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Long-term exposure of human U87 glioblastoma cells to polyethylene microplastics: Investigating the potential cancer progression

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ABSTRACT

Precancerous cells are present in all human bodies. Various environmental triggers can promote the development of cancer. Microplastics, an emerging concern, may potentially act as one such trigger, contributing to cancer initiation or progression. Studies have confirmed the presence of microplastics within the human body. This raises concerns about their potential toxicity and health risks. In the present study, we aimed to investigate the impact of polyethylene microplastics (PE-MPs) within the size range of 37–75 microns on glioblastoma cancer cells. Initially, we assessed the short-term effects of six different concentrations of PE-MPs (20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.62 mg/mL) on the U87 glioblastoma cell line. The results demonstrated that PE-MPs exposure led to an increase in cell proliferation compared to the untreated control group. Based on these findings, we decided to further explore the long-term effects of PE-MPs on U87 cancer cells. To evaluate the long-term effects, U87 glioblastoma cells were continuously exposed to 0.005 g of PE-MPs over an extended period of 26 days. Chronic exposure to PE-MPs significantly increased the proliferative and migratory capacities of U87 cells compared to the unexposed control group. Furthermore, continuous PE-MPs exposure altered the behavior and morphological characteristics of U87 cells. These cells exhibited a propensity to aggregate and form colonies within the culture flask. The formation of spheroid structures was also observed in the PE-MPs-exposed cell population. The results of this research indicate that polyethylene microplastics can promote the progression of glioblastoma cancer.

1. Introduction

Microplastics (MPs) and nanoplastics (NPs) are ubiquitous environmental pollutants resulting from the degradation of larger plastic waste [11,7]. MPs are defined as plastic particles smaller than 5 millimeters [12], while NPs are those smaller than 100 nanometers [19]. Human exposure to these particles occurs through ingestion, inhalation, and dermal contact [24]. Worldwide, individuals unknowingly consume between 0.1 and 5 g of microplastics weekly, equivalent to the weight of a credit card [25]. MPs and NPs have been detected in various biological fluids, such as blood [18], breast milk [22], sputum [14], and human feces [32]. A significant concern regarding microplastics is their potential to increase cancer risk in humans [27].

The human body constantly generates cells with the potential to become cancerous [28]. Microplastics may potentially trigger increased cancer risks [13]. Recent studies have revealed an increased incidence of colorectal cancer among workers in the plastic and rubber industries, suggesting a potential link between occupational exposure to plastic-related compounds and cancer risk [30]. Moreover, microplastic particles have been detected in various cancerous tissues, including lung, gastric, colorectal, and cervical tumors. This raises significant concerns about the potential role of microplastics in carcinogenesis and necessitates further investigation into the relationship between microplastic exposure and cancer development [31,33].

Recent studies have revealed a potential link between microplastic exposure and the accelerated proliferation of cancer cells. In a study led

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by Kim et al., polystyrene microplastics significantly enhanced the proliferation of gastric cancer cells [17]. Furthermore, a separate investigation demonstrated a similar increase in the proliferation of skin cancer cells following exposure to microplastics [29]. Experimental evidence suggests that microplastics and nanoplastics may exacerbate cancer progression. In a study using Bhas 42 cells, exposure to polyethylene terephthalate (PET) nanoplastics at a concentration of 200 μg/mL for 21 days demonstrated non-genotoxic tumor-promoting effects [8]. Additionally, exposure of GES-1 cells to polystyrene microplastics (PS-MPs) for 24 h led to increased expression of tumor-related proteins and redox-dependent activation of the β-catenin/YAP signaling pathway, suggesting a novel toxic mechanism and potential carcinogenic effects of PS-MPs [6].

Cellular motility represents the initial phase of the metastatic cascade and serves as a critical determinant of neoplastic aggression and disease progression. Alarming evidence suggests that microplastics may contribute to increased metastasis in cancer cells. Short-term exposure to polystyrene microplastics has been shown to increase metastasis in colorectal cancer cells [3]. Furthermore, in a study led by Jun Hyung Park, the potential role of polypropylene microplastics (PPMPs) in promoting breast cancer metastasis was explored. By exposing MDA-MB-231 breast cancer cells to PPMP concentrations of 1.6 mg/mL for 24 h, the researchers uncovered a concerning link between PPMP exposure and the upregulation of metastasis-associated genes and cytokines [20]. Additionally, the impact of microplastics on cancer has been evaluated in animal models. Chen et al. examined the effects of polystyrene nanoplastics (PS-NPs) on epithelial ovarian cancer (EOC) progression using a mouse model. The mice were exposed to 100 nm PS-NPs at a 10 mg/L concentration in their drinking water for 27 days. The in vivo results demonstrated that oral exposure to PS-NPs significantly increased tumor weights and volumes compared to control mice not exposed to PS-NPs [4].

Our research investigates the impact of polythene microplastic (PE-MPs) exposure on glioblastoma cancer cells. We observed a concerning trend, glioblastoma cells continuously exposed to microplastics for 26 days exhibited significantly more aggressive behavior compared to the control group. These findings suggest a potential link between microplastics and heightened glioblastoma malignancy. This study highlights the need for further research into the potential carcinogenic and tumor-promoting effects of microplastic long-term exposure.

2. Materials and methods

2.1. Polyethylene microplastics preparation and characterization

Polyethylene powder was procured from Petrochemical, Iran (Lorestan Petrochemical Grade 62N07). A two-stage sieving process was employed to separate different sizes of polyethylene microplastics (PE-MPs). Initially, a 75-micron sieve was used to remove larger polyethylene particles. Subsequently, the remaining PE-MPs was passed through a 37-micron sieve, resulting in a fraction of polyethylene microplastics with sizes ranging between 37 and 75 microns. The morphology of polyethylene microplastics was examined using both light microscopy and scanning electron microscopy. Additionally, the chemical structure of PE-MPs was determined using Raman spectroscopy (Teksan company, model Takram P50C0R10, Iran) with a wavelength of 532 nm and a laser power of 0.5–70 milliwatts.

2.2. Cell lines and cell cultures

Human U87 glioblastoma cells was obtained from Tehran University of Medical Sciences (TUMS, Iran). U87 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Anacell, Iran) supplemented with 10 % fetal bovine serum (FBS; Anacell, Iran) and 1 % penicillin/streptomycin (1 % Pen-Strep; Bioidea, Iran) at 37 °C in a humidified 95 % air and 5 % CO_2 atmosphere. The cells were passaged when they reached 80 % confluence using 0.25 % Trypsin-EDTA (0.25 % Trypsin-EDTA; Bioidea, Iran) [21,26].

2.3. Effect of short-term PE-MPs exposure on U87 cell proliferation

The MTT assay was employed to evaluate the short-term effects of microplastics on the proliferation of U87 cells. For this purpose, U87 cells were seeded at a density of 5000 cells per well in 96-well plates and incubated for 24 h to facilitate adherence to the well surface. Following the initial incubation period, the cells were exposed to six different concentrations of polyethylene microplastics (20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.62 mg/mL). After a 72-h incubation period, 5 mg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Aldrich, Germany) was dissolved in 1 mL of phosphate-buffered saline (PBS, Merck Company, Germany). Subsequently, 10 microliters of this solution were added to each well. The plate was then transferred to an incubator and incubated for an additional 4 h. Following this incubation, the culture medium was carefully removed, and 100 microliters of dimethyl sulfoxide (DMSO, Merck Company, Germany) were added to each well. DMSO was employed to dissolve the formazan crystals, resulting in a purple solution. The solution absorbance was read at 570 nm and 630 nm with a microplate reader (Biotech, Winooski, VT). The experiment included 3 replicates and was repeated in two independent experiments.

2.4. Protocol for long-term exposing U87 cells to PE-MPs

We designed an experimental study to evaluate the long-term effects of polyethylene microplastics exposure on the U87 glioblastoma cell line. U87 glioblastoma cells were cultured in two 25 cm^2 tissue culture flasks (SPL, Korea), one designated as the control group and the other as the PE-MPs-exposed group. To prepare the PE-MPs-exposed group, 0.005 g of polyethylene microplastics were weighed and subsequently sterilized under UV irradiation for 30 min. Sterilized microplastics were transferred to a sterile falcon 15 tube under a laminar flow hood. Thereafter, one milliliter of complete culture medium was added to the tube, and the microplastics were thoroughly dispersed by pipetting. The microplastic suspension was then gently added to the PE-MPs-exposed flask. In this study, U87 glioblastoma cells were exposed to polyethylene microplastics continuously for 26 days. In this study, U87 glioblastoma cells were continuously exposed to polyethylene microplastics for 26 days. During the 26-day exposure period, when the cells in the treatment and control flasks reached 80 % confluence, they were simultaneously passaged using 0.25 % Trypsin-EDTA. After each passage, cell counting was performed, and 200,000 cells were transferred to a new flask. For the treatment group, 0.005 g of polyethylene microplastic was added to each new flask during every passage according to the protocol. This process ensured consistent exposure to microplastics throughout the experiment.

2.5. Migration assay

To assess the impact of microplastics polyethylene (PE) on cellular migration, U87 cells were subjected to persistent PE exposure for 26 days, and their migration was compared to that of unexposed U87 control cells. Both groups of cells were seeded at 200,000 cells per well in a 12-well plate (SPL, Korea) containing DMEM supplemented with 10 % FBS and 1 % penicillin-streptomycin. The cells were incubated overnight to allow for adhesion and the formation of a confluent monolayer. Subsequently, a vertical scratch was created in each well using a 200 μL pipette tip. The wells were then washed twice with phosphate-buffered saline (PBS) to remove any detached cells and debris before the addition of fresh DMEM supplemented with 5 % FBS. After a 24-h incubation period to allow cell migration into the scratch area, brightfield images were captured. The area of the gap between the migrating cell fronts was measured at both 0 h and 24 h post-scratch

using ImageJ software to assess the extent of scratch closure.

2.6. Effect of long-term PE-MPs exposure on U87 cell proliferation

The MTT assay was employed to assess the growth of U87 cells exposed to microplastics polyethylene for 23 days in comparison to the control group. To examine the impact of polyethylene microplastic exposure on U87 cell growth, 5000 cells were seeded in 96-well plates for the PE-MPs-exposed group and control groups. After a 72-h incubation period, the proliferation of PE-MPs-exposed U87 cells and the control group was evaluated using the MTT assay, as described in Section 2.3. The experiment included 8 replicates and was repeated in two independent experiments.

2.7. Statistical analysis

To ensure reliability, the experiment comprised at least two independent trials, each with three replicates. Statistical significance was determined using one-way ANOVA and Student's t-tests for data analysis.

3. Results

3.1. Morphological features and chemical composition of PE-MPs

The morphology of PE-MPs was characterized using scanning electron microscopy (SEM) and light microscopy (Fig. 1a,b,c,d). PE-MPs had a polyhedral shape with protrusions on their surface. Raman spectroscopy analysis validated the existence of functional groups of polymeric polyethylene within the polyethylene microplastics (Fig. 1e). The most notable identification peaks in polyethylene were the C-C stretching vibration peaks at 1061 and 1127 cm^{-1}, as well as the CH2 bending vibration peaks at 1297 and 1441 cm^{-1}, relative to the CH2 stretching vibration peak at 2886 cm^{-1} [23].

3.2. Proliferation response to short-term exposure of PE-MPs

At the beginning of this research, the short-term effects of microplastic exposure on the proliferation of U87 cells were investigated using the MTT assay over a 72-h period. The cells were treated with varying concentrations of polyethylene microplastic (20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.62 mg/mL) to assess the impact on proliferation (Fig. 2). The MTT assay results revealed an increase in the proliferation of U87 cells exposed to microplastic compared to the untreated control group. Notably, at the highest concentration of 20 mg/mL, the observed increase in proliferation was statistically significant, with a p-value of 0.0247 obtained from a one-way ANOVA multiple comparison analysis. The findings of another study align with our research. In a study examining the effect of microplastics on skin cancer cells, the proliferation rate of cancer cells significantly increased in MTT assays at 24, 48, and 72 h compared to the control group [29].

3.3. PE-MPs-exposed U87 cells exhibited tendency to form clusters

Over a period of 26 days and across 10 passages, U87 cells were exposed to polyethylene microplastics. The concentration of PE-MPs was 0.005 grams per passage. After each passage, the culture medium was replaced, and 0.005 g of new microplastic particles were added to maintain a consistent exposure level. By day 21 and at the 8th passage, cells exposed to PE-MPs exhibited a marked tendency to aggregate and form clusters (Fig. 3b,c), while this aggregation behavior was not observed in the control group cells (Fig. 3a). The cells were passaged on day 21 and were again exposed to 0.005 g of microplastics at the 9th passage until the 23rd day. During these 48 h of exposure to PE-MPs, colonies of U87 cells were observed forming (Fig. 3e,f). These colonies were not present in the control group cells (Fig. 3d). On day 23, the U87 cells were passaged for the 10th time and re-exposed to 0.005 g of microplastics. The cells were exposed to these fresh microplastics for 72 h until day 26. During this time period, colonies of U87 cells were observed forming again (Fig. 3h), in contrast to the control group where no colonies formed (Fig. 3g). In a study conducted to evaluate the effect of nanoplastics on tumor promotion, Bhas 42 cells developed more transformed foci after 21 days of exposure to PET nanoplastics compared to the control group[8]. These results suggest that the long-term effects of nanoplastics and microplastics can lead to tumor promotion.

3.4. Collective migration of U87 cells exposed to PE-MPs

As described in Section 3.2, U87 cells exposed to microplastics displayed a tendency to form colonies and clusters. Notably, around these colonies, collective migration and morphological changes of U87 cells were observed on days 23 and 26. This collective migratory behavior of cells was not witnessed in the control group, which did not receive microplastic exposure (Fig. 4a,b).

3.5. PE-MPs induced spheroid formation in U87 Cells

Between days 23 and 26, when the cells were exposed to microplastics for 72 h, spheroids formed and detached from the bottom of the flask. Polyethylene microplastics adhered to the surface of these floating spheroids. The spheroids formed perfectly round shapes and floated on the surface of the culture medium (Fig. 5a,b,c).

3.6. Effect of PE-MPs on U87 cells migration

The scratch assay was employed to examine the impact of PE-MPs on the migratory behavior of U87 cells. Cell migration was observed in both control and PE-MPs-exposed U87 cell groups. However, the migration of U87 cells exposed to polyethylene microplastics was significantly enhanced compared to the control group (Fig. 6a-d). Quantitative analysis revealed that PE-MPs-exposed U87 cells filled 45.42 % ± 2.21 of the scratched area, whereas control cells filled only 14.39 % ± 7.06 (Fig. 6e). This difference was statistically significant, with a p-value of 0.0272 obtained from an unpaired t-test. Notably, in the scratch assay, PE-MPs-exposed U87 cells displayed collective movement and formed cell aggregates within the scratched area (Fig. 6d). Migration of gastric cancer cells exposed to polystyrene microplastics for four weeks has been reported in previous studies [17]. Additionally, upregulation of genes associated with metastasis was observed in breast cancer cells exposed to polypropylene microplastics for 12 h. This evidence indicates that the long-term effects of microplastics may contribute to increased metastasis [20].

3.7. Proliferation response to long-term exposure of PE-MPs

The proliferation rate of U87 cells exposed to polyethylene microplastics for 23 days was evaluated and compared to a control group over a 72-h period using an MTT assay (Fig. 7). Cells exposed to PE-MPs exhibited a significant increase in proliferation rate compared to the control group. The mean proliferation of PE-MPs-exposed U87 cells was 140.4 % ± 13.63, significantly higher than the control group, which showed a mean proliferation of 100.1 % ± 18.47. This difference was statistically significant, with a p-value of 0.0002 obtained from an unpaired t-test. The findings of Kim et al. indicate that exposure of gastric cancer cells to polystyrene microplastics for four weeks resulted in a significant increase in cell proliferation compared to the control group [17]. These results suggest that prolonged exposure to microplastics promotes the proliferation of cancer cells.

4. Discussion

Microplastics have become a major health concern in recent years.

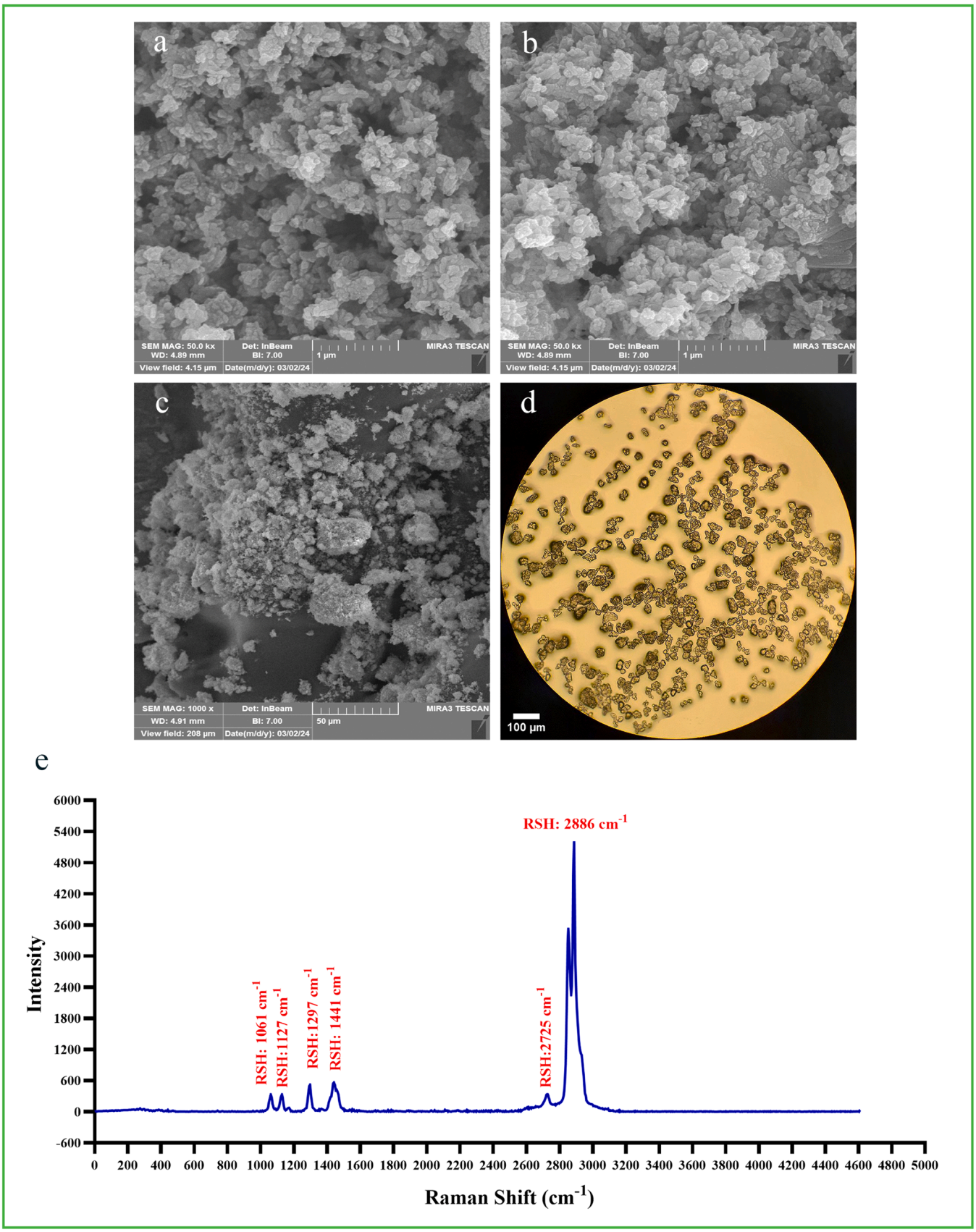

Fig. 1. Characterization of PE-MPs. (a-c) Scanning electron microscopy (SEM) images reveal that the PE-MPs have a polyhedral shape with protrusions on their surface. (d) Light microscopy image of polyethylene microplastics captured using a 10x objective lens. (e) Raman spectrum of polyethylene microplastics exhibiting characteristic peaks, including C-C stretching vibrations at 1061 and 1127 cm^{-1}, CH2 bending vibrations at 1297 and 1441 cm^{-1}, and CH2 stretching peak at 2886 cm^{-1}.

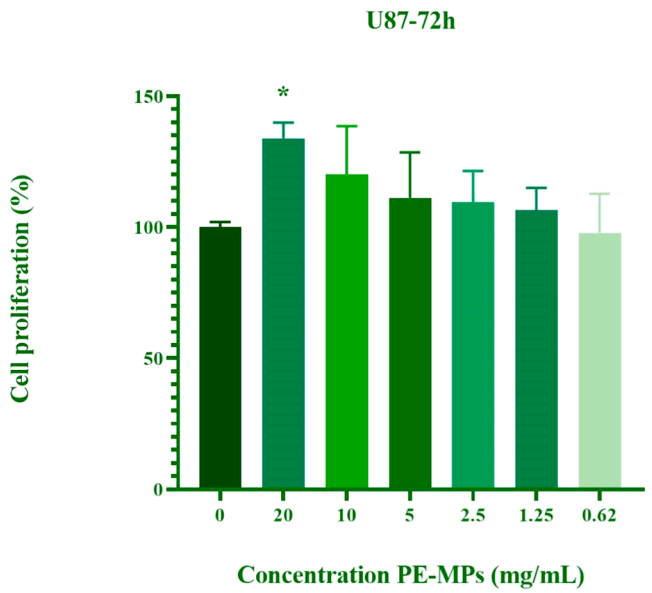

Fig. 2. U87 cell proliferation after 3-day PE-MPs exposure. Cells treated with various concentrations (20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.62 mg/mL) exhibited enhanced proliferation compared to the untreated control group, with the increase being statistically significant at the highest concentration of 20 mg/mL.

These tiny plastic particles can easily enter the human body through food, water, and air [2]. The alarming reality is that microplastics can traverse the intricate defenses of the blood-brain barrier, amassing in brain tissues and potentially disrupting neurological functions [10]. The human body continuously produces potentially neoplastic cells that express tumor-associated neoantigens. However, the immune system has evolved to recognize these abnormal cells by detecting their unique neoantigens. This allows the immune system to specifically target and destroy precancerous cells before they can develop into malignant tumors [28]. There is concern that microplastics could prompt precancerous cells in the human body to progress into malignant cancers. Moreover, recent research has detected microplastics in various tumor tissues, including lung, gastric, colorectal, and cervical tumors. This finding underscores the need for further investigation into the relationship between cancer and microplastics [31,33]. To gain a better understanding of the potential effects of microplastics, we conducted a 26-day study exposing U87 cancer cells to 0.005 grams of polyethylene microplastics.

The potential impact of microplastics on cells is influenced by the type of polymer as well as the size and concentrations [16,5]. Polyethylene was selected as the microplastic for this research due to its ubiquity in both environmental settings and everyday products [15]. Globally, humans could potentially be ingesting an average of 0.1–5 g of microplastics per week [25].

In our study, exposure of U87 cells to 0.005 g of polyethylene microplastic for 26 days significantly enhanced their ability to form spheroids and colonies, a phenomenon not observed in the control group. In fact, exposure of U87 cells to polyethylene microplastics led to an increased tendency for cell aggregation and forming large clusters. This aggregation was accompanied by collective migration and morphological changes of U87 cells. Barguilla et al. exposed prone-to-transform mouse embryonic fibroblast (MEF) cells to polystyrene nanoplastics for six months. The study revealed that long-term exposure led to the development of an advanced tumoral phenotype in these cells, characterized by anchorage-independent growth and increased migration ability [1]. Moreover, a study on nanoplastics and tumor promotion found that Bhas 42 cells exposed to PET nanoplastics for 21 days developed more transformed foci than unexposed cells. This suggests that long-term nanoplastic exposure may contribute to tumor promotion [8]. Animal studies have revealed alarming effects of microplastics and nanoplastics on tumor growth. Exposure to drinking water containing polystyrene nanoplastics for 27 days led to significant increases in both tumor volume and mass in mice [4]. This evidence shows that microplastics and nanoplastics increase the aggressive behavior of cancer cells.

In our research using the MTT assay demonstrated that U87 cells exposed to microplastics exhibited enhanced proliferation in comparison to the control group. Similar observations of enhanced proliferation in cancer cells exposed to microplastics have been documented in various studies. In a study conducted by Kim and colleagues, five different human gastric cancer cell lines (AGS, MKN1, MKN45, NCI-N87, and KATOIII) were exposed to microplastics polystyrene (PS-MPs) particles at a concentration of 8.61 × 10^5 particles per mL for four weeks. MTT assay results revealed a significant increase in proliferation in all five cell lines following PS-MPs exposure [17]. Wang et al. also found similar results regarding skin cancer cells. Their MTT result showed that microplastics exposure stimulated the proliferation of A431 and SCL-1 cells in a dose-dependent manner over a 72-h period [29].

The spread of cancer cells from the primary tumor to distant sites in the body, known as metastasis. Metastasis is enabled by cancer cell migration and invasion, which is the major cause of death in cancer patients [9]. In our study, the scratch cell assay results indicated that cell migration was significantly enhanced in U87 cells exposed to 0.005 g of polyethylene microplastic for 26 days compared to the control group. Research conducted by Kim et al. on human gastric cancer cell lines exposed to microplastics for four weeks revealed enhanced cell migration [17]. Similarly, Barguilla et al. involving polystyrene nanoplastics and MEF cells prone to transformed growth, supported the observation of cell migration after a six-month exposure period [1]. Moreover, when HCT116 human colon cancer cells were exposed to polystyrene microplastics for 48 h, their migration speed increased compared to the control group [3]. In contrast, a study by Park et al. found that exposing breast cancer cell lines MDA-MB-231 and MCF-7–1.6 mg/mL of polypropylene microplastics for 12 h did not affect cell migration. However, the microplastic exposure led to an upregulation of genes associated with metastasis in these cells [20]. Probably, the duration of microplastic exposure plays a crucial role in driving cell migration and metastasis.

The potential role of microplastics in cancer promotion is a subject of increasing scientific interest and debate. Current research has identified several potential links, but significant knowledge gaps remain. Future studies should focus on elucidating the precise mechanisms and cellular behaviors involved in microplastic-induced carcinogenesis. This approach will help resolve existing uncertainties and provide a more comprehensive understanding of the issue.

5. Conclusion

The results of our short-term experiments showed a dose-dependent increase in cell proliferation when U87 cells were exposed to various concentrations of PE-MPs. Building on these initial observations, our long-term study, conducted over 26 days, provided even more striking results. Our results showed that long-term exposure to polyethylene microplastics significantly increased the migratory capacity of U87 cells compared to untreated control cells. Additionally, PE-MPs-exposed U87 cells exhibited greater proliferation compared to the control group not exposed to microplastics. We observed a tendency for PE-MPs-exposed cells to aggregate and form colonies within the culture flask. These observations raise important questions about the potential role of microplastics as environmental factors influencing cancer development and progression. Future research should aim to elucidate the molecular mechanisms underlying the observed effects. This could involve comprehensive gene expression analysis, investigation of key signaling pathways involved in cancer progression, and examination of potential

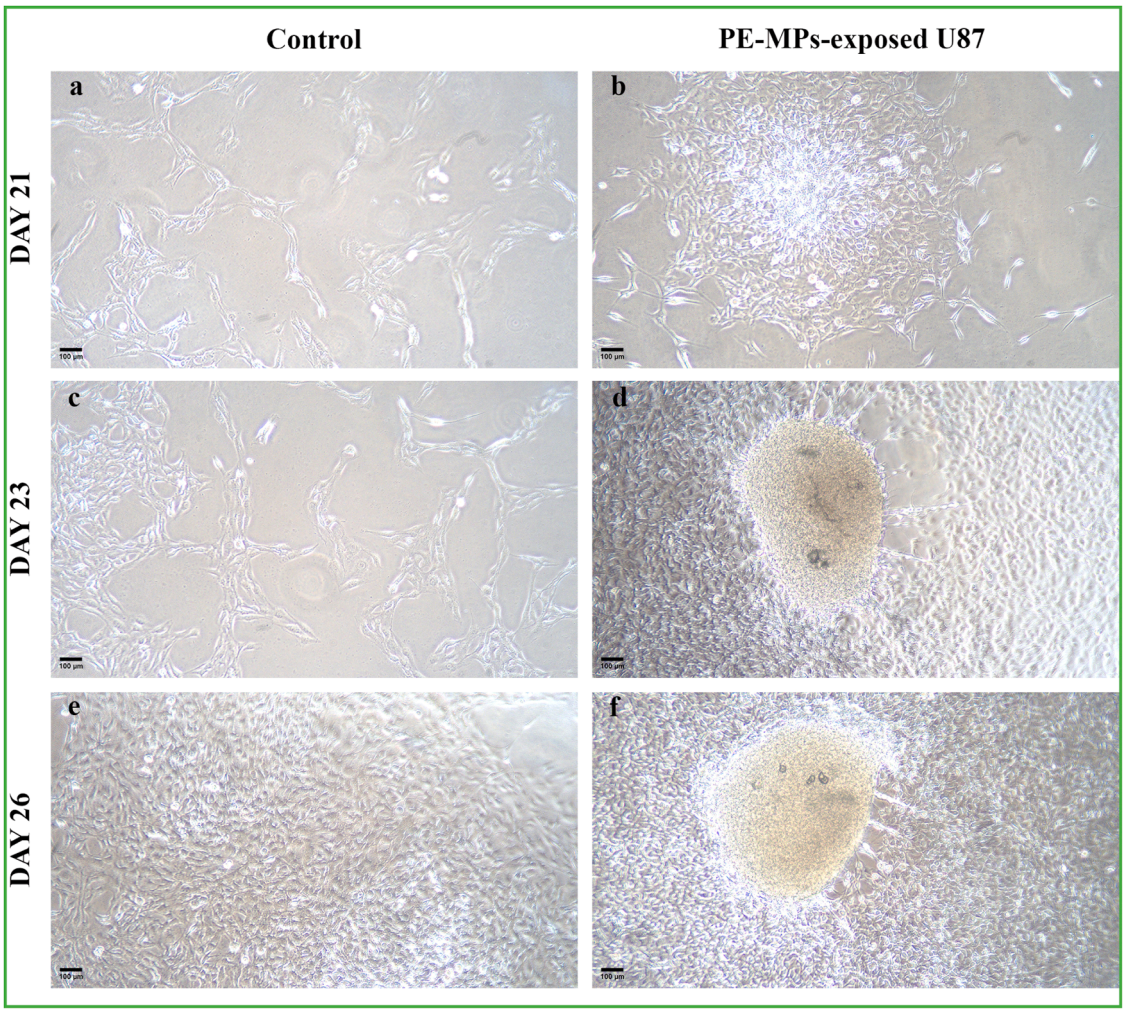

Fig. 3. The tendency of U87 cells treated with PE-MPs to form colonies. (a, d, g) Show the control group of U87 cells not exposed to polyethylene microplastics. (b, c) Show U87 cells after 21 days and 8 passages of exposure to PE-MPs, exhibiting a distinct tendency to form colonies. (e, f) Illustrate U87 cells after 23 days and 9 passages of exposure to polyethylene microplastics, with colonies formed. (h) Shows U87 cells after 26 days and 10 passages of PE-MPs exposure, where large colonies have formed, similar to those observed at day 23.

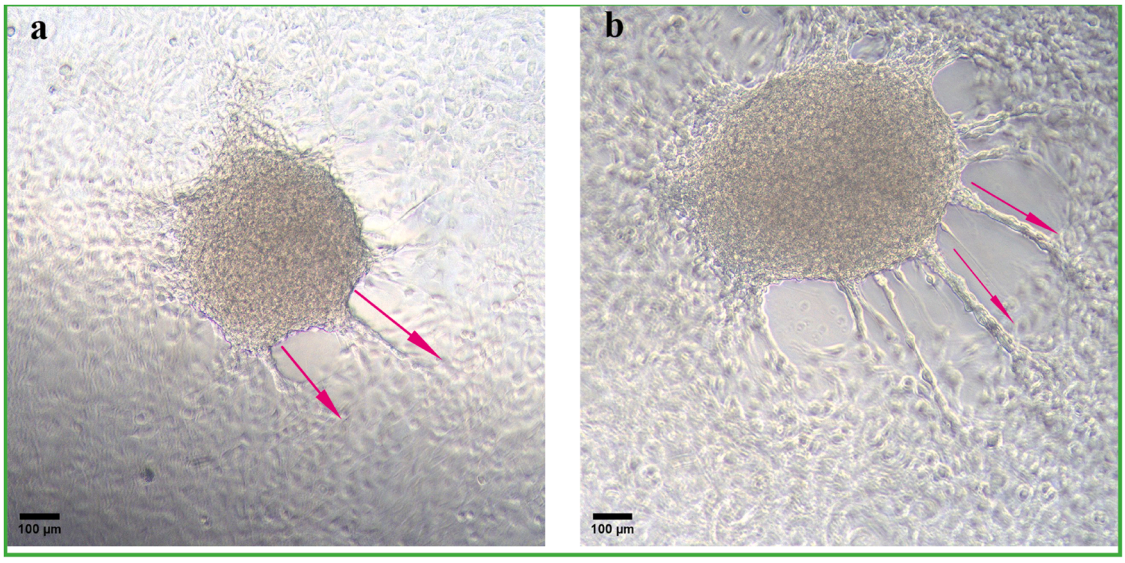

Fig. 4. Collective migration and morphological changes in PE-MPs-exposed U87 cells. (a, b) On day 23, PE-MPs-exposed U87 cells exhibit collective migration, as indicated by the arrows. The migrating cells also display morphological alterations, deviating from their typical shape and appearance.

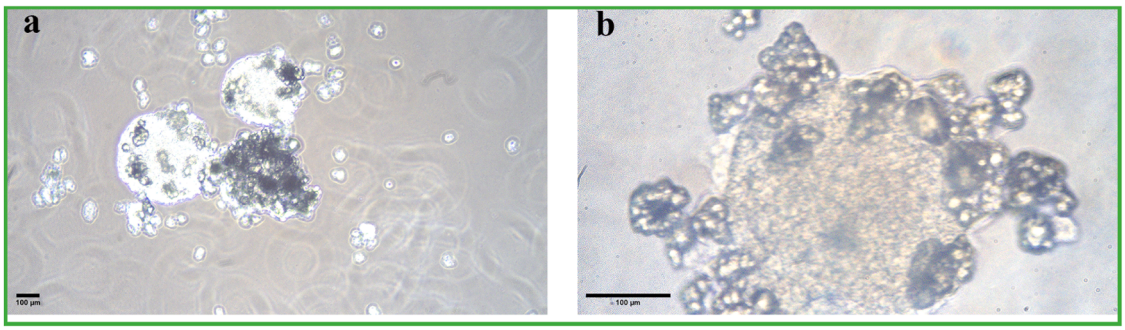

Fig. 5. Spheroid formation in PE-MPs-exposed U87 cells. (a-c) On the 26th day, spheroids formed by PE-MPs-exposed U87 cells are observed floating in the culture medium. Polyethylene microplastics are visibly attached to their surface.

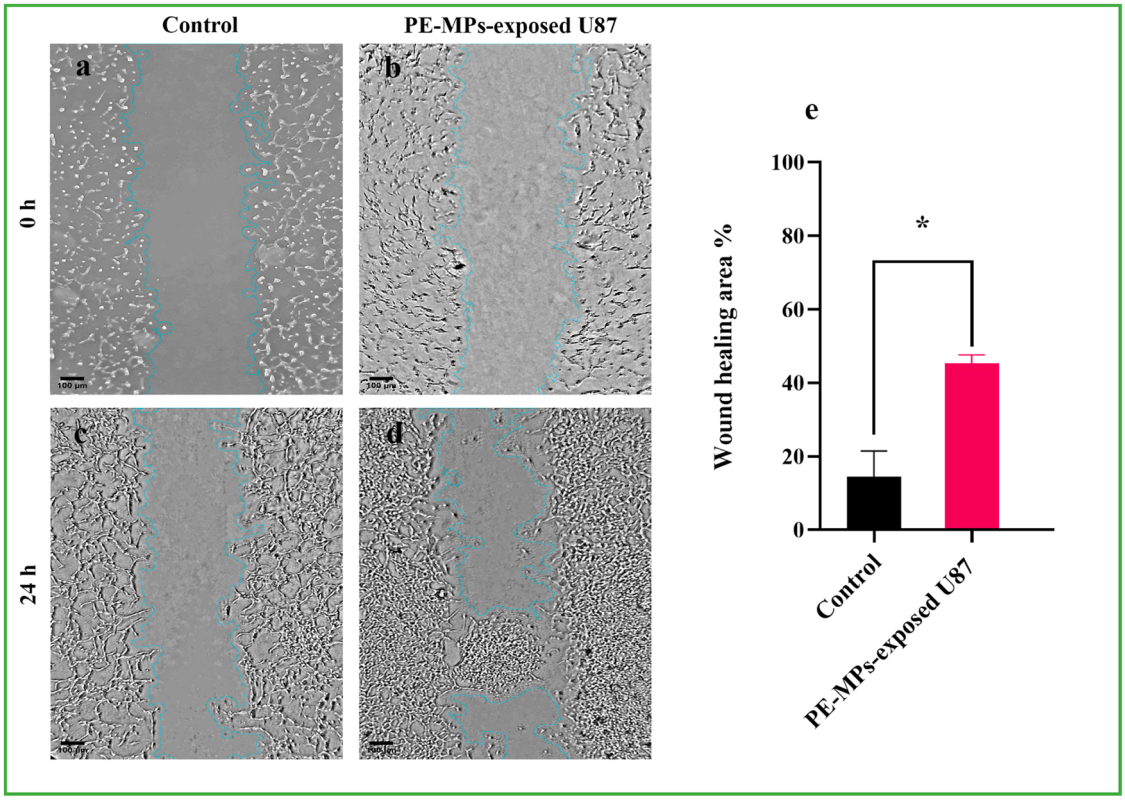

Fig. 6. Migration of U87 cells after 26 days of PE-MPs exposure, assessed by scratch assay. (a) Control U87 cells and (b) PE-MPs-exposed U87 cells at 0 h, immediately after creating the scratch. (c, d) Both cell groups 24 h post-scratch, with (d) showing the aggregation of PE-MPs-exposed U87 cells at the scratch area. (e) Quantification of the scratch assay revealed that PE-MPs-exposed U87 cells exhibited a significantly higher percentage (45.42 %) of cells migrating into and filling the scratched area compared to control cells (14.39 %).

epigenetic modifications induced by PE-MPs exposure. Additionally, exploring the impact of different microplastic characteristics such as size, shape, and polymer type could provide crucial information about structure-activity relationships and help identify the most harmful forms of microplastics.

CRediT authorship contribution statement

Akram Ahvaraki: Validation, Methodology, Investigation. **Parisasadat Rafazi:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Hamed Haghi-Aminjan:** Validation, Methodology, Investigation, Funding acquisition, Conceptualization. **Zeinab Bagheri:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Mahban Rahimifard:** Validation, Methodology, Investigation.

Declaration of Generative AI and AI-assisted technologies in the writing process

The manuscript's text has been refined using artificial intelligence tools solely for language enhancement. All research findings, data analysis, and interpretations remain the original work of the authors, unaltered by AI assistance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

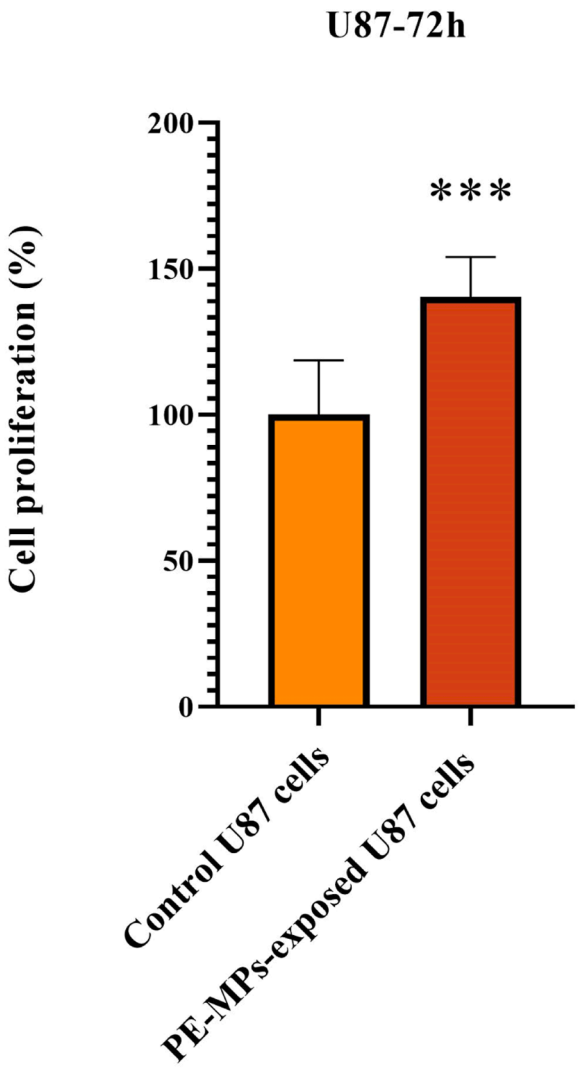

Fig. 7. U87 cell proliferation after 23-day PE-MPs exposure. Chronic PE-MPs exposure enhanced U87 proliferation rate. MTT assay over 72 h showed mean proliferation of 140.4 % ± 13.63 for PE- PE-MPs-exposed U87 cells and 100.1 % ± 18.47 for control.

Data availability

Data will be made available on request.

Acknowledgment

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