PROSPECTS
IN PHARMACEUTICAL SCIENCES

Prospects in Pharmaceutical Sciences, 24(3), 18-27
https://prospects.wum.edu.pl/

Original Article

GOLD NANOPARTICLES AS MULTIFACTORIAL MODULATORS OF PANCREATIC CANCER CELL BEHAVIOR

Małgorzata Lasota[1,2*], Adam Kozik[2], Milena Serwatka[2], Anna Wiśniewska[3]

[1] *Center for Biomedicine and Interdisciplinary Sciences, Faculty of Medicine, Jagiellonian University Medical College, 31-531 Krakow, Poland;*

[2] *Student Scientific Group of Targeted Therapy and Supramolecular Systems, Faculty of Medicine, Jagiellonian University Medical College, 31-531 Krakow, Poland;*

[3] *Chair of Pharmacology, Faculty of Medicine, Jagiellonian University Medical College, 31-531 Krakow, Poland.*

* Correspondence, e-mail: malgorzata.lasota@uj.edu.pl

Received: 30.03.2026 / Revised: 12.04.2026 /Accepted: 12.04.2026 / Published: 01.08.2026

ABSTRACT

Gold nanoparticles (AuNPs) have emerged as promising multifunctional agents in cancer therapy due to their unique physicochemical properties and intrinsic biological activity. In this study, we investigated the effects of 5 nm AuNPs on pancreatic cancer cell lines (PANC-1 and BxPC-3) and non-malignant pancreatic cells (HPDE6c7), focusing on proliferation, apoptosis, migration, invasion and selected signaling pathways. AuNPs induced a dose-dependent decrease in cell viability and promoted apoptosis in both cancer cell lines, with a more pronounced effect observed in PANC-1 cells. Furthermore, AuNP treatment significantly inhibited cell migration and invasion, indicating suppression of metastatic potential. On the contrary, non-malignant pancreatic cells exhibited reduced sensitivity, although measurable cytotoxic effects were still observed. Interestingly, despite strong phenotypic changes, AuNPs did not significantly alter AKT or ERK1/2 phosphorylation, suggesting that their anticancer activity is not mediated by classical EGFR-dependent signaling pathways. Instead, the observed effects are likely driven by multifactorial mechanisms, including oxidative stress, cytoskeletal remodeling, and alterations in intracellular trafficking. In general, these findings highlight AuNPs as active modulators of cancer cell behavior and support their potential as a new therapeutic platform in pancreatic cancer. However, further optimization is required to improve selectivity and define their safety profile.

KEYWORDS: gold nanoparticles, pancreatic cancer, drug delivery systems.

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, second among all causes of death, and accounts for more than 600 000 deaths in the United States alone in 2025 [1]. In Europe, cancer is responsible for approximately 1.9 million deaths annually, representing a major public health burden in the region [2]. In Poland, cancer is also the second leading cause of death, accounting for almost 100,000 deaths per year, with a steadily increasing incidence rate [3]. Despite substantial advances in therapeutic strategies, the clinical treatment of cancer continues to face significant limitations. Surgical resection remains the cornerstone of treatment; however, it is often not feasible in advanced stages and is associated with the risk of residual disease and recurrence. Conventional therapies, including chemotherapy and radiation therapy, are also limited by systemic toxicity, lack of selectivity, and resistance development, significantly compromising their overall effectiveness [4].

These limitations are particularly pronounced in pancreatic cancer, especially pancreatic ductal adenocarcinoma (PDAC), which is characterized by an exceptionally poor prognosis, aggressive progression, and high resistance to conventional therapies [5–7]. The dense stromal microenvironment, together with complex tumor–stroma interactions, significantly affects drug delivery and promotes tumor survival and metastasis [8]. Furthermore, key molecular processes, such as the epithelial-mesenchymal transition (EMT) and aberrant activation of signaling pathways, including MAPK/ERK, contribute to chemoresistance and disease progression, further complicating therapeutic management [9,10].

In response to these challenges, increasing attention has been directed toward the development of targeted drug delivery systems (DDSs), which aim to enhance drug

accumulation at the tumor site while minimizing systemic toxicity. These systems enable controlled and sustained drug release, thereby maintaining therapeutic drug concentrations and improving treatment efficacy, particularly for agents with a narrow therapeutic index [11,12]. Moreover, DDS can be engineered to respond to specific stimuli, such as pH, temperature, or light, enabling site-specific drug release and improved targeting of tumor tissues [11,13].

Nanoparticles have emerged as a promising class of DDS due to their unique physicochemical properties, including improved drug stability, enhanced solubility, and prolonged circulation time. Their nanoscale size allows preferential accumulation within tumor tissues through the enhanced permeability and retention effect (EPR), while surface functionalization with targeting ligands facilitates selective binding to cancer cells and receptor-mediated internalisation [14,15]. These features make nanoparticles particularly attractive for oncological applications.

Among various nanomaterials, gold nanoparticles (AuNPs) have attracted considerable interest due to their chemical stability, biocompatibility, and ease of synthesis and functionalization [16–18]. Their high surface-to-volume ratio enables efficient drug loading and conjugation with target molecules, while surface modifications, such as polyethylene glycol (PEG), improve pharmacokinetics and reduce immunogenicity [17,19]. In addition to serving as drug carriers, AuNPs have intrinsic biological activity and have been shown to exert direct anticancer effects.

A growing body of evidence indicates that AuNPs can inhibit cancer cell proliferation, induce apoptosis, and suppress migration and invasion. In pancreatic cancer models, citrate-stabilized AuNPs have been shown to significantly reduce cell migration and colony formation, while simultaneously improving sensitivity to chemotherapeutic agents such as gemcitabine [9]. Targeted AuNP systems, including cetuximab-conjugated nanoparticles, have demonstrated selective delivery of cytotoxic agents to EGFR-expressing pancreatic cancer cells, resulting in enhanced therapeutic efficacy *in vitro* and *in vivo* [20,21]. Furthermore, AuNP-based platforms have been successfully used in combination with external stimuli, such as radiofrequency fields, to induce tumor destruction through thermal mechanisms [22,23].

At the molecular level, the anticancer effects of AuNPs are through multiple interconnected pathways [24]. These include, among others, inhibition of MAPK/ERK signaling and modulation of EMT-related markers [9], as well as induction of oxidative stress through increased reactive oxygen species (ROS) production [25]. Such alterations may lead to the activation of apoptotic pathways, including caspase-dependent cell death [25], and contribute to the reduction in the invasive potential of cancer cells. Additionally, AuNPs have been shown to modulate the tumor microenvironment by disrupting interactions between cancer cells and stromal components, further inhibiting tumor progression [8].

Despite these promising findings, the clinical translation of AuNP-based therapies remains limited. Although preclinical studies indicate favorable safety profiles, concerns persist about long-term accumulation in organs such as the liver and spleen, as well as variability in biological responses depending on nanoparticle size, surface chemistry, and functionalization [26,27]. Therefore, further systematic evaluation of well-defined nanoparticle systems in relevant tumor models is warranted.

In this study, we investigated the effect of 5 nm gold nanoparticles on selected pancreatic cancer cell lines, with particular emphasis on their effects on cell proliferation, apoptosis, migration, and invasion, as well as on selected signaling pathways.

2. Materials and methods

2.1. Sample preparation

Gold nanoparticles (AuNPs; nominal diameter 5 nm) were purchased from Sigma-Aldrich (St. Louis, MO, USA) as colloidal suspension in phosphate-buffered saline (PBS). Prior to use, the nanoparticle suspension was gently vortexed to ensure homogeneity. For protein functionalization, bovine serum albumin (BSA) was added to AuNP suspension to a final concentration of 1% (w/v), followed by incubation under gentle agitation at room temperature for 30 min to allow protein adsorption on the nanoparticle surface. This approach was used to improve colloidal stability and to mimic protein corona formation under biological conditions, as albumin is known to readily adsorb onto gold nanoparticle surfaces and modulate their physicochemical and biological properties [28,29]. This procedure was performed to enhance colloidal stability. Following incubation, the BSA-functionalized AuNPs were used directly for subsequent *in vitro* experiments without further purification.

2.2. Cell culture

The human pancreatic cancer cell lines (PANC-1 and BxPC-3) and the human pancreatic duct epithelial cells (HPDE6c7) were purchased from ATCC (Manassas, VA, USA). PANC-1 cells were maintained in DMEM, and BxPC-3 cells in RPMI-1640, both supplemented with 10% FBS (EURx Sp. z o.o., Gdansk, Poland). The normal HPDE6c7 line was cultured in Keratinocyte Serum-Free Growth Medium enriched with 50 µg/mL bovine pituitary extract and 5 ng/mL EGF. All culture media were further supplemented with 1% penicillin/streptomycin (Biowest, Riverside, MO, USA). All cells were grown at 37°C in a humidified atmosphere containing 5% CO_2 and were passaged two to three times per week.

2.3. Cell metabolic viability assay

Cells were seeded into 96-well plates at a density of 10^4 cells/well and allowed to adhere for 24 h. Subsequently, the culture medium was replaced with fresh medium containing AuNPs at increasing concentrations. Following 48 h incubation under standard conditions, cell viability was determined using the MTS assay (Promega, Madison, WI, USA). Absorbance was recorded at 490 nm using an Epoch Microplate Spectrophotometer (BioTek Instruments Inc., USA), and the percentage of viable cells was calculated as previously described [30].

2.4. Cell Survival Analysis

Cell survival was assessed using the PE Annexin V Apoptosis Detection Kit I (BD Biosciences, San Jose, CA, USA), following the manufacturer's protocol. Flow cytometry analysis was conducted on a BD FACSCanto instrument (Becton Dickinson, New York, NY, USA).

2.5. Cell Cytotoxicity

To evaluate the cytotoxic effects of the investigated compounds, LDH release into the culture media was measured after 24 h of incubation. The LDH assay (Thermo Fisher Scientific, Waltham, MA, USA) was performed according to the manufacturer's instructions as previously described [31].

2.6. Scratch Assay

Confluent monolayers of PANC-1 and BxPC-3 cells were initially incubated in DMEM or RPMI-1640 media, respectively, supplemented with 0.5% BSA for 24 h to induce serum starvation. A mechanical wound was then created in each well by applying a perpendicular scratch with a sterile pipette tip, followed by a PBS wash to eliminate detached cells. Subsequently, the cells were treated with AuNPs and incubated for an additional 24 h. Wound closure was documented using an AxioVert A1 light microscope equipped with an automated scanning stage and ZEN Pro 2.3 software (Carl Zeiss, Oberkochen, Germany). Image analysis was performed using Fiji ImageJ software, version 1.54f (National Institutes of Health, Bethesda, MD, USA).

2.7. Invasion Assay

The invasive potential of pancreatic cancer cells was evaluated using BD BioCoat™ Matrigel invasion inserts (BD Biosciences, San Jose, CA, USA). PANC-1 and BxPC-3 cells (2×10^4 cells in 0.5 mL) were seeded into the upper chambers in starvation medium (0.5% BSA) and treated with AuNPs at IC_{50} concentration. Cells were allowed to invade through the Matrigel layer toward a chemoattractant (complete medium with 10% FBS) in the lower chamber. After 24 h, cells that had successfully transmigrated to the underside of the filters were visualized using Wright's stain (Sigma-Aldrich, St. Louis, MO, USA) and quantified.

2.8. Protein Extraction and Automated Capillary Immunoblotting Analysis

To activate EGFR signaling, the cells were stimulated with epidermal growth factor (EGF, 50 ng/mL) for 5 min, followed by a 30-minute incubation with AuNPs prior to protein harvest.

Protein expression analysis was performed via capillary Western blotting using the Jess™ Simple Western system (ProteinSimple, San Jose, CA, USA) according to the manufacturer's instructions. Briefly, after washing with PBS, cells were lysed in RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with a protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO, USA). The lysates were scraped, collected, and centrifuged at 10 000 rpm for 10 min at 4°C. Total protein concentration was determined using the DC Protein Assay Kit (BIO-RAD, Hercules, CA, USA).

Samples were diluted to total protein concentrations of 600 ng/µL in 1x fluorescent master mix (EZ Standard Pack I; ProteinSimple), with 5 µL loaded per well. The following primary antibodies were employed: mouse anti-SDHA (Abcam, ab14715; 1:150); rabbit anti-Akt (Cell Signaling, #9272; 1:100); rabbit anti-phospho-Akt (Cell Signaling, #4060; 1:25); rabbit anti-p44/42 MAPK (ERK1/2) (Cell Signaling, #4695; 1:75); mouse anti-phospho-p44/42 MAPK (Thr202/Tyr204) (Cell Signaling, #9106; 1:15).

Detection was performed using system-specific secondary antibodies: anti-mouse (042–205), anti-rabbit (042–206), and anti-mouse NIR (043-821). Chemiluminescence and NIR fluorescence digital images were captured and analyzed using Compass Simple Western software (ProteinSimple).

2.9. Statistical analysis

Unless specified otherwise, data are expressed as the mean ± SD calculated from at least three independent experiments. Statistical analysis was performed by one-way ANOVA with Dunnett's post-hoc test or Student's t-test using GraphPad 5.01 software. Differences with a value of $p < 0.05$ were considered statistically significant. The IC_{50} values were determined by fitting a logistic sigmoidal model to the AuNP dose-response data using the Levenberg-Marquardt iteration algorithm in OriginPro 2025b (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Gold nanoparticles modulate proliferation, cell death, and selected properties of pancreatic cancer cells

To comprehensively evaluate the biological effects of 5 nm gold nanoparticles (AuNPs) on pancreatic cancer progression, a series of functional assays were performed in PANC-1 and BxPC-3 cell lines, including analyses of cell viability, apoptosis, migration, and invasion (Fig. 1A–F).

Antiproliferative activity of AuNPs was assessed using the MTS assay after 48 h of incubation (Fig. 1A). In both cell lines, treatment resulted in a dose-dependent decrease in cell viability, confirming inhibition of metabolic activity. The dose-response curves were fitted using a logistic model, resulting in IC_{50} values of 0.13 nM for PANC-1 and 0.75 nM for BxPC-3 cells, indicating a greater sensitivity of PANC-1 cells to AuNP treatment (Fig. 1A).

To investigate whether reduced viability was associated with cell death induction, Annexin V/propidium iodide staining was performed after 48 h (Fig. 1B). In PANC-1 cells, AuNP treatment led to a marked reduction in viable cells, accompanied by a substantial increase in early and late apoptotic populations, with only a minimal contribution of necrosis. In BxPC-3 cells, a moderate decrease in viability was also observed, associated with an increase in apoptotic fractions; however, in contrast to PANC-1, this response was accompanied by a more pronounced necrotic component, indicating differences in the mode of cell death between the two cell lines.

The effect of AuNPs on cell migration was evaluated using a scratch assay (Fig. 1C, D). Under control conditions, cells exhibited efficient wound closure, particularly BxPC-3 cells, reflecting high basal migratory capacity. Treatment with AuNPs resulted in a strong inhibition of migration in both models, as demonstrated by a substantial reduction in scratch closure compared to control conditions.

The impact of AuNPs on invasive behavior was further assessed using a Matrigel invasion assay (Fig. 1E, F). In PANC-1 cells, AuNP treatment led to a pronounced decrease in the number of invading cells, indicating a strong suppression of the invasive potential. In BxPC-3 cells, the inhibitory effect on invasion was less pronounced but still evident, suggesting a cell line dependent response to nanoparticle exposure.

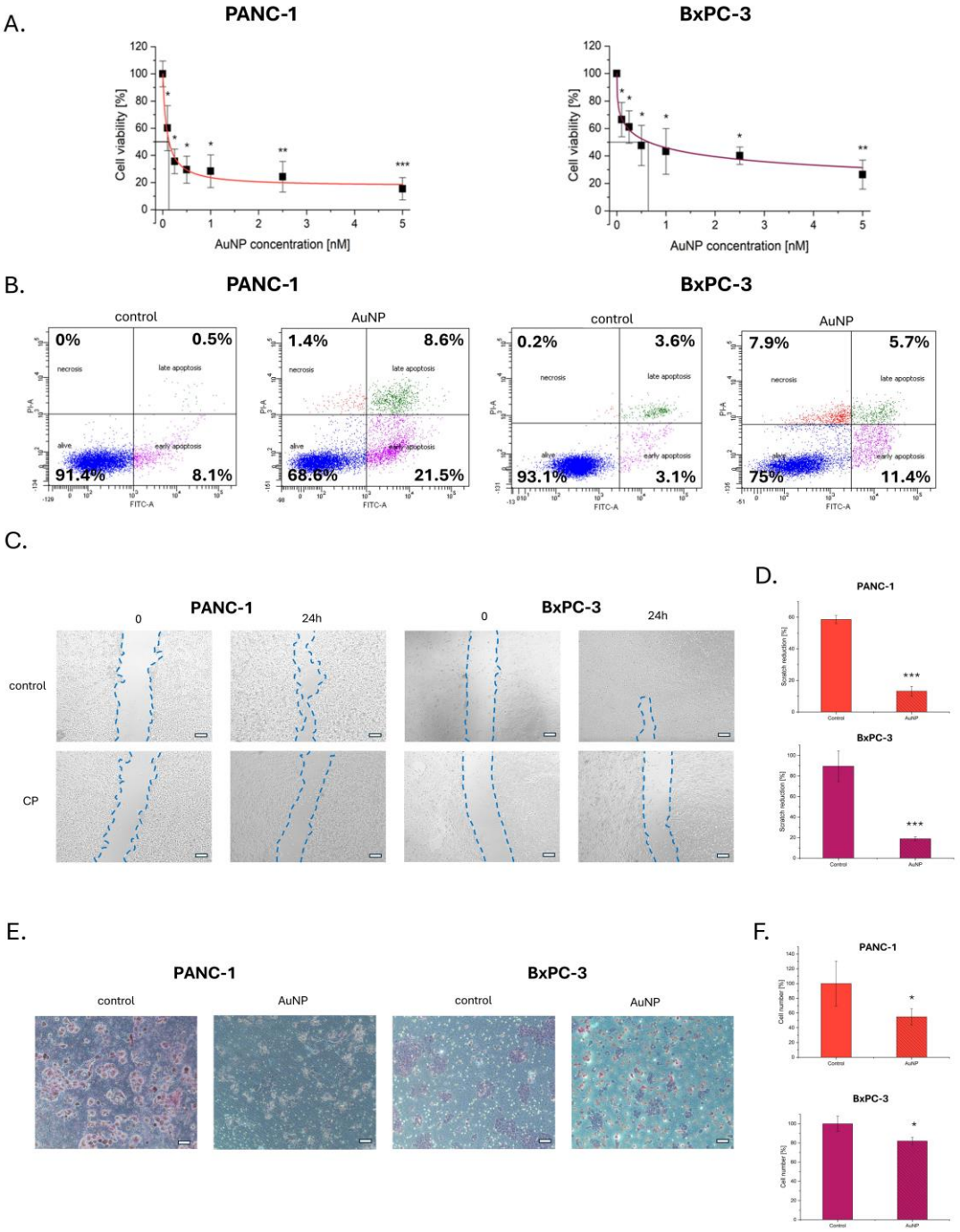

Fig. 1. Gold nanoparticles (AuNPs, 5 nm) exert multidimensional anticancer effects by inhibiting proliferation, inducing apoptosis, and suppressing the migration and invasion of pancreatic cancer cell lines (PANC-1 and BxPC-3). Dose-dependent decrease in cell viability following 48 h of exposure to selected concentrations of AuNPs (0.1–5 nM), assessed using the MTS assay. Nonlinear regression analysis fitting a logistic model to the dose-response data, utilized to determine the half-maximal inhibitory concentration IC_{50} values for both investigated cell lines (**A**). Flow cytometric analysis of cell death induction evaluated after 48 h of AuNP treatment. Representative scatter plots display Annexin V-FITC and propidium iodide (PI) double staining. The respective quadrants represent living cells, early apoptosis, late apoptosis, and necrosis (**B**). Representative optical microscopy images from the *in vitro* scratch wound healing assay used to evaluate cell migration. Images were captured at 0 h and 24 h post-scratching in control and AuNP-treated groups. Dashed blue lines delineate the migration front of the cells into the wounded area. Scale bars: 200 µm (**C**). Quantitative analysis of the wound closure rate (scratch reduction) expressed as a percentage of the initial wound area (**D**). Representative micrographs of the Matrigel transwell invasion assay. Scale bars: 200 µm (**E**). Quantitative evaluation of the invasive capacity, represented as the relative number of invading cells normalized to the untreated control (100%) (**F**). All quantitative data are presented as the mean ± standard error (SE) from at least three independent experiments. Statistical significance was determined using one-way ANOVA with Dunnett's post-hoc test for multiple comparisons (panel A) and Student's t-test for pairwise comparisons between control and treated groups (panels E and F): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Collectively, these results demonstrate that AuNPs exert multidimensional anticancer effects, including inhibition of proliferation, induction of cell death, and suppression of migration and invasion. In particular, the magnitude and nature of these effects differ between cell lines, with PANC-1 cells showing higher sensitivity in terms of proliferation and invasion, while BxPC-3 cells exhibit a more heterogeneous cell death response with a detectable necrotic component.

3.2. Effects of Gold Nanoparticles on Selected EGFR-Dependent Signaling Pathways

To investigate whether the observed biological effects of AuNPs are related to the modulation of key signaling pathways, we analyzed the activation status of AKT and ERK1/2 using a capillary immunoassay (Jess system) (Fig. 2).

In PANC-1 cells, the ratio of phosphorylated AKT to total AKT remained essentially unchanged after AuNP administration compared to control conditions, indicating that the experimental setup used had no significant effect on AKT pathway activation. Similarly, analysis of ERK1/2 signaling revealed no statistically significant differences in the ratio of phospho-ERK to total ERK after AuNP exposure.

In BxPC-3 cells, quantification of ERK1/2 activation showed a trend towards decreased phosphorylation in AuNP-treated samples; however, this effect did not reach statistical significance. Importantly, phosphorylated AKT levels in this cell line were below the detection threshold, preventing a reliable calculation of the pAKT/AKT ratio under experimental conditions.

Taken together, these results indicate that AuNP-induced inhibition of proliferation, migration, and invasion is not associated with significant modulation of key AKT or ERK1/2 signaling pathways under the conditions studied. This suggests that the observed antitumor effects may be mediated by alternative mechanisms, potentially involving cytoskeletal remodeling or oxidative stress, rather than by direct suppression of classical EGFR signaling.

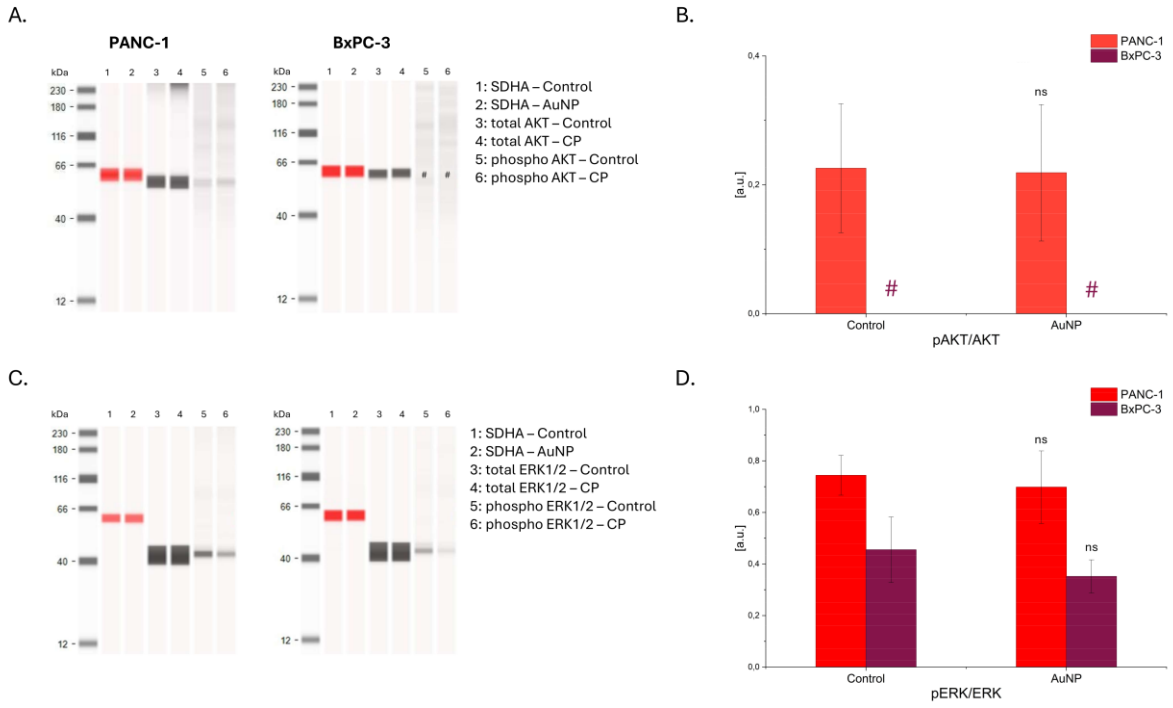

Fig. 2. Effects of gold nanoparticles (AuNPs) on AKT and ERK1/2 phosphorylation in PANC-1 and BxPC-3 cell lines. Representative capillary electropherograms obtained using the Jess Simple Western system are shown for total AKT and phospho-AKT **(A)**, as well as total ERK1/2 and phospho-ERK1/2 **(C)**. Corresponding bar graphs present quantitative analysis of the phospho-AKT to total AKT **(B)** and phospho-ERK1/2 to total ERK1/2 **(D)** ratios. All values were normalized to SDHA, which served as the loading control. Data represent mean ± SD. Statistical significance between control and AuNP-treated samples was evaluated using Student's t-test: ns – non-significant ($p > 0.05$). The hash symbol (#) indicates that phosphorylated AKT levels in BxPC-3 cells were below the detection threshold.

3.3. Effects of Gold Nanoparticles on Non-Malignant Pancreatic Cells

To assess the safety profile of AuNPs and determine whether their anticancer activity is selective against malignant cells, we evaluated their effects on nontumorigenic human pancreatic ductal epithelial cells (HPDE6c7) using viability, apoptosis, and cytotoxicity assays (Fig. 3A-C).

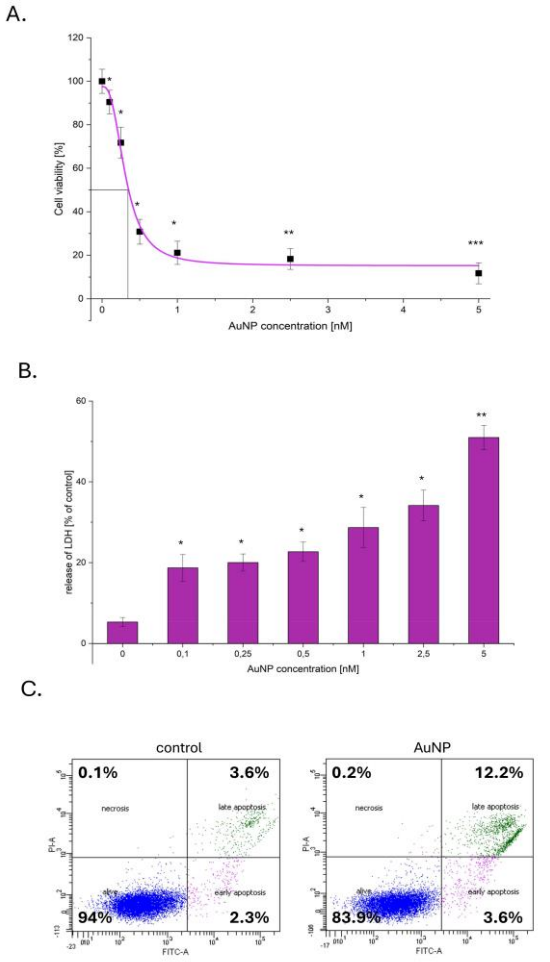

Fig. 3. Effect of gold nanoparticles (AuNPs) on non-malignant human pancreatic ductal epithelial cells (HPDE6c7). The dose-dependent effect of AuNPs on the viability of HPDE6c7 cells after 48 h incubation assessed by MTS assay. Cell viability curve fitted with a nonlinear regression model; the lines indicate the IC_{50} value **(A)**. The cytotoxic influence of AuNPs evaluated by LDH test (results presented as the percentage of LDH released relative to control) **(B).** The effect of AuNPs on apoptosis and necrosis of HPDE6c7 cells determined by Annexin V/propidium iodide staining using flow cytometry **(C).** Statistical significance between untreated and treated samples was evaluated using one-way ANOVA with Dunnett's post-hoc test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the control group.

Cell viability was assessed using the MTS assay after 48 h of incubation (Fig. 3A). AuNP exposure resulted in a dose-dependent decrease in metabolic activity in HPDE6c7 cells. Nonlinear regression analysis yielded an IC_{50} value of 0.35 nM, indicating that non-malignant cells are also susceptible to AuNP-induced growth inhibition, although the effect appeared less pronounced compared to highly sensitive PANC-1 cells.

To further characterize the mode of cell death, Annexin V/propidium iodide staining was performed (Fig. 3C). Under control conditions, most cells remained viable, with only minor fractions undergoing apoptosis. After AuNP treatment, a moderate reduction in viable cells was observed, accompanied by an increase in apoptotic populations, particularly within the late apoptotic fraction. Necrosis remained negligible, suggesting that cell death in HPDE6c7 cells occurs predominantly through apoptosis rather than membrane-disruptive mechanisms.

Cytotoxicity was also evaluated using the LDH release assay (Fig. 3B), which reflects loss of membrane integrity. Treatment with AuNP resulted in a progressive increase in LDH release with increasing concentrations, indicating a dose-dependent induction of cellular damage. However, the gradual nature of this increase, in conjunction with the low necrotic fraction observed in flow cytometry, suggests that membrane disruption is not the primary mechanism of cell death, but rather a secondary consequence of ongoing cellular stress.

Collectively, these findings demonstrate that while AuNPs exhibit clear cytotoxic and antiproliferative effects in non-malignant pancreatic cells, the mode of action appears to be predominantly apoptotic and concentration dependent. Importantly, comparison with cancer cell models indicates differential sensitivity between malignant and non-malignant cells, supporting a partially selective anticancer profile of AuNPs, although with a relatively narrow therapeutic window.

4. Discussion

Gold nanoparticles (AuNPs) have gained increasing attention as multifunctional agents in cancer therapy due to their ability to modulate cellular behavior through mechanisms extending beyond classical receptor-mediated signaling pathways. In the present study, we demonstrate that 5 nm AuNPs significantly reduce cell viability, induce cell death, and strongly inhibit migration and invasion in pancreatic cancer cell lines, while exerting comparatively moderate effects on non-malignant pancreatic cells.

The dose-dependent decrease in cell viability observed in PANC-1 and BxPC-3 cells is consistent with previous studies reporting the cytotoxic effects of AuNPs. The size is a critical determinant of biological activity, as smaller particles exhibit enhanced cellular uptake and increased surface reactivity. Pan et al. demonstrated that ultrasmall AuNPs (1 to 2 nm) exhibit pronounced cytotoxicity due to their ability to penetrate intracellular compartments and interact with biomolecules [32]. Although the particles used in this study (5 nm) are larger, they remain within a highly bioactive range, which is reflected in the low nanomolar IC_{50} values, particularly in PANC-1 cells.

The pro-apoptotic effects detected by flow cytometry further support previous findings that AuNPs can activate cell death pathways. Several studies have shown that AuNPs induce apoptosis through mitochondrial dysfunction, oxidative stress, and the activation of caspase cascades.

For example, Liu et al. reported that AuNP-induced generation of reactive oxygen species (ROS) leads to mitochondrial damage and apoptosis [25]. Similarly, Arvizo et al. demonstrated that AuNPs can interfere with cellular redox balance and induce apoptosis in cancer cells [33]. In our study, the presence of both apoptotic and necrotic cell populations suggests that AuNPs trigger multiple cell death mechanisms, likely reflecting differences in cellular susceptibility and stress response pathways between PANC-1 and BxPC-3 cells.

A key finding of this study is the strong inhibition of migration and invasion after AuNP treatment. Both PANC-1 and BxPC-3 cells exhibited a substantial reduction in wound closure and invasive capacity, indicating a significant impairment of metastatic potential. These results are consistent with previous studies demonstrating antimigratory and anti-invasive effects of AuNPs in pancreatic cancer models. Huai et al. reported that citrate-stabilised AuNPs reduce migration and colony formation through modulation of epithelial–mesenchymal transition (EMT)-associated pathways [9], while Saha et al. showed that AuNPs can disrupt tumour–stroma interactions and reprogram the tumor microenvironment, thereby inhibiting tumor progression [8]. The magnitude of the observed effects suggests that AuNPs interfere with the fundamental processes that govern cell motility.

Interestingly, despite these pronounced phenotypic changes, our capillary-based protein analysis did not reveal statistically significant alterations in AKT or ERK1/2 phosphorylation. In PANC-1 cells, the pAKT/AKT ratio remained largely unchanged, while in BxPC-3 cells phospho-AKT levels were below detection under the applied experimental conditions. Similarly, ERK activation exhibited only minor, nonsignificant changes. These findings suggest that the biological effects of AuNPs may not be mediated by acute inhibition of EGFR downstream pathways, at least under the short-term conditions examined.

This apparent discrepancy between pronounced phenotypic effects and limited modulation of canonical signaling pathways highlights a fundamental characteristic of nanoparticle-cell interactions. Unlike conventional targeted therapeutics, AuNPs exert their biological activity through complex physicochemical mechanisms rather than selective inhibition of single molecular targets.

Importantly, in the present study, AuNPs were functionalized with bovine serum albumin (BSA), which may significantly influence their physicochemical behavior and biological interactions. Albumin is known to readily adsorb onto gold nanoparticle surfaces, forming a protein corona that enhances colloidal stability and reduces aggregation under physiological conditions. Previous studies have demonstrated that adsorption of serum albumin onto AuNPs alters their hydrodynamic size, surface properties, and interaction with biological systems [28]. Therefore, the observed biological effects may result not only from the intrinsic properties of AuNPs, but also from the presence of a protein corona that modulates nanoparticle–cell interactions, cellular uptake, and intracellular trafficking. This aspect should be considered when interpreting the results and comparing them with studies using non-functionalized nanoparticles.

One potential mechanism involves a direct interaction with the cytoskeleton. AuNPs have been associated with alterations in cellular nanomechanical properties, including increased stiffness, which correlates with reduced migratory and invasive potential. These alterations have been demonstrated using approaches based on atomic force microscopy, linking cytoskeletal remodeling with decreased metastatic capacity [34].

Another important mechanism is the induction of oxidative stress. AuNPs can increase intracellular levels of reactive oxygen species (ROS), leading to oxidative damage to proteins, lipids, and nucleic acids, thereby affecting essential cellular processes. This phenomenon has been well documented for small-sized nanoparticles and is associated with mitochondrial dysfunction and the activation of apoptosis pathways [25,32].

In addition, AuNPs can influence membrane-associated processes and intracellular trafficking. Due to their nanoscale dimensions and surface properties, nanoparticles interact with cellular membranes and accumulate within endocytic compartments, potentially altering receptor distribution and downstream signaling independently of direct kinase inhibition [35,36].

Intracellular localization further contributes to AuNP-mediated effects. Studies have demonstrated that nanoparticles accumulate in lysosomes and mitochondria, where they can induce localized dysfunction and contribute to cytotoxicity by altering organelle integrity [37,38].

The lack of significant modulation of AKT and ERK phosphorylation observed in our study may also reflect the molecular background of pancreatic cancer cells. Constitutive activation of KRAS signaling, a hallmark of pancreatic ductal adenocarcinoma, reduces dependence on upstream receptor-mediated pathways such as EGFR. Consequently, substantial phenotypic effects may occur without detectable changes in downstream phosphorylation events [39,40].

The differential responses observed between PANC-1 and BxPC-3 cells may be further explained by their distinct genetic and molecular backgrounds. PANC-1 cells harbor activating mutations in KRAS, which result in constitutive downstream signaling and reduced dependence on upstream receptor-mediated pathways such as EGFR. In contrast, BxPC-3 cells are characterized by wild-type KRAS status, making them more reliant on receptor-level signaling for proliferation and survival. These differences may contribute to the observed variability in sensitivity to AuNP treatment, as well as the limited changes in AKT and ERK phosphorylation despite pronounced phenotypic effects [39,41].

Importantly, this distinction supports the concept that nanoparticle-mediated effects are not strictly dependent on selected signaling inhibition, but rather arise from broader physicochemical interactions that can affect cells independently of their mutational status. This may explain why significant alterations in viability, migration, and invasion were observed even in the absence of strong modulation of classical signaling pathways.

Importantly, our study also evaluated the effects of AuNPs on non-malignant pancreatic cells (HPDE6c7). Although AuNPs reduced viability and increased LDH release in these cells, the effects were less pronounced compared to cancer cell lines, suggesting a degree of

selectivity. This observation is consistent with previous reports indicating that the response of normal cells depends on cellular context and nanoparticle physicochemical properties [36,42]. However, the observed cytotoxicity at higher concentrations highlights the need for careful dose optimization in potential therapeutic applications.

Taken together, our results demonstrate that AuNPs exert a robust inhibitory effect on key hallmarks of pancreatic cancer progression, including proliferation, survival, migration, and invasion. Importantly, these effects appear to arise from complex, multifactorial mechanisms that extend beyond the simple inhibition of signaling pathways. This positions AuNPs not only as passive drug carriers, but also as active modulators of cancer cell behavior. These findings further support the concept that AuNPs act as multifactorial modulators of cancer cell behavior, exerting their effects through integrated physicochemical interactions rather than through selective inhibition of individual signaling pathways.

However, the observed effects on normal pancreatic cells highlight the need for further optimization, particularly with respect to targeting specificity and dosing strategies. Future studies should focus on functionalization approaches that enhance tumor selectivity, as well as *in vivo* validation, to better define the therapeutic window and long-term safety profile.

5. Conclusions

This study demonstrates that 5 nm gold nanoparticles exert significant anticancer effects in pancreatic cancer models by reducing cell viability, inducing apoptosis, and strongly inhibiting migration and invasion. Importantly, these functional effects occur in the absence of pronounced alterations in AKT and ERK1/2 signaling, suggesting that AuNPs act through complex, non-classical mechanisms involving physicochemical interactions and modulation of cellular architecture.

The differential sensitivity observed between cancer and non-malignant cells indicates a degree of selectivity; however, the measurable impact on normal pancreatic cells underscores the need for further refinement of nanoparticle-based strategies to improve safety profiles.

Overall, our findings support the concept that AuNPs represent a promising and mechanistically distinct therapeutic platform in pancreatic cancer, with the potential to complement existing treatment modalities. Future investigations should aim to enhance tumor specificity, elucidate underlying molecular mechanisms, and validate these effects in physiologically relevant *in vivo* models.

Author Contributions: Conceptualization – M.L.; methodology – M.L., A.W.; validation – M.L.; investigation - M.L., A.K., M.S., A.W.; resources – M.L.; data curation – M.L., A.K., A.W.; writing—original draft preparation – M.L., A.K, M.S.; visualization – A.K.; supervision – M.L.; project administration – M.L.; funding acquisition – M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the state budget allocated by the Minister of Science under the program "Student Scientific Groups Create Innovations" (Studenckie Koła Naukowe Tworzą Innowacje), grant number SKN/SP/601857/2024.

Conflicts of Interest: The authors declare no conflict of interest.

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