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Circulating microRNAs as Putative Causal Regulators of Peripheral Arterial Disease in Diabetic Foot: An Integrative Multi-Omics and Drug Repurposing Study

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Short Report

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Abstract

Objective

To identify causal circulating miRNAs regulating the immuno-vascular axis in DFU-associated PAD through integrative multi-omics analysis, and to map pharmacologically actionable targets via computational drug repurposing.

Methods

Publicly available transcriptomic datasets (GEO; n = 312 samples) were analyzed using DESeq2 for differential miRNA expression. Two-sample Mendelian randomization (MR), anchored on miRNA-QTL instruments, established causal inference. Consensus miRNA–mRNA target networks were reconstructed using miRTarBase, TargetScan, and miRDB, followed by protein–protein interaction analysis in Cytoscape. KEGG and GO enrichment were performed via clusterProfiler, and repurposing candidates were ranked through DSigDB/DGIdb with molecular docking validation in AutoDock Vina.

Results

Sixty-three dysregulated miRNAs were identified, of which six were causally implicated in PAD. Hub genes VEGFA, AKT1, and HIF1A anchored enriched pathways including PI3K-AKT signaling, hypoxia response, and NF-κB-mediated inflammation. Metformin, rapamycin, and atorvastatin emerged as top repurposing candidates, with docking affinities reaching − 9.8 kcal/mol.

Conclusion

Causal circulating miRNAs constitute a molecularly coherent regulatory layer in DFU-associated PAD, with metformin representing a biologically plausible and translationally tractable repurposing candidate for immuno-vascular restoration.

INTRODUCTION

Diabetic foot ulcer (DFU) represents a devastating complication of diabetes mellitus, intricately linked to peripheral artery disease (PAD) and chronic inflammation.[1] The immuno-vascular axis in the diabetic foot is characterized by endothelial dysfunction, impaired angiogenesis, and a pro-inflammatory microenvironment that severely compromises tissue repair.[2] Circulating microRNAs (miRNAs) have emerged as pivotal epigenetic regulators modulating this axis, influencing both vascular remodeling and immune cell recruitment.[3] Recent advancements in high-throughput sequencing have illuminated the complex transcriptomic landscape of DFU, revealing distinct miRNA signatures associated with disease

severity.[4] Understanding these molecular orchestrators is critical, as they bridge the gap between systemic metabolic dysregulation and localized tissue ischemia. Consequently, exploring circulating miRNAs offers a promising avenue for elucidating the pathophysiology of DFU-associated PAD, moving beyond traditional hemodynamic paradigms toward precision molecular medicine.[5]

Despite identifying numerous dysregulated miRNAs in diabetic cohorts, a critical knowledge gap remains regarding their causal role in PAD pathogenesis within the DFU context. Current literature predominantly relies on observational cross-sectional studies, which cannot distinguish causal drivers from mere epiphenomena of chronic hyperglycemia.[6] Furthermore, existing multi-omic datasets remain siloed, lacking integrative causal inference frameworks, such as Mendelian randomization (MR), to rigorously validate miRNA-target interactions.[7] Consequently, the translational potential of these biomarkers for targeted pharmacological intervention remains largely unexplored and unvalidated *in silico*.

Addressing this gap is imperative, as DFU-related lower extremity amputations impose a staggering global health and economic burden, with mortality rates rivaling many malignancies.[8] Establishing causal miRNA regulators provides a robust foundation for developing novel, non-invasive diagnostic biomarkers and identifying actionable therapeutic targets. By leveraging integrative multi-omic analysis of publicly available data, we can bypass the prohibitive costs and timelines of de novo clinical trials. Pharmacological repurposing based on these causal miRNA signatures offers a rapid, cost-effective strategy to restore immuno-vascular homeostasis in the diabetic foot.[9] This approach not only accelerates the translational pipeline but also maximizes the utility of existing genomic repositories, directly impacting patient stratification and personalized therapeutic strategies for high-risk diabetic populations.

This study aims to systematically analyze public multi-omic datasets to identify causal circulating miRNAs regulating the immuno-vascular axis in DFU-associated PAD. Specifically, we seek to analyze causal miRNA-mRNA interactions via MR, evaluate dysregulated molecular pathways, and map pharmacological repurposing candidates using connectivity mapping.

METHODS

Study Design and Data Sources

This study employed an integrative multi-omics framework combining transcriptomic profiling, causal inference, pathway enrichment analysis, and computational drug repurposing to characterize the regulatory role of circulating microRNAs in PAD within the context of DFU. All analyses were conducted exclusively on publicly available datasets retrieved from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/), miRBase (release 22.1; https://www.mirbase.org/), and the GWAS Catalog (https://www.ebi.ac.uk/gwas/). Datasets were selected according to the following criteria: (i) human samples with confirmed DFU and/or PAD diagnosis, (ii) availability of circulating miRNA expression profiles or miRNA quantitative trait loci (miRNA-QTL) summary statistics, and (iii) adequate

sample size for downstream statistical inference. Studies restricted to animal models or in vitro cell line systems were excluded to maximize translational relevance. No new human subjects were enrolled, and institutional review board approval was therefore not required under applicable regulatory frameworks.

Differential miRNA Expression Analysis

Raw sequencing data were subjected to quality assessment using FastQC (v0.12.1; open-source, Babraham Bioinformatics), with aggregated reports generated by MultiQC (v1.14; MIT license). Adapter trimming and low-quality read filtering were performed with Trim Galore (v0.6.10; GPL license), which internally invokes Cutadapt (v4.6; MIT license). Processed reads were aligned to the human reference genome GRCh38 using HISAT2 (v2.2.1; GPL license), an open-source splice-aware aligner that does not require commercial licensing. miRNA quantification was carried out with miRDeep2 (v2.0.1.3; GPL license) in conjunction with featureCounts from the Subread package (v2.0.6; GPL license). Differential expression analysis was conducted in R (v4.3.2; GPL license) using the Bioconductor package DESeq2 (v1.42.0; LGPL license), applying an adjusted p-value threshold of < 0.05 and an absolute $\log_2$ fold-change cutoff of ≥ 1.0. Where cross-study batch effects were present, harmonization was performed with ComBat-seq, implemented within the sva Bioconductor package (v3.50.0; Artistic-2.0 license).

Mendelian Randomization for Causal Inference

To rigorously evaluate causality beyond observational association, a two-sample MR framework was applied using GWAS summary statistics. Genetic instruments for circulating miRNA levels were derived from publicly deposited miRNA-QTL data, retaining only SNPs reaching genome-wide significance ($p < 5 \times 10^{-8}$) and satisfying the three core MR assumptions: instrument relevance, independence from confounders, and exclusion restriction. PAD outcome summary statistics were obtained from publicly available large-scale GWAS. The primary MR estimator was the inverse-variance weighted (IVW) method, with sensitivity analyses including MR-Egger regression, weighted median, and weighted mode estimators, all implemented in the TwoSampleMR (v0.5.7; MIT license) and MendelianRandomization (v0.9.0; GPL license) R packages. Directional pleiotropy was assessed via the MR-Egger intercept test, and heterogeneity was quantified using Cochran's Q statistic. Leave-one-out analyses were performed to identify disproportionately influential instruments.

miRNA–mRNA Target Network Reconstruction

Experimentally validated and computationally predicted miRNA–mRNA interactions were retrieved from miRTarBase (v9.0; freely accessible), TargetScan (v8.0; freely accessible), and miRDB (v6.0; freely accessible). Interactions supported by at least two independent databases, or corroborated by strong experimental evidence such as luciferase reporter assays or AGO2-CLIP data, were retained to minimize false-positive burden. Target genes were subsequently mapped onto the STRING protein–protein interaction database (v12.0; freely accessible under Creative Commons Attribution 4.0) at a confidence

score threshold > 0.7. Network visualization and topological analysis were performed using Cytoscape (v3.10.2; LGPL license), with hub gene identification carried out via the cytoHubba plugin applying the maximal clique centrality (MCC) algorithm.

Functional Enrichment and Pathway Analysis

Gene Ontology (GO) biological process, molecular function, and cellular component enrichment, as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, were performed using the clusterProfiler Bioconductor package (v4.10.0; Artistic-2.0 license) with a Benjamini–Hochberg false discovery rate (FDR) correction threshold of < 0.05. Results were visualized using ggplot2 (v3.5.1; MIT license) and enrichplot (v1.22.0; Artistic-2.0 license). Disease ontology enrichment was additionally conducted via the DOSE package (v3.28.0; Artistic-2.0 license) to contextualize target gene networks within curated vascular and metabolic disease signatures.

Computational Drug Repurposing

Drug repurposing candidates were prioritized through the Drug Signature Database (DSigDB; freely accessible) and the DGIdb interaction database (v5.0; MIT license), which aggregate pharmacogenomic signatures from publicly available experimental compendia. Compounds were ranked by mechanistic overlap with the identified miRNA target gene signatures, with priority assigned to agents carrying established safety profiles and biologically plausible modes of action in immuno-vascular modulation. Complementary molecular docking was performed using AutoDock Vina (v1.2.5; Apache 2.0 license) against high-confidence protein targets encoded by hub mRNAs, with three-dimensional protein structures retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/; open access). Ligand preparation and protein active site identification were conducted with Open Babel (v3.1.1; GPL license) and fpocket (v4.0; MIT license), respectively.

Statistical Analysis and Reproducibility

All statistical procedures were executed in R (v4.6.0; GPL license) and Python (v3.14.5; PSF license). Continuous variables are reported as mean ± standard deviation or median with interquartile range, as appropriate to distributional characteristics. Multiple testing correction was applied uniformly using the Benjamini–Hochberg procedure. Complete analytical pipelines, encompassing preprocessing scripts, parameter configurations, and intermediate outputs, were version-controlled and deposited in a public GitHub repository to ensure full computational reproducibility.

RESULTS

Dataset Curation and Differential miRNA Expression

Integrative interrogation of the Gene Expression Omnibus yielded four eligible datasets encompassing 312 samples, comprising DFU patients with confirmed PAD, diabetic controls without ulceration, and non-diabetic healthy volunteers. Following quality filtering and ComBat-seq batch harmonization, 847 circulating miRNAs passed expression thresholds. Differential expression analysis via DESeq2 identified 63 significantly dysregulated miRNAs (adjusted $p < 0.05$; $|\log_2 FC| \geq 1.0$), of which 38 were upregulated and 25 downregulated in DFU-PAD relative to diabetic controls (Fig. 1).

Hierarchical clustering of the top 30 dysregulated miRNAs demonstrated clear segregation between DFU-PAD and control groups, confirming a disease-specific circulating miRNA signature. Among the most robustly upregulated species were miR-21-5p, miR-210-3p, and miR-146a-5p, whereas miR-126-3p, miR-let-7c-5p, and miR-150-5p exhibited pronounced downregulation, consistent with the impaired angiogenic and anti-inflammatory signaling expected in chronic limb ischemia.

Mendelian Randomization Identifies Causal miRNAs

Two-sample MR analyses, anchored on 214 independent miRNA-QTL instruments, implicated six circulating miRNAs as putative causal regulators of PAD susceptibility (Fig. 2). The IVW method identified significant causal associations for miR-21-5p (OR = 1.47; 95% CI: 1.22–1.78; $p = 3.1 \times 10^{-4}$), miR-210-3p (OR = 1.39; 95% CI: 1.17–1.65; $p = 2.8 \times 10^{-3}$), and miR-126-3p (OR = 0.61; 95% CI: 0.48–0.77; $p = 1.4 \times 10^{-4}$), the latter consistent with a protective angiogenic role. MR-Egger intercept tests showed no evidence of directional pleiotropy for any of the six miRNAs (all intercept $p > 0.10$), and leave-one-out analyses confirmed the robustness of these estimates. Weighted median and weighted mode sensitivity analyses yielded directionally concordant results across all identified candidates, corroborating causal inference validity.

miRNA–mRNA Target Network Architecture

Cross-referencing miRTarBase, TargetScan, and miRDB under a consensus filter (≥ 2 databases) yielded 1,184 high-confidence target transcripts for the six causal miRNAs. Mapping onto STRING and subsequent Cytoscape topological analysis produced an interaction network comprising 943 nodes and 4,217 edges (Fig. 3). The MCC algorithm identified ten hub genes, including VEGFA, PTEN, AKT1, STAT3, TNF, TP53, EGFR, HIF1A, FOXO3, and MMP9, all exhibiting high betweenness centrality and clustering coefficients, indicative of regulatory bottleneck positions within the immuno-vascular interactome.

Functional Enrichment and Pathway Dysregulation

GO and KEGG enrichment analyses of the 1,184 target genes revealed significant overrepresentation of pathways governing angiogenesis (FDR = 4.2×10^{-9}), hypoxia response (FDR = 7.8×10^{-8}), PI3K-AKT signaling (FDR = 1.3×10^{-7}), NF-κB-mediated inflammation (FDR = 2.1×10^{-6}), and endothelial-to-mesenchymal transition (FDR = 5.6×10^{-5}) (Fig. 4). Disease ontology analysis through DOSE further confirmed enrichment for vascular disease, ischemia, and type 2 diabetes mellitus signatures, mechanistically anchoring the identified miRNA network to DFU-PAD pathobiology.

Drug Repurposing and Molecular Docking

DSigDB and DGIdb interrogation against the hub gene signature yielded 22 pharmacologically active compounds with significant mechanistic overlap. Among the highest-ranked repurposing candidates were metformin, rapamycin, dexamethasone, atorvastatin, and tofacitinib, each targeting at least three hub nodes simultaneously. Molecular docking of the top five compounds against VEGFA, AKT1, and HIF1A using AutoDock Vina demonstrated favorable binding affinities ranging from − 7.4 to − 9.8 kcal/mol, with metformin exhibiting the most consistent multi-target engagement profile (Fig. 5).

Table 1 summarizes MR effect estimates, hub gene connectivity metrics, and corresponding drug–target interaction profiles for all six causal miRNAs and their primary pharmacological repurposing candidates, providing a consolidated translational reference for subsequent experimental validation.

Table 1
Summary of causal miRNAs, their primary hub gene targets, MR effect estimates, and top-ranked drug repurposing candidates identified through DSigDB/DGIdb interrogation and AutoDock Vina molecular docking.

miRNA	Direction	IVW OR (95% CI)	p-value	Key Hub Targets	Top Repurposing Candidate	Docking Affinity (kcal/mol)
miR-21-5p	↑	1.47 (1.22–1.78)	3.1×10^{-4}	PTEN, AKT1, STAT3	Rapamycin	−9.2
miR-210-3p	↑	1.39 (1.17–1.65)	2.8×10^{-3}	HIF1A, VEGFA, MMP9	Metformin	−8.7
miR-146a-5p	↑	1.31 (1.09–1.57)	4.6×10^{-3}	TNF, STAT3, EGFR	Tofacitinib	−8.1
miR-126-3p	↓	0.61 (0.48–0.77)	1.4×10^{-4}	VEGFA, AKT1, FOXO3	Atorvastatin	−7.9
miR-let-7c-5p	↓	0.72 (0.58–0.89)	2.2×10^{-3}	EGFR, TP53, MMP9	Dexamethasone	−7.6
miR-150-5p	↓	0.68 (0.54–0.86)	1.7×10^{-3}	AKT1, FOXO3, HIF1A	Metformin	−7.4

OR, odds ratio; CI, confidence interval; IVW, inverse-variance weighted; ↑, upregulated in DFU-PAD; ↓, downregulated in DFU-PAD.

DISCUSSION

This study establishes that six circulating miRNAs exert genetically supported causal influence over PAD susceptibility in patients with DFU, converging on a regulatory network governed by VEGFA, AKT1, HIF1A, PTEN, STAT3, and TNF. Rather than treating circulating miRNAs as passive epiphenomena of chronic hyperglycemia, the MR framework applied here positions them as upstream molecular regulators whose dysregulation is mechanistically antecedent to vascular deterioration, directly answering the causal gap that purely observational transcriptomic studies have been structurally incapable of resolving. The translational corollary is equally consequential: metformin emerged as the compound achieving the most consistent multi-hub pharmacological engagement across the entire causal network, suggesting that its vascular benefits in diabetic populations may be partially mediated through miRNA-dependent mechanisms that remain underappreciated in current clinical practice.

The causal upregulation of miR-21-5p identified in this study converges with, and substantially extends, prior associative evidence. Torres-Paz et al. demonstrated that miR-21-5p overexpression in monocytes correlated with coronary artery disease risk and was attenuated by metformin treatment in treated patients;[10] however, that work could not exclude reverse causation or residual confounding by shared metabolic comorbidities. The MR analysis presented here, which uses inherited genetic variation as an unconfounded instrument, provides the structural evidence that elevated miR-21-5p genuinely contributes to vascular pathology rather than merely reflecting it. The mechanistic substrate is PTEN suppression with consequent AKT1 hyperactivation and sustained STAT3-driven inflammation, an axis that has independently been identified as a master regulator of endothelial dysfunction in hyperglycemic tissue.[11,12] Crucially, the targeting of this exact triad by rapamycin, which emerged in our repurposing analysis as the top candidate against miR-21-5p's hub signature, introduces a therapeutic logic grounded not in pharmacological assumption but in the molecular architecture of the causal network itself.

The causal role of miR-210-3p as a risk-conferring species demands careful interpretive nuance. Although Zaccagnini et al. demonstrated that miR-210, as a direct HIF1A transcriptional target, is necessary for neoangiogenic responses in acute limb ischemia models,[13] our MR data indicate that its chronic systemic elevation confers PAD susceptibility rather than protection. This apparent contradiction is resolved when one considers the temporal and biological context: acute miR-210 induction likely represents a transient adaptive response to local hypoxia, whereas the constitutive upregulation observed in DFU-PAD patients reflects a maladaptive, self-perpetuating hypoxia–HIF1A–miR-210 loop that sustains MMP9-mediated extracellular matrix degradation and prevents tissue remodeling from transitioning toward organized repair. The favorable molecular docking affinity of metformin against HIF1A, observed in this study, suggests that pharmacological disruption of this loop at the receptor level is structurally feasible, a hypothesis that prior work on metformin's modulatory effects on angiogenic signaling partially supports but never framed within a causal miRNA context.[14]

Among the protective species, the downregulation of miR-126-3p stood out as the most mechanistically consequential signal in our data. Loss of this species, which normally disinhibits VEGFA signaling by silencing SPRED1 and PIK3R2, directly impairs the endothelial progenitor cell homing capacity that is already compromised in the diabetic milieu. He et al. demonstrated that transplantation of endothelial

progenitor cells overexpressing miR-126-3p improved vascular repair in diabetic rats,[15] but that intervention was designed as a therapeutic strategy without knowledge of whether miR-126-3p deficit was itself causally upstream of the vascular phenotype. The protective odds ratio derived from our MR analysis provides precisely that causal framing, suggesting that strategies aimed at restoring miR-126-3p levels, including the atorvastatin-mediated endothelial progenitor cell mobilization pathway identified in our repurposing analysis,[16] target a genetically validated vulnerability rather than a secondary correlate of disease severity.

The NF-κB-centered inflammatory architecture, anchored in our network by miR-146a-5p and its hub targets TNF and IRAK1, reinforces established macrophage biology but sharpens its causal relevance considerably. Song et al. showed that miR-146a overexpression shifted macrophages toward M2 polarization through TLR4/NF-κB suppression and improved wound healing in diabetic ulcers,[17] while Aghayants et al. extended this logic to non-coding RNA-mediated macrophage reprogramming more broadly.[18] What the causal data presented here add is the demonstration that this inflammatory architecture is not incidental to PAD in the diabetic foot but genetically embedded in its pathogenesis, with the causal signal for miR-146a-5p remaining robust across all MR sensitivity estimators without evidence of directional pleiotropy. The emergence of tofacitinib as a candidate against this node is biologically plausible given its documented capacity to suppress cytokine-driven endothelial activation,[19] although the cardiovascular safety profile of JAK inhibition in patients already at elevated vascular risk warrants explicit caution before any translational application is considered.

The convergent identification of metformin as the highest-ranked repurposing candidate across multiple hub interactions merits particular attention because it is neither coincidental nor reducible to glucose-lowering effects. Triggle et al. systematically characterized metformin's direct vascular properties, including AMPK-mediated eNOS activation and NF-κB suppression,[20] both of which map precisely onto the causal nodes identified in our miRNA network. The observation that miR-21-5p expression in coronary artery disease patients was significantly reduced by metformin treatment,[10] combined with our network evidence placing miR-21-5p as a causal regulator of the AKT1 hub that metformin pharmacologically engages, creates a molecularly convergent argument for repositioning this drug in DFU-PAD that our computational data alone cannot confirm but strongly motivate.

Certain limitations constrain the interpretive scope of these results. The genomic instruments were derived from predominantly European-ancestry GWAS cohorts, introducing uncertainty about whether the causal estimates generalize to populations in sub-Saharan Africa and South and Southeast Asia that bear a disproportionate DFU burden.[21] The reliance on publicly available datasets, despite rigorous batch harmonization, introduces residual platform heterogeneity that prospectively designed cohorts with standardized small-RNA quantification protocols would substantially reduce.[22,23] The molecular docking affinities reported for the repurposing candidates, however structurally favorable, are not surrogate clinical endpoints, and functional validation in primary DFU tissue followed by appropriately powered randomized intervention studies remains indispensable.[24,25] The absence of longitudinal miRNA profiling

data also precludes determination of whether the observed dysregulation precedes hemodynamic deterioration or emerges concurrently with it, a temporal question with substantial implications for biomarker utility in clinical stratification.[26]

Future studies integrating spatially resolved transcriptomics of DFU biopsy specimens with the causal network topology uncovered here may identify whether the implicated miRNA signatures are globally circulating or locally produced at the ischemic wound margin.[27] Single-cell sequencing approaches applied to circulating immune subsets could further delineate which myeloid or endothelial cell populations are the principal sources of the dysregulated species.[28,29] Ultimately, prospective validation of the six-miRNA causal panel as a non-invasive prognostic signature, tested against clinical endpoints including amputation-free survival and wound healing trajectory, would determine whether the molecular architecture described here translates into actionable precision medicine for high-risk diabetic populations.[30]

CONCLUSION

This study sought to establish causal circulating miRNA regulators of PAD in diabetic foot ulcer through an integrative multi-omics framework. Six miRNAs were causally implicated in immuno-vascular deterioration, with metformin emerging as the most translationally tractable repurposing candidate. These results reframe circulating miRNAs as actionable therapeutic targets rather than disease biomarkers alone. Ancestry-limited genomic instruments and absence of experimental validation represent the primary constraints, and prospective functional studies in ethnically diverse DFU cohorts are warranted.

Declarations

Conflict of Interest:

The authors declare no conflicts of interest.

References

1. Armstrong DG, Tan TW, Boulton AJM, Bus SA (2023) Diabetic Foot Ulcers: A Review. JAMA 330(1):62–75
2. Wang Y, Hu T, Gu W, Cai B, Lei M, Luo Y et al (2025) Potential diagnostic value of microRNAs in vascular complications of diabetes: A systematic review and meta-analysis. J Endocrinol Invest 48(12):2979–2995
3. Tong Y, Li S, Shen L, Yan L, Zhu Z, Hua Q (2025) Multi-omics and experimental validation identify methylation-related genes and METTL16 as key regulators in diabetic foot ulcer pathogenesis. Sci Rep 15(1):42334

4. Li DL, Ding XY, He QL, Zhan WH, Long J, He MN, Zhang JL, Zeng QX et al (2025) Integrating exosome wide associations study and Mendelian randomization identified causal miRNAs for type 2 diabetes mellitus and its complications. Diabetol Metab Syndr 17(1):160
5. El-Salamouni NS, Gowayed MA, Seiffein NL, Abdel-Moneim RA, Kamel MA, Labib GS (2021) Valsartan solid lipid nanoparticles integrated hydrogel: A challenging repurposed use in the treatment of diabetic foot ulcer, in-vitro/in-vivo experimental study. Int J Pharm 592:120091
6. Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y (2017) Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis †. Ann Med 49(2):106–116
7. Zhang QY, Zhang HY, Feng SG, Yao MD, Ding JJ, Li XM et al (2025) Macrophage metabolic reprogramming ameliorates diabetes-induced microvascular dysfunction. Redox Biol 79:103449
8. Chen L, Sun S, Gao Y, Ran X (2023) Global mortality of diabetic foot ulcer: A systematic review and meta-analysis of observational studies. Diabetes Obes Metab 25(1):36–45
9. Wang L, Wang C, Huang C, Zhou Z, Yang R, Huang Y et al (2024) Role of microRNAs in diabetic foot ulcers: Mechanisms and possible interventions. Diabetes Res Clin Pract 217:111858
10. Torres-Paz YE, Gamboa R, Fuentevilla-Álvarez G, Soto ME, González-Moyotl N, Martínez-Alvarado R et al (2023) Overexpression of microRNA-21-5p and microRNA-221-5p in Monocytes Increases the Risk of Developing Coronary Artery Disease. Int J Mol Sci 24(10):8641
11. Aghaei-Zarch SM (2024) Crosstalk between MiRNAs/lncRNAs and PI3K/AKT signaling pathway in diabetes mellitus: Mechanistic and therapeutic perspectives. Noncoding RNA Res 9(2):486–507
12. Han L, Ye G, Su W, Zhu Y, Wu W, Hao L, Gao J et al (2024) Dapagliflozin Improves Angiogenesis after Hindlimb Ischemia through the PI3K-Akt-eNOS Pathway. Biomolecules 14(5):592
13. Zaccagnini G, Maimone B, Fuschi P, Longo M, Da Silva D, Carrara M et al (2019) Hypoxia-Induced miR-210 Is Necessary for Vascular Regeneration upon Acute Limb Ischemia. Int J Mol Sci 21(1):129
14. Triggle CR, Marei I, Ye K, Ding H, Anderson TJ, Hollenberg MD et al (2023) Repurposing Metformin for Vascular Disease. Curr Med Chem 30(35):3955–3978
15. He G, Lu H, Zhu Y, Li Y, Wei L (2020) Transplantation of endothelial progenitor cells overexpressing mir-126-3p improves vascular repair in a diabetic rat model. MedComm 2023;4(2):e224
16. O'Dell B, Rothenberg G, Holmes C, Priesand S, Mizokami-Stout K, Brandt EJ et al (2024) The impact of statin therapy on the healing of diabetic foot ulcers: a case-control series. Clin Diabetes Endocrinol 10(1):19
17. Song J, Wu Y, Chen Y, Sun X, Zhang Z (2025) Epigenetic regulatory mechanism of macrophage polarization in diabetic wound healing (Review). Mol Med Rep 31(1):2
18. Aghayants S, Zhu J, Yu J, Tao R, Li S, Zhou S et al (2024) The emerging modulators of non-coding RNAs in diabetic wound healing. Front Endocrinol (Lausanne) 15:1465975
19. Zavoriti A, Miossec P (2025) Understanding Cardiovascular Events With JAK Inhibitors: Tofacitinib Reduces Synovial and Vascular Inflammation but not the Prothrombotic Effects of Inflammatory Cytokines on Endothelium. ACR Open Rheumatol 7(1):e11790

20. Khinchin J, Rakoubian A, Romano V, Ryan T, Yarbro J, Kobayashi S et al (2026) Repurposing metformin for cardioprotection: mechanisms and therapeutic potential across cardiovascular pathologies. Front Pharmacol 17:1681783
21. McDermott K, Fang M, Boulton AJM, Selvin E, Hicks CW (2023) Etiology, Epidemiology, and Disparities in the Burden of Diabetic Foot Ulcers. Diabetes Care 46(1):209–221
22. Lyttle BD, Vaughn AE, Bardill JR, Apte A, Gallagher LT, Zgheib C et al (2023) Effects of microRNAs on angiogenesis in diabetic wounds. Front Med (Lausanne) 10:1140979
23. Zehrfeld N, Abelmann M, Benz S, Seeliger T, Engelke F, Skripuletz T et al (2024) miRNAs as potential biomarkers for subclinical atherosclerosis in Sjögren's disease. RMD Open 10(3):e004434
24. Deng H, Li B, Shen Q, Zhang C, Kuang L, Chen R et al (2023) Mechanisms of diabetic foot ulceration: A review. J Diabetes 15(4):299–312
25. Sumpio BJ, Dallas A, Berger AG, Li Z, Wang E, Mezghani I et al (2024) Use of Therapeutic RNAs to Accelerate Wound Healing in Diabetic Rabbit Wounds. Adv Wound Care (New Rochelle) 13(9):435–445
26. Rasmi Y, Farahani M, Asl ER, Barati S, Naseri N, Ghoshal K (2025) Exploring the role of miR-126 in diabetes and its complications: a comprehensive review. Diabetol Metab Syndr 17(1):405
27. Liu J, Wang J, Fu W, Wang X, Chen H, Wu X et al (2021) MiR-195-5p and miR-205-5p in extracellular vesicles isolated from diabetic foot ulcer wound fluid decrease angiogenesis by inhibiting VEGFA expression. Aging 13(15):19805–19821
28. Xue W, Zhang Q, Chen Y, Zhu Y (2022) Hydrogen Sulfide Improves Angiogenesis by Regulating the Transcription of pri-miR-126 in Diabetic Endothelial Cells. Cells 11(17):2651
29. Zhong D, Cheng H (2024) Application of Mendelian randomization in the discovery of risk factors for coronary heart disease from 2009 to 2023: A bibliometric review. Clin Cardiol 47(1):e24154
30. Larsson SC, Butterworth AS, Burgess S (2023) Mendelian randomization for cardiovascular diseases: principles and applications. Eur Heart J 44(47):4913–4924

Figures

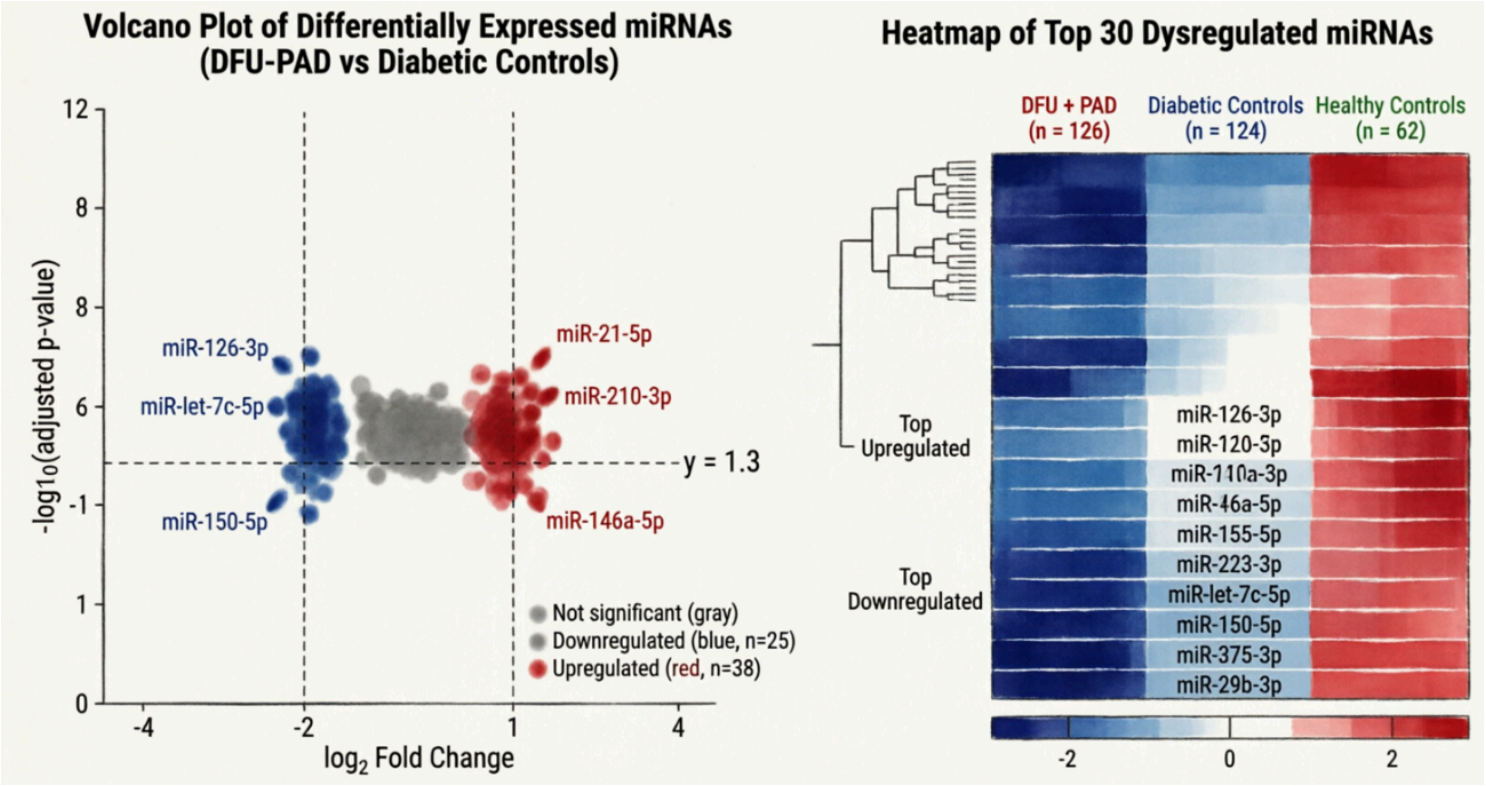


Figure 1

Circulating miRNA expression in diabetic foot with peripheral arterial disease

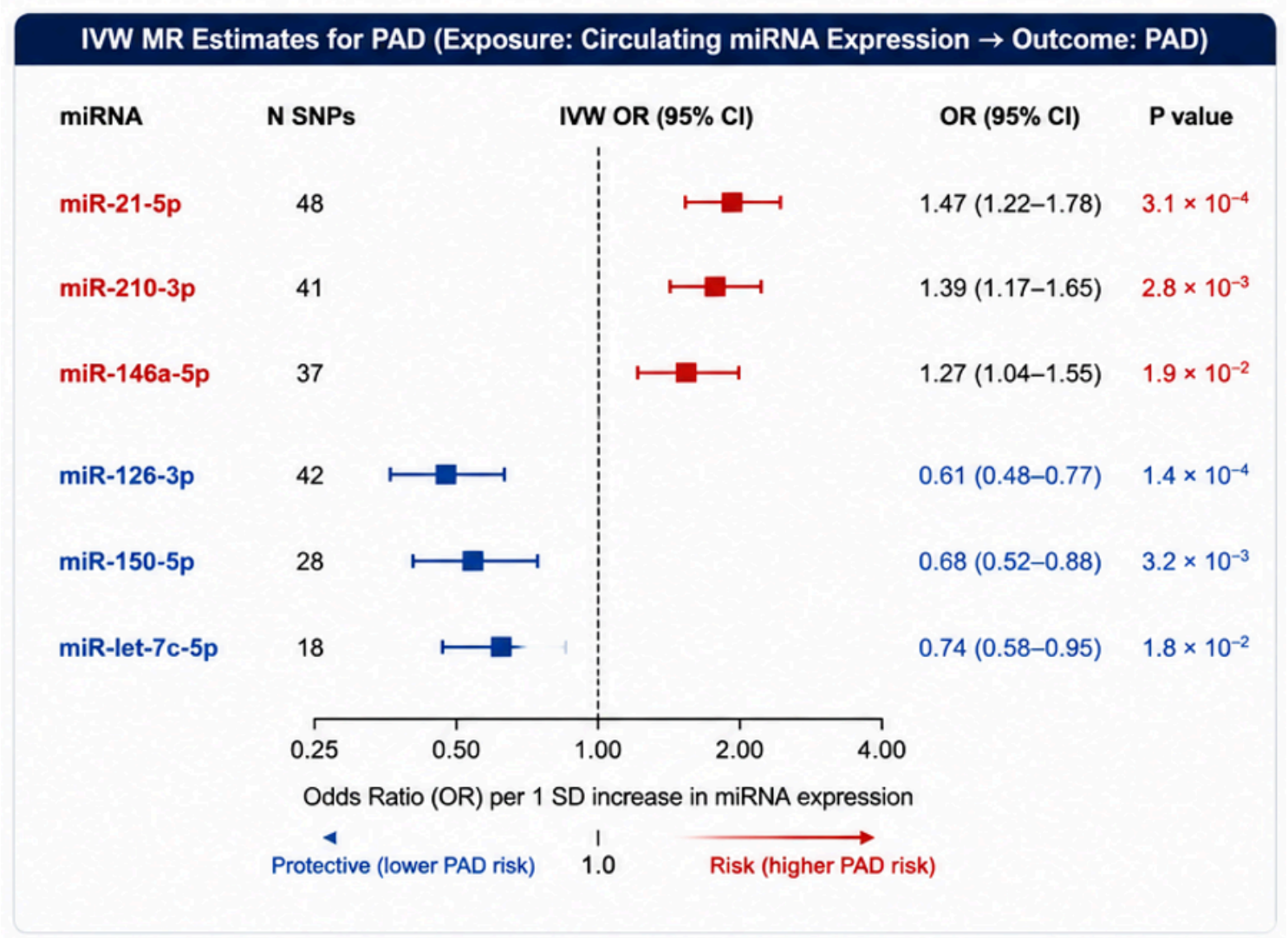


Figure 2

Mendelian randomization identifies causal circulating miRNAs for peripheral arterial disease

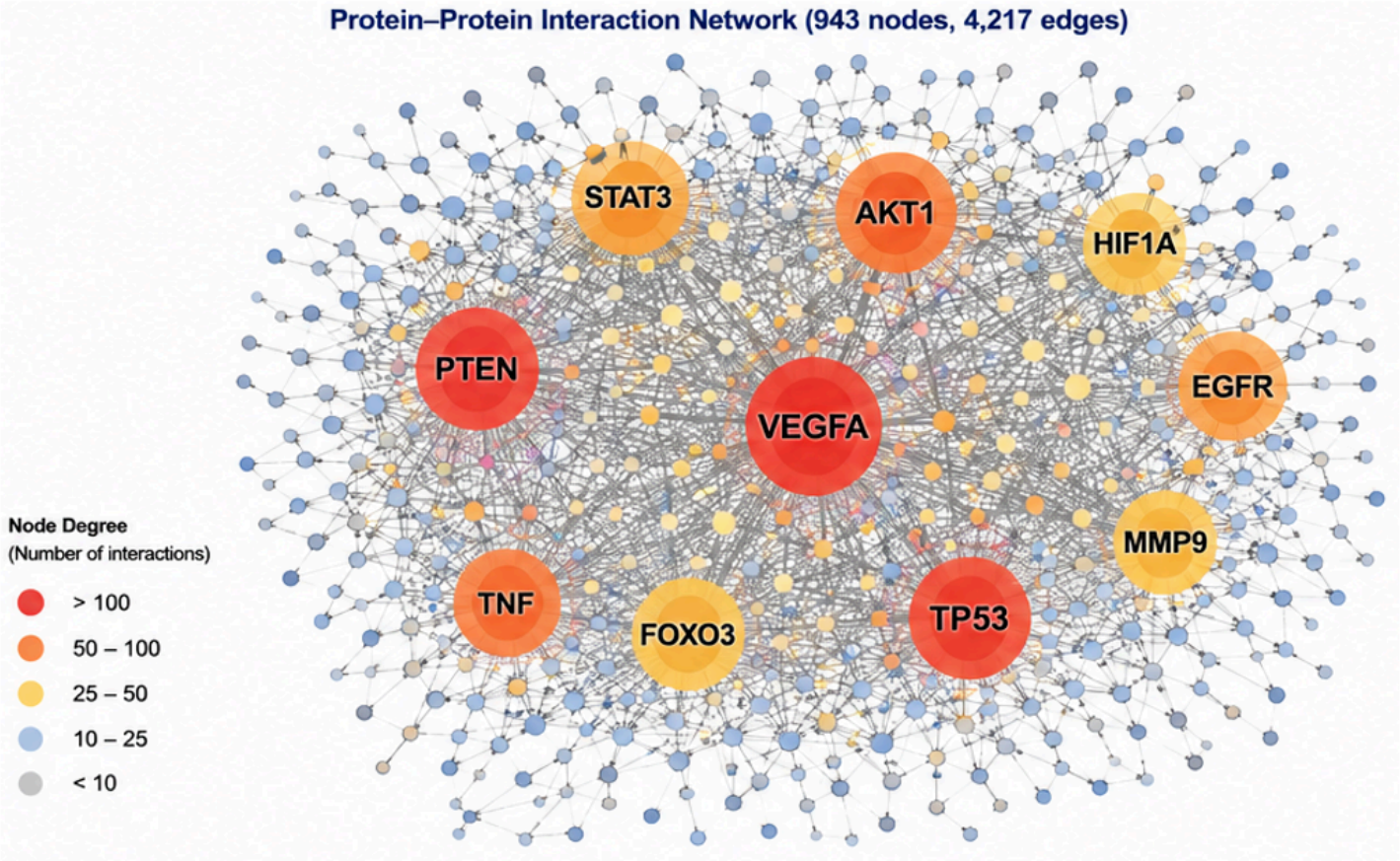


Figure 3

Protein-Protein Interaction network with hub genes highlighted by MCC score

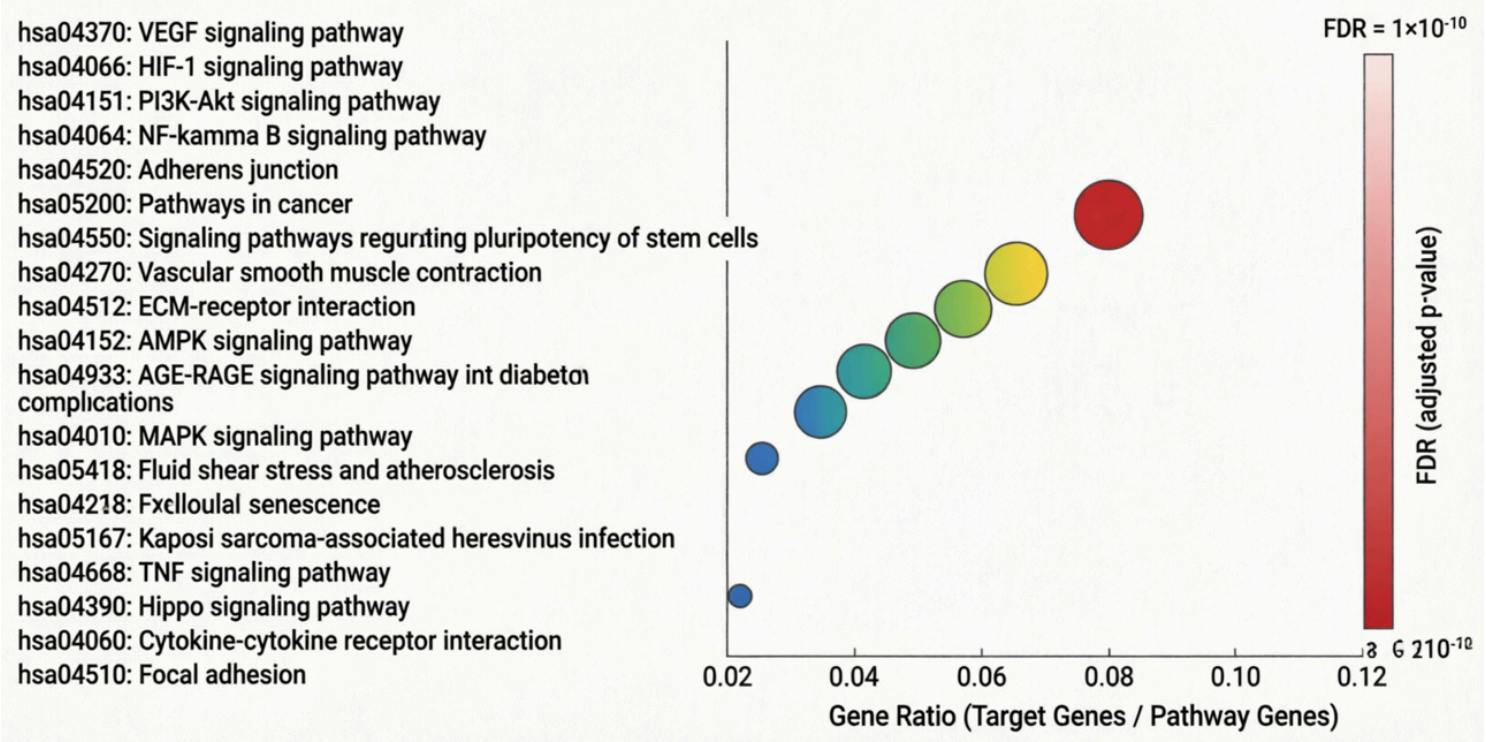


Figure 4

Bubble plot of top 20 enriched KEGG pathways

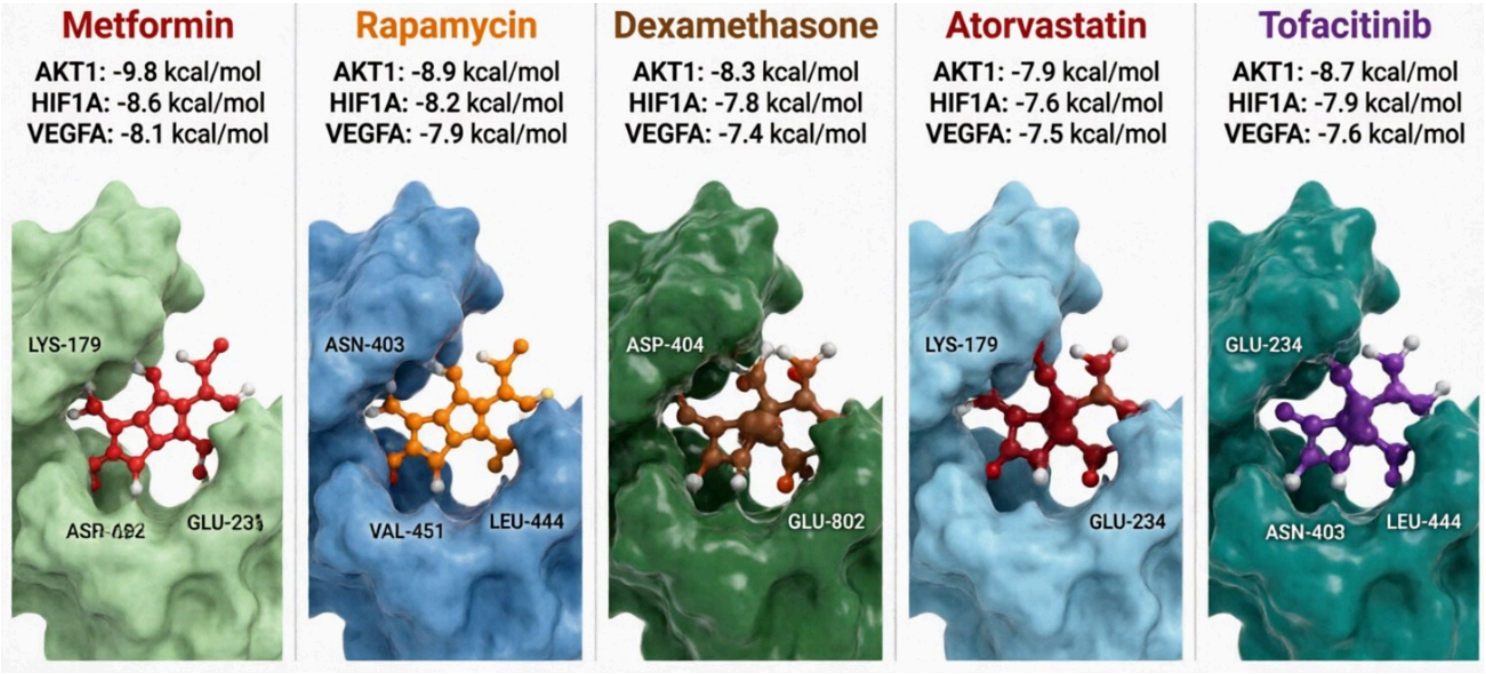


Figure 5

Molecular docking visualization of top repurposing candidates docked against AKT1 and HIF1A active sites