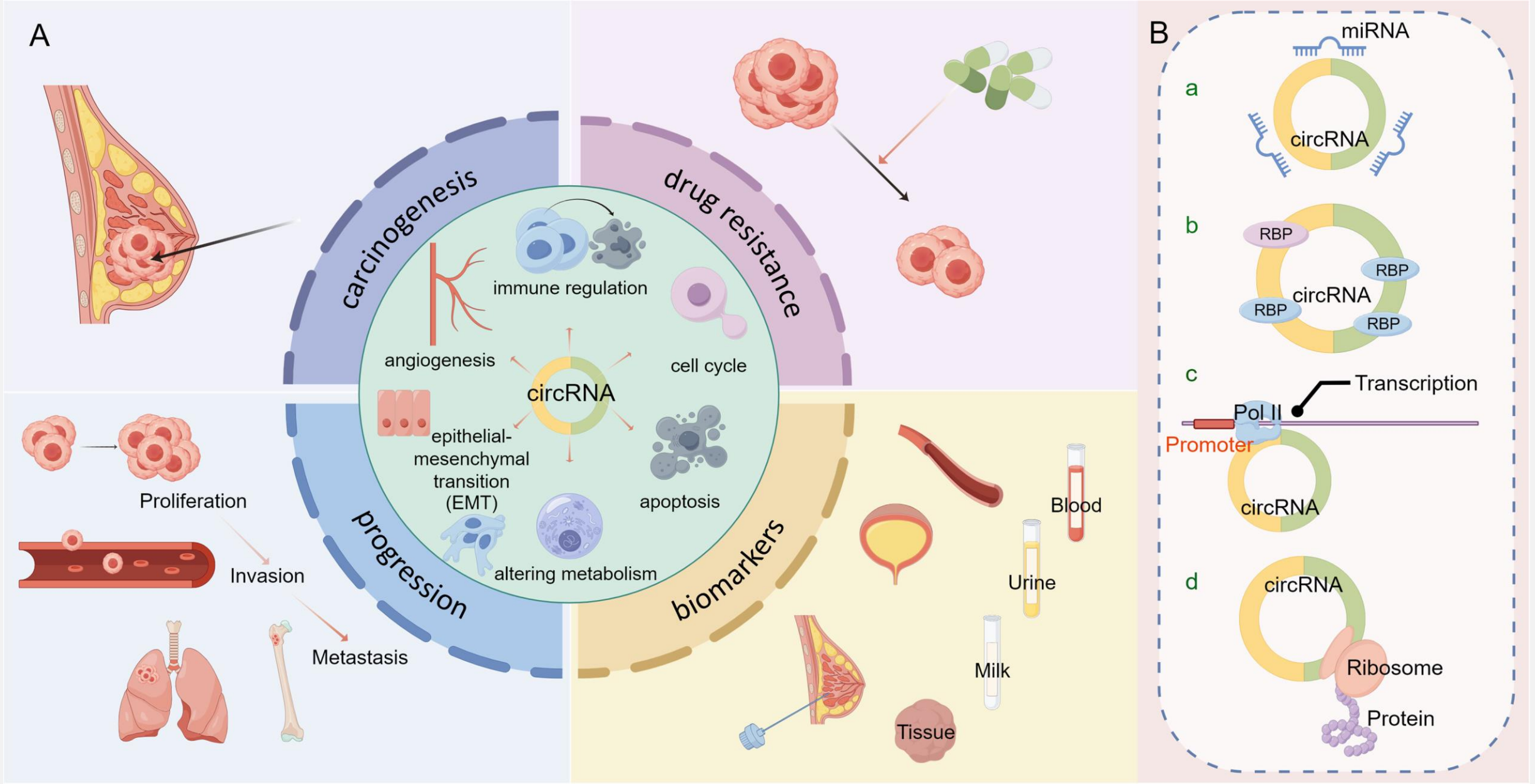
Mechanism:

- ✓ circRNAs regulate signaling pathways principally by acting as sponges for microRNAs (miRNAs), preventing miRNAs from repressing their target mRNAs, thereby modulating gene expression.
- ✓ They also interact with RNA-binding proteins (RBPs) that affect signal transduction protein stability and activity.
- ✓ Some circRNAs directly bind signaling molecules or transcription factors to alter pathway activity.

Example pathways impacted by circRNAs:

- PI3K/Akt Pathway: circRNAs can promote or suppress cancer cell survival and growth by regulating this pathway.
- MAPK/ERK Pathway: Affected circRNAs modulate cell proliferation and differentiation signals.
- Wnt/β-catenin Pathway: circRNA involvement influences development and tumorigenesis.
- Notch Signaling: circRNAs modulate this pathway critical in cell fate decisions.


Extracellular matrix
Cytoplasm
p110
p85
PI3K
PIP2
PIP3
PDK1
Thr308
P
P
Ser473
AKT
Circ_0008285
Circ-IL4R
Circ_0026344
Circ_ 0001313
Circ-CDYL
mTOR,
GSK-3β,
P53,
CREB,
P27,
NF-κB
Gene expression
Nucleus
Proliferation,
angiogenesis
differentiation,
migration,
invasion,
apoptosis



Applications of circRNAs

Diagnostic Applications of circRNAs

- circRNAs are emerging as valuable biomarkers for a variety of diseases due to their unique structure and functional stability:

- High stability and abundance in body fluids like blood, serum, saliva, urine, and semen make circRNAs ideal for non-invasive diagnostic markers.
- In neurodegenerative diseases, specific circRNA expression patterns correlate with disease progression, offering potential for early diagnosis and prognosis.
- In cancer, circRNAs regulate gene expression by sponging microRNAs or interacting with RNA-binding proteins, and their altered expression levels serve as biomarkers for cancers such as colorectal, lung, breast, gastric, liver, and pancreatic cancers.
- In cardiovascular diseases, circRNAs influence pathways related to heart function and disease, presenting as potential diagnostic markers.
- The specificity and tissue expression patterns of circRNAs enable stage-specific monitoring of disease dynamics.

Diagnostic Applications of circRNAs

Diagnostic Applications of circRNAs in Liver Fibrosis Patients

- ✓ circRNAs show promising potential as non-invasive biomarkers for diagnosis and staging of liver fibrosis due to their high stability and presence in body fluids like blood.
- ✓ Specific circRNAs have been identified with altered expression in fibrotic liver tissues and serum of patients with liver fibrosis, including those caused by chronic hepatitis or other hepatic injuries.
- ✓ circRNAs regulate fibrosis-related signaling pathways (e.g., TGF-β, Wnt, and MAPK pathways) and affect the activation of hepatic stellate cells, key players in liver fibrogenesis.
- ✓ Detection of fibrosis-associated circRNAs in serum can help monitor disease progression and response to therapy without invasive liver biopsy.
- ✓ Researchers have found circRNAs highly correlated with fibrosis stages and clinical outcomes, underscoring their potential as reliable diagnostic and prognostic tools for liver fibrosis.

Presence of circRNAs in Body Fluids as Non-Invasive Biomarkers

❑ Circular RNAs (circRNAs) are emerging as promising non-invasive biomarkers due to their stability and detectability in various body fluids such as:

- ✓ Blood (plasma, serum)
- ✓ Urine
- ✓ Saliva
- ✓ Other body fluids

- ➢ Detection of specific circRNAs in urine samples has been shown useful for diagnosing renal cell carcinoma and differentiating malignant from benign tumors.
- ➢ circRNAs in plasma/serum distinguish cancer patients from healthy individuals in various cancers, such as lung, breast, colorectal, and liver cancers.

Their circular structure makes them highly resistant to exonuclease degradation, allowing them to remain stable and detectable in these fluids even under harsh conditions.

This stability enables the use of circRNAs in liquid biopsy approaches for early diagnosis, prognosis, and monitoring of diseases without the need for invasive tissue biopsies.

Therapeutic Targets and Inhibition/Use of circRNAs

- circRNAs serve as therapeutic targets by modulating their levels to correct dysregulated gene expression in diseases such as cancer and fibrosis.

- Strategies include inhibiting oncogenic circRNAs or restoring tumor-suppressor circRNAs through antisense oligonucleotides or RNA interference.

- circRNAs can also be utilized as therapeutic molecules themselves, given their stability and regulatory functions.

Therapeutic Targets and Inhibition/Use of circRNAs

- exonuclease degradation, enhancing stability compared to linear RNAs.
- circRNAs exhibit longer half-life in biological environments, making them superior in maintaining therapeutic function over time.
- Their structure allows efficient and targeted delivery using advanced nanocarriers such as lipid nanoparticles (LNPs), improving drug bioavailability and reducing off-target effects.
- circRNAs can interact with disease-related miRNAs and proteins, providing versatile tools for gene expression regulation therapeutics.
- This stability reduces the need for frequent dosing and potentially improves treatment efficacy and patient compliance.

Bioinformatics and Identification of circRNAs

Use of Data Mining and Bioinformatics for circRNA Discovery and Analysis

- circRNAs are identified from high-throughput RNA sequencing (RNA-seq) data using advanced computational methods that detect back-spliced junctions unique to circRNAs.
- Bioinformatics techniques include quality control and preprocessing of RNA-seq reads, alignment to reference genomes, and detection of circular RNA reads using specialized algorithms.
- Differential expression analysis helps identify circRNAs with significant changes between different biological conditions or disease states.
- Functional annotation and pathway analysis enable understanding of biological roles and regulatory networks involving circRNAs.

Bioinformatics and Identification of circRNAs

Commonly Used Bioinformatics Tools for circRNA Identification

- ✓ CIRI2 and find_circ: Detect circular RNA candidates by identifying back-splice junction reads.
- ✓ circExplorer2: Comprehensive circRNA analysis including detection, expression quantification, and annotation.
- ✓ DCC (Detection of CircRNAs from Chimeric Transcripts): Focuses on chimeric read detection for circRNAs.
- ✓ RNA-seq Aligners: STAR, BWA-MEM for high-quality read mapping required prior to circRNA detection.
- ✓ DESeq2/edgeR: For differential expression analysis of circRNAs.
- ✓ Functional Enrichment Tools: DAVID, GO, KEGG pathway analysis for biological interpretation.

Advantages and Limitations of circRNAs in Medicine

Advantages

- High Stability
- Reduced Immunogenicity
- Efficient Protein Expression
- Simpler Production Process
- Innovative Delivery Systems
- Therapeutic Potential

Limitations and Challenges

- Technical Challenges
- Functional Characterization
- Delivery Efficiency
- Regulatory and Safety Concerns
- Standardization Issues

Summary - Importance of circRNAs in Disease Diagnosis and Therapy

- Circular RNAs (circRNAs) are stable, covalently closed RNA molecules with diverse biological functions and exceptional stability, making them promising tools in medicine.
- circRNAs serve as accurate, non-invasive biomarkers for early diagnosis of various diseases, including cancers, neurodegenerative disorders, and cardiovascular diseases.
- Their high stability in body fluids and ability to regulate gene expression underscore their potential in therapeutic applications, such as targeted drug delivery and novel RNA-based therapies.
- Advances in bioinformatics, sequencing technologies, and nanodelivery systems have accelerated circRNA research, opening new avenues for precision medicine.
- Despite their potential, further functional studies and clinical trials are necessary to fully realize their diagnostic and therapeutic utility.

REFRENCE

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