Synergistic Antitumor and Immunotherapeutic Targeted Nanoparticles for Cancer Therapy

*M. Khoobchandani[1, 2], A. Raphael Karikachery[1, 2], V. C. Thipe[1-3], K. K. Katti[1, 2], K. V. Katti[1-3]

[1]Department of Radiology, University of Missouri, Columbia, MO 65212, USA
[2]Institute of Green Nanotechnology, University of Missouri, Columbia, MO 65212, USA
[3]Department of Chemistry, University of Missouri, Columbia, MO 65201, USA

Gold and palladium nanoparticles (AuNPs and PdNPs) are well known for their antitumor efficacy (1-3). Phytochemicals expresses unique receptor-binding affinity towards different tumor tissues (breast, prostate and pancreatic) (4). Hence, encapsulation of immunomodulatory phytochemicals on gold and palladium nanoparticles will amplify their immunomodulatory features and are therefore expected to overcome some of the limitations found in traditional therapeutic and diagnostic agents (5).

Consequently, we focused on developing a new generation of phytochemical-encapsulated metallic nanoparticles to produce immunomodulatory cancer therapy agents. Naringenin (Ng) conjugated AuNPs were synthesized for exploring their antitumor efficacy. Naringenin is obtained from the Typha Capensis plant extract (6). Cancer therapeutic applications of resveratrol conjugated PdNPs was also explored. Resveratrol (Res) *trans*-3,5,4′-trihydroxystilbene is a highly attractive phytochemical available in abundance in grapes that has been extensively investigated for its efficacy in disease prevention and cancer treatment (7).

In Prof. Katti's laboratory we have been developing Green Nanotechnology based phytochemical conjugated metallic nanoparticles by mixing the metal salt with the respective phyto-extracts in an aqueous solution. Gold nanoparticles were synthesized by addition of a 0.1 M solution of sodium tetrachloroaurate(III) dihydrate ($NaAuCl_4.2H_2O$) to naringenin in distilled water [In press Keenau's Thesis]. Palladium nanoparticles were synthesized by addition of a 0.1 M solution of sodium tetrachloropalladate(II) (Na_2PdCl_4) to resveratrol in distilled water [Veli's Thesis]. The solutions were then stirred overnight for optimization. The nanoparticle solutions were then purified by centrifugation and stored at 4°C until further use.

Naringenin gold nanoparticles (Ng-AuNPs) and resveratrol palladium nanoparticles (Res-PdNPs) were characterized by UV visible spectrophotometry and Dynamic Light Scattering (DLS) measurements at Professor. Katti's laboratory in the Department of Radiology, Institute of Green Nanotechnology (University of Missouri, School of Medicine). Both the nanoparticles were further characterized for their core size and cellular internalization by Transmission Electron Microscopy (TEM) (University of Missouri, Electron Microscopy Core) [In press Keenau and Veli Thesis].

Spectrophotometric analysis was performed for the Ng-AuNPs and Res-PdNPs as the first step to confirm the presence of gold and palladium nanoparticles respectively [In press Keenau and Veli Thesis]. UV-visible spectrometry yielded surface plasmon resonance absorption peak at 540 nm for Ng-AuNPs corresponding to the presence of gold nanoparticle. The disappearance of the Pd(II) ions peak at 420 nm confirmed the successful synthesis of the Res-PdNPs. The hydrodynamic size of the synthesized nanoparticles was measured by DLS using a NanoZeta sizer [In press, Keenau and Veli

Thesis]. The average hydrodynamic size of Ng-AuNPs and Res-PdNPs was 21±1 nm and 50±3 nm respectively. The Polydispersity Index (PDI) of Ng-AuNPs and Res-PdNPs was 0.4±0.1 nm and 0.3±0.1 nm respectively indicating a narrow size distribution. Zeta potential was measured to determine the surface forces/charge and stability of the metallic nanoparticles. Ng-AuNPs and Res-PdNPs yielded an average zeta potential of -33±1 mV and -38±1 mV respectively conferring a high degree of stability for both the formulations.

TEM imaging was used to visualize and confirm the presence of gold and palladium nanoparticles in both the Ng-AuNPs and Res-PdNPs formulations respectively [In press, Keenau and Veli Thesis]. The morphology of the nanoparticles were spherically shaped with a consistent observable size. Size distribution histograms yielded a particle size range with a calculated average size of 13±6 nm and 13±4 for Ng-AuNPs and Res-PdNPs respectively. The internalization and localization of the Ng-AuNPs within prostate cancer (PC-3) cells was also confirmed by TEM. TEM image analysis indicated cellular internalization through endocytic pathway (Figure 1-3).

Acknowledgement: We would like to acknowledge and express our sincere gratitude to Prof. Kattesh V. Katti for all the support and guidance for this research. We also want to thank Kavita K. Katti for her assistance and support for this work. A very special acknowledgement to Keenau Pearce from University of Western Cape, Cape Town, South Africa for Ng-AuNPs synthesis and characterization.

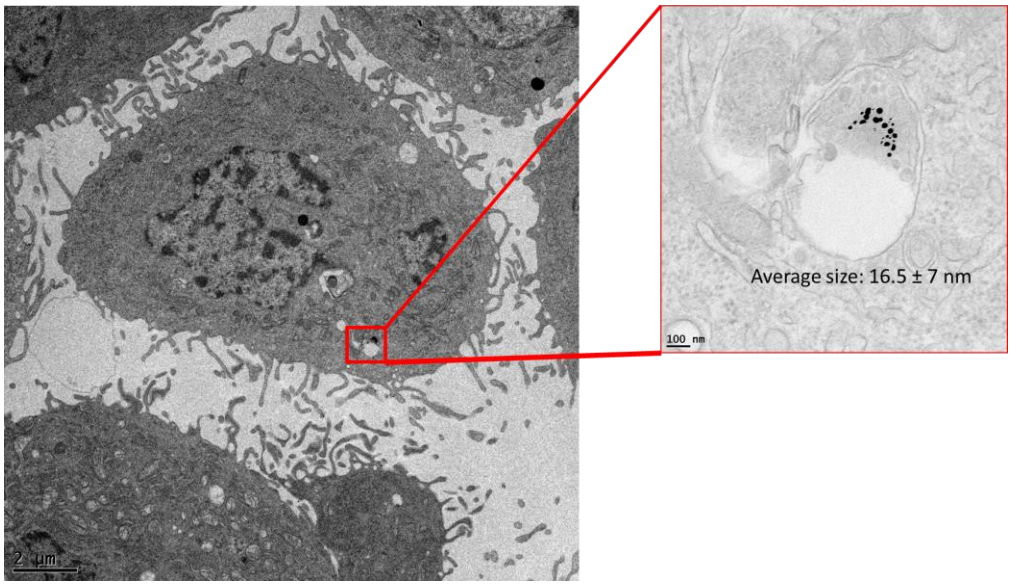

Fig. 1. Cellular uptake of Ng-AuNPs within PC-3 cells at concentration 100 ug/mL with 2 hr incubation time, images captured by TEM.

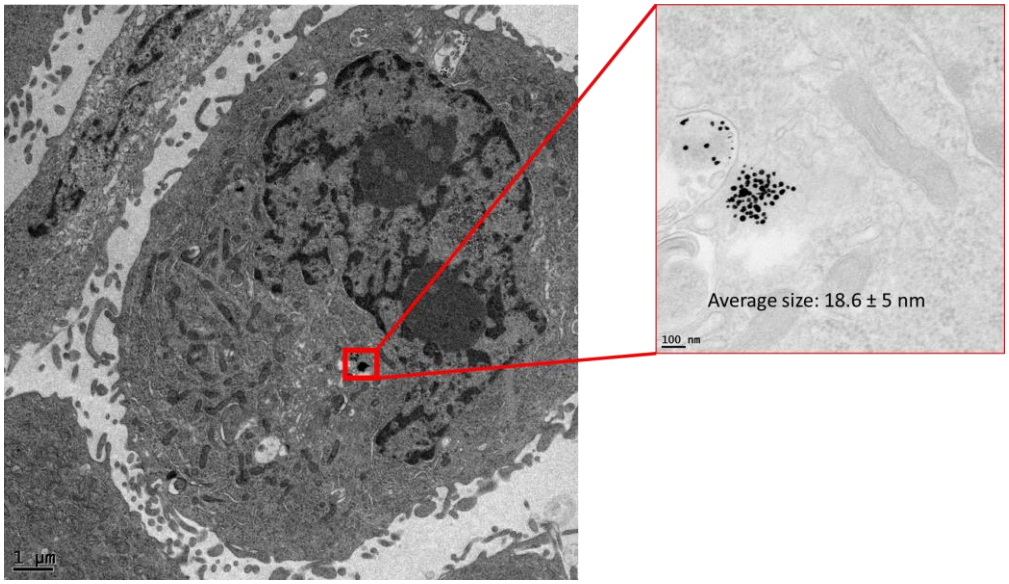

Fig. 2. Cellular uptake of Ng-AuNPs within PC-3 cells at concentration 100 ug/mL with 6 hr incubation time, images captured by TEM.

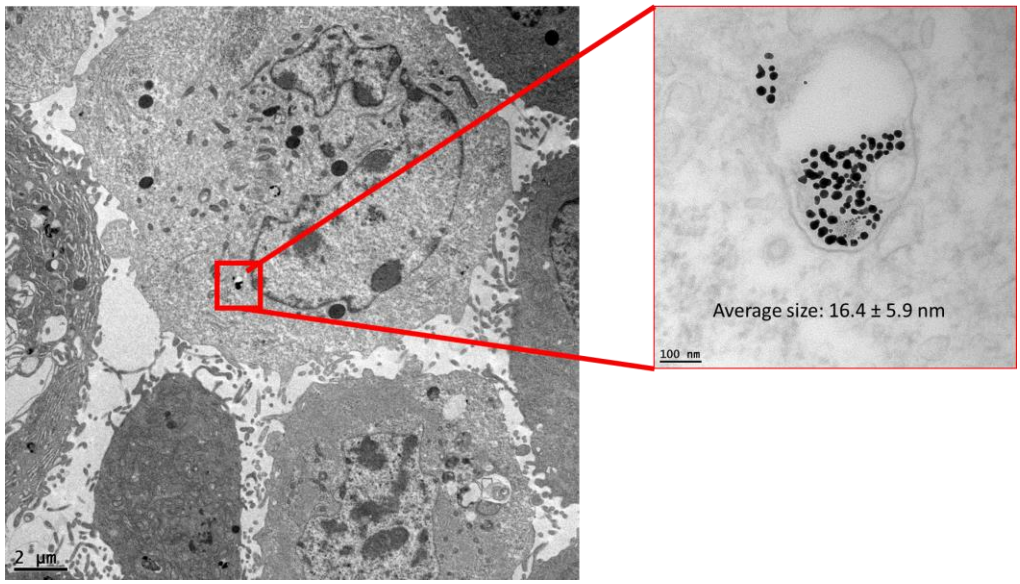

Fig. 3. Cellular uptake of Ng-AuNPs within PC-3 cells at concentration 100 ug/mL with 24 hr incubation time, images captured by TEM.

References:

1. Katti, K.V.*, et al. Prostate tumor therapy advances in nuclear medicine: green nanotechnology toward the design of tumor specific radioactive gold nanoparticles.* J Radioanal Nucl Ch, 2018. 318(3):1737-1747.
2. Al-Yasiri, A.Y.*, et al. Mangiferin functionalized radioactive gold nanoparticles (MGF-(198)AuNPs) in prostate tumor therapy: green nanotechnology for production, in vivo tumor retention and evaluation of therapeutic efficacy.* Dalton T, 2017. 46(42):14561-14571.
3. Khoobchandani, M., Katti, K., Maxwell, A., Fay, W.P., Katti, K.V. *Laminin Receptor-Avid Nanotherapeutic EGCg-AuNPs as a Potential Alternative Therapeutic Approach to Prevent Restenosis.* Int J Mol Sci, 2016. 17(3).
4. Vacha, R., Martinez-Veracoechea, F.J., Frenkel, D. *Receptor-Mediated Endocytosis of Nanoparticles of Various Shapes*. Nano Lett, 2011. 11(12):5391-5395.
5. Jiao, Q., Li, L.W., Mu, Q.X., Zhang, Q., *Immunomodulation of Nanoparticles in Nanomedicine Applications.* Biomed Res Int, 2014.
6. Ghezal, N., et al., *Stimulatory effect on pea of Typha Angustifolia L. extracts and their chemical composition.* J Plant Nutr, 2014. 40(14):1993-2005.
7. Carter, L.G., D'Orazio, J.A., Pearson, K.J. *Resveratrol and cancer: focus on in vivo evidence*. Endocr-Relat Cancer, 2014. 21(3):R209-R225.
8. Thipe, V. C. PhD. Dissertation, University of Missouri, 2019.
9. Pearce, K. PhD. Dissertation, University of Western Cape (in preparation).