Recent Studies & Advances in Breast Cancer

Chapter 2

Magic Sized Quantum Dots as a Theranostic Tool for Breast Cancer

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Abstract

The discovery of quantum dots has sparked the research on biological imaging with many *in vitro* and *in vivo* applications, due to their incredible photostability and greater luminescence. Recently, the new magic sized quantum dots (MSQD) have surpassed most of the problems related to labeling, especially because of several important characteristics: the ultra-small size (<2 nm) that allows passive absorption into cells, the variable functional surface engineering that permits conjugation to different molecules for selectivity improvement, the highly biocompatible nature due to its colloidal synthesis, the luminescence tuning capability in different wave lengths, and the low complexity of the system, which have led us to a new level of bioimaging. In this review, we demonstrate the potential applications of MSQD conjugated to three novel molecules as theranostic tools for both diagnosis and treatment of triple negative breast cancer.

Keywords: Magic Sized Quantum Dots; Bioconjugation; Biocompatibility; Specific probe; Breast Cancer; PhospolipasesA2; Pepstatin A.

1. Introduction

Quantum dots have revolutionized biological imaging, but an important pitfall preventing its use for *in vivo* imaging and as therapeutic tools is their cytotoxicity effects. Recently, a new class of ultra-small quantum dots with very high stability and low toxicity has been developed by our group, which has been successfully exploited as a theranostic tool. This chapter presents the proof-of-concept with novel applications of MSQD in breast cancer diagnostics and therapeutics, which will be explored here in.

1.1. Quantum dots as specific probes

In the area of biological labeling, the great applicability of the quantum dots (QDs) occurs because they present several advantages over the traditional organic fluorophores, such as, long fluorescence life, high photo-resistance and chroma-degradation [1,2]. However, its cytotoxicity is a highly studied subject because these QDs show high cytotoxicity.

Magic sized quantum dots (MSQDs) are a category of quantum dots that have extremely small sizes (≤ 2 nm), stability of size and luminescence in function of time, present a broad emission spectrum, that allows their detection in the most diverse channels in the fluorescence microscopes [3]. In addition to these several advantages over traditional quantum dots, we have demonstrated that is possible control their biocompatibility and specificity during the synthesis [4]. Therefore, the use of these MSQDs is extremely important in monitoring applications of biological assays as a function of time.

The dispersion of QDs in biological fluids and conjugation with biological molecules consists of coating the surface of the QDs molecules containing specific chemical groups aiming at their use as luminescent probes. In our recent work, we performed functionalization process in which we demonstrated the formation of the CdS shell around CdSe QDs as a function of the synthesis temperature [3], and the concentration of stabilizer with external thiol group [5]. These methodologies were developed to increase the luminescence and biocompatibility of the QDs, since we will show that the cytotoxicity is related to the amount of Cd2$^+$ ions adsorbed on the surface of the CdSe QDs.

The use of QDs as specific probes in biological and biomedical applications is important because is possible to attach on the your surface a variety of biomolecules, including nucleic acids, proteins (avidin/streptavidin, albumin and antibodies), polysaccharides, and peptides. Several methods of bioconjugation have been used; however, the characteristics of biological molecules must be taking into consideration for specific coupling strategies. Biomolecules are generally conjugated on QDs' surface by the following bioconjugation methodologies: 1) cross-linked covalent bond: bonds the carboxyl groups on the surface of the PQs to the amine moieties present in the biomolecule using 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide

(EDC) [6] or N-hydroxysulfosuccinimide (NHS) [7]; 2) adsorption or non-covalent self-as-sembly, using protein engineering (polyhistidine(His) [8-11]. Further more, due to the high ratio surface volume is possible to couple several different kinds of biomolecules in a single QD where each has a specific function that ensures the multifunctionality of QDs [12].

1.2. Carcinogenesis and Cancer

The functioning of the human body can be considered as a society, whose members are cells, which reproduce themselves through cellular divisions and are organized in sets (tissues) that collaborate with each other. Thus, to coordinate this behavior the cells send, receive, and interpret a sophisticated set of signals. Each cell behaves in a way, being able to divide, dif-ferentiate or die. Any molecular change that disrupts this harmonious behavior can result in problems for the body. In the human body, many cells constantly mutate, and sometimes such a mutation can result in a certain selective advantage to a cell, allowing it to grow and divide more vigorously and survive more easily than other cells, becoming the founder of a mutant clone that grows out of the normal context [13].

Carcinogenesis is a complex process, in which normal cells progress to cells with neo-plastic phenotypes. This is a multi step process describes like somatic evolution conducted by mutations and alterations of DNA. The tumor cells confers interactions where cell-cell contact is lost, followed by inadequate growth, hypoxia, ischemia, senescence, resistance to apoptosis, and self-sustaining growth. Innumerable mechanisms for invasion and metastasis have been shown elsewhere [14,15].

1.2.1. Breast Cancer

Breast cancer is generally a malignant tumor that has developed from cells in the breast. This cancer is genetically and clinically heterogeneous and is always caused by genetic muta-tions on the DNA: although only 5-10% of these mutations are hereditary, the majority (90-95%) is caused by abnormalities originating of lifestyle and environmental factors [16-19].

In this context, breast cancer classification systems have been developed to organize this heterogeneity. The advances in genomics have led to elucidation of the subtypes of breast cancer based on the molecular profile of three markers: estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor type 2 (HER2). This classification is critical for choosing the appropriate treatment of patients, and for the development of new therapeutic strategies [17,20,21].

The most aggressive subtype of breast cancer, called triple negative breast cancer (TNBC) is characterized by lack of the three markers expression: ER, PR and HER2. As a consequence, TNBC cells are insensitive to hormone or HER2-targeted therapies and the

treatment options for these patients is chemotherapy, surgery and radiation therapy. Thus, the investigation of TNBC-targeted therapy is imperative and the cell culture model has become a useful tool for understanding the cancer functionality and provides interesting information about the biology of the disease becoming a good candidate for development of specific targeted therapy. Here, we investigated the efficacy of Pepstatin A and Phospholipases BnSP-6 and BThtX-II as a TNBC treatment.

1.2.1.1. Pepstatin A

Pepstatin A is an aspartic protease inhibitor that acts specifically in the inhibition of Cathepsin D catalytic activity in triple negative breast cancer [22]. Cathepsin D is a protease that is currently investigated as a biomarker among triple negative breast cancers. In addition, Cathepsin D inhibition is essential to reduce breast cancer cells aggressiveness, since it is involved in proteolytic events responsible for breast cancer progression and metastasis [23].

Evidences suggest that Pepstatin A induced apoptosis and autophagy processes in triple negative breast cancer cell, while reducing proliferation, migration and invasion [24]. Cathepsin D inhibition is also crucial to increase sensitivity of breast cancer cells to chemotherapy, because it may protect cancer cells from chemotherapeutic agents [25]. So, it is important to investigate the bioconjugation of Pepstatin A with MSQD to allow its tracking within the cell and, in addition, to verify the specificity of this treatment for triple negative breast cancer cells.

1.2.1.2. Snake venom phospolipase A2

Snake venoms constitute a mixture of bioactive components that are involved not only in envenomation pathophysiology but also in the development of new drugs. Different enzymatic and non-enzymatic proteins such as phospholipases A2 (PLA_2) are present in the venom composition, with PLA_2 being responsible for specifically catalyzing the hydrolysis of the sn-2 acyl groups of membrane phospholipids to release arachidonic acid and lysophospholipids [26,27]. The replacement of the amino acid aspartate by a lysine in position 49 provokes loss of enzymatic activity of these PLA_2, and therefore these variants have been referred to as Lys49 PLA_2 homologues capable of disrupting the integrity of membranes and provoking many pharmacological effects [28-31]. These proteins have been studied for a long time and this last decade many studies have demonstrated their therapeutic potential as promise models for therapeutic agents design, since numerous studies have demonstrated the action microbicides, antitumor, antiplatelet and antiangiogenesis activities, although many advances have been made in cancer therapy, the search for new drugs from natural resources is one important topic of biomedical research [32-34].

1.2.1.2.1. BnSP-6 – Lys49 PLA$_2$ from *Bothrops pauloensis*

PLA2-BnSP-6 has a molecular mass of 13,420 Da, with 122 amino acid residues in its-dimeric form. It has a high content of basic and hydrophobic amino acids and isoelectric point (pI)of 8.6. This phospholipase also has no phospholipase or coagulant activity [35,36].

In this context, recently our group published a work showing the in vitro antitumor effect of BnSP-6, a Lys 49 PLA$_2$ isolated from Bothropspauloensis venom on human breast cancer MDA-MB-231 cells [34]. In this work, we demonstrated that BnSP-6 caused a dose-dependent cytotoxicity and inhibited cell adhesion of human breast cancer MDA-MB-231 cells. Interestingly, cytotoxic activity of BnSP-6 was significantly lower against MCF10A, a non-tumorigenic breast cell line. BnSP-6 stimulated the autophagy process and induced both early and late apoptosis. Apoptosis of MDA-MB-231 cells were also confirmed by up-regulation of different genes related to the apoptosis pathway, such as TNF, TNFRSF10B, TNFRSF1A and CASP8 and decreased expression of anti-apoptotic genes (BCL2 and BCL2L). In addition, BnSP-6 caused a remarkable increase in gene expression of BRCA2 and TP53 tumor suppressors. Finally, BnSP-6 induced down-regulation of Angiopoietin 1 gene, a potent pro-angiogenic factor, and inhibited adhesion and migration of MDA-MB-231 cells, suggesting pharmaceutical applications of this PLA$_2$ as an anti-angiogenic and anti-metastatic agent [34].

1.2.1.2.2   BthTX-II- Phospolipase A2-Asp49 of *Bothrops jararacussu*

Several constituents of Bothropsjararacussu venom have already been isolated, and biochemically and functionally characterized. Among them, two PLA$_2$s named Bothropstoxin I (BthTx-I) and Bothropstoxin II (BthTx-II) [37] have been intensively explored. The BthTx-II is a basic Asp49 phospholipase A2 with 13,976 Da and 120 amino acid residues [38-40]. This toxin has several biological effects, including myotoxicity, edematogenic activity, low phospholipase activity, induces platelet aggregation and presents hypotensive activity [39-41] PLA2 BthTx-I has already been explored for its antitumor potential by our research group [42]. We are currently investigating its action on breast cancer cells.

2. Results and Discussions

2.1. Pepstatin A

CdSe/CdS MSQDs were incubated with MCF-10A and MDA-MB-231. The internalization of MSQDs was visualized under a confocal fluorescence microscopy.
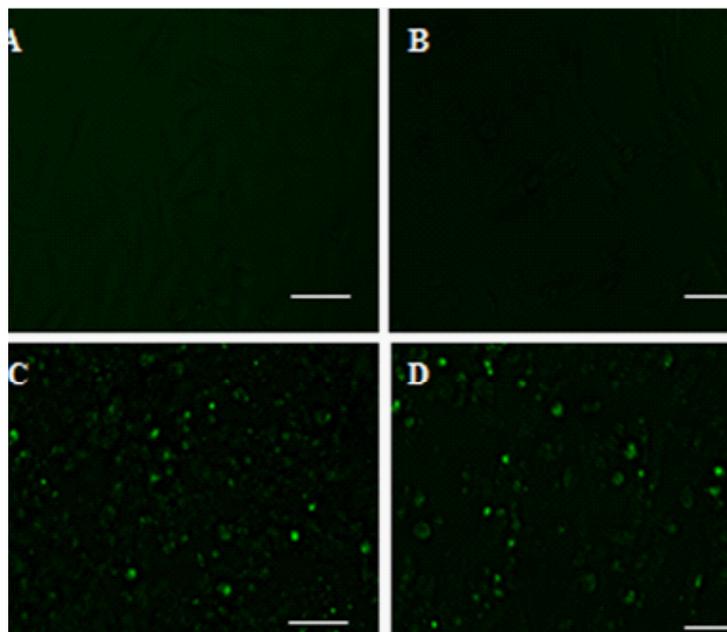
Figure 1: Pepstatin A effect in breast cells. A) MCF-10 A normal breast cells with quantum dots; B) MDA-MB-231 triple negative breast cancer cells treated only with MSQDs; C) MDA-MB-231 triple negative breast cancer cells treated with 1μm of MSQDs bioconjugated with Pepstatin A. D) MDA-MB-231 triple negative breast cancer cells treated with 10μM of MSQDs bioconjugated with Pepstatin A. The bar corresponds to 200 μm.

In the **Figure 1** observed a green fluorescence of the MSQDs bioconjugated to Pepstatin A in two doses (1μm. - **Figure 1C**, and 10μm - **Figure 1D**) was detected inside the cytosolic compartment of MDA-MB-231 cells in 6h after treatment. Notably, we verified that CdSe/CdS MSQDs alone is not internalized both in MCF-10A (**Figure 1A**) and MDA-MB-231 (**Figure 2B**).

Our present study suggests that Pepstatin A bioconjugated with MSQDs could be a novel and specific treatment for triple negative breast cancer since it was able to penetrate specifically at MDA-MB-231 in a short period (6 hours). However, for clinical use, we suggestan additional investigation with *in vivo* models to better understand the pharmacokinetics, toxicity, tissue distribution and MSQD elimination.

2.2 Phospholipases

2.2.1 BnSP-6
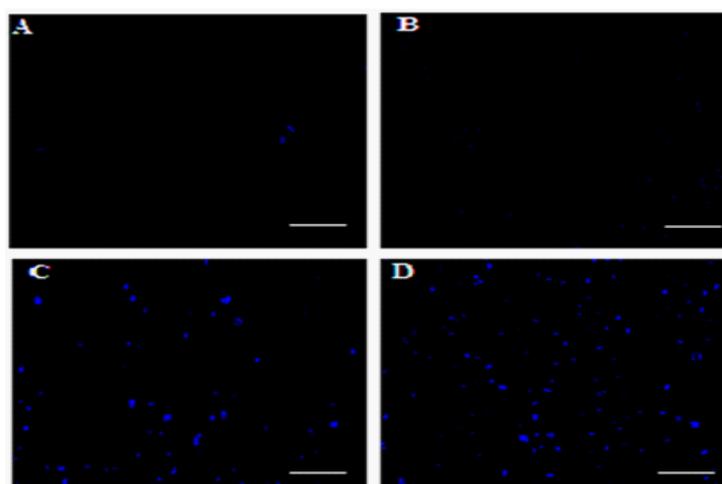
Figure 2: BnSP-6 effect in breast cells. A) MCF-10 A normal breast cells with quantum dots; B) MDA-MB-231 triple negative breast cancer cells treated only with MSQDs; C) MDA-MB-231 triple negative breast cancer cells treated with 1μg/ml of MSQDs bioconjugated with BnSP-6. D) MDA-MB-231 triple negative breast cancer cells treated with 50μg/ml of MSQDs bioconjugated with BnSP-6. The bar corresponds to 200 μm..

As shown in Figure 2, the blue fluorescence of the MSQDs bioconjugated with BnSP-6 in two doses (1μg/ml - Figure 2C,and 50μg/ml - Figure 2D) was detected inside the cytosolic compartment of MDA-MB-231 cells after3h treatment. Notably, we verified that MSQDs alone is not internalized both in MCF-10A (Figure 1A) and MDA-MB-231 (Figure 1B).

Our present study suggests that BnSP-6 coupled to the MSQD could be a recent and specific treatment for triple negative breast cancer since it was able to penetrate specifically at MDA-MB-231immediately after treatment. However, more studies must show itsclinical use. We also suggestin vivo models for additional investigation todemonstrate its pharmacokinetics, toxicity, tissue distribution and MSQD elimination.

2.2.2. BThtX-II
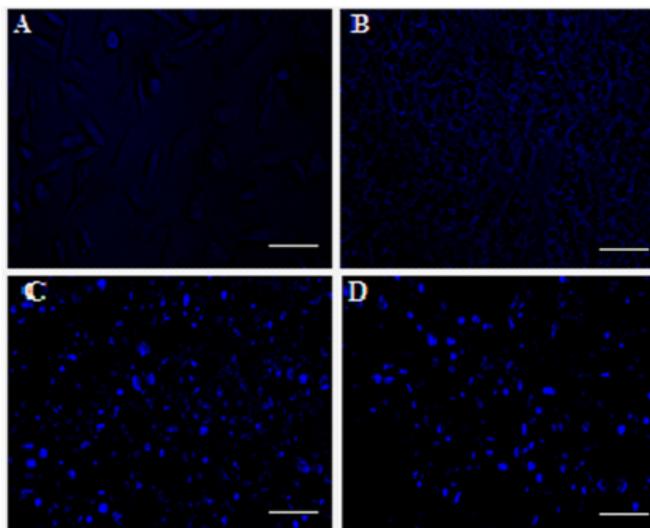
Figure 3: BThtX-II effect in breast cells. A) MCF-10 A normal breast cells with quantum dots; B) MDA-MB-231 triple negative breast cancer cells treated only with MSQDs; C) MDA-MB-231 triple negative breast cancer cells treated with 1μg/ml of MSQDs bioconjugated with BThtX-II. D) MDA-MB-231 triple negative breast cancer cells treated with 50μg/ml of MSQDs bioconjugated with BThtX-II. The bar corresponds to 200 μm..

Similarly to the BnSP-6 effect shown in **Figure 2**, the blue fluorescence from MSQD bioconjugated to BthTx-II in two doses (1μg/ml - **Figure 3C**,and 50μg/ml - **Figure 3D**) was detected inside the cells'nucleicompartment of MDA-MB-231 cells 3h after treatment. Notably, we verified that the MSQD alone was not internalized in both MCF-10A (**Figure 2A**) and MDA-MB-231 (**Figure 2B**).

Our present study suggests that BthTX-II bioconjugation to the MSQD could be a noveland specific treatment for triple negative breast cancer, since it was able to specifically penetrate MDA-MB-231 cells immediately after treatment. However, new studies are under way in order to demonstrate the PLA2 applications as promising drug against triple negative breast cancer.

3. Conclusion

In conclusion, our review has successfully demonstrated the exploitation of possible therapies for triple negative breast cancers with unexplored theranostic tools, specifically by tracking three major tumor targets coupled with CdSe/CdS MSQDs in tumor cells, Pepstatin A, BnSP-6 and BthTx-II, which may become potential therapeutic strategies in the near future.

4. Acknowledgments

5. References

1. Deerinck TJ. The application of fluorescent quantum dots to confocal, multiphoton, and electron microscopic imaging. Toxicol Pathol. 2008; 36(1): 112-116.

2. Resch-Genger U, Grabolle M, Cavaliere-Jaricot S, Nitschke R, and Nann T. Quantum dots versus organic dyes as fluorescent labels. Nat Methods. 2008; 5(9): 763-775.

3. Silva ACA, Neto ESF, da Silva SW, Morais PC, and Dantas NO. Modified Phonon Confinement Model and Its Application to CdSe/CdS Core–Shell Magic-Sized Quantum Dots Synthesized in Aqueous Solution by a New Route. The Journal of Physical Chemistry C. 2013; 117(4): 1904-1914.

4. Silva AC, da Silva SW, Morais PC, and Dantas NO. Shell thickness modulation in ultrasmall CdSe/CdS(x)Se(1-x)/CdS core/shell quantum dots via 1-thioglycerol. ACS Nano. 2014; 8(2): 1913-1922.

5. Silva AC, Silva MJ, da Luz FA, Silva DP, de Deus SL, and Dantas NO. Controlling the cytotoxicity of CdSe magic-sized quantum dots as a function of surface defect density. Nano Lett. 2014; 14(9): 5452-5457.

6. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, Ge N, Peale F, and Bruchez MP. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol. 2003; 21(1): 41-46.

7. Han H-y, Sheng Z-h, and Liang J-g. A novel method for the preparation of water-soluble and small-size CdSe quantum dots. Materials Letters. 2006; 60(29): 3782-3785.

8. Hainfeld JF, Liu W, Halsey CM, Freimuth P, and Powell RD. Ni-NTA-gold clusters target His-tagged proteins. J Struct Biol. 1999; 127(2): 185-198.

9. Goldman ER, Anderson GP, Tran PT, Mattoussi H, Charles PT, and Mauro JM. Conjugation of Luminescent Quantum Dots with Antibodies Using an Engineered Adaptor Protein To Provide New Reagents for Fluoroimmuno assays. Analytical Chemistry. 2002; 74(4): 841-847.

10. Mattoussi H, Mauro JM, Goldman ER, Green TM, Anderson GP, Sundar VC, and Bawendi MG. Bioconjugation of Highly Luminescent Colloidal CdSe–ZnS Quantum Dots with an Engineered Two-Domain Recombinant Protein. Physica Status Solidi (b). 2001; 224(1): 277-283.

11. Mattoussi H, Mauro JM, Goldman ER, Anderson GP, Sundar VC, Mikulec FV, and Bawendi MG. Self-Assembly of

CdSe−ZnS Quantum Dot Bioconjugates Using an Engineered Recombinant Protein. Journal of the American Chemical Society. 2000; 122(49): 12142-12150.

12. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, and Weiss S. Quantum Dots for Live Cells, in Vivo Imaging, and Diagnostics. Science. 2005; 307(5709): 538-544.

13. Alberts BJ A; Lewis J; Ralf M; Roberts K; Walter P. Biologia Molecular da Céula. Artmed 2010.

14. Karin M, and Greten FR. NF-kappa B: linking inflammation and immunity to cancer development and progression. Nature Reviews Immunology. 2005; 5(10): 749-759.

15. Vendramini-Costa DB, and Carvalho JE. Molecular link mechanisms between inflammation and cancer. Current Pharmaceutical Design. 2012; 18(26): 3831-3852.

16. INCa. Instituto Nacional do Câncer. Tipos de Câncer. http://www2.inca.gov.br/wps/wcm/connect/tiposdecancer/ site/home. Accessed May, 2015.

17. Correa Geyer F, and Reis-Filho JS. Microarray-based gene expression profiling as a clinical tool for breast cancer management: are we there yet? International Journal of Surgical Pathology. 2009; 17(4): 285-302.

18. Badve S, Dabbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, et al. Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Modern pathology : an Official Journal of the United States and Canadian Academy of Pathology, Inc. 2011; 24(2): 157-167.

19. Pierobon M, and Frankenfeld CL. Obesity as a risk factor for triple-negative breast cancers: a systematic review and meta-analysis. Breast Cancer Research and Treatment. 2013; 137(1): 307-314.

20. Weigelt B, Peterse JL, and van 't Veer LJ. Breast cancer metastasis: markers and models. Nature Reviews Cancer. 2005; 5(8): 591-602.

21. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, and Ellis IO. Prognostic markers in triple-negative breast cancer. Cancer. 2007; 109(1): 25-32.

22. Beaujouin M, and Liaudet-Coopman E. Cathepsin D over expressed by cancer cells can enhance apoptosis-dependent chemo-sensitivity independently of its catalytic activity. Advances in Experimental Medicine and Biology. 2008; 617(453-61.

23. Anantaraju HS, Battu MB, Viswanadha S, Sriram D, and Yogeeswari P. Cathepsin D inhibitors as potential therapeutics for breast cancer treatment: Molecular docking and bioevaluation against triple-negative and triple-positive breast cancers. Molecular Diversity. 2016; 20(2): 521-535.

24. Zóia MAP, Azevedo FVPV, Rodovalho VR, Cordeiro AO, Mota STS, Silva ACA, Dantas NO, and Goulart LR. Annexin A1 cleavage by Cathepsin D is a critical mechanism in triple negative breast cancer. Manuscript under submission. 2017.

25. Huang L, Liu Z, Chen S, Liu Y, and Shao Z. A prognostic model for triple-negative breast cancer patients based on node status, cathepsin-D and Ki-67 index. PloS one. 2013; 8(12): e83081.

26. Arni RK, Ward RJ. Phospholipase A2—a structural review. Toxicon. 1996; 34(8):827-841.

27. Kini RM. Excitement ahead: structure, function and mechanism of snake venom phospholipase A2 enzymes. Toxicon. 2003; 42(8): 827-840.

28. Diaz C, Gutierrez JM, Lomonte B, and Gene JA. The effect of myotoxins isolated from Bothrops snake venoms on multilamellar liposomes: relationship to phospholipase A2, anticoagulant and myotoxic activities. Biochim Biophys Acta. 1991; 1070(2): 455-460.

29. Rufini S, Cesaroni P, Desideri A, Farias R, Gubensek F, Gutierrez JM, Luly P, Massoud R, Morero R, and Pedersen JZ. Calcium ion independent membrane leakage induced by phospholipase-like myotoxins. Biochemistry. 1992; 31(49): 12424-12430.

30. Lomonte B, Angulo Y, and Santamaria C. Comparative study of synthetic peptides corresponding to region 115-129 in Lys49 myotoxic phospholipases A2 from snake venoms. Toxicon. 2003; 42(3): 307-312.

31. Lomonte B, and Rangel J. Snake venom Lys49 myotoxins: From phospholipases A(2) to non-enzymatic membrane disruptors. Toxicon. 2012; 60(4): 520-530.

32. Calderon LA, Sobrinho JC, Zaqueo KD, de Moura AA, Grabner AN, Mazzi MV, Marcussi S, Nomizo A, Fernandes CF, Zuliani JP, et al. Antitumoral activity of snake venom proteins: new trends in cancer therapy. Biomed Res Int. 2014; (203639.

33. Rodrigues VM, Lopes DS, Castanheira LE, Gimenes SN, Naves de Souza DL, Ache DC, Borges IP, Yoneyama KA, and Rodrigues RS. Bothrops pauloensis snake venom toxins: the search for new therapeutic models. Curr Top Med Chem. 2015; 15(7): 670-684.

34. Azevedo FV, Lopes DS, Cirilo Gimenes SN, Ache DC, Vecchi L, Alves PT, Guimaraes Dde O, Rodrigues RS, Goulart LR, Rodrigues Vde M, et al. Human breast cancer cell death induced by BnSP-6, a Lys-49 PLA(2) homologue from Bothrops pauloensis venom. International Journal of Biological Macromolecules. 2016; 82 (671-7.

35. Rodrigues VM, Soares AM, Mancin AC, Fontes MR, Homsi-Brandeburgo MI, and Giglio JR. Geographic variations in the composition of myotoxins from Bothrops neuwiedi snake venoms: biochemical characterization and biological activity. Comparative biochemistry and physiology Part A, Molecular & integrative physiology. 1998; 121(3): 215-222.

36. Magro AJ, Soares AM, Giglio JR, and Fontes MR. Crystal structures of BnSP-7 and BnSP-6, two Lys49-phospholipases A(2): quaternary structure and inhibition mechanism insights. Biochemical and Biophysical Research Communications. 2003; 311(3): 713-720.

37. Homsi-Brandeburgo MI, Queiroz LS, Santo-Neto H, Rodrigues-Simioni L, and Giglio JR. Fractionation of Bothrops jararacussu snake venom: partial chemical characterization and biological activity of bothropstoxin. Toxicon. 1988; 26(7): 615-627.

38. Correa LC, Marchi-Salvador DP, Cintra AC, Soares AM, and Fontes MR. Preliminary X-ray crystallographic studies of BthTX-II, a myotoxic Asp49-phospholipase A2 with low catalytic activity from Bothrops jararacussu venom. Acta crystallographica Section F, Structural Biology and Crystallization Communications. 2006; 62(Pt 8): 765-767.

39. Gutierrez JM, Nunez J, Diaz C, Cintra AC, Homsi-Brandeburgo MI, and Giglio JR. Skeletal muscle degeneration and regeneration after injection of bothropstoxin-II, a phospholipase A2 isolated from the venom of the snake Bothrops jararacussu. Experimental and Molecular Pathology. 1991; 55(3): 217-229.

40. Murakami MT, Lourenzoni MR, Arruda EZ, Tomaz MA, Vicoti MM, Abrego JR, Melo PA, and Arni RK. Biochemical and structural investigations of Bothropstoxin-II, a myotoxic Asp49 phospholipase A2 from Bothrops jararacussu venom. Protein and Peptide Letters. 2008; 15(9): 1002-1008.

41. Andriao-Escarso SH, Soares AM, Fontes MR, Fuly AL, Correa FM, Rosa JC, Greene LJ, and Giglio JR. Structural and functional characterization of an acidic platelet aggregation inhibitor and hypotensive phospholipase A(2) from Bothrops jararacussu snake venom. Biochemical Pharmacology. 2002; 64(4): 723-732.

42. Gebrim LC, Marcussi S, Menaldo DL, de Menezes CS, Nomizo A, Hamaguchi A, Silveira-Lacerda EP, Homsi-Brandeburgo MI, Sampaio SV, Soares AM, et al. Antitumor effects of snake venom chemically modified Lys49 phospholipase A2-like BthTX-I and a synthetic peptide derived from its C-terminal region. Biologicals : Journal of the International Association of Biological Standardization. 2009; 37(4): 222-229.