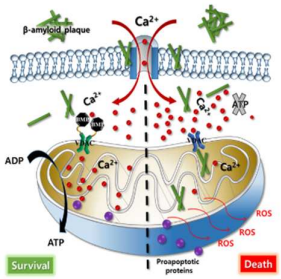
RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

A table of contents entry

The capture of VDAC2 channel with BMPs-VDAC2 antibody complexes significantly decreases the expressed intracellular calcium levels induced by Aβ.



Journal Name

RSCPublishing

COMMUNICATION

Effect of Magnetic Modulation of Mitochondrial Voltage-Dependent Anion Channel 2 against beta-Amyloid induced Neurotoxicity

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Sujin Lee,[a] Junghoon Lee,[a] Sang Jun Sim,[b] and Misun Cha[*b]

Magnetic capture of mitochondrial VDAC2 with bacterial magnetic particles (BMPs) conjugated VDAC2 antibody (BMPs-Ab) was significantly decreasing the expressed intracellular calcium levels and neurotoxicity induced by Aβ. This magnetic modulation of mitochondrial VDAC play key role in various Ca^{2+} flux pathways should provide attractive targets for future development of AD treatments.

Mitochondria are the governors of both cell life (e.g. energy generation) and cell death. Some regulation of both of these functions occurs at the level of the outer membrane in that it controls the flow of metabolites and the release of intermembrane space proteins into the cytosol. The VDAC proteins, pore-forming proteins predominantly found in the outer mitochondrial membrane, are the major pathways for anions, cations, ATP, Ca^{2+} and metabolites flux through the outer membrane. [1, 2] These can be regulated in many ways and the integration of these regulatory inputs allows mitochondrial metabolism to be adjusted to changing cellular conditions. [3, 4]

Ca^{2+} is known to synchronize mitochondrial metabolism as well as intracellular Ca^{2+} signalling is fundamental to neuronal physiology and viability. Therefore Ca^{2+} signalling have become a major focus of study in multifactorial neurodegenerative diseases such as Alzheimer disease (AD). [5, 6] In AD, neurotoxic mechanisms that are associated with β-amyloid (Aβ) include mitochondria dysfunctions cause disturbances in calcium homeostasis. [7] It suggests that control of intracellular calcium flux can prevent or inhibit fatal injury caused by Aβ-induced neurotoxicity.

[a] *School of Mechanical and Aerospace Engineering, Seoul National University, Seoul, 151-744, South Korea.*
[b] *Department of Chemical Engineering and Biological Engineering, Korea University, Seoul, 136-701, South Korea.*
[*] *Address correspondence to: Misun Cha, Fax:+82-2-926-6102; Tel: +82-2-3290-3686; E-mail:cmsbestok@gmail.com*

† Electronic Supplementary Information (ESI) available: Experimental methods and additional proofs. See DOI: 10.1039/c000000x/

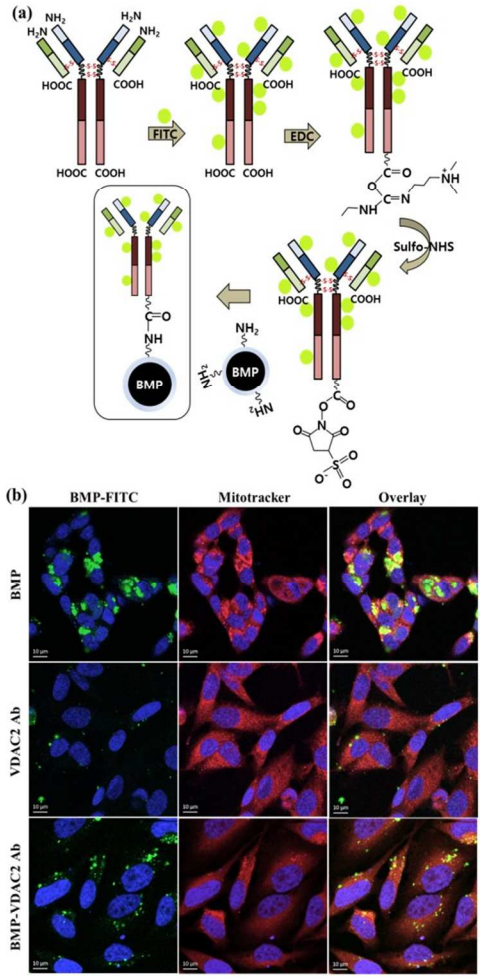


Fig.1 (a) Schematic of the BMP-VDAC2 Ab complex, (b) Specific targeting of BMP-VDAC2 Ab complex to mitochondria inside SH-SY5Y cells; blue: nucleus, red: mitochondria, green: BMP; scale bar: 10 μm.

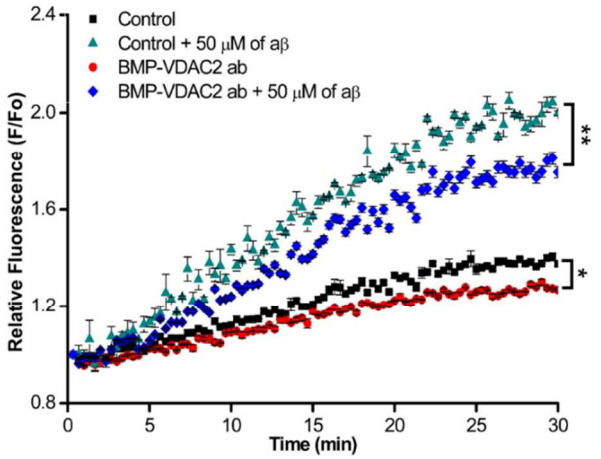


Fig. 2. Effect of BMP-VDAC2 Ab complex on amyloid beta-induced Ca^{2+} influx in the SH-SY5Y cells. Control: VDAC2 untargeted, BMP-VDAC2 ab: VDAC2 targeted cells, Control + 50 μM of aβ: VDAC2 untargeted cells under Aβ treated condition, BMP-VDAC2 ab + 50 μM of aβ: VDAC2 targeted cells with BMPs-Ab under Aβ treated condition. (*p<0.05 and **p<0.01, n=3) Calibration method for fluo-3 fluorescence signals to [Ca^{2+}] is described in supplementary information (materials and methods section and fig. S5). [19]

Here, we demonstrate that the magnetic modulation of mitochondrial VDAC2, which is the only mammalian-specific isoform among VDAC isoforms, can contribute to protect the neurodegenerative disease attenuating the changes in the intracellular calcium levels that were induced by beta-amyloid. In this study, BMPs originated from *Magnetospirillum* sp. AMB-1 directly conjugated with VDAC2 antibody using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) linker which is used to couple carboxyl groups to primary amines (Fig. 1(a)).

BMPs-VDAC2 antibody complexes (BMPs-Ab) introduced into SH-SY5Y cells, human derived neuroblasts which are often used as *in vitro* models of neuronal function and differentiation. As shown in Fig 1(b), most of BMPs-Ab were successfully internalized into SH-ST5Y cells and observed with correlation to mitochondria (yellow color). In contrast, BMPs without VDAC-2 antibody were randomly distributed inside cells, with no particular mitochondrial localization. Because of their many roles in energy production, cell-death regulation, and cell signalling transduction, mitochondria have been considered an important target for therapeutic treatment of various diseases.[8, 9] Thereby, the efficient internalization and specific mitochondrial VDAC2 targeting of the BMP-Ab is promising for mitochondria specific manipulation of cell function.

Aβ, a hallmark of AD, destabilizes intracellular Ca^{2+} homeostasis, resulting in an elevation of intracellular free Ca^{2+} concentration. [6, 10, 11] We investigated that the effect of magnetically modulated VDAC2 on the change of intracellular Ca^{2+} levels induced by Aβ (Fig. 2). SH-SY5Y cells were loaded with 5 μM Fluo-3 AM (Sigma-Aldrich Co., USA) for
30 min, and then the changes in the level of Ca^{2+} before and after treatment with Aβ were measured by 488-nm laser source to excite Fluo-3. Fluorescence intensity reflecting intracellular Ca^{2+} concentration was measured by the Microplate Reader (Victor 3, Perkin Elmer, USA). As shown in Fig. 2, intracellular calcium levels of VDAC2 targeted SH-SY5Y cells with BMPs-Ab moderately decreased compare to those of untargeted cells when the Aβ was not treated.

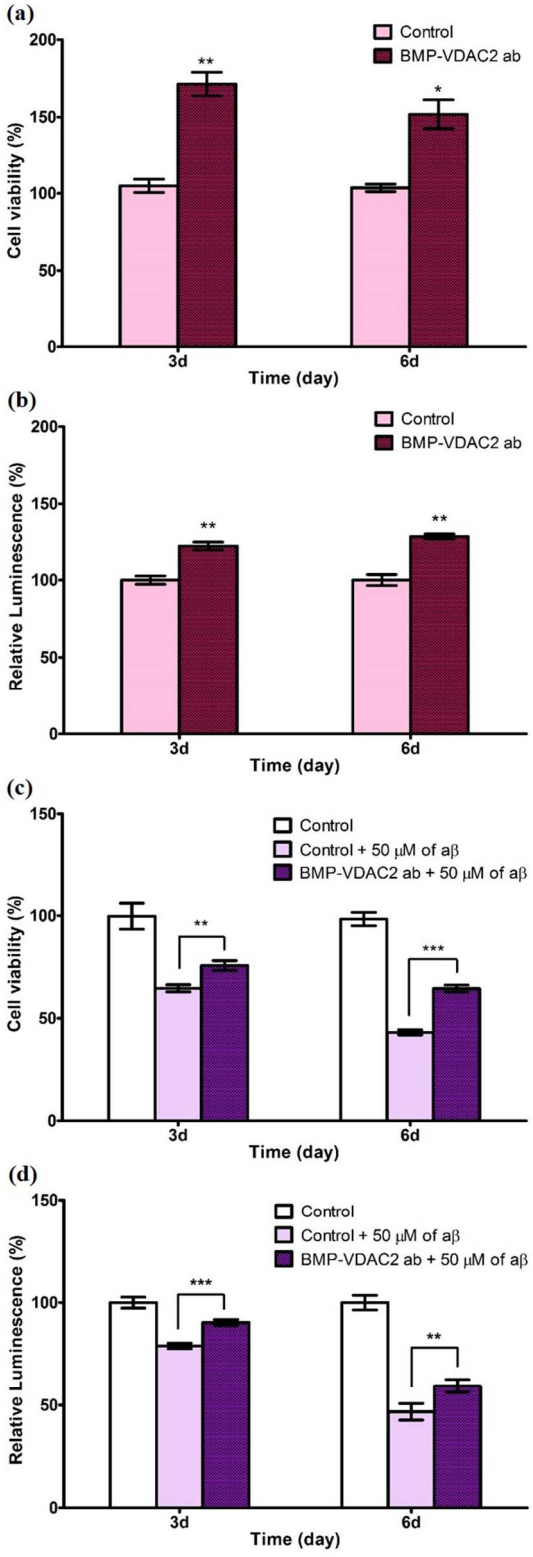


Fig. 3. Effect of BMP-VDAC2 Ab complex on cell growth by (a) MTS assay and (b) ATP level. Under the amyloid-beta induced neurotoxic condition, effect of BMP-VDAC2 Ab complex on cell growth by (c) MTS assay and (d) ATP level. Control: VDAC2 untargeted cells, BMP-VDAC2 ab: VDAC2 targeted cells with BMPs, Control + 50 μM of aβ: amyloid-beta treated VDAC2 untargeted cells, and BMP-VDAC2 ab + 50 μM of aβ: amyloid-beta treated VDAC2 targeted cells. (*p<0.05, **p<0.01, and ***p<0.001, n=4)

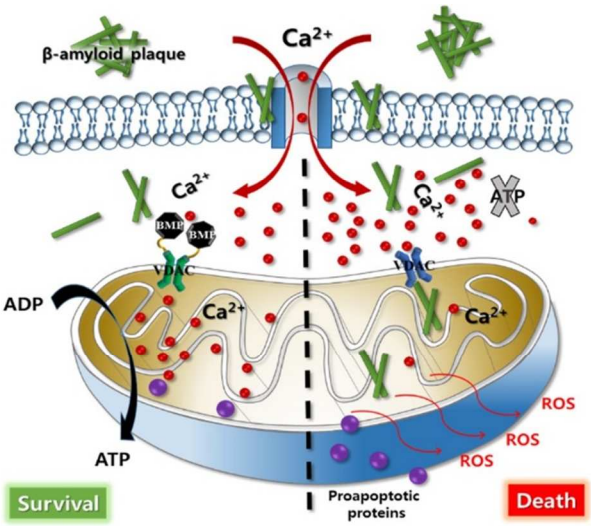


Fig. 4. Schematic map of mitochondrial Ca^{2+} transporters in the amyloid-beta induced toxicity. Aβ results in elevated cytosolic calcium levels and localize to mitochondrial membranes. Aβ in mitochondria inhibits mitochondrial ATP production and other energy-dependent functions, and releases calcium stored in mitochondria, thereby further deregulating neuronal calcium signaling. Finally, a release of proapoptotic proteins from damaged mitochondria results in neuronal injury. However, the magnetic modulation of VDAC2 should block the localization of Aβ to the mitochondria, whereas promote the Ca^{2+} uptake into mitochondria within the threshold. Mitochondria produce ATP and maintain other functions.

After treatment of Aβ, however, capture of VDAC2 with BMPs-Ab was found to more significantly decrease the expressed intracellular calcium levels when compare to those of untargeted cells.

Ca^{2+} is an interesting second messenger which can initiate both cellular life and death pathways in mitochondria. Mitochondria accumulate Ca^{2+} for cellular bioenergetics metabolism and suppression of mitochondrial motility within the cell. Excessive Ca^{2+} uptake into mitochondria often leads to mitochondrial membrane permeabilization and induction of apoptosis.

Interestingly, magnetic modulation of VDAC2 considerably increases the proliferation of SH-ST5Y cells, up to 50% for 3 days culture (Fig. 3(a)). The increased growth can be ascribed to the ATP content of the VDAC2 targeted cells that improved about 30% higher compared with that of VDAC2 untargeted cells (Fig. 3(b)). These results were reconfirmed with the change of cell viability in the presence of Aβ (25–35), which destabilize intracellular Ca^{2+} homeostasis. As shown in Fig. 3(c) and (d), Aβ treatment induced lethal cell death, however, magnetic capture of VDAC2 with BMPs-Ab significantly reduced Aβ-induced toxicity in SH-SY5Y cells.

Ca^{2+} signalling causes transient changes in cytosolic Ca^{2+} concentration. Mitochondria rapidly take up Ca^{2+} when a physiological stimulus elicits an increase in cytosolic Ca^{2+} concentrations. This uptake machinery allows mitochondria to act as "Ca^{2+} buffers" to maintain the normal homeostasis. Aβ destabilize intracellular Ca^{2+} homeostasis as well as localize to mitochondrial membranes, block the transport of nuclear-encoded mitochondrial proteins to mitochondria, interact with mitochondrial proteins, disrupt the electron transport chain, increase reactive oxygen species (ROS) production, cause mitochondrial damage, and eventually induce the neurodegeneration or cell death (Fig. 4 Right).[12-14] The magnetic modulation of VDAC2 should block the localization of Aβ to the mitochondria, whereas promote the Ca^{2+} uptake into mitochondria within the threshold. Consequently, mitochondria should be able to maintain its functions like the ATP production and other energy-dependent function (Fig. 4, Left).

In the previous studies, we reports that magnetic stimulation can lead to changes in a wide range of cellular properties, such as cell shape, cytoskeletal organization, and cell fate. [15, 16] And also, some other groups demonstrated that activation of ion channels is possible by using nanoscale magnetic particles. [17-19] Magnetic modulation of mitochondrial VDAC plays key role in various Ca^{2+} influx and efflux pathways should provide attractive targets for future development of AD treatments.

Conclusions

BMPs-VDAC2 antibody complexes (BMPs-Ab) introduced into SH-SY5Y cells were successfully internalized into SH-SY5Y cells. The capture of VDAC2 with BMPs-Ab was significantly decreasing the expressed intracellular calcium levels induced by Aβ. This magnetic modulation of VDAC2 considerably increases the proliferation and reduced Aβ-induced toxicity in SH-SY5Y. These results suggest that magnetic modulation of VDAC-2 is able to protect the neurodegenerative disease attenuating the changes in the intracellular calcium levels that were induced by Aβ.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A3010079).

Notes and references

1 M. Y. Vyssokikh and D. Brdiczka, *Acta. Biochim. Pol.*, 2003, **50**, 389.
2 D. Gincel, H. Zaid and V. Shoshan-Barmatz, *Biochem. J.*, 2001, **358**, 147.
3 V. Shoshan-Barmatz and D. Gincel, *Cell Biochem. Biophys.,* 2003, **39**, 279.
4 T. K. Rostovtseva, W. Tan and M. Colombini, *J. Bioenerg. Biomembr.*, 2005, **37**, 129.
5 M. J. Berridge, *Neuron*, 1998, **21**, 13.
6 Z. S. Khachaturian, *Neurobiol. Aging*, 1987, **8**, 345.
7 G. Csordás, A. P. Thomas and G. Hajnóczky, *EMBO*, 1999, **18**, 96.
8 T. J. Preston, A. Abadi, L. Wilson and C. Singh, *Adv. Drug Delivery Rev.*, 2001, **49** , 45.
9 L. Galluzzi, O. Kepp, C. Trojel-Hansen and G. Kroemer, *Circ. Res.*, 2012, **111**, 1198.
10 H. L. Pahl, and P. A. Baeuerle, *FEBS Lett.*, 1996, **392**, 129.

11 A. Deshpande, E. Mina, C. Glabe, and J. Busciglio, *J. Neurosci*., 2006, **26**, 6011.
12 L. Devi, B. M. Prabhu, D. F. Galati, N. G. Avadhani, and H. K. Anandatheerthavarada, *Journal of Neuroscience*, 2006, **26**, 9057.
13 P. H. Reddy, *Antioxidants and Redox Signaling*, 2007, **9**, 1647.
14 H. K. Anandatheerthavarada, G. Biswas, M. A. Robin, and N. G. Avadhani, *Journal of Cell Biology*, 2003, **161**, 41.
15 J. Shin, C. Yoo, J. Lee, and M. Cha, *Biomaterials*, 2012, **33(22)**, 5650-5657
16 J. Choi, J. Shin, J. Lee, and M. Cha, *Chem. Commun.* 2012, **48(60)**, 7474–7476.
17 R. J. Mannix, S. Kumar, F. Cassiola, M. Montoya-Zavala, E. Feinstein, M. Prentiss and D. E. Ingber, *Nat. Nanotechnol.*, 2008, **3**, 139.
18 S. Hughes, S. McBain, J. Dobson and A. J. El Haj, *J. R. Soc. Interface*, 2008, **5**, 855.
19. J. E. Merritt et al, *Biochem J.*, 1990, **269(2)**, 513–519.

