CD.42 - Identification of novel class of VEGF inhibitors using virtual screening

Erika Piccirillo[1], Lilian C. Alecrim[1], Antonia Tavares do Amaral[2], Ricardo Jóse Giordano[1]
[1]Bioquímica, Instituto de Química, Universidade de São Paulo (Sao Paulo, Brasil), [2]Química Fundamental, Instituto de Química, Universidade de São Paulo (Sao Paulo, Brasil)

Inhibition of angiogenesis, the formation of new blood vessels from pre-existing ones, is a reality and an important therapeutic option for patients suffering from oncological and ocular diseases. Most angiogenesis inhibitors target the VEGF pathway, the main factor responsible for initiating and maintaining the neovascularization process. Although effective, there are challenges to anti-VEGF therapy, such as side effects and drug resistance. Our group has shown that small molecules targeting the VEGF receptors might be an important alternative for a novel class of VEGF inhibitors (Michaloski, et al., Sci Adv, 2016). Here, we show that small organic compound mimetic of this peptide identified by virtual screening (VS) inhibit angiogenesis and might be an important drug lead for the development of novel angiogenesis inhibitors. Crystal structure of VEGFR-1 complex with VEGF (PDB 1FLT) was used to dock a pre-filtered subset of the ZINC database ($7.8x10^{6}$ molecules) with FRED (v. 3.3.03, OpenEye Scientific). Docking poses with good fit were further minimizaded and visually inspected using VIDA (v. 4.1.1, OpenEye Scientific). Most promising compounds were purchased, and their anti-VEGF activity evaluated using *in vitro* and *in vivo* angiogenesis assays (VEGF induced-cell proliferation/migration; aorta-ring and oxygen-induced retinopathy — OIR). The VS campaign suggested 29 possible VEGF inhibitors. Three of them were purchased and tested as anti-VEGF inhibitors. One compound (V2) selectively inhibits the VEGF-induced proliferation of endothelial cells over epithelial ones. V2 also inhibits two tumor cells responsive to VEGF (CAKI-1 and U87). We also analyzed V2 using two angiogenesis models: aorta-ring and OIR. V2 inhibits the neovascularization in both models. Finally, V2 showed low acute toxicity in mice. Altogether, these results suggested that V2 might be an important drug lead for the development of novel anti-VEGF inhibitors.

Keywords: Angiogenesis, Drug Discovery, Virtual Screening
Supported by: FAPESP (2018/24678-0), CNPq, CAPES, CEPID-REDOXOMA

CD.43 - Optimization of Lipid Nanoparticle Formulations for DNA delivery in cardiomyocytes

Sérgio Scalzo[1], Anderson K. Santos[1], Heloísa Athaydes[1], Pedro A. Costa[2], Pedro H. D. M. Prazeres[2], Lays C. Guimaraes[1], Mário de Morais e Silva[1], Marco T. R. Alves[1], Celso T. R. Viana[1], Alice Pereira Rodrigues[1], Frederic Frezard[1], Silvia Guatimosim[1], Pedro Guimaraes[1]
[1]Department of Physiology and Biophysics, [2]Department of General Pathology, Institute of Biological Sciences, Universidade Federal de Minas Gerais (Minas Gerais, Brasil)

Gene therapy is a promising approach to be applied in cardiac regeneration after myocardial infarction and gene correction for inherited cardiomyopathies. One of the greatest challenges faced is the gene delivery vector. Here, we developed a library of lipid nanoparticles (LNPs) containing plasmid DNA (pDNA) for enhanced transfection efficiency in cardiomyocytes. Identify an optimized LNP formulation to enhance gene expression in cardiomyocytes *in vitro* and *in vivo*. pDNA encoding GFP was encapsulated in LNPs consisting of varying lipid molar ratios via rapid microfluidic mixing. LNPs were characterized using DLS, zeta potential and cryo-TEM. pKa of LNPs was assessed via fluorescent reagent 6-(ptoluidinyl) naphthalene-2-sulfonic acid. pDNA concentration was determined using a NanoDrop and encapsulation efficiency was obtained through Qubit dsDNA HS Assay Kit. Primary culture of cardiomyocytes was treated with LNPs at pDNA dosages of 0.00625-0.8µg *in vitro*. C57/BL6 mice were treated via tail vein injection with LNPs at a dose of 10 µg total of pDNA to determine in vivo GFP expression. Cell viability was assessed by resazurin reduction method. GFP fluorescence was carried out in confocal and Cytation 5. 90-120nm LNPs containing pDNA were formulated via microfluidic mixing. Encapsulation efficiency varied from 71% to 94%. Lead LNP induced higher than 60% transfection efficiency after 24h and 80% after 48h, which remained until day 8. LNPs formulated with higher DOPE and lower cholesterol molar ratio exhibited enhanced GFP expression in cardiomyocytes. In addition, LNP with pKa closer to endosomal pH has shown higher GFP expression and cellular uptake, without cell toxicity. *In vivo*, lead LNP was able to induce significant gene expression in heart tissue 7 days after intravenous injection, with negligible toxicity. Collectively, the use of our LNPs holds promise to improve pDNA delivery and transfection efficiency for the treatment of cardiovascular diseases.

Keywords: Lipid nanoparticle, DNA delivery, Heart, **Supported by:** CNPq, CAPES and FAPEMIG