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De Novo Gold Nanoparticles Activate P53 by Inhibiting NF-Kb Signalling in Breast Cancer Cells

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Abstract: The aim of this study was to make de novo gold nanoparticles (Au(0)NPs) that turn on p53 and turn off NF-kB signaling in SKBR3 breast cancer cells. The chemical method was used to make the erythromycin-based Au(0)NPs. Authentic techniques were used to figure out what these Au(0)NPs were like. In the end, relative gene expression studies were used to treat SKBR3 breast cancer cells with these Au(0)NPs as a nanomedicine. When Au(0)NPs were present, the levels of caspases 3, 8, and 9 changed, p53 was turned on, and NF-kB was turned off at the same time. Compared to normal breast cells, the number of breast cancer cells (SKBR3) that could live was cut down (CRL-4010). Gene expressions of caspases also showed that the data were correct. When AuNPs were used to treat breast cancer cells, it was found that p53 and NF-kB had the opposite relationship. The study laid out a first step for using newly made AuNPs as a chemotherapeutic agent to treat SKBR3 cells.

Keywords: Gold nanoparticles, Erythromycin, Gene expression, Cell Viability, Breast Cancer

Introduction

Patients with breast cancer are also more likely to become resistant to chemotherapy, and relapses of the disease are widely accepted (Sung et al., 2021). Following the development of multidrug resistance that is complicated due to many factors that cause, is a major problem for breast cancer treatment (Nedeljkovic & Damjanovic, 2019).

Researchers are interested in chemotherapies that use nanoparticles. When gold nanoparticles (AuNPs) are used to treat cancer, the results have been very positive (Mousavi et al., 2007; Shreyash et al., 2021). Not only do these particles stop the growth of cancers (Safdar et al., 2022; Safdar et al., 2019) other than normal cells, but they induce apoptosis that is the main goal of cancer drugs (López-Barrera et al., 2021), and you can easily see how they work by looking at caspases. Also, the action of gold nanoparticles on the tumour suppressor p53 (López-Barrera et al., 2021) that is involved in the apoptosis pathways (Kanamori et al., 2021). It is also known that NF-kB work inversely to p53, and breast cancer cells have too much of it (Ge et al., 2021). So, stopping NF-kB could be used as a chemotherapeutic target. Also, in some studies it is found that the p53 and NF-kB followed different pathways due to change of drugs in the tumor cells (Gottstein et al., 2013). Furthermore, it had been seen that if gene expression changes had been done in the p53 gene then it led to change NF-kB and finally induce different cancers (Fadaka et al., 2021). Even though there are some drugs that selectively turn on p53 or turn off NF-kB with multiple side effects. Therefore, it is a need of time for specific nano-drugs that can control different pathways to inhibit breast cancers. So, a major goal for making new cancer drugs is to turn on p53 and shut down the NF-kB pathway (Arshad et al., 2019; Safdar & Ozaslan, 2022; Safdar et al., 2021).
The purpose of this study was to synthesize de novo gold nanoparticles activate p53 by inhibiting NF-kB signalling in breast cancer cell lines by using MTT and qRT-PCR assays.

Method

Preparation of gold nanoparticles

For the first time, it was shown that the er-AuNPs were made with the help of erythromycin (er) as a reducing agent and a capping agent. For the making of er-AuNPs, 0.5 mL of a 0.01 M solution of HAuCl4 salts was put in a glass beaker and put on a hotplate at 100 °C for 20 minutes. Then, 0.1 mL of a 0.01 M solution of NaOH was added, and the mixture was left for 1 hour to develop its properties. The solution proceeded from yellow to purple, which shows that the AuNPs were there. Also, UV Vis, XRD, and FT-IR were used to find out more about these newly made AuNPs. Lastly, SKBR3 cell lines were used to test the cytotoxicity of these nanoparticles. The average size of AuNPs was between 10 and 50 nm. These particles were made in culture media with 0–200 g/mL of serum or no supplements.

Cell Culture

Human breast epithelium CRL-4010 and breast cancer cell line SKBR3 were grown in Dulbecco's modified Eagle's medium with 10% foetal calf serum and antibiotic-antimycotic solution (100 U/mL penicillin, 0.1 mg/ml streptomycin, and 0.25 g/mL amphotericin B). Cells were grown in a sterile, humidified, 37°C environment with 5% CO2 until they reached full growth.

Cell Viability

Researchers used the MTT assay to measure how alive the cells were. In a 96-well plate with 3000 cells per well, er-AuNP was put in front of cells for 24 hours. Used media was thrown away and replaced with 10 mg/mL of MTT reagent and 100 L of new media (1:9). Another hour of incubation at 37 °C was given to the chromogen reaction to take place. The cells were washed with PBS, and 100 L/well of dimethyl sulfoxide was added to dissolve the formazan crystals. A microplate spectrophotometer was used to measure the absorbance at a wavelength of 505 nm (Molecular Devices). At least three separate experiments with at least duplicate samples were done.

qRT-PCR

Cells were grown in a 25 cm^2 flask, and the NucleoZOL method was used to get the total RNA from the cells (Macherey-Nagel, Germany). In a PCR thermal cycler, a "Precision Reverse Transcription Kit" (made by Qiagen in Germany) was used to do the reverse transcription (Applied BioSystems, USA). By comparing the transcript levels of NF-kB, p53, and caspases to the level of the housekeeping gene GAPDH, real-time PCR was used to find out how these levels changed. The reactions went through 40 cycles with 94 °C for 30 sec, 55-60°C for 45 sec, and 72 °C for 45 sec along with a final extension step of 72 °C for 4 min. Changes in gene expression between groups were found using the method called $2^{-\Delta\Delta CT}$.

Results

After 24 hours, the amount of AuNP were used to treat CRL-4010 and SKBR3 cells induced and evaluate the cell viability. But the response to treatment was stronger in SKBR2 cancer cells than in normal cells. Starting at a concentration of 50 g/ml, when AuNP was added to SKBR3 cells, the IC50 of the cells went down. Cancer cells treated with AuNP showed clear signs of cell death, while normal breast cells lost much less cell viability (Fig. 1). So, we knew that AuNPs were selective for cancer cells because at a higher dose of 150 µg/ml, only 50% of the normal CRL-4010 cells were still alive.

In this study, we investigated how er-AuNP affected the various genes including p53, NF-kB and caspases in CRL-4010 and SKBR3 cell lines. These results showed that Casp3, Casp8, and Casp9 were higher in er-AuNP treated CRL-4010 cells compared to untreated control cells, but p53 and NF-kB gene expression was almost the same in both groups of cells. On the other hand, the mRNA transcripts for p53, Casp3, Casp8, and Casp9 went up a lot in the SKBR3 cells. But when AuNP was added to SKBR3 cells, NF-kB expression went down (Fig. 2). All of the genes selected were affected by the change in mRNA levels in SKBR3 cells.

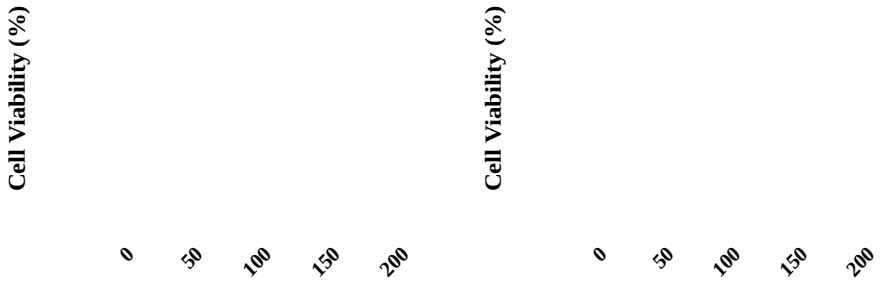

Figure. 1. Compared to CRL-4010 cell line, SKBR3 cell line was decreased when AuNPs were added. MTT showed that SKBR3 were more decreased than CRL-4010 and after being exposed to 0-200g/mL AuNP concentrations. All the data is represented in the form of *P 0.01; **P 0.001; ***P 0.0001.

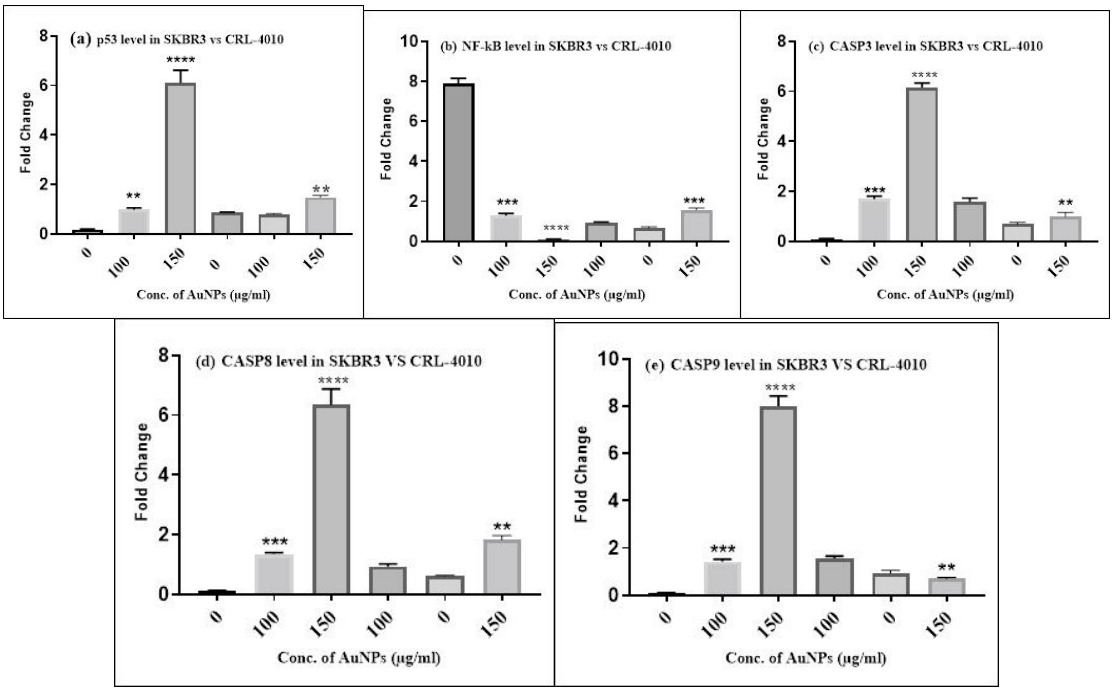

Figure. 2. When AuNP was added to SKBR3 for 24 hours, the p53 gene gene expression increased and the NF-kB gene expression dropped down. The amounts of targeted genes (p53, caspases 3, 8, and 9, and NF-kB) mRNA transcript continued to persist in CRL-4010 (control) cells. The levels of p53, caspases 3, 8, and 9, as well as the levels of NF-kB mRNA gene transcripts, were significantly changed in SKBR3 breast cancer cells. *P≤0.05, **P≤0.01 (Figure. 2a-e).

Discussion

Due to their unique ability to sense tumours, AuNPs have become very promising materials for treating malignant tumours. Because of this, AuNPs have been thought of for a long time as a possible tool for diagnosing and treating different types of cancer. In this study, we talked about the likely way that newly made er-AuNPs induce apoptosis in SKBR3 cells and how exposure to er-AuNPs changes in the targeted genes particularly p53 and NF-kB (Fig. 1&2). Previously, different nanoparticles were used by some researchers to show that these mechanisms were at work in human hepatocellular carcinoma cells (Ahmadian et al., 2018). It is also known that AuNPs have less toxicity as compared to other nanoparticles so they can be used as chemotherapeutics (Khan et al., 2021). Also, this study made it clear that 50-99 ug/ml concentrations of these nanoparticles were best on SKBR3 cells at the same concentration (cancer cells). Other concentrations, like 150 and 200 ug/ml, were very harmful to both healthy and cancerous cells. That was the real reason we did not used them in gene expression studies on both normal and cancer cells. Researchers may need to look into this more.

Nanomaterials also have a special property called cell-dependent cytotoxicity, which has been seen in HT29 and HeLa (Shejawal et al., 2021), MCF-7 (Uzma et al., 2020), and A459 (Niloy et al., 2021) cancer cells.

It is a known fact that the apoptosis is followed by both pathways (intrinsic and extrinsic) (Rajendran et al., 2021) that led to the planned death of the cells. The cytochrome C is a key protein that attach to Apaf-1 (Bock & Tait, 2020) that is released when a cell dies through the intrinsic pathway. Apaf1 turns on caspase 9, which turns on caspase 3, which is a very important part of cell death (Bock & Tait, 2020). Death receptors, on the other hand, turn on caspase 8, which then turns on caspase 3 (Muscari et al., 2020). The intrinsic pathway is now turned on by caspase 3 (Muscari et al., 2020; Safdar et al., 2020; Vo-Dinh et al., 2005). In this study, mRNA expression (caspase 9, 8, and 3) of the targeted genes were very low in normal CRL-4010 cells but it was more than four folds in cancerous SKBR3 cells. This shows that AuNPs work more specifically on cancer cells (Fig. 2). This meant that AuNPs sped up both the internal and external apoptotic cascades in breast cancer cells, which led to the death of the cells. Even when cancer cells were only exposed to 50 M of AuNPs, the expression of caspase 3 went up by a large amount (Safdar et al., 2021). So far as we know, this is the first report that shows how er-AuNPs affect both apoptotic cascades in breast cancer cells. Still, more research is needed to figure out how AuNPs cause cell death at the molecular level. One problem with this study was that it only used in-vitro experiments. More research will be needed to see how AuNPs work in in-vivo models before they can be used as a treatment for breast cancer.

Conclusion

So, these results showed that the newly synthesised and characterised er-AuNPs are a new and promising nanomaterial for a robust evidence-based analysis of chemotherapeutics. Using er-AuNPs could be a smart way to figure out where breast cancer could be spreading. Using relative gene expression studies, changes in caspases 3, 8, and 9 as well as dependence on p53 and NF-kB were found to be higher in SKBR3 than in normal breast cells (CRL-4010).

Recommendations

The study laid out the first step toward using er-AuNPs as a chemotherapeutic and suggested that more research to be done. Also, we recommend the nano-ELISA for further protein analysis.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Acknowledgements or Notes

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