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Synthesis and Characterization of Gold/Silver Bimetallic Nanoparticles Using Trastuzumab: An Enhanced Anti-Cancer Activity

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Abstract: Bimetallic nanoparticles are getting a lot of attention in medicine and society because they might have interesting physical and synthetic properties, such as higher affinity, a lower molecular weight, and a larger surface area. Trastuzumab capped gold/silver bimetallic nanoparticles (Au/Ag-BNPs) were prepared for the purpose of enhancing the effects of Au/Ag-BNPs on SKBR3 breast cancer cells in order to reduce trastuzumab resistance. Au/Ag-BNPs were produced chemically. These Au/Ag-BNPs were characterized using various techniques. UV-Visible (UV-Vis) spectroscopy were used to monitor Plasmon absorption maxima at 462 nm. X-ray powder diffraction (XRD) pattern was utilized to confirm the crystalline nature of the BNPs. Lastly, Fourier transformed infrared (FT-IR) spectroscopy was used to investigate the bonding patterns of the NPs. In SKBR3 human breast cancer cells, the biological characteristics of these NPs were exemplary. Based on the results, we concluded that these gold/silver bimetallic nanoparticles have anticancer potential and can serve as an alternative to decrease the trastuzumab resistance of SKBR3 breast cancer cells. However, additional research is required to corroborate these results.

Keywords: Bimetallic nanoparticles, Trastuzumab resistance, SKBR3 cells, Apoptosis

Introduction

Nanotechnology has gotten people interested in engineering, farming, medicine, and other fields (Safdar & Ozaslan, 2022; Safdar et al., 2021; Safdar et al., 2022; Safdar, Ozaslan, et al., 2019). In order to protect human and environmental health, research will concentrate on safer synthetic processes that use green methods, such as renewability, the use of non-toxic chemicals, the use of less energy, and time-conserving approaches (Mageswari et al., 2016). Nanoparticles with different sizes between 1 and 100 nm have been found to be very effective and have better results in a wide range of areas (Mejia et al., 2021). This is because they are very potent, have a wide base, can act as catalysts, absorb light, and have a robust compatibility. Metal NPs have also been shown to have good attributes in the areas of catalysis, mechanics, magnetism, temperature, light, photoelectrochemistry, and biology (Safdar & Ozaslan, 2022; Safdar et al., 2021; Safdar et al., 2022; Safdar, Ozaslan, et al., 2019). Metal NPs have been used as bacteriostatic agents, drug carriers, drug delivery, to treat cancer, for biosensing, and to speed up chemical reactions (Surapaneni et al., 2018). Scientists have used many different ways to make nanoparticles such as physical, chemical, and biological methods (Uzma et al., 2020). In the past, bimetallic nanoparticles have been developed and employed for a variety of biomedical applications, such as imaging, luminous tagging, labelling, and drug transporters (Smith et al., 2013). By regulating the diffusion of Cu into Au, atomically ordered intermetallic nanocrystals have been prepared using Au-Cu nanoparticles (Sra & Schaak, 2004). This concept offers a novel method for the fabrication of one-of-a-kind bimetallic nanoparticles. Au-Ni nanorods have been engineered as selectively functionalized DNA vehicles (Salem et al., 2003). Bimetallic silver/nickel nanoparticles excelled common catalysts owing to their relatively small size, novel physicochemical properties, and electrostatic impacts (Karaman, 2022). Trastuzumab

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(Herceptin) is a monoclonal antibody that is used to treat breast cancer and stomach cancer (Rebischung et al., 2005). It is only used to treat cancers that have HER2 receptors. It can be used by itself or with other medicines for chemotherapy (Safdar et al., 2020; Safdar et al., 2019). One of the major problems with using trastuzumab to treat people with breast cancer is trastuzumab resistance (Maximiano et al., 2016). In the last ten years, many tests have been done to figure out how trastuzumab resistance happens with or without extra drugs, but they failed yet (Maximiano et al., 2016). To best our knowledge, there is no study related to synthesize and characterize trastuzumab capped gold/silver bimetallic nanoparticles (Au/Ag BNPs) to target HER2 receptors via MTT, relative gene and protein expressions on SKBR3 breast cancer cells. The aimed of this study was to synthesize, characterize and analyze novel trastuzumab capped gold/silver bimetallic nanoparticles (Au/Ag BNPs) to target HER2 receptors via MTT and relative gene expressions on SKBR3 breast cancer cells.

Methods

Bimetallic Au/Ag nanoparticles

In order to generate 1.5 mM solutions of gold and silver salt, 0.021 g of AuSO4 and 0.022 g of AgNO3 were dissolved in 100 mL of deionized water in two separate measuring flasks. A 500 mL flask was filled with 40 mL of a binary salt aqueous solution (1.5 mM) and 1 mL of trastuzumab solution. The flask was set on a hot plate at 55-50 °C and 1300 rpm for an hour, and a magnetic stirrer was used to keep the mixture moving. Because metal salts were quickly broken down, bimetallic nanoparticles were made, and the colour of Ag/Au BNPs changed from light yellow to dark yellow as they formed. Several analytical techniques were used to find out about the size, shape, form, crystallinity, and potential of dried bimetallic nanoparticles. The Ag/Au BNPs were used as a nanomedicine for SKBR3 breast cancer cell treatment. These nanoparticles were characterized by various techniques such as UV Visible, FTIR, SEM, TEM and XRD.

Cell culture

Breast cancer cell line (SKBR3) and immortal breast epithelial cell line (CRL-4010) were cultured and grown in the petri dished under laboratory optimized conditions. When they reached on the optimum condition, then they were harvested and RNA was extracted (Safdar & Ozaslan, 2022).

Cell Viability

Cell viability was assessed using the MTT assay according to the protocol mentioned on the kit. Cells grown at a density of 3000 cells/well were exposure to Au/AgNPs, for 24 h in a 96 well plate. Absorbance was measured at 505 nm wavelength using a microplate spectrophotometer (Molecular Devices).

Analysis of gene expression

A reverse transcription assay kit was used to obtain cDNA (Qiagen, Germany). HER2, and GAPDH gene expression levels (housekeeping gene). 2.5 Statistical analysis Corbet Rotor-Gene software was used to examine the data collected. Considerable downregulation was defined as a fold change (2Ct) value of 0.1 to 0.5, whereas significant upregulation was defined as a value of >2.0.

Results and Discussion

After treatment with Au/AgNPs, the expression level of HER2 and cell survival of both cell lines were assessed using the MTT test and quantitative Real-time PCR (qRT-PCR). It was shown that Au/Ag bimetallic NPs reduced the levels of HER2 expression in the SKBR3 breast cancer cell line. However, prior to receiving therapy with Au/AgNPs, the SKBR3 breast cancer cell line had significant high levels of HER2 expression. Additionally, the downregulation of NF-kB and simultaneous overexpression of p53 following Au/AgNPs therapy revealed that HER2 receptors were downregulated through reduced Trastuzumab resistance. This study is one of the first of its kind since it shows a connection between the NF-B, p53, and HER2 following treatment with Au/Ag bimetalic NPs (Fig. 1 & 2).

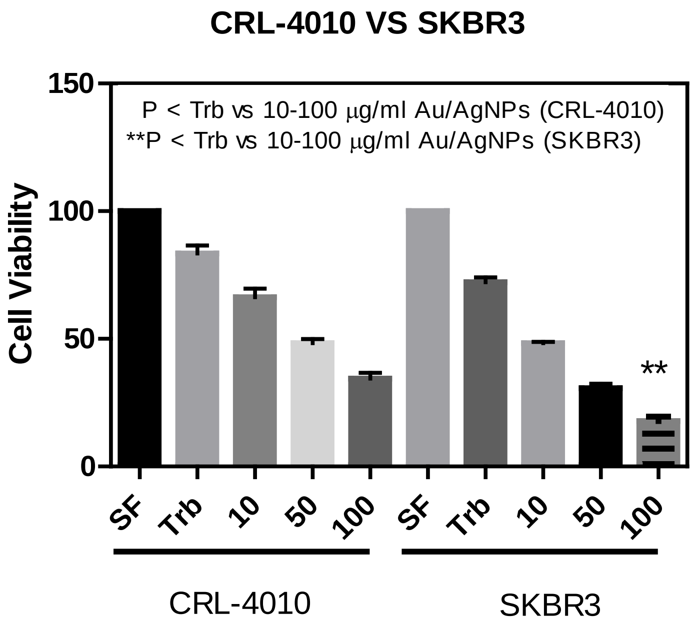

Figure 1. Au/AgNPs has decreased significantly the living cells in SKBR3 when compared with CRL-4010 cells. The 10-100µg/mL Au/AgNPs concentrations were exposed to both cell lines via MTT assay. The results have been expressed as $^{*}P \leq 0.01$, and $^{**}P \leq 0.001$.

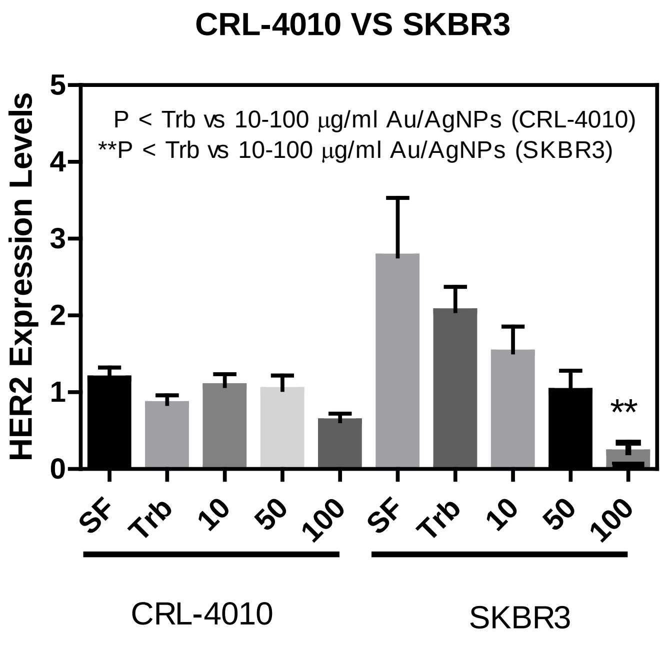

Figure. 2. HER2 expression levels are overexpressed in SKBR3 cell lines. Au/AgNPs has decreased HER2 gene expression levels significantly in SKBR3 when compared with CRL-4010 cells. The 10-100µg/mL Au/AgNPs concentrations were exposed to both cancer and normal cell lines via qRTPCR assay. The results have been expressed as $^{*}P \leq 0.01$, and $^{**}P \leq 0.001$.

HER2-positive breast cancer cells have been targeted with metallic nanoparticles (Dziawer et al., 2019; Maximiano et al., 2016; Rebischung et al., 2005). However, because of the focus on cellular absorption, we deemed the same results as (Kulhari et al., 2016) to be noteworthy and worth addressing in this study. The cellular absorption of trastuzumab was investigated in parallel (Kulhari et al., 2016). The results were compared to the SKBR3 HER2-positive breast cancer cell line and the CRL4010 cell line (Wege et al., 2014). The uptake efficiency of nanoparticles in combination with trastuzumab was compared. The fluorescence intensity was higher in the nanoparticles, indicating that increased uptake occurred due to the targeting mechanism, which could lead to a reduction in SKBR3 malignancies in women (Mi et al., 2012). As a result, these bimetallic nanoparticles are crucial for future use in nanomedicine applications.

Conclusion

The expression levels of HER2 in breast cancer cell line (SKBR3) were increased/decreased on a particular concentration (10-100µg/mL) of gold and silver bimetallic nanoparticles. In addition, the downregulation of HER2 after Au/AgNPs treatment displayed a particular pathway. These results showed that expression levels of NFκB, p53 and HER2 genes were modified via Au/Ag bimetallic treatments.

Recommendations

This article will lead the scientists to focus on HER2 overexpression control via newly synthesized bimetallic nanoparticles.

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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