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Ispinesib Mesylate-Induced Oxidative Stress Via Mir-30e-5p/BCL2L11 Axis in Acute Myocardial Infarction: A Comprehensive Bioinformatics and Experimental Validation Investigation

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ABSTRACT

Objective: In this study, we used molecular biology, cell biology and other techniques to reveal the relationship between miR-30e-5p and hypoxia-induced CA16 apoptosis at the molecular and cellular levels, further improve the molecular mechanism of acute myocardial infarction, and verify the target role of BCL2L11 in acute myocardial infarction through bioinformatics, so as to analyze and predict the therapeutic drugs for acute myocardial infarction, and provide early diagnosis and treatment targets for the prevention and treatment of acute myocardial infarction in clinical practice.

Methods: In this study, the datasets in the Gene Expression Comprehensive (GEO) database were used to conduct in-depth analysis of key genes by various methods, such as differential analysis, Wien analysis and weighted correlation network analysis (WGCNA). Subsequently, the correlation between the correlation factors and key genes was further analyzed. In addition, real-time quantitative polymerase chain reaction (RT-qPCR) and lentiviral transfection experiments were carried out, miRNA-mRNA networks were constructed based on miRBase databases, three-dimensional structures were predicted with the help of RNAfold and Vfold3D databases, and drug sensitivity analysis was performed using RNAactDrug databases.

Results: Through classification, WGCNA clustering and Wien screening analysis, we successfully identified two differentially expressed genes closely related to apoptosis: PTEN and BCL2L11. The results of real-time quantitative polymerase chain reaction (RT-qPCR) and lentiviral infection experiments verified that the expression of BCL2L11 was consistent with the results of previous analysis. Finally, through miRNA-mRNA network and drug susceptibility analysis, we found that Ispinesib Mesylate, Bleomycin (50 uM)/miR-141-3p/BCL2L11 axis may be effective strategies for the treatment or prevention of acute myocardial infarction.

Conclusion: In this study, the key genes were analyzed and verified by cell culture and apoptosis assays. Subsequently, through a database of multiple drug targets, the concepts of Ispinesib Mesylate and Bleomycin (50 uM) / miR-141-3p / BCL2L11 axis were proposed for the first time, and two drugs of Ispinesib Mesylate and Bleomycin (50 uM) were predicted, and their 2D and 3D molecular structures were drawn, in order to provide a new perspective for the treatment and prevention of acute myocardial infarction.

INTRODUCTION

Acute myocardial infarction (AM) is a widespread and prevalent condition. Among them, acute myocardial infarction not only has a high incidence rate, but also has a mortality risk that cannot be ignored Zhang et al. (2024). The mechanism of apoptosis has a profound impact on the pathophysiological processes of almost all human diseases. Hypoxia can be caused by the combined effects of the internal and external environment, which is a central problem in the field of apoptosis biology He et al. (2024). Under normal conditions, hypoxia plays a leading role in maintaining cellular and mitochondrial signaling and function. However, if hypoxia is not effectively controlled, it may induce oxidative tissue and cell damage, leading to more severe apoptosis Amaro-Prellezo E et al. (2024), Liang et al. (2024), Gasecka et al. (2024), Lin et al. (2024). However, biomarkers of apoptosis and their molecular mechanisms remain to be further clarified Feng et al. (2024), Wu et al. (2024), Aries et al. (2023).

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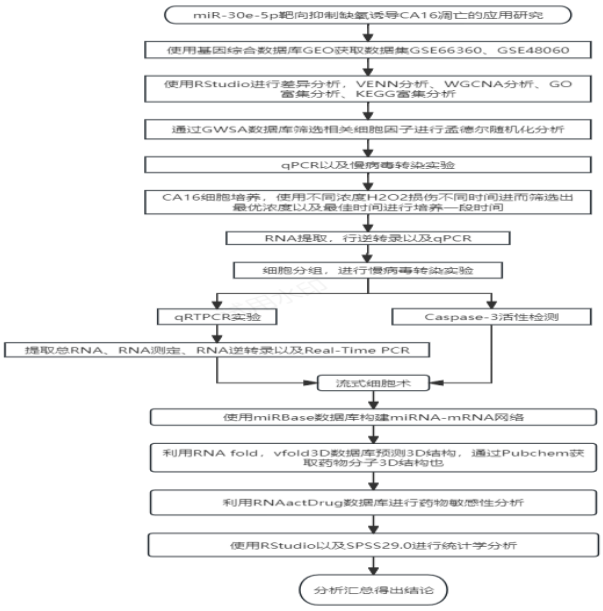
To this end, our research team obtained a large number of sequencing data on acute myocardial infarction from gene expression databases (GEOs) Hou et al. (2023), Edgar et al. (2002). Using differential analysis and generalized redundant network analysis (WGCNA), we were able to identify differentially expressed genes associated with apoptosis Langfelder et al. (2008).

Subsequently, we used cell-based experiments to perform real-time quantitative polymerase chain reaction (RT-qPCR) and experimental validation of pivot genes Mendelian randomization I, Melinda C. Mills et al. (2020), Zheng et al. (2017), Katan et al. (1986), Smith Finally et al. (2003). Finally, we constructed a miRNA-mRNA regulatory network to screen a novel drug for the prevention and treatment of acute myocardial infarction Correia de Sousa et al. (2019), as shown in Figure 1.



SUBSTANCE AND PROCEDURES

Gene Collection and Processing

Gene expression datasets related to acute myocardial infarction were extracted and analyzed. The data comes from the Gene Expression Synthesis (GEO) database of the Google Bioinformatics Institute (NCBI) at http://www.ncbi.nlm.nih.gov/geo/. To ensure the accuracy and reliability of the data, we selected only gene expression datasets that included normal individuals (N) as well as patients with osteoporosis (OP), while excluding all non-human samples. Specifically, we selected two datasets, GSE66360 (N = 50, PA = 49) and GSE48060 (N = 21, PA = 31), for in-depth analysis.

Differential expression of genes

Multiplex MRI was studied on the transcriptome data of peripheral blood mononuclear cells (PBMCs) in 49 patients with acute myocardial infarction (AMI) and 50 healthy individuals, i.e.,

GSE66360 details. At the same time, the standardization was carried out, and the PCA method was used to reveal the differential expression patterns between the two groups of samples, and the volcano map and heat map techniques were used (the screening conditions were log2FC greater than 1 and p-value less than 0.05).

In addition, we cross-compared 1604 differentially expressed genes (DEGs) with 378 oligosaccharides and sulfatides related genes (OSRGs) and demonstrated the complex association between them through a Venn diagram.

Co-expression analysis

These genes and samples were screened for possible abnormalities using the goodSamplesGenes method in the R software package WGCNA. The WGCNA algorithm was used to construct a scale-free co-expression network, in which the β parameter was set to 7 and the sensitivity was set to 3. On this basis, those modules with a correlation coefficient of less than 0.25 between genes in the module were combined.

GO and KEGG enrichment analysis of key module characteristic genes

With the help of WGCNA deep cluster analysis technology, key modules with high correlation with phenotypic performance were screened out, and a detailed list of all genes was obtained.

The "clusterProfile" extension package of R programming language is used to carry out multi-dimensional in-depth analysis and research of GO and KEGG for all genes in the green module.

Identification and correlation analysis of pivot genes

The Venn diagram was used to show the interaction between the key module genes of WGCNA,

the genes related to apoptosis and the differentially expressed genes of acute myocardial infarction, and finally two important hub genes: PTEN and BCL2L11 were screened.

Cell experiments

1. In vitro culture of cardiomyocytes: First, human-derived cardiomyocytes (derived from type CA16) were routinely resuscitated and inoculated into a Petri dish containing fetal bovine serum and Dulbecco's Modified Eagle Medium (DMEM) medium with a mass fraction of 9%, and a suspension of penicillin and streptomycin with a volume fraction of 1% was added to ensure a good bacterial environment.

2. Group treatment of cardiomyocytes: Cardiomyocytes CA16 in the logarithmic growth phase were divided into four groups according to different treatment methods: normal group (N), hypoxia group (H), overexpression control group (NC+H) and miR-30e-5p+H group (miR+H).

3. Induction method of hypoxia treatment: normal group N group does not need to be treated; Group H only needed to be incubated for 24 hours after hypoxia treatment; Both the NC+H and miR+H groups were stably infected and loaded with lentivirus, so they were placed at 37°C for continuous culture.

4. CCK8 cell viability assay:

AC16 cardiomyocytes that are in good growth condition are picked out and then properly digested with trypsin in order to obtain a homogeneous cell suspension, and the plates are placed in the incubator and incubated for 1 h. During this time, the absorbance (OD) at 450 nm is determined using a microplate reader. Then, the viability of the cells is calculated according to the relevant formula.

5. Cell scratch test pour about 5×10^5 cells into each well, use a pipette tip or a sterile toothpick to scratch the surface of the cell layer at right angles to the cell plane according to the pre-set marking line direction, and add the corresponding drug medium or serum-free medium to each group of cells according to the requirements of the experimental design. Open the taken images using ImageJ software and assess the growth of the cells by averaging the distance between the cells.

6. Transwell Migration Experiment: Place a well-prepared Transwell insert into a designated 24-well plate. After the above work is completed, the appropriate amount of serum-free medium should be appropriately supplemented in the upper room, and then placed in a room temperature environment for a reasonable period of time. Finally, the remaining medium was completely extracted by pasteurized tube, so that the sample digestion process could be carried out smoothly.

7. Flow cytometry to detect apoptosis: prepare in vitro HR cell models, digest them, discard the supernatant after centrifugation, and detect the apoptosis rate of CA16 cardiomyocytes in group C, group H and N groups N groups within 1 hour. FlowJo software was used to analyze the proportion and number of different cell states (mainly early apoptosis, late apoptosis and death).

Discuss miRNA-mRNA and drug prediction networks

The miRNA-mRNA network model was constructed with the help of the miRBase database, and the TargetScan database (URL https://www.targetscan.org/) was used to display the binding sites of BCL2L11 and miRNA with high confidence.

Use the RNAactDrug database to reveal potential associations between drug sensitivity and RNA molecules, including mRNA, lncRNA, and miRNA. The three-dimensional structure of the drug molecule was obtained by Pubchem using the RNAfold database and the Vfold3D database to predict the three-dimensional structure of the target miRNA.

Statistical methods

All raw data processing is done by R software (version 4.2.1). At the same time, the t-test or Wilcoxon's rank-sum test is used. When there are more than two independent groups, the Kruskal-Walli's test is used to assess the differences. All p-values were set to be bilateral, with $p<0.05$ as the threshold for statistical significance.

Ethical Review Process

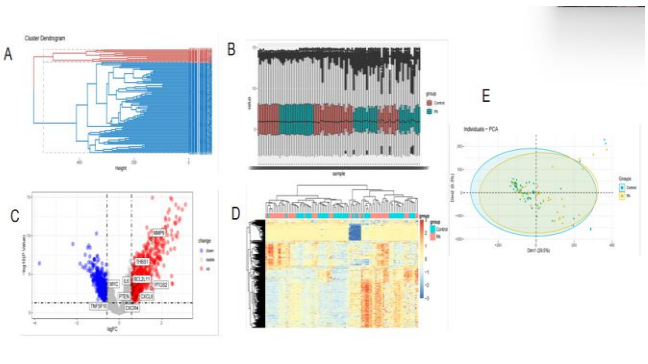
This research did not involve any human experimentation; hence no informed consent form was necessitated.

CONSEQUENTIALITY

Differentially expressed genes associated with apoptosis

An in-depth study of the RNA-seq dataset of Peripheral Blood Mononuclear Cells (PBMCs) from 50 healthy subjects and 49 patients with acute myocardial infarction (AMI) was GSE66360. During preliminary data analysis, we found that all 99 samples exhibited good homogeneity, however, there was significant heterogeneity between the two groups (see Figure 2. A）。 In view of this, we used differential expression analysis to reveal the biological differences between the two groups of samples, and finally identified a total of 1605 genes with varying degrees of expression changes (see Figure 2. B), these genes are presented in the form of a volcano map (see Figure 2. C) and is also shown as a heat map (see Figure 2. In addition, we obtained a list of 378 genes closely related to apoptosis through literature review, and screened 55 apoptosis-related genes that were differentially expressed in the two groups of samples.

Figure 2:



A. Cluster analysis of data set samples; The differentially expressed genes related to oxidative stress between B. ami group and normal group; C. Volcano map for differential gene analysis; D. Heat map of differential gene analysis; E. PCA analysis of data set samples.

Figure 2

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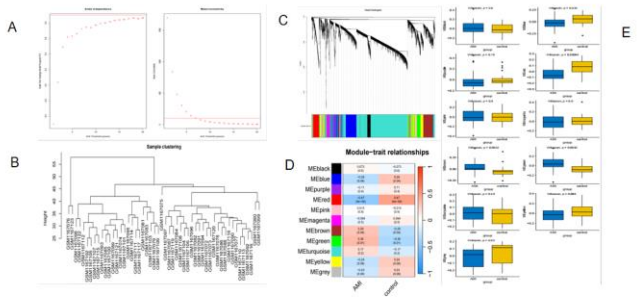
Investigation of crucial modules employing WGCNA methodology

In the study of GSE48060 (sample size N=21, acute myocardial infarction AMI=31), we carefully selected the optimal soft threshold parameter β set the value to 6 as the basis for subsequent in-depth analysis (see Figure 3. A ）。 Presented by cluster diagram (see Figure 3. B), further revealing the interconnectedness of the seven modules, and the use of color intuitively reflects the distance between them. (See Figure 3. C), where the green module was significantly associated with acute myocardial infarction (AMI) levels (see Fig. 3.D and Fig. 3.E), we decided to conduct a more in-depth follow-up analysis of this green module.

Figure 3:



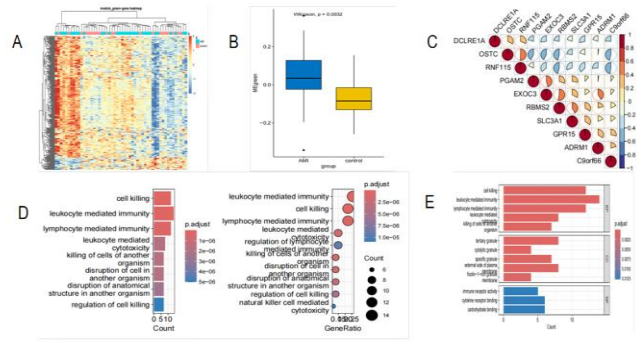
A. The scale independence of different soft thresholds and the scale-free fitting index of different soft thresholds (X-axis) were analyzed (Y-axis); Average connectivity of different soft thresholds. B. Cluster analysis diagram of WGCNA analysis; C. Construct a gene sample cluster tree drawn by coexpression network based on the optimal soft threshold; D. Correlation heat maps showing the correlation and importance between the module and clinical features of interest; E. Gene co-expression in different modules

Figure 3

GO and KEGG pathway enrichment analysis pertaining to the green module

A total of 1084 genes were used for further bioinformatics analysis in the third category of green modules obtained by the WGCNA method (see Figure 4. A) to reveal the roles and mechanisms that these genes may play. Specifically, we performed a tuple cluster analysis of the genes contained in these green modules (see Figure 4. B, partial gene expression is shown in Figure 4. C), which mainly includes the correlation analysis of biological processes and functions at different levels.

Figure 4:



A. Heat map of modules of interest B. Gene expression of key modules analyzed by GCNA C. Pie chart of gene expression of some genes of key modules analyzed by GCNA d.Column chart + bubble chart of KEGG enrichment analysis of key modules analyzed by GCNA e.Column chart of GO enrichment analysis of key modules analyzed by GCNA

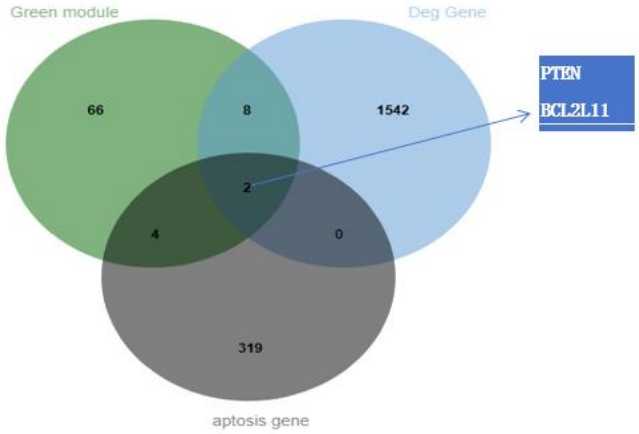
Figure 4

It was found that these genes are closely related to many cellular functions, including cell killing, leukocyte-mediated immune response, lymphocyte-mediated immune response, leukocyte-mediated cytotoxicity, and cell killing in other organisms. In addition, we also performed an in-depth analysis of these genes using the KEGG database, and the results of the analysis are shown in Figure 4. D、4. E）。

Pivot gene identification

After comprehensive systematic analysis of differentially expressed AMI genes, apoptosis-related genes, and green module genes, we successfully identified two key genes, BCL2L11 and PTEN (see Figure 5).

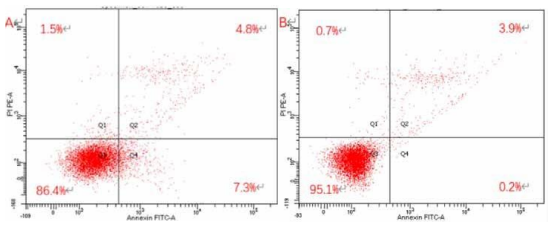
Figure 5: Identification of pivot genes



Experimental validation

AC16 cardiomyocytes were treated with H2O2 at concentrations of 0 μM, 50 μM and 100 μM, and the treated cells were continued to be cultured in a 37°C incubator containing 5% CO2, and the activity of cardiomyocytes was determined after 0 h (control group), 12 h and 24 h respectively, and the cell viability was calculated as follows: cell viability = (OD value of H2O2 treatment group - OD value of blank group) (/ OD value of control group - OD value of blank group), and the results showed that the concentration was 50 μM and 100 μM Cell viability decreased significantly after H2O2 injury, and decreased even more significantly after 100μM H2O2 injury.

Figure 6: Apoptosis of cells in AC16 groups at different concentrations

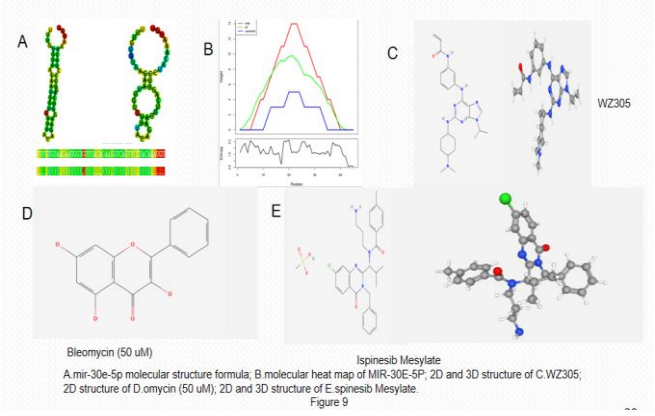


Flow cytometry analysis was used to obtain quadrant plots, Q2 quadrant represents apoptotic cells, Q4 quadrant represents advanced apoptotic cells, and the results showed a $P<0.05$, the difference was statistically significant.

miRNA–mRNA networks and drug prediction

Detailed data integration and analysis were carried out from the miRbase database, and then the miRNA network image with BCL2L11 as the focus of regulation was drawn, and more accurate miRNA sequencing technology was used to conduct in-depth research on the normal group and acute myocardial infarction (AMI) group in clinical samples, and it is worth noting that miR-30e-5p and BCL2L11 mRNA were studied The conserved binding sites between the sites are provided by the TargetScan database (see Figure 7.A, Figure 7.B). At the same time, the RNAactDrug database showed that the sensitivity of Ispinesib Mesylate, Bleomycin (50 uM) and other drugs showed a significant positive correlation with the expression level of BCL2L11, while WZ3105 showed a significant negative correlation with it. In addition, we obtained the 3D structure information of Ispinesib Mesylate, Bleomycin, and WZ3105 from the PubChem database, and predicted the 3D structure of miR-30e-5p through the RNAfold and Vfold3D databases. Finally, the corresponding molecular mechanism model was successfully constructed (see Fig. 7. C, Figure 7. D, Figure 7. E）。

Figure 7:



Acute myocardial infarction (AMI) is the most prevalent cardiovascular condition worldwide, affecting more than 800 million people worldwide Stelzer et al. (2016), Ainiwan et al. (2023). With the acceleration of people's life in modern society and the intensification of pressure in all aspects, diseases that should belong to the elderly group have begun to spread to the younger group Jin et al. (2023). From a pathological point of view, the occurrence of AMI is most likely significantly influenced by hypoxia and apoptosis Hendler-Neumark et al. (2023), Wang et al (2023), Maries et al. (2023). It has been proposed that apoptosis may be one of the key risk factors for AMI, which may interfere with cell differentiation and regeneration, and thus become an important driver of disease progression Zhang et al. (2023), Li et al. (2024). However, there are still many unknowns about the biological markers of apoptosis and their molecular mechanisms in AMI Alkhalil et al. (2023).

To this end, our team collected data from single-cell AMI samples, and after careful classification, WGCNA clustering, and Venn screening analysis, we successfully identified two differentially expressed genes closely related to apoptosis: PTEN and BCL2L11 Miao et al. (2023).Finally, we constructed a network model that included the miR-30e-5p/BCL2L11 axis and identified two novel drugs that are promising for the prevention and treatment of AMI through drug susceptibility analysis: Ispinesib Mesylate and Bleomycin (50 uM) Liu et al. (2023).

In this study, WGCNA analysis revealed an important gene module called "green". The genes in this module are significantly associated with cell killing, leukocyte-mediated immune response, lymphocyte-mediated immune response, leukocyte-mediated cytotoxicity, and cell killing in other organisms Ran et al. (2023).

In this study, apoptosis assays revealed a significant increase in the expression of the BCL2L11 gene in the acute myocardial infarction (AMI) patient population Liu et al. (2022). This phenomenon is confirmed by the growing evidence that the interrelationship between microRNAs (miRNAs) and targets plays an important role in cardiovascular disease processes such as AMI. Among them, miR-30e-5p, as one of the direct targets of miR-30e-5p, can affect the degree of activation of BCL2L11, thereby exacerbating myocardial injury caused by AMI Almaghrbi et al. (2023). In this study, BCL2L11 was officially confirmed as the primary regulator of miR-30e-5p. BCL2L11 is not only a key regulator of protein synthesis and cell growth, but also an important driver of myocardial ischemic injury. Therefore, we speculate that miR-30e-5p may further accelerate the progress of AMI by regulating the expression of BCL2L11 Kang et al. (2023).

In order to further explore the upstream molecular mechanism of the BCL2L11, we constructed a miRNA-mRNA network and integrated it with the experimental results of miRNA-seq, and found that the expression of miR-30e-5p in the AMI patient population showed a significant downward trend, which coincided with the high expression of BCL2L11 in the AMI patient population, and the existence of a stable binding site with BCL2L11 mRNA Venugopal et al. (2022).

In addition, drug susceptibility analysis showed a significant positive correlation between the expression of Ispinesib Mesylate and Bleomycin (50 uM) and miR-30,

while the expression of Ispinesib Mesylate and Bleomycin (50 uM) and BCL2L11 showed a significant negative correlation Cheng et al. (2022). This suggests that Ispinesib Mesylate and Bleomycin (50 uM) may inhibit the development of acute myocardial infarction and mediate the degradation of BCL2L11 mRNA by affecting the expression of miR-30e-5p.

Although our study has shed new light on the intrinsic association of Isopinesib Mesylate and Bleomycin (50 uM) with the miR-141-3p and BCL2L11 molecular signaling pathways, as well as their key role in apoptosis in patients with acute myocardial infarction, we must also recognize some of the limitations and challenges of this study Chen et al. (2022). First, due to the limited sample size, it is necessary to collect and integrate data from more sources to improve the reliability and accuracy of the study. Second, in order to ensure the confidence of the analysis results, we need to conduct further validation by self-testing samples. Third, although we have preliminarily verified the expression levels of related genes, more in-depth experimental exploration of their specific regulatory mechanisms is needed Zhu et al. (2022). In addition, it is worth noting that some studies suggest that genetic factors may contribute to significant differences in trait performance between different races and between individuals within the same race. Therefore, we must fully consider the diversity and complexity of genetic structures among different populations in predicting, diagnosing, intervening, and assessing the risk of acute myocardial infarction Zhao et al. (2022).

CONCLUSION

Until present, there has been an absence of comprehensive investigation into the interplay among Ispinesib Mesylate, Bleomycin (concentration 50μM), miR-30e-5p, and BCL2L11's involvement in inducing apoptosis during acute myocardial infarction. The groundbreaking findings attained through this study undeniably offer a novel perspective for comprehending the mechanism underlying apoptosis in this condition. Consequently, it was discovered that BCL2L11 could potentially serve as a novel biomarker for the management and mitigation of acute myocardial infarction. Nevertheless, further exploration is required to gain a deeper understanding of the precise molecular mechanisms employed by BCL2L11 in controlling the evolution of acute myocardial infarction.

DECLARATIONS

Author contributions

Ningxia Wu and Fei Li were major contributors in methodology, investigation, and writing.

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Competing interests

The authors declare no competing interests.

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Ethical Approval

Not applicable.

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