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Use of an innovative system and nanotechnology-based strategy for therapeutic applications of Gla-rich protein (GRP)

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Agarose Gel Electrophoresis (AGE), SDS-PAGE, Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA). Zeta-potential values were estimated by Ferguson Plot Analysis of the AGE runs or were determined by Electrophoretic Light Scattering (ELS). Computational methods were applied to the analysis of electrophoretic mobility as observed in video sampling of the AGE runs.
Results: Size and concentration of the synthesized AuNPs was determined by UV-vis spectroscopy. rom a Langmuir Isotherm fitting of the observed bioconjugation curves, binding constants were obtained for AuNP-MUA conjugation with BSA ($1.5 \times 10^{-2} \pm 0.1$), or Fib ($34.3 \times 10^{-2} \pm 1.2$); and for AuNP-CALNN conjugation with Fib ($51.2 \times 10^{-2} \pm 4.7$). AuNP-CALNN conjugation with BSA lead to band smearing on AGE, not allowing an evaluation of the binding process. Overnight competitive incubation of a protein mixture with AuNP-MUA, favoured Fib adsorption over BSA.
Discussion and conclusions: Electrostatic protein conjugates show a robust structure in AGE runs, with generally low band dispersion, especially for AuNP-MUA conjugates. Video analysis revealed protein corona stabilization and a constant band migration velocity. Binding affinity constants and competitive behaviour confirmed a higher affinity for Fib conjugation over BSA to the functionalized AuNPs. This higher binding affinity for Fib can be explained by its higher surface contact area and a possible cooperativity mechanism, deserving further analysis.

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Use of an innovative system and nanotechnology-based strategy for therapeutic applications of Gla-rich protein (GRP)

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Introduction: Gla-rich protein (GRP) is a vitamin K-dependent protein (VKDP) acting as a calcification inhibitor and anti-inflammatory agent in cardiovascular and articular systems, and THP1 monocyte/macrophage cells [1,2]. Calcification and inflammation processes are known to be involved in the etiology of several calcification-related chronic inflammatory diseases such as atherosclerosis, CKD and osteoarthritis, in a complex bi-directional interplay that drives disease progression. Here, we developed an innovative system to produce human γ-carboxylated GRP (cGRP), and a nanotechnology strategy based on GRP loading into extracellular vesicles (EVs) as a gold standard delivery system for GRP in therapeutic applications.
Materials and methods: Human GRP protein was co-expressed with γ-carboxylase enzyme (GGCX), vitamin K oxidoreductase (GGCX) and furin, in the insect cell baculovirus system in the presence of vitamin K. GRP released in the cell culture media was characterized by mass spectrometry based techniques and Western blot analysis. EVs released by the insect cells overexpressing GRP were isolated by ultracentrifugation, and characterized for GRP content through TEM-immunogold staining, Western blot, ELISA, qPCR. Functional assays using isolated EVs containing GRP were performed in primary vascular smooth muscle cells (VSMCs) and THP1 monocyte/macrophage cells, for anti-mineralizing and anti-inflammatory screening.

Results: GRP released in the cell culture media when co-expressed with GGCX, VKOR and furin in the presence of vitamin K, is processed at the pro-peptide and contain Gla residues. EVs released by the insect cells in this system were shown to be loaded with GRP protein and mRNA, and capable of reducing ECM calcium deposition of calcifying VSMCs and the production of TNFα in THP1 monocyte/macrophage cells stimulated with LPS.
Discussion and conclusions: While the successful production of human cGRP constitutes a major achievement, this innovative methodology will open new opportunities for the production of other biological active VKDPs. Furthermore, EVs loaded with GRP were shown to have anti-mineralizing and anti-inflammatory properties, with promising therapeutic potentialities for calcification-related chronic inflammatory diseases.

KEYWORDS Calcification; inflammation; extracellular vesicles (EVs); therapeutics

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Production of breast cancer cell lines expressing viral gene VIF

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Introduction: Virion Infectivity factor (Vif) is a protein required for lentiviruses successful infection of human target cells, through the degradation of host restriction factors, APOBEC3 [1]. In the last years, the activity of APOBEC3 deaminases has also been associated with mutation patterns found in cancer cells, including breast cancer cells [2]. We hypothesize that Vif protein can be used to inhibit APOBEC3 avoiding breast cancer progression and resistance to chemotherapy. This hypothesis should be tested in breast cancer cells expressing Vif proteins from HIV-1 (Vif1), and HIV-2 (Vif2) treated with chemotherapy agents. The aim of this work was the production of breast cell lines expressing constitutively Vif1 and Vif 2 proteins, using lentiviruses as a gene-delivering system.
Materials and methods: Vif1 and Vif2 delivering lentivirus vectors were obtained using the fourth generation lentiviral packaging systems (Clontech). Briefly, Vif 1 and Vif 2 genes were cloned in a bicistronic lentiviral expression vector (pLVX-IRES-zsGreen1) which allowed the co-expression of Vif and the reporter gene (ZsGreen) from a single mRNA transcript. VSV-G pseudotyped lentiviruses were produced by transfection of packaging cells, Lenti-X 293T cells (Clontech). Viruses were then used to transduce MCF10A and HCC1806 breast cells. Transduced cells stably expressing fluorescent marker were isolated by Fluorescence Activated Cell Sorting (FACS-AriaIII). The isolated cells were expanded and characterized. RNA was extracted from the cell lines MCF10A-Vif and HCC1806-Vif, and cDNA synthesis was performed. Expression of Vif was confirmed by PCR amplification of cDNA and APOBEC3G, 3B and 3C expression levels were also confirmed by qRT-PCR (TaqMan Technology).
Results: We obtained high-titer of Vif delivering lentiviruses used to transduce the breast cells. FACS allowed us to obtain sorting populations with highly purified transduced cells (sorting populations with 97 to 99% purity). Characterization of the new cell populations showed that lentiviral constructs integrated either Vif1 or Vif2 genes into genomic DNA. Moreover, Vif genes are expressed in these populations with no significant effect on the mRNA levels of APOBEC3G, 3C, and 3B (~1.5 to 2 fold increases).
Discussion and conclusions: The genomic DNA of MCF10A (normal breast cell line) and HCC1806 (breast cancer cell line) were modified with Vif gene (and ZsGreen gene) integration generating new stable breast cell lines. In the near