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*Research article*

# Identification of phosphoinositide-associated microRNAs reveals candidates with potential roles in cancer signaling

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**Abstract:** MicroRNAs (miRNAs) are key post-transcriptional regulators of gene expression and have been associated with pathological processes, including oncogenesis. In this study, the interaction between miRNAs and phosphoinositides, particularly phosphatidylinositol 4,5-bisphosphate (PIP2), was explored by pull-down assays, followed by RNA sequencing (RNA-seq) analysis in HeLa cells. Four miRNAs with specific affinity toward PIP2 and PI4P were identified, including hsa-miR-3181-1, hsa-miR-4720, hsa-miR-142, and hsa-miR-940. Structural analysis suggested that the interaction with lipids is mediated by secondary conformations rather than by sequence homology. Furthermore, two of these miRNAs were present in human blood fractions, with particular emphasis on hsa-miR-142, whose plasma distribution suggests a potential role as a phosphoinositide-mediated long-range signaling molecule. These miRNAs have been associated with tumor-related processes, suggesting a potential, although not yet confirmed, relevance as noninvasive biomarkers or therapeutic targets in cancer.

**Keywords:** microRNA; phosphoinositides; cancer; bloods cells; PIP2; PI4P

## 1. Introduction

In the early 1990s, several scientists independently observed in different eukaryotic organisms, through transgenic co-expression experiments or viral infection, an intriguing phenomenon of RNA-mediated protein synthesis inhibition [1].

The regulatory effects of these RNA molecules redefined the view of RNA as a simple messenger. Early studies described the phenomenon as "cosuppression" in plants, as "post-transcriptional gene silencing" in nematodes, or as " repression " in fungi, but none of them suspected that RNA was the key factor until the identification of the first microRNA (miRNA) in the nematode *Caenorhabditis elegans* in 1993 [2]. To this day, more than 30 years later, there are many advances in the knowledge of these molecules, but there is a huge gap to be filled.

miRNAs are small non-coding RNA molecules that regulate gene expression post-transcriptionally [3]. A single miRNA can act on hundreds of mRNAs and influence the expression of numerous genes, often involved in a functional interaction pathway [4].

This fine-tuned regulation of gene expression enables miRNAs to play crucial roles in diverse biological processes, including development, cell differentiation, proliferation, and apoptosis. Alterations in miRNA expression have been widely associated with cancer pathogenesis, acting as oncogenes or tumor suppressors depending on the context and their specific molecular targets [5].

miRNAs exert their function in the cytosol, normally binding to the 3'-UTR region of the target messenger, thereby preventing gene expression. However, in recent years, it has been observed that miRNAs can also act within the nucleus, forming RISC complexes that are thought to differ slightly from cytoplasmic ones in their composition and in the binding affinity of their components. Nuclear RISC complexes can regulate transcription, alternative splicing, or the expression of ribosomal RNAs in the nucleolus [3].

On the other hand, phosphatidylinositol 4,5-bisphosphate (PIP2) is a membrane phospholipid that participates in multiple cellular processes, such as intracellular signaling, cytoskeletal organization, and plasma membrane dynamics. PIP2 serves as a precursor for the generation of second messengers and as an anchoring site for proteins containing specific domains, thus modulating cellular signaling pathways [6].

PIP2, traditionally associated with functions at the plasma membrane, also plays pivotal roles in the cell nucleus. In this compartment, PIP2 is not only involved in regulating chromatin structure and nucleoskeletal dynamics, but also modulates key processes such as gene transcription, RNA processing, and epigenetic remodeling. PIP2 has been shown to interact with transcription factors, RNA polymerase II, and components of the splicing complex, suggesting a direct influence on gene expression [7]. Furthermore, nuclear PIP2 associates with RNA- and protein-rich domains, pointing to a possible role as a platform for the biogenesis and trafficking of non-coding RNAs, including miRNAs.

The presence of miRNAs in bodily fluids, such as blood, has opened new possibilities for their use as noninvasive biomarkers in cancer diagnosis and prognosis. The detection of specific miRNAs that interact with PIP2 in blood samples could reflect active tumor processes and offer information on disease dynamics [8]. The possibility that these interactions facilitate the transport of miRNAs through bodily fluids to specific sites of action raises new hypotheses about the mechanisms of intercellular communication in cancer.

Research on the interaction between ncRNAs and phospholipids has been growing in recent years. For example, Lin et al. [9] demonstrated that a lncRNA called long intergenic non-coding kinase activation RNA (LINK A, also LINC01139) interacts directly and with AKT and PIP3. The LINK A-AKT and -PIP3 interactions facilitate AKT recruitment and subsequent activation.

Jiménez-Ramírez et al. [10] demonstrated that snR191 interacts with PIP2 in vitro. PIP2 is coupled to snR191 in a stem-loop-stem motif. Six hydrogen bonds across four nucleotides mediated the PIP2-snR191 interaction. Finally, mutations in snR191 affected structural folding.

We aimed to explore the interaction between miRNAs and PIP2, considering their possible involvement in oncogenesis and their potential as blood-based biomarkers. We used a database of experimental interactions between miRNAs and PIP2 to identify relevant candidates and examine their expression in blood samples using bioinformatics analysis. We also discussed possible mechanisms by which these interactions could facilitate the transport of miRNAs through body fluids to specific sites of action, thus contributing to tumor progression.

## 2. Materials and methods

### *2.1. Extraction of nuclear RNA*

Nuclear RNA was isolated from HeLa cell line cultures grown to 70% confluence in a total volume of 500 mL. Cells were harvested by centrifugation at 300 × g for 1 minute at 4°C and subsequently washed three times with cold PBS buffer saline. After PBS was removed, cell lysis was performed by adding 3 mL of buffer (50 mM Tris-HCl pH 7, 150 mM NaCl, 2 mM $MgCl_2$, 0.5% NP-40, and RNase blocker (Agilent Technologies) in a 1:100 µL ratio, keeping the mixture cold. The cells were resuspended and centrifuged again at 1000 × g at 4°C to separate the cytoplasmic fraction, which was discarded.

The obtained nuclear pellet was washed once more with 3 ml of the same buffer and centrifuged under the same conditions. Subsequently, the supernatant was removed, and the pellet was resuspended in 1.2 mL of lysis buffer, being transferred to a clean tube. To this fraction, 2.4 mL of Ribozol ™ (N580200ML, Avantor) and 2.4 mL of chloroform were added, mixing vigorously by vortexing before centrifuging at 3500 × g for 5 minutes. Only the upper aqueous phase was recovered, which was precipitated with isopropanol (0.7 mL per ml of the recovered volume), followed by centrifugation at 3500 × g for 15 minutes at 4°C. Finally, the pellet was washed with 80% ethanol, air-dried, and dissolved in RNase- free water adjusted to pH 7.0.

The concentration and purity of all extracted RNAs were determined by spectrophotometry using a Nanodrop device. Subsequently, 600 µg of RNA from each sample was individually treated with RNase -free DNase I (New England BioLabs, catalog no. M0303S) to eliminate possible genomic DNA contamination.

### *2.2. RNA-phosphoinositide binding assay*

Pull-down tests were performed to determine the binding between extracted RNAs and phosphoinositides; in this case, the interaction with PIP2 and PI4P (phosphatidylinositol 4-phosphate) was tested. For this purpose, specific microspheres coated with PIP2 (P-B045a) and PI4P (#P-B004a) were used, along with their corresponding controls (cat. no. PB000). PIP2, PIP4, and PB000 were obtained from Echelon Biosciences Inc.

Using a pull-down assay, a pre-clearance was performed to reduce nonspecific interactions: 200 µg of total RNA was incubated with 100 µL of control microspheres (without lipid coating) and 1 µL of RNase inhibitor for 5 minutes at room temperature. Subsequently, the unretained RNA was separated by centrifugation at 100 × g for 1 minute.

In parallel, 100 µL of lipid-coated microspheres were prewashed using RNase -free PBS.

Precleared RNA was then added to these functionalized microspheres, and the mixture was incubated for 30 min in refrigeration with constant agitation. This procedure was repeated for the lipid-coated and control microspheres.

After incubation, the microspheres were centrifuged at 100 × g for 1 minute to remove unbound RNA, followed by three consecutive washes with 500 µL of RNase -free PBS, using a new tube at each step to avoid cross-contamination.

For the recovery of RNA bound to lipids, 100 µL of RNase -free PBS, 200 µL of Ribozol, and 200 µL of chloroform were added. The mixture was homogenized and centrifuged at 3500 × g for 10 minutes. The obtained aqueous phase was precipitated with 0.7 volumes of isopropanol, and the resulting pellet was washed with 80% ethanol. Finally, the purified RNA was stored at −80°C until further use.

### *2.3. RNA sequencing and data analysis*

As described in our other work [11], RNA libraries were sequenced on the Illumina platform NovaSeq 6000 using 150-base paired reads. Raw reads were then quality controlled, and those meeting established criteria were aligned to the human reference genome using the STAR aligner. Raw reads were filtered by removing sequences containing adaptors, reads with more than 10% undetermined bases ($N > 10\%$), and reads in which over 50% of bases had a Qscore $\leq 5$.

Gene expression levels were quantified using RSEM software [12], which estimates transcriptomic abundance from alignments. Expression data were normalized using the Trimmed Mean of M (TMM) method, which corrects for differences in sequencing depth between samples. Differential expression analysis was performed using the EdgeR package in the R environment [13]. Genes that presented a $\log_2$ (Fold Change) greater than 1 and an adjusted p value less than 0.001 were considered significantly enriched.

The RNA-seq raw data obtained were deposited in the National Center of Biotechnology Information (NCBI), with the BioProject accession: PRJNA937906. The accession numbers for BioSamples are SAMN33421421-Hs_INPUT, SAMN33421422-Hs_CONTROL, SAMN33421423-Hs_PIP2, SAMN33421424-Hs_P4.

### *2.4. Identification of PIP2 and PI4P-binding miRNAs*

Phosphoinositide-interacting ncRNA databases obtained miRNAs that interact with PIP2 and PI4P were selected. miRNA structures were constructed in PDB format. The 3D structure was constructed from the nucleotide sequence using the RNAcomposer online server (https://rnacomposer.cs.put.poznan.pl/). It was subsequently complemented with the molecular smile tool, using the structure editing option of the UCSF Chimera 1.14 Molecular Graphics software. Multiple sequence alignments were performed using the ClustalW / ClustalX program [14], which enables the progressive and efficient alignment of RNA sequences.

### *2.5. Molecular modeling and docking analysis*

The molecular structures of the phosphoinositides PIP2 and PI4P were obtained from the PubChem database (CID: PIP2 – [53480255], PI4P – [9547150]) in .sdf format and subsequently converted to .pdb for use in structural analysis. The sequences of the differentially expressed miRNAs (hsa-miR-940, hsa-miR-142, hsa-miR-3180 and hsa-miR-4720) were three-dimensionally modeled using RNAComposer.

Molecular docking analysis between miRNAs and phosphoinositides was performed using HDOCK Server, a hybrid docking software that enables modeling RNA-ligand interactions without requiring prior crystallographic structures. For each miRNA, multiple complexes with PIP2 and PI4P were generated, and the model with the best energy and steric conformation scores was selected.

The docking scores are calculated by our knowledge-based iterative scoring function ITScorePP or ITScorePR. A more negative docking score means a more possible binding model, but the score should not be treated as the true binding affinity of two molecules because it has not been calibrated to the experimental data.

Confidence Score: Given that the protein-protein/RNA/DNA complexes in the PDB normally have a docking score of around −200 or better, we have empirically defined a docking score-dependent confidence score to indicate the binding likeliness of two molecules as follows,

$$\text{Confidence score} = 1.0/[1.0+e0.02*(\text{Docking\_Score}+150)]$$

Roughly, when the confidence score was above 0.7, the two molecules would be very likely to bind; when the confidence score was between 0.5 and 0.7, the two molecules would be likely to bind; and when the confidence score was below 0.5, the two molecules would be unlikely to bind. Nevertheless, the confidence score here should be used carefully due to its empirical nature.

Ligand rmsd: The ligand RMSDs were calculated by comparing the ligands in the docking models with the input or modeled structures. Therefore, the ligand RMSD was not necessarily a metric of the accuracy for the corresponding model. Visualization was performed using UCSF Chimera v1.16 software.

### *2.6. Analysis of miRNA presence in blood*

To evaluate the presence of miRNAs identified as interacting with PIP2 and PI4P in human blood samples, the miR-BLOOD database (https://www.mirblood.org/) was consulted, which compiles miRNA expression profiles detected experimentally in different blood fractions, such as plasma, serum, mononuclear cells, erythrocytes, and exosomes. miRNAs simultaneously present in our experimental database and in miR-BLOOD were identified, with the aim of exploring their possible function as molecules transported by biological fluids towards specific sites of action. The data obtained were organized and visualized using graphs prepared in GraphPad software. Prism version 8.0 (GraphPad Software, San Diego, CA, USA) enabled us to represent the distribution of these miRNAs in the different cell types and blood compartments.

## 3. Results

In this study, beads coated with PIP2 and PI4P were used to determine their interaction with the extracted RNA sample. Sequencing was then performed, revealing a library that included mRNA and ncRNA. Differential expression analysis comparing human cells treated with PIP2 or PI4P versus control revealed a total of 5949 transcripts, 2924 corresponding to the PIP2 condition and 3025 to the

PI4P condition. The criteria for defining significantly differentially linked genes (DBGs) were a padj < 0.001 and a $log^2$ (Fold Change) > 1.

In both treatments, mRNA and lncRNA were the predominant categories. However, differences were observed in the abundance of certain ncRNAs, suggesting possible phosphoinositide-type-specific effects (Table 1).

**Table 1.** Classification of differentially up-expressed RNAs by transcript type.

| RNA Type | PIP2 Condition | PI4P Condition | Total |
|---|---|---|---|
| Protein coding | 2555 | 2671 | 5226 |
| Antisense | 101 | 95 | 196 |
| lincRNA | 95 | 86 | 181 |
| Pseudogene | 94 | 90 | 184 |
| Processed transcript | 59 | 66 | 125 |
| Sense intronic | 10 | 7 | 17 |
| Sense overlapping | 8 | 7 | 15 |
| Polymorphic pseudogene | 1 | 1 | 2 |
| IG_V pseudogene | 1 | 0 | 1 |
| IG_C gene | 0 | 2 | 2 |

To focus the analysis on miRNA-type ncRNAs, we filtered the data on overexpressed transcripts, selecting only those annotations classified as "microRNA" or that included the standard "hsa-miR" nomenclature in their official identifier. This strategy resulted in the identification of four miRNAs that showed significant overexpression in at least one of the conditions analyzed. The four miRNAs identified were hsa-miR-3180-1, hsa-miR-4720, hsa-miR-142, and hsa-miR-940. They showed significantly higher expression levels under conditions treated with PIP2 and/or PI4P compared to the control.

Under PIP2 condition, the miRNAs hsa-miR-3180-1 and hsa-miR-4720 were upregulated, while under the PI4P condition, the miRNAs hsa-miR-4720, hsa-miR-142, and hsa-miR-940 were upregulated. The statistical significance padj (qvalue) and corrected pvalue showed that where the smaller the p-adjusted is, the more significant the differentially expressed genes. The fold-change of the miRNA addressed in this work is described in Table 2.

**Table 2.** The statistical significance padj (qvalue) and the fold-change of the miRNA upregulated under PIP2 and PI4P conditions.

| miRNA name | PIP2 vs Control | | PI4P vs Control | |
|---|---|---|---|---|
| | Padj (qvalue) | log2FoldChange | Padj (qvalue) | log2FoldChange |
| hsa-miR-3180-1 | 7.44E-05 | 7.6524 | - | - |
| hsa-miR-4720 | 3.30E-07 | 8.6726 | 0.00010098 | 7.5994 |
| hsa-miR-142 | - | - | 0.00031636 | 7.3694 |
| hsa-miR-940 | - | - | 3.15E-07 | 8.3959 |

Our RNA-seq analysis identified four miRNAs enriched in the phosphoinositide-pull-down fraction. All showed positive log2 fold-changes, indicating strong enrichment compared with the control fraction.

Differential binding quantification was represented by a heat map generated with RStudio, which showed a distinct enrichment pattern of these miRNAs in the presence of PIP2 and PI4P compared to control beads (Figure 1c). This result suggests a non-random interaction, possibly mediated by structural or sequence features of the identified miRNAs.

UCSF Chimera 1.14 software and a multiple alignment of their primary sequences was performed with ClustalW (Figure 1a).

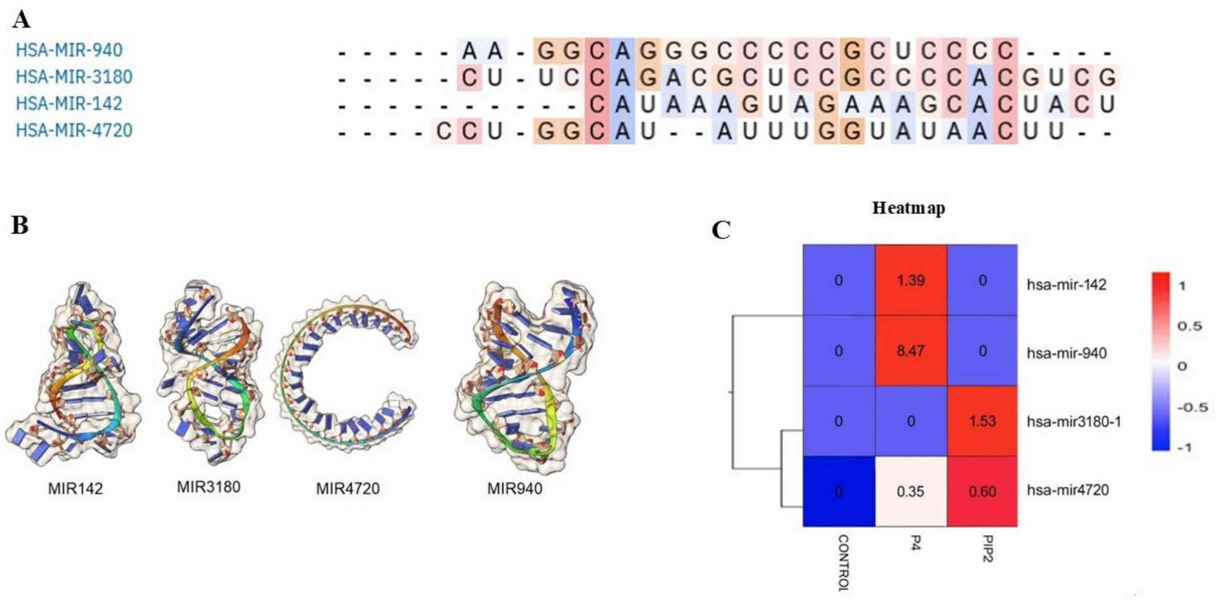
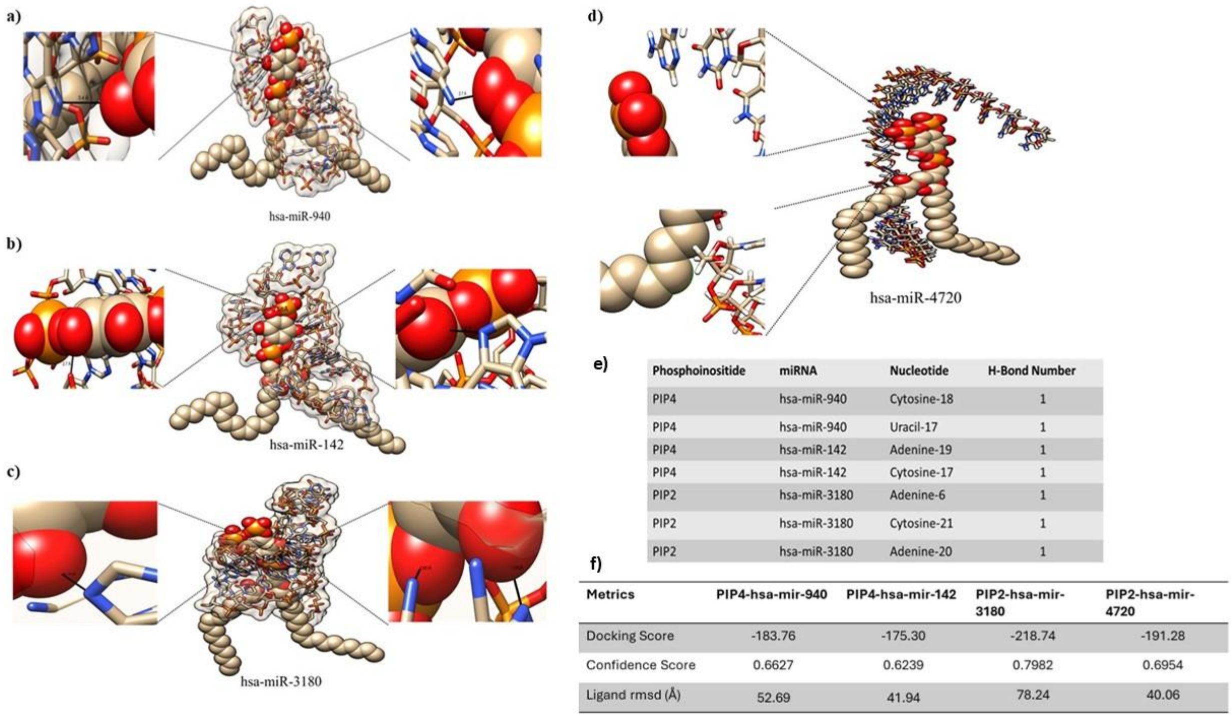



**Figure 1.** miRNAs interact with PIP2 and PI4P (A) A multiple sequence alignment of the four identified microRNAs with affinity toward phosphoinositides (hsa-miR-940, hsa-miR-3180, hsa-miR-142, and hsa-miR-4720), indicating partial conservation in central regions. (B) Three-dimensional modeling of the secondary structure of the microRNAs using UCSF Chimera 1.14, showing the hairpin-like conformations that could favor the interaction with lipids. (C) Heatmap of relative enrichment of microRNAs in pull-down assays with microspheres coated with PIP2, PI4P, and lipid-free control.

### *3.1. Structural interaction between miRNAs and phosphoinositides: Docking analysis*

To model the structural interaction between these miRNAs and the phosphoinositides PIP2 and PI4P, molecular docking studies were performed. A docking protocol using the HDOCK tool was used, yielding high-affinity interaction complexes visualized with UCSF Chimera 1.14.

Figure 2 shows the most representative docking complexes between the selected miRNAs and the lipids PIP2 and PI4P, demonstrating the most relevant interactions at the atomic level.




| Phosphoinositide | miRNA | Nucleotide | H-Bond Number |
|---|---|---|---|
| PIP4 | hsa-miR-940 | Cytosine-18 | 1 |
| PIP4 | hsa-miR-940 | Uracil-17 | 1 |
| PIP4 | hsa-miR-142 | Adenine-19 | 1 |
| PIP4 | hsa-miR-142 | Cytosine-17 | 1 |
| PIP2 | hsa-miR-3180 | Adenine-6 | 1 |
| PIP2 | hsa-miR-3180 | Cytosine-21 | 1 |
| PIP2 | hsa-miR-3180 | Adenine-20 | 1 |

| Metrics | PIP4-hsa-mir-940 | PIP4-hsa-mir-142 | PIP2-hsa-mir-3180 | PIP2-hsa-mir-4720 |
|---|---|---|---|---|
| Docking Score | -183.76 | -175.30 | -218.74 | -191.28 |
| Confidence Score | 0.6627 | 0.6239 | 0.7982 | 0.6954 |
| Ligand rmsd (Å) | 52.69 | 41.94 | 78.24 | 40.06 |

**Figure 2.** Molecular docking modeling between selected human miRNAs and phosphoinositides PIP2 and PI4P. (a) hsa-miR-940-PI4P docking modeling, (b) hsa-miR-142-PI4P docking modeling, (c) hsa-miR-3180-PIP2 docking modeling, and (d) hsa-miR-4720-PIP2 docking modeling. Interaction regions are shown by zooming in on the contact sites between the phosphoinositide (colored spheres) and residues of the miRNA (represented as sticks). The interaction occurs predominantly through the polar head of the phosphoinositide, suggesting a key role of this region in molecular. (e) Summary table of the observed hydrogen bond interactions, indicating the type of phosphoinositide, the corresponding miRNA, the nucleotide involved in the interaction, and the number of hydrogen bonds detected. (f). Docking results showing predicted binding scores, confidence scores, and ligand RMSD values for miRNA–phosphoinositide complexes obtained with the HDOCK server. Lower docking scores and higher confidence values indicate more favorable predicted interactions.

The molecular docking analysis was performed to evaluate the potential interaction between the selected miRNAs (hsa-miR-142, hsa-miR-940, hsa-miR-3180, and hsa-miR-4720) and phosphoinositides (PIP2 and PI4P). The docking procedure yielded scores ranging from –165 to –208, with confidence values between 0.68 and 0.82 (Figure 2f). These results suggest geometrically and energetically favorable complexes, particularly for hsa-miR-142 and hsa-miR-940, which exhibited the most negative docking scores and the highest confidence levels. The lowest ligand RMSD values indicated consistent binding poses across docking models, supporting the stability of the predicted conformations. Altogether, these computational results indicate that the analyzed miRNAs could possess structural compatibility for interacting with phosphoinositides at the molecular level.

For hsa-miR-940: PI4P and hsa-miR-142: PI4P complexes likely displayed direct interactions via hydrogen bonds between the miRNA nucleotides and the lipid phosphate groups. In hsa-miR-940, hydrogen bonds were observed between cytosine-18 and uracil-17 with the PI4P head (Figure 2a), while in hsa-miR-142, the interactions were established via adenine-19 and cytosine-17 (Figure 2b). The interatomic distances ranged from 2.7 to 3.0 Å, consistent with stable H-bonds.

On the other hand, the miRNA hsa-miR-3180 showed preferential interaction with PIP2 (Figure 2c), establishing hydrogen bonds with Adenine-6, Adenine-20, and Cytosine-21, also in the polar head region. This suggests a relative specificity of certain miRNAs toward different phosphoinositides, possibly mediated by the pattern and position of charge on the phosphate groups.

In the case of hsa-miR-4720, no modeled hydrogen bonds were identified in the complex with PI4P (Figure 2d). However, steric analysis revealed proximal interactions through hydrophobic contacts and complementary surface couplings.

### *3.2. iRNAs interacting with Phosphoinositides in blood components and their relationship with cancer*

As part of the functional analysis and to explore the potential circulating role of these miRNAs, a search was performed in the miR-BLOOD database. Two of the four identified miRNAs were present in different human blood fractions, including plasma, erythrocytes, and mononuclear cells. These data were graphically represented using GraphPad. Prism 8, demonstrating that the expression of these miRNAs in blood is consistent and ubiquitous in multiple cellular and extracellular compartments.

In the miRNA expression analysis of human blood components, distinct molecular profiles were observed for hsa-mir-142 and hsa-mir-940 (Figure 3a). hsa-mir-940 showed expression restricted to eosinophils, suggesting a specific function possibly related to type 2 immune responses. The microRNA hsa-mir-142 showed high and ubiquitous expression in most hematopoietic cell populations, consistent with its known role in regulating immune development and function. Notably, hsa-mir-142 also showed considerable expression in plasma, suggesting that this microRNA actively circulates extracellularly.

The microRNA hsa-mir-142 showed high and ubiquitous expression in most hematopoietic cell populations, consistent with its known role in regulating immune development and function. Notably, hsa-mir-142 also showed significant expression in plasma, suggesting that this microRNA actively circulates extracellularly.

Research suggests that miRNAs are aberrantly expressed in various cancer types. Many miRNAs have been observed to be either over- or under-expressed in cancer samples compared to their counterparts in healthy tissue.

In this context, the two microRNAs identified as present in blood cells and interacting with phosphoinositides (hsa-miR-142 and hsa-miR-940) have been reported in the context of cancer, either as diagnostic or prognostic biomarkers or as key regulators of tumor processes such as proliferation, migration, and immune evasion (Figure 3b).

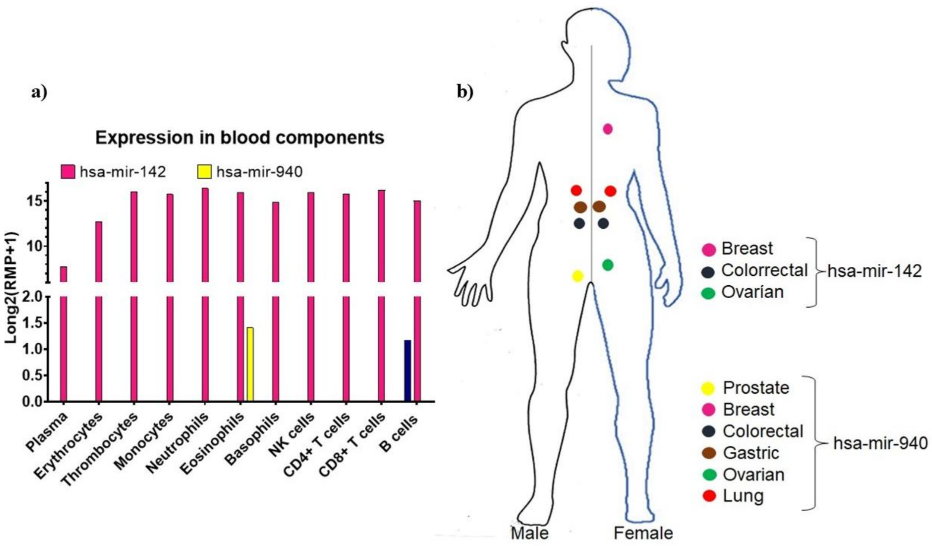



**Figure 3.** miRNAs Associated with Cancer and Expression in Blood Components. (A) Expression profile of hsa-miR-142 and hsa-miR-940 in different cellular components of human blood. hsa-miR-142 is ubiquitously expressed, and hsa-miR-940 in eosinophils. (B) Documented association of the two miRNAs with different types of cancer.

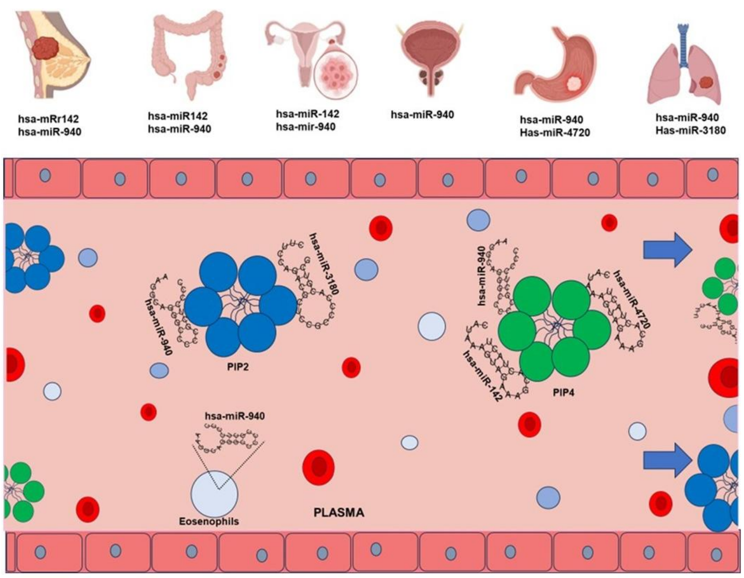



**Figure 4.** Proposed mechanism of extracellular transport of phosphoinositide-associated miRNAs in blood. Schematic representation of the interaction between the microRNAs hsa-miR-142 and hsa-miR-940 with PI4P (green) in the plasma environment. Interactions are shown in immune cells (eosinophils) and in circulating plasma, suggesting phosphoinositide-mediated stabilization and transport mechanisms. Of note is hsa-miR-142, which associates with PI4P in plasma, indicating a potential role in long-range extracellular signaling. The cancer types with which these miRNAs are associated are indicated at the top, reinforcing their potential as noninvasive biomarkers and therapeutic targets in oncology.

Figure 4 proposes a general mechanism by which hs-miR-142 could utilize PIP2 as an extracellular transport vehicle, enabling it to circulate in plasma and potentially reach target tissues. This property distinguishes miR-142 from other miRNAs such as miR-1587 or miR-940, which appear to be confined to cellular compartments. The ability of miR-142 to disseminate via plasma through its interaction with PIP2 could represent a possible mechanism involved in long-range molecular communication, which may be relevant in oncogenic contexts, although this remains hypothetical.

## 4. Discussion

### *4.1. Structural basis of miRNA–phosphoinositide association*

miRNAs are known for their ability to form stable secondary structures, such as hairpins and imperfect duplexes, which play a crucial role in their stability, function, and molecular recognition. These structural conformations are not only essential for their incorporation into complexes such as RISC, but can also facilitate interactions with other molecules, including phosphoinositides [ 15].

The structural similarity between certain miRNAs and RNA aptamers, which are known for their ability to bind to a variety of molecules including lipids, supports the hypothesis that the secondary structure of miRNAs might enable specific interactions with phosphoinositides [16]. This binding ability could have functional implications for miRNA subcellular localization, stability, and involvement in cancer-related signaling pathways.

In this context, the structural prediction made using UCSF Chimera 1.14 for the miRNAs identified in this study revealed conformations compatible with potential PIP2 and PI4P binding sites. These observations suggest that, in addition to their primary sequence, the secondary structure of miRNAs may be a key determinant of their ability to interact with phosphoinositides, possibly mediating their subcellular localization, transport, or stability in contexts such as the plasma or vesicular environment.

The four miRNAs analyzed did not show significant homology in their primary sequences after multiple alignments with ClustalW (Figure 1a); however, as mentioned, each one presented characteristic secondary structures that could facilitate interactions with phosphoinositides. This observation suggests that the binding capacity to PIP2 and PI4P does not necessarily depend on a conserved sequence, but on individual structural conformations compatible with lipid interaction.

This finding opens the possibility that the affinity of certain miRNAs toward lipids such as PIP2 and PI4P is conditioned not only by their sequence, but also by structural aspects that are poorly characterized.

In all cases, the most stable interactions were on the polar head of the phosphoinositides, consistent with the hydrophilic nature of the phosphate groups and their role in electrostatic and hydrogen-bonding interactions.

As a general pattern, the lipid was observed to fit into a cleft formed by the miRNA secondary structure, indicating that, in the absence of hydrogen bonds, other weak van der Waals-type interactions may predominate to stabilize the complex.

### 4.2. Potential role of lipid association in extracellular stability and transport

A very interesting approach for the diagnosis of certain human genetic diseases is the study of circulating miRNAs in body fluids [17]. It is known that some miRNAs can be secreted into the extracellular medium in pathological contexts and that they are also quite stable thanks to a series of mechanisms that protect them from the numerous RNases in blood plasma, which, although their presence is necessary for defense against pathogens or tumors, can also degrade miRNAs [18].

This plasma presence may be consistent with a possible association with phosphoinositides or other lipid structures, which could act as stabilization and transport vehicles, although this remains to be experimentally validated. Since it has been shown that this miRNA can interact directly with PIP2 and PI4P, it is plausible that hsa-mir-142 is incorporated into extracellular vesicles, lipid micelles, or other phosphoinositide-rich complexes that facilitate its release and systemic distribution. This hypothesis acquires relevance in the context of intercellular signaling mediated by miRNAs and opens the possibility that hsa-mir-142 exerts paracrine or endocrine functions modulated by its interaction with membrane components such as phosphoinositides.

Taken together, these data highlight the specificity of microRNA expression in distinct hematological compartments and suggest differential secretion and transport mechanisms, particularly in the case of hsa-mir-142, where its detection in plasma supports a possible functional interaction with phosphoinositides as part of its extracellular dynamics.

### 4.3. Relevance in cancer and previously reported oncogenic contexts

The first report of differentially expressed miRNAs in cancer included miR-15 and miR-16, where a simultaneous reduction of these two miRNAs was observed in cancer samples (leukemia), compared to normal tissues [19].

Consistent with these observations, the global decrease in miRNA by inhibition of the miRNA processing machinery stimulates cellular transformation and tumorigenesis in vivo. This implies that miRNA alteration is not simply a result of tumorigenesis but actively contributes to cancer development [19].

Despite the overall reduction of miRNAs in cancers, several miRNAs are upregulated, some of which undoubtedly play oncogenic roles. For example, hsa miR21 has been widely studied in lung cancer since its overexpression inhibits the expression of some genes, causing an increase in cell multiplication and migration and a decrease in apoptosis [20]. Overexpression of miR-55 in colorectal cancer is related to resistance to several chemotherapeutic drugs, promoting the survival, proliferation, and epithelial-mesenchymal transition (EMT) of tumor cells by repressing tumor suppressors and activating oncogenes [21].

Research has shown the involvement of hsa-miR-940 in multiple cancer types. Ma et al. reported that reduced levels of miR-940 promote tumorigenesis in nasopharyngeal carcinoma cells [22]. Similarly, Rajendiran et al. demonstrated that miR-940 suppresses cell migratory and invasive capacity, decreases their anchorage-independent growth, and promotes E-cadherin expression in the context of prostate cancer [23]. In addition, a significant decrease in its expression has been observed in tumor tissues and cell lines derived from hepatocarcinoma [24].

hsa-miR-142 is a highly evolutionarily conserved microRNA with a wide diversity of target genes involved in key physiological functions. Its predominant expression in hematopoietic cells has motivated numerous studies, establishing its involvement in different subtypes of leukemias and lymphomas. Through the modulation of epigenetic factors and signaling pathways associated with tumor development, miR-142 exerts control over multiple genes regulated in a dependent manner [25].

Its immunomodulatory capacity has been documented in different types of cancer, highlighting its relevance in the mechanisms of immunosuppression and in the escape of tumor cells from immune surveillance [26]. In the case of breast cancer, it has been observed that hsa-miR-142 induces apoptosis and blocks the cell cycle in the G2/M phase. This effect is related to its direct interaction with the 3' untranslated region (3' UTR) of the HMGA2 gene, which encodes an oncoembryonic protein frequently overexpressed in various types of cancer, including breast cancer [27].

In this context, the two microRNAs identified as present in blood cells and interacting with phosphoinositides (hsa-miR-142 and hsa-miR-940) have been reported in the context of cancer, either as diagnostic or prognostic biomarkers or as key regulators of tumor processes, such as proliferation, migration, and immune evasion.

## 5. Conclusions

In this study, we identified and characterized a subpopulation of microRNAs competent in interacting with phosphoinositides, PIP2 and PI4P, opening a new regulatory pathway for these molecules in both cellular and extracellular environments. The detection of this set of miRNAs, such as hsa-miR-142, in blood plasma and their interaction with lipids suggests a novel mechanism for their transport and protection, which may play a role in intercellular signaling**.** Whether these interactions contribute to cancer-related processes remains a possibility that needs to be experimentally validated**.**

## Author contributions

Conceptualization: JV; Methodology: AB, JV, JC, EC: Data Analysis: JV, AB, JC; Investigation: JV, JC; Writing-review and editing: EC, AB. All authors have read and agreed to the published version of the manuscript.

## Use of Generative-AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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## Conflict of interest

The authors declare no conflict of interest in this paper.

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