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Expression alterations of the KRAS gene associated with AgNPs

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Abstract

Objective

Nanotechnology has had a significant impact on medicine, cosmetics, and food packaging via the utilization of artificially created nanomaterials like AgNPs. Due to their potent antibacterial properties, AgNPs are popular nanoparticles in consumer and medical products. This investigation sought to explore the effects of AgNPs on the expression of the Kirsten rat sarcoma (KRAS) viral gene in the liver and spleen of mice to evaluate the molecular safety of exposure to AgNPs.

Materials and Methods

A total of 56 mice were assigned to seven different groups (8 mice in each). Three groups were treated with AgNPs every day at 0.25, 0.5, and 1 mg per kg for one week. Remaining three groups were given the same amount of treatment for 14 days. One group functioned as an untreated counterpart. After the treatment, the spleen and liver tissues were excised. Later, total RNA was isolated using the method of Junqueira and Carneiro, and cDNA was made using the Applied Biosystem Kit (Product No. 4387406) for the measurement of gene expression in quantitative form.

Results

The expression of the KRAS gene changed with the amount and length of time spent in AgNP. The expression of KRAS was decreased in all of the AgNP-treated in comparison to the control liver's tissue. On the other hand, the KRAS expression in the spleen of the treated animals was consistently higher than that of the controls. These modifications were more significant at higher concentrations and longer durations.

Conclusions

The results demonstrated that AgNP publicity altered the expression of KRAS genes in a tissue-unique way: downregulation was observed inside the liver, and upregulation changed into found inside the spleen. These distinctive classes of expression suggest that AgNPs can influence cell communication pathways depending on the features of the organ and the microenvironment. Specifically, the sustained growth in KRAS in the spleen following the injection of AgNPs, particularly at higher doses and with a longer period of publicity, has caused large concerns regarding the ability of the injection to lead to a cancerous manner in related tissues, which are immune-associated. These findings display the need for careful consideration of AgNP's utilization, specifically in merchandise meant for long-term or worldwide use. Future planned investigations have to discover the capacity mechanisms through which AgNPs affect KRAS, assess the capability of long-term consequences of pathology, and determine the safe limits of exposure. Comprehensive molecular safety evaluation is crucial to ensuring the responsible development and usage of silver-primarily based technologies in scientific and commercial enterprise settings.

Keywords: β-ACTIN, gene expression, liver, silver nanoparticles, spleen

Paper Type: Research Paper.

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Introduction

The manufacturing and utilization of nanoparticles have enhanced appreciably over recent years, in general due to two motives: simple manufacturing of nanoparticles and their increasing range of applications (Al-Taee, 2020; Naief et al., 2023). Specifically, silver nanoparticles have come to be more popular most of the numerous forms of nanoparticles. Their sizeable reputation is attributed to their multiple homes, which include broad-spectrum antimicrobial interest in opposition to many microbial species (Tiàn et al., 2018; Kŭmar et al., 2018; Kadhum and Hussein, 2020), the incredibly low cost in their synthesis, and the flexibility to produce exceptionally sized and fashioned marketers (Sohn et al., 2015; Rajapantulu and Bandyopadhyaya, 2021). Today, AgNPs are not unusual in lots of everyday merchandise like textiles, meals containers, wound dressings, and personal care products (Wei et al., 2015; MćGillicuddy et al., 2017). However, despite the numerous advantages of AgNPs, worries about their safety, mostly due to the capability of releasing (Ag^+), which might be poisonous to organisms (Ýue et al., 2017; Padhye et al., 2023). Despite the not unusual fact that the Ag^+ ions release is accountable for the toxicity of AgNPs, some studies have observed a link among the two (Sorensen et al., 2015; Mat Lazim et al., 2023). Other scientists have even stated that the toxicity of metal AgNPs is greater than that of the ions themselves (Sakamoto et al., 2015; Pakrashi et al., 2017; Abramenko et al., 2018). The complexity indicates that the organic results of AgNPs are complicated and require further molecular research. An interesting gene here is Kirsten's viral oncogene for rats (KRAS), this gene is a proto-oncogene that encodes a small GTPase which takes part in the cell proliferation, differentiation, and survival. Alterations of KRAS are found in many human cancers; hence, it is an important target for research and development in oncology (Cox, 2014). Overexpression or mutation of KRAS has been associated with tumorigenesis, metastasis, and angiogenesis in cancer (Mahmood *et al*., 2019; Gökmen *et al*., 2023). Therefore, expression studies on environmental influences such as nanoparticles on KRAS provide implications both for toxicology as well as therapy. AgNPs showed anti-cancer activities, which involved down-regulation of cancer genes like KRAS. For example, reverse transcriptase-qPCR has proven that AgNPs substantially diminished the KRAS expression in treated cancer cells (Bin Saeed et al., 2023). On the other hand, increasing worries nearby the disadvantages of doses or long periods of exposure to chemical synthesis AgNPs, which can lead to cell toxicity and loss of efficacy as a treatment option (Srisaisap & Boonserm, 2024). As a result, while performing future studies in molecular terms for safety applications against cancer therapy applications, epigenetic modifications should also be taken into consideration. NPs can carry out gene expression modification through several recent studies on epigenetic mechanisms concerning DNA methylation and histone tail modifications, along with chromatin restructuring and noncoding RNAs like microRNAs and

long noncoding RNAs. The control of epigenetics is very complex, and the factors that contribute to it are diets, infections, climate conditions, and even xenobiotics (Amiri Roudbar et al., 2020). Such interactions occur on multiple levels and significantly define the expression profiles at genes that modulate phenotype and disease susceptibility (Safàei et al., 2022; Alavi et al., 2022). Gene regulation in eukaryotes managed tissue-specifically as well as time-specifically. At any given type of tissue, only a limited number of actively expressed genes; their expression pattern varies with developmental stage and health status (Hajalizadeh et al., 2021; Heidarpour et al., 2011). Both intrinsic factors, like chromatin architecture, as well as extrinsic elements such as inter-tissue signals, participate in this spatial and temporal control (Shokri et al., 2023). Epigenetic modifications serve as a mechanism through which environmental factors can trigger inheritable modifications in gene expression without changing the DNA sequence. Studies carried out reveal that maternal nutritional status during gestation has an imprinting effect on offspring epigenetics, at least regarding development, metabolism, and disease predisposition. Exposure of organisms to environmental stressors such as toxins and pathogens is also capable of inducing epigenetic modifications that affect gene expression and cellular behavior. This learning holds great value. Knowledge of epigenetics may improve livestock breeding, enhance productivity, and improve disease resistance (increased health resilience). Epigenetic marks are in use for forecasting health results and performance traits in diverse species (livestock included). The basic genetic research has now shifted towards the gene and protein identification related to specific traits, and then studying these genes and proteins via their chromosomal and cellular analysis (Bordbar et al., 2022). Increasing knowledge about the regulation of genes plus their response to environmental factors holds great promise for new therapeutic innovations that would enhance health in animals as well as humans (Arabpour et al., 2021; Mohammadabadi et al., 2022b; Louay et al., 2020). Therefore, this paper attempted a molecular safety evaluation of silver nanoparticles by investigating the impact of such particles on KRAS expression in mouse liver and spleen. The tissues were selected based on previously reported sensitivity to nanoparticle assays, along with their primary role in detoxification as well as immune regulation. This study tries to know the exact molecular impacts of AgNPs by looking at how different levels and times of exposure to AgNPs affect the KRAS gene expression in the various tissues; this will aid in giving better info about their safety.

Material and methods

AgNPs preparation: The silver nanoparticles (AgNPs) used in this research have been previously utilized in the preceding observe by using Nasir et al. (2016) and described in detail.

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Spherical AgNPs with an ordinary length of 40 ± 5nm were created using an environmentally friendly technique that utilized the extract of olives as the reducing agent and D-sorbitol as the stabilizer (Nasir et al., 2016; Nasir et al., 2020). The shape, length distribution, and balance of the synthesized nanoparticles were evaluated, and they were stored in a dark, wrapped box at 37 ̊C till they were used in this experiment to prevent the formation of clusters and photodegradation.

Animals used in experiments: Adult male mice (weight variety: 24-36 g; age: 8-10 weeks) were involved in this study. The mice were housed in a monitored condition with a temperature. Of 23-25°C with a 12 hr. mild-dark cycle (Balls, 2022). The mice have been given a week to adapt to the laboratory's conditions before the experiments started (Klimek & Rogalska, 2021). A total of 56 mice had been assigned to 7 agencies of 8 mice each, these businesses were housed in separate cages. All mice were permitted to eat the standard pelleted feed and drink water freely at some point during the experimental period. The businesses that obtained remedies had been segregated into:

Group 1: 0.250 mg of AgNP / 7 days, Group 2: 0.500 mg of AgNP / 7 days, Group 3: 1,000 mg of AgNP / 7 days, Group 4: 0.250 mg of AgNP / 14 days, Group 5: 0.500 mg of AgNP / 14 days, Group 6: 1,000 mg of AgNP/ 14 days, Group 7 (control group): 0.500 mg of distilled water for 14 days.

Tissue sampling: All mice were sacrificed 24 hours after the completion of their respective treatments using cervical dislocation as the euthanasia method (Balls, 2022). Hepatic and splenic tissues were immediately harvested for downstream molecular and histopathological analyses.

RNA extraction and histological processing: Tissue samples from the spleen and liver were processed for histopathological analysis following the approach described by Junqueira and Carneiro (Mescher, 2024). Tissue fragments were cut into small to medium-sized pieces with a thickness of 5 to 10 µm using a precise microscope that rotates.

cDNA synthesis: Whole RNA was isolated from the tissues (liver and spleen), and cDNA using the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (Cat.No.4387406). The reactions were conducted in a thermal cycler according to the protocol outlined by the manufacturer, maintaining the temperature and reaction time as described in Table 1. The cDNA that was synthesized was kept at −20°C for analysis of the gene expression.

Primer design and qPCR analysis: Primers for the intended gene (KRAS) and a housekeeping gene (β-ACTIN) were created using the Primer3Plus software (Table 2). β-ACTIN was chosen as the housekeeping gene to equalize the amount of the target gene (Li et al., 2010). The comparative Ct method (ΔΔCt) was employed to assess the expression ratio of the target

gene. This approach contrasts the Ct values of the treated samples with those of the control samples and normalizes the latter to the internal reference gene β-ACTIN (Ali et al., 2022).

Table 1. Reverse transcription thermal profile

Temperature (°C)	Heated Lid	S-1	S-2	S-3	S-4	S-5
Time (min.)	—	10	10	60	5	∞
Value	111	25	37	42	75	4

Table 2. Primer sequences and melting temperatures for KRAS and β-ACTIN

Gene	Primer	Sequence (5′ → 3′)	Tm (°C)
KRAS	Forward	AGGCCTGCTGAAAATGACTG	63.9
KRAS	Reverse	TCTGAATTAGCTGTATCGTCAAGG	65.9
β-ACTIN	Forward	CCTGAACTACCTTGCAGCTC	60.0
β-ACTIN	Reverse	ACTCCTGCTTGCTGATCCAC	63.0

qPCR output: Gene expression was evaluated through quantitative PCR, and results were visualized using amplification plots. Figure 1 illustrates the threshold cycle (Ct) values for each sample across all groups.

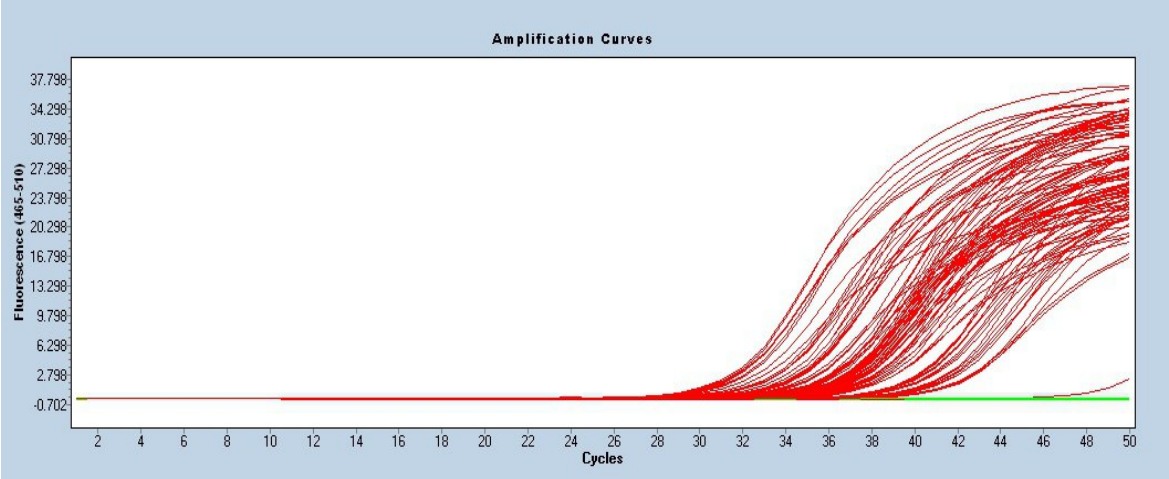

Figure 1. CT values of KRAS and β-ACTIN gene expression across experimental groups

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Results and discussion

The results of this investigation demonstrated that the KRAS gene expression in the liver and spleen increased significantly as a function of the concentration and duration of AgNP treatment. Also, the treatment group and the control group had different results (Table 3).

Table 3. The effects of one and two weeks of exposure to AgNPs in the spleen and liver tissues

Dose of AgNPs	Liver (Mean ± SE)	Spleen (Mean ± SE)
0.25 mg/kg bw / 1 week	0.01268 ± 0.0045 ^{bc}	0.0107 ± 0.0006 ^{bc}
0.5 mg/kg bw / 1 week	0.0118 ± 0.0030 ^{bc}	0.0254 ± 0.0041 ^{ab}
1.0 mg/kg bw / 1 week	0.0114 ± 0.0043 ^{bc}	0.0306 ± 0.0070 ^{a}
0.25 mg/kg bw / 2 weeks	0.0272 ± 0.0062 ^{a}	0.0172 ± 0.0087 ^{abc}
0.5 mg/kg bw / 2 weeks	0.0083 ± 0.0045 ^{bc}	0.0079 ± 0.0032 ^{c}
1.0 mg/kg bw / 2 weeks	0.0024 ± 0.0005 ^{c}	0.0068 ± 0.0051 ^{c}
Control	0.0145 ± 0.0022 ^{b}	0.0099 ± 0.0016 ^{bc}
LSD	**0.0117 ***	**0.0159 ***

* Significant at $P < 0.05$. Different superscripts in each row designate significant differences between columns.

Administration of the lowest AgNP dose (0.25 mg/kg) for two weeks led to the highest hepatic KRAS gene expression value (0.0272), while the lowest value (0.0024) was recorded in the group that received the highest dose (1 mg/kg) for two weeks. This finding suggests that prolonged high-dose exposure may suppress KRAS gene expression in liver tissue, potentially indicating toxic or regulatory suppression mechanisms (Figure 2). In splenic tissue, the highest KRAS gene expression (0.0306) was observed following one-week exposure to 1 mg/kg AgNPs. On the contrary, the lowest splenic expression level (0.0068) was noted in the group that received the same dose for two weeks, reflecting a time-dependent decline despite high initial upregulation (Figure 3). Increasing proof supports the molecular toxicity of silver-based nanoparticles at low concentrations (Shen et al., 2015). Their outcomes on overall fitness, especially concerning the immune system, are documented (Shi et al., 2024). Our results confirmed a giant association between AgNP publicity and the expression of KRAS genes in tissue, particularly within the liver. Notably, the expression level of the KRAS gene inside the livers of mice that had been exposed

to one mg/kg AgNP for one week and 0.5-1 mg/kg AgNP for two weeks became substantially decrease.

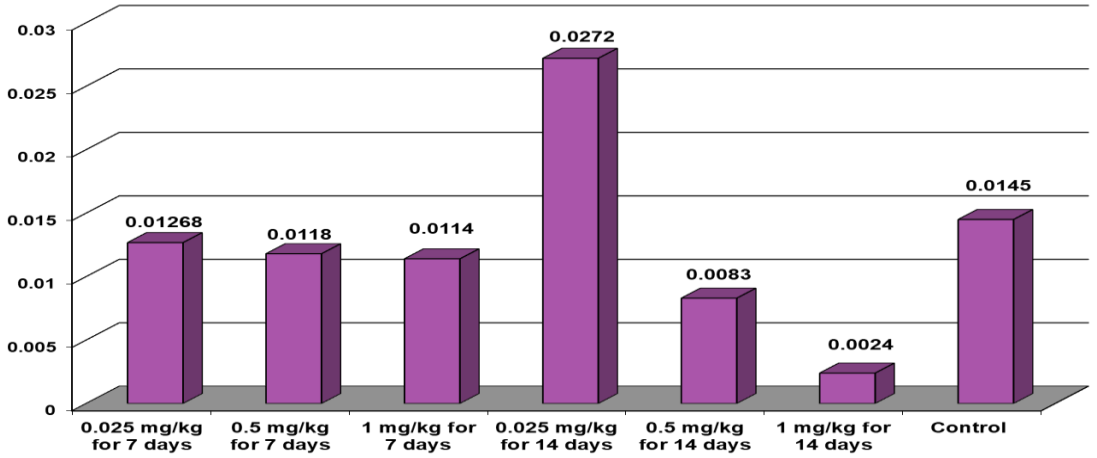

Figure 2. Effect of AgNP treatments on hepatic KRAS gene expression

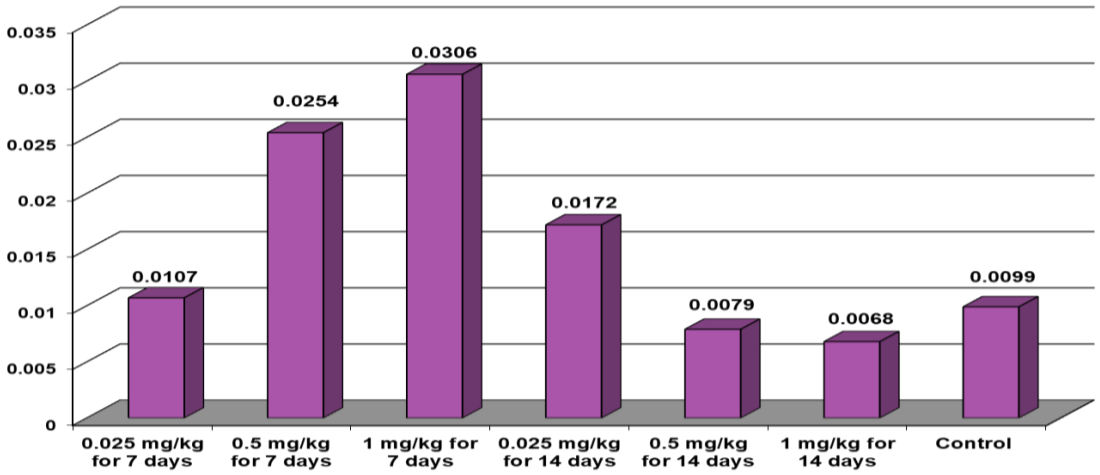

Figure 3. Effect of silver nanoparticle (AgNP) treatments on spleen KRAS gene expression in exclusive observation

Hepatocellular carcinoma (HCC) is the maximum not common shape of number one liver cancer and represents approximately 90% of all tumors inside the liver (Djuraeva et al., 2025). This malignancy is often related to the contamination of HBV and/or genetic adjustments in the RAS gene circle of relatives, such as mutations the Arzumanyan et al., 2013; Najm et al., 2020; Kouroumalis et al., 2023). KRAS mutations are in particular common in the liver's RAS family, they're not common than HRAS and NRAS mutations in this family of genes. Approximately 7% of all cancers have this type of mutation (Camonis et al., 2024). Previous investigations concur

with our findings. For instance, Bin Saeed et al. (2023) documented that AgNPs decreased the KRAS oncogene expression in most cancerous cells. Similarly, Xu et al. (2024) documented that silver nanoparticles had a sizable effect on lowering KRAS ranges. Elechalawar's team (2023) confirmed that nanoparticles can inhibit the activity of KRAS, which consequences in the suppression of tumor growth in cancers that have an imperative function of KRAS activation. In this research, the degrees of KRAS inside the liver tissues of the three experimental organizations were much lower than the control organization. Additionally, the average expression degree of KRAS in the three different-week agencies becomes constantly lower than the manipulate organization. These findings support the inhibitory role of AgNPs on the expression of KRAS inside the liver, this effect is mainly mentioned while the particles are uncovered for a long time.

On the other hand, the expression of KRAS within the spleen turned into associated with the AgNP dose throughout the one-week publicity duration. The KRAS gene expression tiers within the spleen of mice were zero.25 mg/kg AgNP for one week, and people dealt with 0.5-1 mg/kg AgNP for 2 weeks have been lower than those of the managed institution. However, a trendy in KRAS expression changed into observed in the one-week publicity group, which suggests that the duration of publicity has a great impact on the regulation of genes. KRAS is a sizable oncoprotein that participates in controlling the proliferation, transformation, and cellular specialization via various internalized pathways (Ma et al., 2019; Najm, 2019). Its improved expression is related to improved tumor growth and endothelial mobile development (Zhou et al., 2024; Nasir et al., 2025). As a result, the improved stage of KRAS located inside the spleen of mice handled with AgNPs for a restrained time period has brought about issues approximately its ability to sell cancer in other related organs. Despite the shortage of direct evidence that hyperlinks AgNP-prompted KRAS over expression to splenic most cancers, several studies have confirmed that AgNPs can reason great splenic harm. Rubab et al. (2023) found big changes in hematological tendencies, oxidative pressure signs, and microanatomy in rats that have been exposed to greenish artificial AgNPs.

Histological analysis demonstrated the presence of hemorrhage, vascular congestion, and an accumulation of brownish color in the purple pulp. These pigment deposits had been notably absent in the manipulated institution, which indicates that the spleen tissue is suffering from AgNP. The facts also tested that the expression stage of the KRAS gene in the spleen of the 3 one-week observe groups changed into higher than that of the manage organization, at the same time as the average expression level of the KRAS in spleen tissue for the 3 two-week observe groups turned into still beneath the manage organization's degree. This temporal shift may also endorse a strain response that results in gene upregulation, observed via a suppression or fatigue mechanism as the length increases.

Additionally, the position of the tumor's microenvironment in the disease process, particularly via genetic and epigenetic modifications, is documented. KRAS is mostly responsible for those changes, specifically in most cancers, which is related to irritation (Hazim et al., 2020; Raheem et al., 2021; Nasir et al., 2022; Abbas AL-Essawi and Mahmood, 2024; Daghman et al., 2024). As KRAS upregulation promotes the improvement of tumors and promotes angiogenesis, our findings support the concept that exposure to AgNPs might also have a role in the early onset of cancerous behavior within the spleen tissue. Despite the lack of specific evidence of a hyperlink between AgNPs and splenic cancer, the observed changes in gene expression and spleen histology have to be studied in extra detail.

Conclusions: This research shows that the expression of KRAS is specifically altered in tissues following exposure to silver particles. Notably, the expression of KRAS in the liver was decreased following increased doses and longer exposures, which suggests that the molecule has a toxic or inhibitory effect. Conversely, a high-dose exposure period of short duration led to an increase in KRAS expression in the spleen, this was of concern because of the potential for it to lead to an immunological or carcinogenic outcome. These results concur with the theory that AgNPs have molecular effects that detrimentally affect the tissue and its function, as well as a stable genome. Future investigations should assess the long-term effects of these alterations and explain the molecular mechanisms behind the AgNP-induced alteration of gene expression.

Conflict of Interest

There is no disagreement in this study.

Authors' Contributions

Gulboy A. Nasir conducted the experiments in the laboratory and recorded the results. Mohammed A. Najm and Huda M. Mahmood had a role in the data analysis, writing the manuscripts, and developing the concept. All authors commented on and approved the ultimate version of the article.

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Ethical Considerations

All ethical guidelines were strictly followed throughout the study. The authors affirm that no data fabrication, falsification, plagiarism, or research misconduct occurred. Ethical approval was granted by the appropriate institutional committee (Approval No. 102, dated March 24, 2024).

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تغییرات بیان ژن KRAS مرتبط با نانوذرات نقره (AgNPs)

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چکیده

هدف: فناوری نانو انقلابی در حوزه‌های مختلف از جمله پزشکی، لوازم آرایشی و بسته‌بندی مواد غذایی ایجاد کرده است، به‌ویژه از طریق استفاده از نانومواد مهندسی‌شده مانند نانوذرات نقره (AgNPs). نانوذرات نقره به دلیل خواص ضد میکروبی قوی، از پرکاربردترین نانوذرات در محصولات مصرفی و زیست‌پزشکی محسوب می‌شوند. این مطالعه با هدف بررسی اثرات نانوذرات نقره بر سطح بیان ژن سرطان‌زای ویروسی Kirsten rat sarcoma (KRAS) در بافت‌های کبد و طحال موش‌ها انجام شد تا ایمنی مولکولی تماس با AgNPs ارزیابی شود.

مواد و روش‌ها: در مجموع ۵۶ موش به‌صورت تصادفی به هفت گروه (n=8 در هر گروه) تقسیم شدند. سه گروه به‌مدت ۷ روز مقادیر ۰/۲۵، ۰/۵ و ۱ میلی‌گرم بر کیلوگرم وزن بدن AgNP دریافت کردند. سه گروه دیگر همان دوزها را به‌مدت ۱۴ روز دریافت کردند. یک گروه نیز به عنوان کنترل بدون درمان باقی ماند. پس از پایان دوره درمان، بافت‌های کبد و طحال جمع‌آوری شد. RNA کل با استفاده از روش جونکویرا و کارنیرو استخراج و سنتز cDNA با استفاده از کیت Applied Biosystem به شماره ۴۳۸۷۴۰۶ برای آنالیز بیان ژن انجام شد.

نتایج: سطح بیان ژن KRAS بسته به دوز و مدت‌زمان تماس با AgNPها متفاوت بود. در بافت کبد، بیان ژن KRAS در همه گروه‌های دریافت‌کننده AgNP نسبت به گروه کنترل کاهش یافت. در مقابل، در بافت طحال، بیان ژن KRAS در تمام گروه‌های دریافت‌کننده به‌طور قابل‌توجهی افزایش داشت. این تغییرات در دوزهای بالاتر و مدت تماس طولانی‌تر شدیدتر بودند.

نتیجه‌گیری: یافته‌ها نشان می‌دهند که تماس با AgNPها باعث تنظیم مجدد بیان ژن KRAS به‌صورت مختص–بافتی می‌شود؛ به‌طوری‌که کاهش بیان در بافت کبد و افزایش بیان در بافت طحال مشاهده شد. این الگوهای متفاوت بیان بیانگر آن هستند که نانوذرات نقره ممکن است مسیرهای پیام‌رسانی سلولی را به شیوه‌ای تحت تأثیر عملکرد اندام‌ها و ریزمحیط‌ها تغییر دهند. به‌ویژه، افزایش مداوم بیان KRAS در طحال پس از مصرف AgNPها، به‌ویژه در دوزهای بالا و تماس طولانی‌مدت نگرانی‌هایی جدی درباره اختلالات ایمنی یا آغاز فرآیندهای سرطان‌زایی در بافت‌های مرتبط با سیستم ایمنی ایجاد می‌کند. این نتایج لزوم ارزیابی دقیق‌تر استفاده از AgNPها را به‌ویژه در محصولاتی که برای کاربرد طولانی‌مدت یا سیستمیک طراحی شده‌اند، نشان می‌دهد. مطالعات بیشتری برای روشن‌سازی مکانیسم‌های زیربنایی تنظیم KRAS توسطAgNP ها، ارزیابی پیامدهای آسیب‌شناسی احتمالی بلندمدت، و تعیین آستانه‌های ایمن تماس ضروری است. تهیه یک پروفایل جامع ایمنی مولکولی برای تضمین توسعه و استفاده مسئولانه از فناوری‌های مبتنی بر نانوذرات نقره در حوزه‌های پزشکی و تجاری حیاتی است.

کلمات کلیدی: بیان ژن، طحال، کبد، نانوذرات نقره، β-ACTIN

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