



Anti-Cancer activity of AuNPs in Breast Cancer (SKBR3) Cell Line and Mammary Epithelial (CRL-4010) Cell Line by the Up Regulation of TRPs and p53

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Abstract: Breast cancer is a metastatic disease with high mortality rate and is regarded as the most abundant malignancy observed in women. In various crucial cellular processes, such as progression and initiation of different cancers (TRPs) Transient receptor potential channels play a significant role. Recently, TLRs are regarded as novel treatment for breast cancer while TRP channels expression are also vital in regulating cellular processes, such as differentiation, angiogenesis, proliferation, migration and apoptosis. This study illustrates that the TRPs and TLRs expression levels can be modified using gold Nano-particles (AuNPs). AuNPs were applied on mammary epithelial cell line (CRL-4010) and breast cancer (SKBR3) cell line. After AuNPs treatment Real-time PCR (qRT-PCR) was performed to evaluate the expression levels of TRPs, NFκB and p53. Gold Nanoparticles (AuNPs) are regarded as promising and emerging agent for cancer therapy as well as Photo-thermal, Radio-Sensitisers and contrast agents also are investigated as drug carriers. The expression levels of TRP1, TRP2, and TRP5 were up regulated in breast cancer cell line SKBR3 due to AuNPs application. In addition, NF-Kb was recorded as significantly down regulated on the other hand simultaneous up regulation of p53 occurred that indicated that these two cellular pathways cross talk each other. This study enlightens the AuNPs implication for the treatment of cancer in association with NFκB, TRPs and p53.

Keywords: TRPs, NFκB, qRT-PCR, SKBR3, CRL-4010, AuNPs, Breast Cancer.

Introduction

Among all the cancers found around the globe in female, Breast cancer is about 23.1% while in USA the account of Breast cancer is about 11-15% [1]. During 2019 top ten malignancies that were reported in SKMCH&RC, breast cancer was ranked as leading malignancy of 7,044 total malignancies 3791 of patients were Breast cancer malignant. Rectum, anus, colon, canal and leukemia Cancers were recorded as 2nd and 3rd most seen malignancies respectively. TLRs and TRPs (Transient receptor potential channels) are related to the activation and growth of tumors. For the modification of TLRs and TRPs expression levels Gold Nanoparticles (AuNPs) are found as effective agents and were applied on (CRL-4010) mammary epithelial and (SKBR3) breast cancer cell lines. To treat breast cancer drugs utilized were not entirely efficient and eventually susceptible to resistance. Thus there is a need of hour to discover the therapeutic agents which are more effective for this disorder. Nanobiotechnology is the recent emerging field owing its applications in industry, agriculture, Medicine and related fields. For the treatment and diagnosis of diseases like cancer Nano-biotechnology is regarded as a great

hope [2]. For of drugs delivery & as biologically active macromolecules, Nano-particles (NPs) own distinctive chemical, surface and physical properties hence are being utilized as drug carriers [3,4]. Unlike small drug molecules AuNPs possess exceptional physiochemical properties that allows them for easy penetration to organism's cells [5]. In a latest preclinical study citrate AuNPs-TNF complex was compared with TNF indicated that the improved toxicity related to TNF and deplorable uptake occur by RES. In tumour specificity, a dramatic advancement was observed with the addition of PEG-Thiol at 6 hour plateauing and active tumour uptake, and a steady decline in spleen and liver concentration over the same time [6]. AgNPs Nanometer scale was manufactured [7] and antiviral, anticancer and antibacterial activity was tested [8,9,10,11-14]. Several synthetic reagents like polyvinyl-pyrrolidone [15], polyethylene glycol [16] and polyvinyl alcohol [17] were utilized for AgNPs stabilization as well as for numerous purposes. AuNPs possess the tiny size having diameter of about 30nm and do not react with O₂ at any temperature thus can easily enter into cell's membranes (Giersig and Mulvaney 1993). AuNPs exhibit the non toxic effects as well as no immunogenicity.

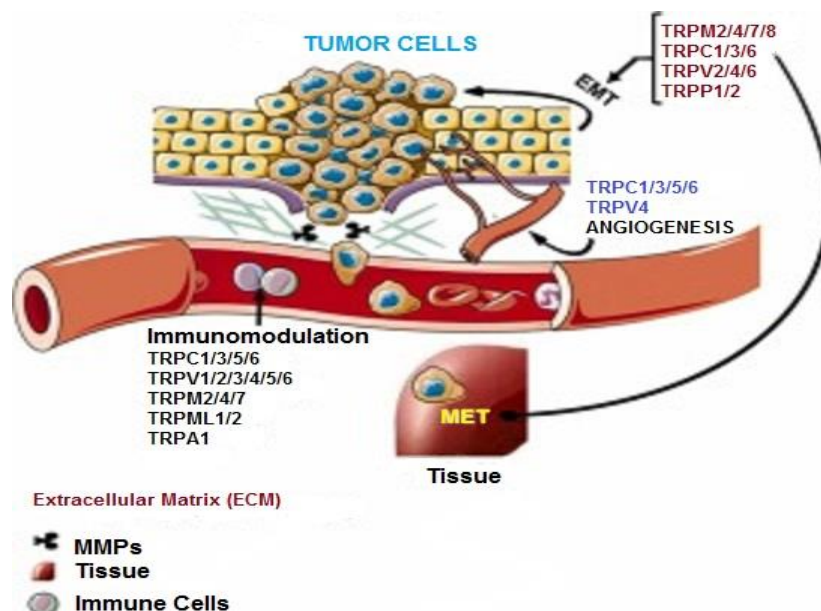


Figure: TRPs and Tumor Cells Immunomodulation

Material and Methods

a. Preparation of AuNPs : AuNPs were synthesized using an antibiotic drug and various techniques like TEM, SEM, UV Visible, XRD and FTIR were used to characterize them. After their synthesis and characterization AuNPs were applied on normal CRL-4010 and SKBR3 breast cancer Cell lines

b. Cell culture: To culture breast epithelial CRL-4010 & Breast cancer SKBR3 cell lines (LGC Promo chem, Teddington, UK) was used and DMEM (Sigma,USA) was used for cell cultivation containing (Sigma,USA) 2mM-glutamine,10% FCS. (100µg/ml) streptomycin and (100U/ml) of penicillin(Sigma,USA).the cultivated cells were treated with Gold Nanoparticles and after 24 hours of treatment total RNA was isolated from the harvested cells

c. Gene expression analysis:Commercially available kits (QIAcube,Qiagen,Germany) were used for total RNA isolation. For the synthesis of cDNA reverse transcription assay kit (Qiagen,Germany) was used. The quantification of Gene expression of various genes like NFkB, p53, GAPDH (house-keeping gene) and TRP1, TRP 2, TRP 5 was recorded by qRT-PCR (Corbet Rotor-Gene 6000 rotary analyzer (Corbett,Australia).

d. Statistical study: Corbet Rotor-Gene software was used for the analysis of collected data. The Fold change values ($2^{-\Delta\Delta Ct}$) were about 0.1 to 0.5 and were considered as significantly down regulated on the other hand >1.5 were up regulated significantly. The expression levels for targeted genes like TRPs, p53 and NFkB of both (SKBR3) breast cancer cell line and (CRL-4010) mammary epithelial cell line were compared

Results and Discussion

After the AuNPs treatment TRPs, NF-kB and p53 expression levels were measured by using Real-time PCR (qRT-PCR). It was observed that there is an up regulation of TRP5, TRP2, TRP1 and p53expression levels while NF-kB was significantly down regulated. The cross talk among vital cellular cascades like NF-kB, TRPs and p53 was indicated after the application of AuNPs treatment and it confirms that, in breast cancer such Onco genes expression can be altered by using AuNPs. The association between TRPs, NFkB, and p53 can also be depicted by this study.

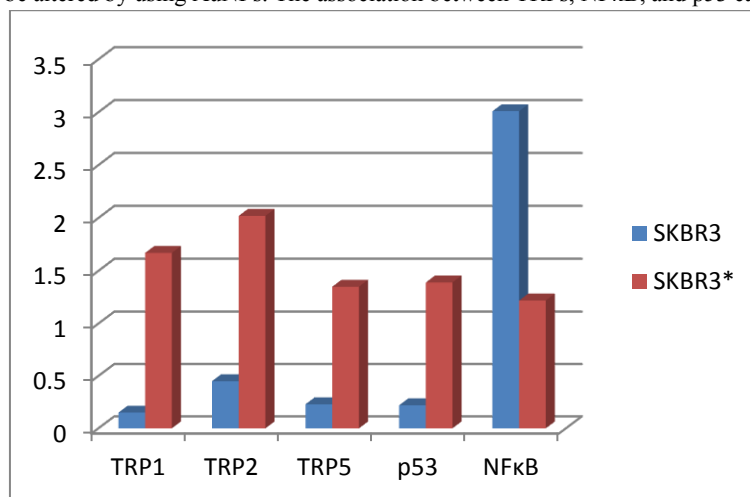


Table1: Effect of AuNPs on SKBR3 Cell Line

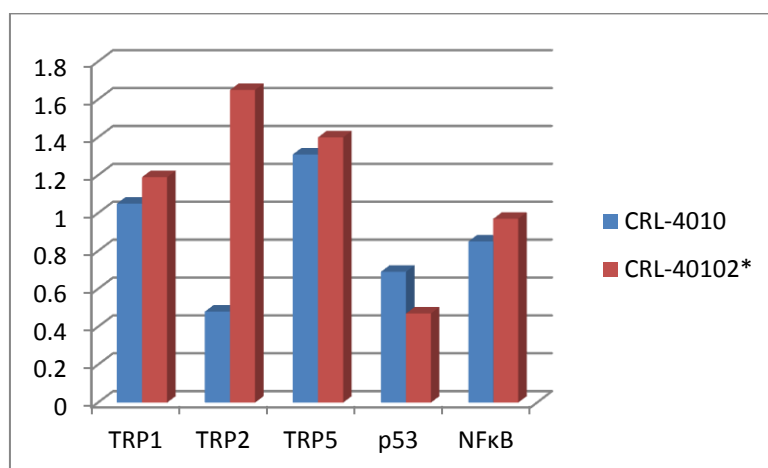


Table2: Effect of AuNPs on CRL-4010 Cell Line

Conclusion

In women the most profuse and metastatic disease observed with higher mortality rate is breast cancer. TLRs Toll- like receptors and TRPs activation indicates the malignant growth and Cancer progression. In this study there an up regulation of p53, TRP5, TRP2 and TRP1 was observed in breast cancer cell line SKBR3 while the Necrotic factor NFkB was down regulated after the treatment of AuNPs using (qRT-PCR) Real-time PCR. The NF-kB down-regulation & simultaneously up-regulation of p53 suggests the crosstalk between such important cellular cascades. AuNPs can be potential and noval therapeutic agents for the treatment of Cancer. The modified expression levels of TRPs, p53 and NFkB demonstrates the effects of AuNPs. However this is a preliminary study on the Anti-Cancer activity of AuNPs and future studies are suggested for better insight.

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