Platelet Microparticles Influence Gene Expression and Modulate Biological Activities of Chronic Myeloid Leukemia Cells (K562)

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Research Article

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Abstract

Background

The current understanding emphasizes the intricate interplay between leukemia and its environment. Platelet microparticles play a crucial role in facilitating intercellular communication and contribute to the complex landscape of cancer pathology. This study aimed to investigate the influence of Platelet microparticles on cell proliferation, apoptosis, and the expression of key genes, including P53, P21, Cyclin D1, Bax, and Bcl-2, within the context of a Chronic Myeloid Leukemia cell line (K562).

Methods and Results

Platelet microparticles were obtained through centrifugation at various speeds, and their concentration was quantified using the BCA assay. To determine the size and immunophenotypic characteristics of the PMPs, both the DLS technique and flow cytometry were employed. Cell proliferation was assessed using the MTT assay, and cell cycle analysis was conducted through DNA content evaluation. Real-time PCR was utilized for gene expression analysis of Bax, Bcl-2, Cyclin D1, P53, and P21. Flow cytometry was employed to examine cell apoptosis. The findings revealed that platelet microparticles have the ability to decrease proliferation of the K562 cell line, while not exerting an impact on apoptosis and cell cycle progression. Analysis through real-time PCR indicated an upregulation in the gene expression of P53, P21, and Bcl-2, accompanied by a downregulation in Bax and Cyclin D1.

Conclusion

This investigation sheds light on the intricate relationship between CML and its microenvironment, particularly the involvement of platelet-derived microparticles. The study underscores the potential of PMPs to influence cell behavior and gene expression, providing a deeper understanding of their role in CML and its therapeutic implications.

1. Introduction

Chronic myeloid leukemia (CML) is a type of blood cancer that accounts for 15% of newly diagnosed cases of adult leukemia. It is caused by a genetic mutation resulting from a translocation between chromosome 9 and chromosome 22, which creates the BCR-ABL gene on chromosome 22[1]. This gene produces a protein that increments cell proliferation and inhibits apoptosis.[2, 3].

Prior to 2000, CML treatment relied on non-specific drugs such as interferon-alpha (IFN-a), hydroxyurea, and busulfan. However, the introduction of tyrosine kinase inhibitors (TKIs) brought about significant changes in the treatment approach, resulting in a remarkable improvement in the 10-year survival rate of individuals with CML. This advancement has elevated the survival rate from a modest 20% to an
impressive range of 80–90%.[1] A major goal of CML therapy is to achieve a stable therapeutic response and to reduce or discontinue TKI use to minimize drug-related toxicity. Ultimately, enhancing the individual's quality of life [4, 5]. Achieving this goal requires a careful study of the various factors that influence the pathophysiology and progression of CML. It is now known that leukemia and other malignancies are closely linked to their microenvironment, with platelets being a key component of the cancer cell microenvironment [6]. Studies have shown that Cancer cells have the ability to trigger platelet activation and initiate the discharge of platelet granules and Platelet microparticles called PMPs [7]. Platelet microparticles (PMPs) are plasma membrane particles that range in size between 100–1000 nm and constitute more than 90% of bloodstream microparticles. PMPs carry biomolecules such as lipids, proteins, growth factors, cytokines, and microRNA [8], and their molecular composition is influenced by the formation process, stimulus type, and agonist of PMP formation.[7]. Research has demonstrated that PMPs play a significant role in intercellular communication and in communication between cells and their environment by transferring chemical and genetic contents to different cells.[9]. While the interaction and effect of PMPs on the biology of various diseases and malignancies have been studied [7], the current knowledge in this field remains incomplete and requires further investigation. In this study, we evaluated the impact of PMPs on vital aspects of K562 cancer cells related to chronic myeloid leukemia (CML), including apoptosis, survival, metabolic activity, proliferation, and gene expression patterns (Bax, Bcl-2, P21, P53, and CyclinD1).

2. Materials and Methods

2.1. Preparation of platelet microparticles

To prepare PMPs, platelet bags were collected from the Kerman blood transfusion center and underwent standard screening tests. The contents of the bags were centrifuged twice at room temperature at 2500 g for 10 minutes to remove white blood cells, erythrocytes, and platelets. The resulting supernatant was then centrifuged at 22000 g for a duration of 30 minutes to precipitate the microparticles. Following this, to remove plasma proteins, the resultant pellets underwent washing with phosphate-buffered saline, followed by dissolution in PBS, and were stored at -70°C.

2.2. Characterization of platelet microparticles

To determine the origin of the PMPs, specific platelet markers (CD42b, CD61) were examined using the flow cytometry method. For this purpose, 100 µL of the PMP solution was added to 10 µL of FITC-conjugated anti-CD61 antibody, PE-conjugated anti-CD42b antibody, and an isotype control, and then incubated at room temperature in the dark for 30 minutes. The results were analyzed using the BD FACSCalibur (USA) and Flomax software. To determine the size of the microparticles, the Malvern Master Sizer 2000 laser diffraction system based on DLS (Dynamic Light Scattering) was used. Laser light was irradiated into a suspension containing microparticles, and the scattered light was detected. The average size of the PMPs was calculated using the software installed in the device.
Protein concentrations in the PMPs were quantified using the BCA method with a standard concentration of bovine serum albumin (BSA).

2.3. Cell culture and microparticles treatment

The K562 cell line was cultured in RPMI 1640 (Roswell Park Memorial Institute) medium. The culture medium was supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and enhanced with penicillin (100 units/mL) and streptomycin (100 µg/mL). Subsequently, the cells were incubated in a humidified environment at 37°C with 5% CO2. To investigate the effect of microparticles on the cells, the cell lines were treated with various concentrations of PMPs (100, 200, 400, 800 µg/mL) for 48 and 72 hours in a 5% CO2 incubator at 37°C. Each experiment was performed three times, both before and after incubating PMPs (platelet-derived microparticles) within the cells. The components of the culture medium were obtained from Gibco Life Technologies, USA.

2.4. Evaluation of cell survival

To assess the influence of PMPs on cell viability, K562 cell lines subjected to PMP treatment were exposed to trypan blue. The cells were initially seeded into a 6-well culture plate at a density of 1 × 105 cells per well and subsequently treated with various concentrations of PMPs (100, 200, 400, 800 µg/mL) for time intervals of 48 and 72 hours. The survival rate of the cells was evaluated using trypan blue solution (Sigma-Aldrich, USA), following the guidelines provided by the manufacturer.

2.5. Cell Proliferation Analysis

The influence of platelet microparticles on cell proliferation was examined using the MTT assay. The MTT cell proliferation assay measures the ability of live cells to reduce yellow tetrazolium salts into blue-colored formazan crystals.

Briefly, K562 cells were added to a 96-well culture plate (5 × 103 cells/well) in triplicate and incubated for 48 hours with PMPs (200, 400, 800 µg/mL). Following the removal of the culture medium, the cells were exposed to an MTT solution (Sigma-Aldrich, USA) and incubated for 4 hours at 37°C. Then, dimethyl sulfoxide (DMSO; Merck, Germany) was added to dissolve the formazan crystals. After 15 minutes of incubation at room temperature, the soluble formazan product was measured using an ELISA reader (at 570 nm) (Bio Tek Elkor 808, USA). Simultaneously with the MTT test, the cells were counted using a hemocytometer (Hausser Scientific, Horsham, PA) under a microscope.

2.6. Apoptosis assay

The impact of platelet microparticles on the K562 cell line apoptosis was assessed by co-staining with FITC and PI. Cells were plated into individual wells of cell culture plates at a density of 5 × 105 cells per well. Subsequent to a 48-hour incubation with 400 µg/mL of PMPs, the cells were harvested. Afterward, the cells were washed with PBS and then stained using the FITC Annexin V Apoptosis Detection Kit II following the manufacturer's instructions (BD Biosciences, USA). The Becton–Dickinson FACS system was employed to distinguish the apoptotic cells. Cells that exhibited Annexin V-positive and PI-negative
staining were categorized as being in the early apoptotic phase, while those displaying positive staining for both Annexin V and PI were deemed to be in the late apoptotic phase. A control group consisted of K562 cells cultured in complete media without PMPs.

2.7. Cell Cycle Assessment

Cell cycle analysis with DNA content was employed to examine the cellular distribution across distinct phases of the cell cycle. To assess how PMPs affect cell cycle progression, $1 \times 10^5$ K562 cells were exposed to PMPs (0 µg/mL and 400 µg/mL) over a 48-hour period. After the incubation, the cells were collected, subjected to two washes with PBS, and then treated with pre-cooled 70% ethanol fixative overnight at -20°C. This step served to immobilize the cells and preserve their DNA content. Following ethanol fixation, additional PBS washes were performed, and a solution containing 0.5 µg/mL RNase in PBS was added to the cell pellet. This mixture was then incubated for a 30-minute period at 37°C. Subsequently, cells were stained with 50 µg/mL propidium iodide (PI) for an additional 30 minutes.

After these preparation steps, a FACScan flow cytometer (provided by Becton Dickinson) was utilized to analyze the cells. The fluorescence signals were used to determine the relative DNA content of each cell. This analysis provided insights into any alterations in cell cycle progression caused by exposure to PMPs.

2.8. Gene expression analysis using real-time PCR amplification

RNA extraction was performed using Trizol Reagent (Invitrogen, USA). The extracted RNA's purity and concentration were assessed by analyzing the ratio of light absorption at wavelengths of 260 nm and 280 nm using a Nano Drop 1000 Spectrophotometer. Additionally, the quality of the extracted RNA was evaluated through a process involving the separation of RNA molecules on an agarose gel using electrophoresis. The reverse transcription (RT) process was carried out using the cDNA Synthesis kit, manufactured by Fermentas (Thermo Fisher Scientific, USA), involving the conversion of RNA into complementary DNA (cDNA).

Real-time PCR experiments were conducted with a total solution volume of 10 µL. This mixture included RealQ Plus 2x Master Mix Green from Amplicon (Denmark), primers at a concentration of 0.5 µM, complementary DNA (cDNA) at a concentration of 70 ng/µL, and water. The amplification process occurred within the RotorGene Q Real-time PCR System made by Qiagen (USA). Specific primers were employed for analyzing certain genes, such as Bax, Bcl2, CyclinD1, P53, P21, and GAPDH. If necessary, the sequences of these primers can be provided upon request.

2.9. Statistical analysis

A two-tailed Student's t-test was utilized to assess the statistical significance of the differences in the experimental variable. A p-value below 0.05 was considered indicative of statistical significance.
Statistical analysis was performed using SPSS version 18.0 software, developed by SPSS, Inc., based in Chicago, IL, USA.

3. Results

3.1. Characterization of platelet microparticles

CD42b and CD61 were assessed as platelet-specific markers through flow cytometry analysis. The analysis showed significant expression of CD42b (96%) and CD61 (99.6%) on the surface of isolated microparticles (Fig. 1). Furthermore, the average size of platelet microparticles was measured using dynamic light scattering (DLS) Malvern Master Sizer, and the results showed an average size of 170 nm (Fig. 2).

3.2. Cell viability assay

K562 cells were subjected to different concentration of PMPs (100, 200, 400, and 800 µg/mL) over periods of 48 and 72 hours. Subsequently, the Trypan blue exclusion assay was utilized to ascertain the percentage of cells that remained viable. As shown in (Fig. 3), there was no notable difference in cell viability between the control cells and cells treated with PMPs.

3.3. Proliferation assessment

The influence of PMPs on K562 cell proliferation was assessed by the MTT cell proliferation assay and a hemocytometer. As shown in (Fig. 4) proliferation of K562 cells treated with PMPs exhibited a notable decrease in a dose- and time-dependent manner (P-value < 0.05).

3.4. Apoptosis assay

To explore the potential cytotoxic effect of PMPs on K562 cells, the cells were treated with PMPs (400 µg/mL) for 72 hours. As shown in (Fig. 5) Following a 48-hour exposure of K562 cells to PMPs, no significant influence on early apoptosis or late apoptosis was observed.

3.5. Influence of PMPs on the Cell Cycle

To investigate the influence of PMPs on the cell cycle pattern of K562 cells, cells were exposed to a concentration of 400 µg/mL of PMPs for 48 hours. The nucleus was stained with propidium iodide (PI) to evaluate the cell cycle, and the intensity of DNA staining was examined by flow cytometry. The P-value obtained from the statistical analysis indicates that PMPs have no impact on cell distribution.( Fig. 6).

3.6. Effect of PMPs on Gene expression

To gain a deeper insight into the molecular mechanism responsible for the decrease in the proliferation of K562 cells, an assessment of the expression of genes related to cell proliferation (P21, P53, and Cyclin D1) was conducted. As presented in (Fig. 7), Following the treatment of K562 cells with PMPs, the expression of the P53 and P21 genes increased significantly. This suggests that the suppressive impact
of PMPs on proliferation may be related to the upregulation of the P21 and P53 genes. Finally, our findings demonstrated a notable decrease in the expression of Cyclin D1 within the treatment group. The expression of the anti-apoptotic gene Bcl-2 increased significantly in PMP-treated K562 cells compared to the control cells (Fig. 8). PMPs exhibited no notable influence on the expression of the Bax gene (Fig. 8) (P-Value = 0.602)

4. Discussion

CML accounts for approximately 15% of leukemia cases diagnosed in the adult population [1]. Recent studies suggest that the prevalence of CML is increasing, and the incidence of this disease is anticipated to increase by about 35-fold between 2030 and 2040[10]. While TKIs have led to significant advances in the treatment of CML, the increased resistance to treatment and the prevalence of the disease highlight the need for more detailed studies on the pathophysiological mechanisms affecting the biology of this leukemia[11]. In this regard, the interaction between malignant cells and their environment is an important research field in identifying cancer biology. Understanding the impact of different environmental components on cancer biology is crucial in designing effective treatments [12]. Various studies have shown that platelets, as one of the most important environmental components, are widely involved in the biology of leukemia, and PMPs have attracted much attention as potential mediators of communications between platelets and cancerous cells [12]. PMPs are the most important mediators of intercellular communication and affect the target cell biology by delivering active molecules such as nucleic acids, proteins, and lipids from platelets to the target cell[13–16]. Despite various studies on the impact of PMPs on cancer cells, current knowledge about the effectiveness of platelet microparticles on leukemic cell biology is largely incomplete and requires more detailed studies. In this study, we investigated the consequences of the interaction of PMPs with malignant cells, focusing on the main aspects of cancer cell biology, including apoptosis, metabolic activity, proliferation, and gene expression involved in cancer progression. Our study indicates that PMPs can impact the behavior of K562 cell line and elicit distinct responses. This is consistent with prior research demonstrating that PMPs can reduce metabolic activity in tumor cells by inhibiting mitochondrial function.[17]. Specifically, our findings are in line with those of Vismara et al, who observed that PMPs significantly decreased the metabolic activity of tumor cells but did not have a significant impact on cell viability[12]. Importantly, our results also support the conclusion that PMPs do not induce apoptosis in cancer cells, in line with earlier studies. In our study, we found that PMPs do not change the cell cycle pattern in K562 cells. However, previous studies have reported different impact of PMPs on the cell cycle progression of various cancer cell lines. For instance, Daniel Cacic showed in a study that PMPs caused major changes in the cell cycle progression of the THP-1 cell line. [18]. Similarly, in another study investigating the effect of platelet microparticles on cell cycle patterns in different cell lines related to breast cancer, it was found that PMPs did not significantly alter the cell cycle pattern in the MDA-MB-231 cell line, while they did alter the cell cycle pattern in the BT-474 and SKBR3 cell lines[12]. The exact mechanism underlying these differential responses is unclear, but it may be related to the genetics of the target cells or the types of compounds transmitted by the PMPs.
This suggests that the response of cancer cells to PMPs may depend on various factors and needs to be further investigated.

Several studies have demonstrated the potential anti-tumor effects of PMPs, including their ability to reduce tumor growth in mice with lung and colon cancer [17]. Furthermore, in vitro experiments have shown that PMPs can impede the proliferation of various cancer cell lines, including human leukemia monocyctic cell line (THP-1) and primary cells isolated from the vein of the umbilical cord (HUVEC) [18, 19]. In line with these findings, our study also showed a significant reduction in the population of K562 cells treated with PMPs.

Previous studies have shown that PMPs transfer their genetic contents, such as miRNAs, to target cells and affect gene expression in vitro [15–22]. In our study, we found that the molecular findings were consistent with the results of reduced cell proliferation in cell culture. Specifically, increased expression of p53 and p21 genes, which are the main genes controlling cell proliferation[23, 24]. Previous studies show that increased expression of the P53 gene is directly related to decreased cell proliferation. The P53 gene reduces cancer cell growth and proliferation through pathways dependent on the gene expression of downstream targets, including P21, as well as genes involved in reducing cellular metabolic activity and inducing aging[23]. Similarly, the P21 gene reduces the growth and proliferation of cancer cells through pathways dependent on and independent of the P53 gene, such as controlling metabolic pathways and inducing aging [24]. In our study, we also found that treatment with PMPs reduced the expression of the Cyclin D1 gene in K562 cells. Reduced expression of the Cyclin D1 gene is associated with decreased growth and proliferation of tumors cells[25]. Moreover, we observed that the gene expression of Bcl-2 increased following exposure of K562 cells with PMPs. Studies have shown that the Bcl-2 gene, in addition to being involved in apoptotic pathways, is associated with cell proliferation, and its expression in some cancer cell lines reduces proliferation[26]. Furthermore, a slight reduction in the Bax gene and an increment in Bcl-2 gene expression in our study could be possible factors in preventing the apoptotic effect of platelet microparticles on K562 cells.

These findings suggest that PMPs affect cancer biology through modulating the expression of genes involved in proliferation, survival, and apoptosis. However, additional studies are necessary to elucidate the clarify mechanisms and potential of PMPs in regulating the gene expression in cancerous cells.

Our study shows that platelet microparticles can reduce the metabolic activity and proliferation of K562 cells while also modulating the expression of genes, thereby affecting their biology. studies have shown that PMPs can result in either cancer progression or suppression of tumor growth.[27] Our findings support the inhibitory role of PMPs on cancer cells in vitro However, the exact mechanism underlying these effects remains unclear, and further studies are needed to elucidate it. Moreover, our study highlights the usefulness of using platelet microparticles, which are abundant and easily separable, for studying cancer biology and developing new treatment protocols.

5. Conclusion
our study provides new insights into the interaction between platelet microparticles (PMPs) and cancer cells. and suggest that PMPs can influence the behavior of cancer cells and elicit distinct responses, these results support the growing body of evidence on the anti-tumor effects of PMPs and highlight the need for further research to understand the underlying mechanisms fully. Moreover, our study underscores the importance of studying the impact of environmental components, such as PMPs, in cancer biology, which may ultimately lead to the development of more effective treatments for leukemia and other types of cancer.

**Abbreviations**

CML; Chronic myeloid leukemia  
PMP; Platelet microparticles  
TKI; Tyrosine kinase inhibitor

**Declarations**

The authors declare that they have no conflict of interest.

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**Author Contributions**

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Fariba Nikravesh. The first draft of the manuscript was written by Dr. Hajar Mardani Valandani and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Ethics approval**

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Vice Chancellor for Research and Technology of Kerman University of Medical Sciences (IR.KMU.REC.1399.420).
Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon request.

References


Figures

**Figure 1**

Characterization of PMPs, Flow cytometry analysis showed high expression of surface-expressed molecules CD61 (99.6%) and CD42b (96%) on PMPs
Figure 2

Size distribution of PMPs derived from platelet; the size distribution of PMPs was determined to be in the range of 170nm
Figure 3

PMP treatment did not produce any noticeable impact on the viability of K562 cells. Various concentrations of PMP (100, 200, 400, 800 μg/mL) were added K562 cells, and the trypan blue exclusion method was employed to assess cell viability following different durations of exposure (average ± standard error, sample size = 3). (P-Value=0.858)

Figure 4

K562 cells were treated with different concentrations of PMP and proliferation was evaluated using MTT and hemocytometer method, after exposure at 48 and 72 hours (mean ± SE, n = 3). PMP treatment, the population of K562 cells decreased in a dose time-dependent manner. Hemocytometer result (P-Value<0.0001, P-Value<0.0001), MTT assay (P-Value=0.001, P-Value=0.005)
Figure 5

PMP had no cytotoxic effect on K562 cells. After being exposed to a concentration of 400 μg/ml PMPs for 48 hours, K562 cells were subsequently analyzed using flow cytometry to evaluate the binding of Annexin V and Annexin PI. PMPs did not result in any notable impact on the apoptosis of K562 cells, (P-Value=0.09)
K562 Cells were exposed to PMPs at a concentration of 400 µg/ml for a 48 hours. The alterations in the distribution of cell cycle phases were evaluated through cell cycle analysis. It was determined that PMPs have no impact on the various phase.
Figure 7

Analysis of Gene Expressions. PMPs stimulated an increase in the expression of the P53 and P21 genes while causing a reduction in the expression of the cyclin D1 gene within K562 cells. The K562 cells were subjected to concentration of 400 µg/ml PMPs for 48 hours. Following this treatment, RNA was extracted and transformed into complementary DNA (cDNA) through a synthesis process. The outcomes of the Sybr-green real-time PCR, utilizing specific primers, have been presented. All recorded values were standardized with respect to the GAPDH gene (sample size = 3, P-Values < 0.01, 0.024, and 0.013)
Analysis of Gene Expressions. PMPs led to an increase in the expression of the Bcl2 gene within K562 cells. However, no notable difference in BAX gene expression was observed between the treatment group and the control groups. The K562 cells were treated with PMPs at a concentration of 400 µg/ml for a span of 48 hours. All recorded values were normalized using the GAPDH gene as a reference (sample size = 3, P-Values = 0.027 and 0.602)