**In vivo Evaluation of Annexin A2-Targeted Combinatorial Nanoparticles to cause Sustained Reduction of Plasminogen Activation in Highly Metastatic Breast Cancer**

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**ABSTRACT SUMMARY**

The anti-angiogenic and fibrinolytic activity of Annexin A2 (AnxA2) has led investigators to examine the regulatory roles of Annexin A2 for therapeutic applications in the treatment of various cancers. In the present study, we have formulated anti-Anx A2 conjugated PLGA nanoparticles loaded with curcumin for targeted delivery to breast cancer cells. Our results show that these nanoparticles are capable of sustained intracellular delivery of curcumin and AnxA2 mediated targeting and reduction of plasmin generation in metastatic breast cancer.

**INTRODUCTION**

Targeting anticancer drugs to their specific molecular targets is a major challenge in cancer therapy. However, advances in biomedical and protein engineering have led to novel nanoparticle targeting approaches. In this study, we used a novel non-covalent insertion of a homo-bifunctional spacer for targeted delivery to various cancer cells. Despite multiple medicinal benefits of curcumin, a potent anti-cancer agent, poor water solubility and low bioavailability pose a major challenge in developing an efficacious formulation for clinical use. In this study, we have developed curcumin loaded PLGA nanoparticles which are surface functionalized to attach targeting molecules. Annexin A2 (AnxA2) has been reported as a potential serum marker for hepatocellular carcinoma¹. It also plays an important role in plasmin generation which affects the invasion and migration². Differential expression of AnxA2 will serve as a potential biomarker in breast cancer progression and PLGA nanoparticles encapsulating curcumin conjugated to monoclonal anti-AnxA2 would serve dual function, help target these nanoparticles at the desired site and inhibit AnxA2 dependent plasmin generation in highly invasive and metastatic breast cancer cells.

**EXPERIMENTAL METHODS**

We investigated the differential expression of AnxA2 by Total Internal Reflectance Fluorescence (TIRF) Microscopy and Confocal Laser Scanning Microscopy (CLSM). Functionalized nanoparticles for antibody conjugation were prepared by w/o/w emulsion solvent evaporation method using crosslinking ligands. Nanoparticles were characterized for particle size, surface morphology and percent antibody attachment. Cell uptake studies with AnxA2 conjugated and unconjugated nanoparticles were carried out to validate targeted nanoparticle delivery in highly metastatic breast cancer cell line, MCF10CA1a. Functional assays such as the plasmin generation assay was carried out in MCF10CA1a cells using AnxA2 conjugated and unconjugated nanoparticles to demonstrate the effect of antibody conjugation on plasminogen-plasmin conversion. Further, the ability of these AnxA2 conjugated curcumin loaded nanoparticles to specifically target tumor tissue was evaluated in a mouse model system using live animal imaging. The therapeutic efficacy of the targeted nanoparticles was determined by tumor regression in xenograft studies.
RESULTS AND DISCUSSION

The differential cell surface expression of AnxA2 in breast cancer progression was established using TIRF and CLSM as seen in Figure 1. This clearly indicates that cell

![Image of differential expression of Annexin A2 in non-malignant and highly malignant breast cancer cell lines.](image1)

surface AnxA2 may be used as a biomarker to target anti-Anx A2 conjugated nanoparticles. We prepared activated PLGA nanoparticles using 3 different ligands. The mean nanoparticle size with BS3 was found to be 189nm. Anti-AnxA2 conjugated curcumin loaded PLGA nanoparticles were spherical and smooth in morphology. Percent antibody attachment was found to be 92.8%. Intra-cellular uptake of Anti-AnxA2 conjugated nanoparticles was higher in highly malignant breast cancer cells than unconjugated nanoparticles. Treatment by AnxA2 conjugated nanoparticles resulted in decrease in the amount of plasmin generation than unconjugated nanoparticles due to a probable decrease in AnxA2-plasminogen binding. Live animal imaging results clearly demonstrate that AnxA2 conjugated nanoparticles showed increased uptake and accumulation in MCF10CA1a

![Image of live animal imaging of tumor bearing mice.](image2)

Figure 2. Live animal imaging of tumor bearing mice
derived xenograft tumors in mice as compared to unconjugated nanoparticles (Figure 2). Therapeutic efficacy of the Anti-AnxA2 conjugated curcumin loaded nanoparticles was determined in xenograft study and results showed ~40% reduction in tumor burden when treated with conjugated curcumin nanoparticles as compared to untreated control animals.

CONCLUSION

The evaluation of targeted anti-Anx A2 conjugated curcumin loaded nanoparticles was successfully carried out in mice. Such an approach would further enhance the development of novel targeted theranostic nanoparticles for breast cancer imaging and therapy.

REFERENCES


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