

## GOLD NANOPARTICLES ENHANCE THE IN VITRO ANTITUMOR ACTIVITY OF KAHALALIDE F

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Natural products and their derivatives have traditionally been a common source of drugs. Cytotoxic peptides are synthesized by a large number of plants and animals<sup>1,2</sup>, and can be attached to gold nanoparticles for delivery.

Herein, we show that the anti-tumor activity of two synthetic analogues of a marine compound is higher when these compounds are delivered as conjugates with gold particles than when delivered alone. Two synthetic epimeric analogues of the marine cyclodepsipeptide Kahalalide-F (KF) (**Figure 1**) were synthesized. This compound is the most bioactive member of the Kahalalide family of peptides. The target for KF has been studied in cultured cells. The presence of the compound appears to cause alterations in cell lysosomes, meaning that lysosomes are a target for KF action<sup>3,4</sup>.

The peptides were then separately conjugated to two different sizes (20 and 40 nm) of colloidal gold nanoparticles in order to study how nanoparticle size related to conjugate activity and were exhaustively characterized using UV-vis spectroscopy, amino acid analysis (AAA), transmission electron microscopy (TEM), electron energy loss spectroscopy (EELS) and X-ray spectroscopy (XPS). **Figure 2** shows the high-resolution TEM micrographs (HRTEM) of gold nanoparticles when uncoated and when coated with P1. The presence of a layer around the nanoparticle core (**Figure 2.b**) corresponding to the peptide was observed following uranyl acetate staining. As observed, the peptide covered the whole surface of the nanoparticle and increased the hydrodynamic size of the peptide-capped nanoparticle.

In addition, EELS (electron energy loss spectroscopy) and XPS (X-ray photoelectron spectroscopy) were used to confirm the presence of S-Au bonds on the surface.

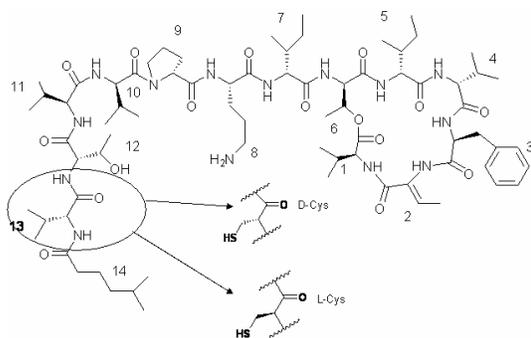
The degree of cytotoxicity of single peptides (P1 and P2), single gold nanoparticle solutions (AuNp-20 and AuNp-40) and their respective conjugates was determined by the WST-1 assay in HeLa tumor cells (**Figure 3**).

The results showed that both gold nanoparticle solutions displayed residual cytotoxicity against HeLa cells, determined in 20 and 30 % inhibition for AuNp-20 and AuNp-40, respectively. Regarding the cytotoxicity of the single peptides, P2 resulted to be less cytotoxic than P1 (10 vs 50 % inhibition, respectively). Additive cytotoxicity was found for P2 conjugates (both, AuNp-20- and AuNp-40-P2) as well as for AuNp-20-P1. However, AuNp-40-P1 resulted in a more than additive cytotoxicity (60 % inhibition) compared to the corresponding single components. This could be the result of the better cell uptake of the former, which would be consistent with the findings of Chithrani et al.<sup>5</sup> The authors reported that among nanoparticles of different sizes, those of 50 nm demonstrated the highest level of uptake by HeLa cells. In agreement with these results, Osaki et al.<sup>6</sup> qualitatively showed that 50 nm nanoparticles entered the cells via receptor-mediated endocytosis more efficiently than smaller ones. We thus considered that the higher toxicity could be a consequence of a synergic effect between the peptide and the nanoparticles.

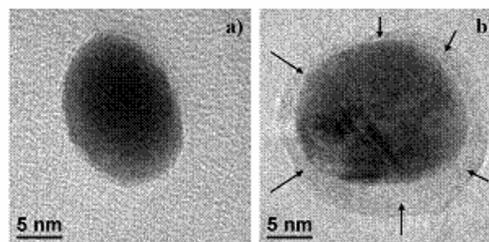
Cellular localization of the gold-conjugates was also studied by TEM (**Figure 4**) and confocal microscopy. Both sizes of conjugated AuNPs were found in the lysosome-like structures in much higher quantities than unconjugated AuNPs. A substantial difference between conjugated and free AuNPs was observed. This may be due to the fact that the peptide directs the AuNPs to the lysosome-like structures. The finding is in agreement with the studies conducted by Garcia-Rocha et al., who determined that Kahalalide F acts through cell lysosomes.

This paper illustrates how the activity of a bioactive molecule such as an anti-tumor peptide can be heightened by conjugation to nanoparticles. Furthermore, it demonstrates that nanoparticles of a certain size can be directed to targets within cells. Additive or more than additive cytotoxic effect was observed when conjugating the peptides and nanoparticles. The nanoparticle acts as a presenter of the anti-tumour agent, concentrating numerous peptide molecules on its surface, which then guide the nanoparticle to the lysosomal compartment.

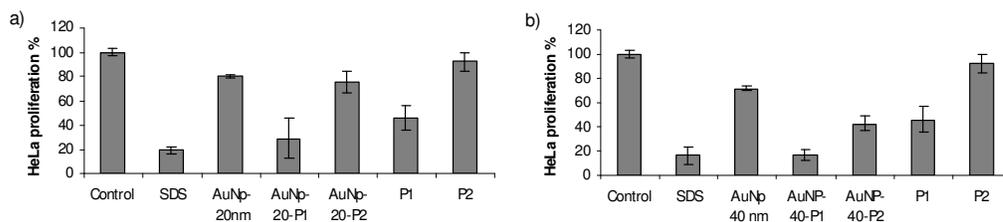
### Figures:



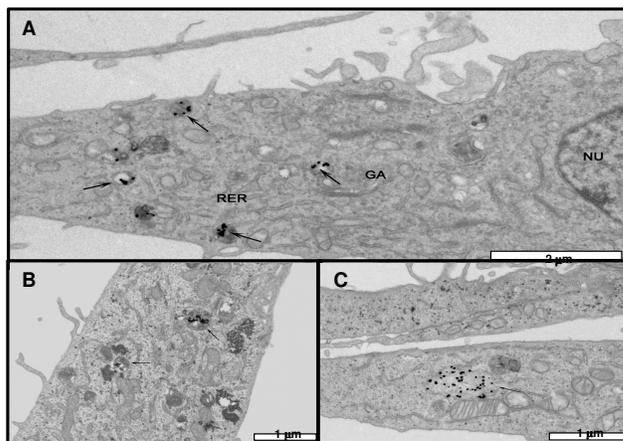
**Figure 1.** Structures of KF, P1 and P2.



**Figure 2.** a) Uncojugated and b) P1-conjugated gold nanoparticles.



**Figure 3.** Anti-proliferation results of the incubation of P1- and P2-conjugated 20 nm (a) and 40 nm (b) gold nanoparticles with HeLa cells for 24 h.



**Figure 4.** TEM images of HeLa cells incubated with a) 20 nm uncojugated nanoparticles, b) 20 nm P1-conjugated nanoparticles, and c) 40 nm P1-conjugated nanoparticles; The arrows indicate the presence of the AuNPs inside lysosome-like structures. NU (nucleus), RER (rough endoplasmic reticulum), GA (Golgi apparatus).

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