Frontiers in Pharmacology, 2017, vol.8, NSEP

Binase Immobilized on halloysite nanotubes exerts enhanced cytotoxicity toward human colon adenocarcinoma cells

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Abstract

© 2017 Khodzhaeva, Makeeva, Ulyanova. Many ribonucleases (RNases) are considered as promising tools for antitumor therapy because of their selective cytotoxicity toward cancer cells. Binase, the RNase from Bacillus pumilus, triggers apoptotic response in cancer cells expressing RAS oncogene which is mutated in a large percentage of prevalent and deadly malignancies including colorectal cancer. The specific antitumor effect of binase toward RAS-transformed cells is due to its direct binding of RAS protein and inhibition of downstream signaling. However, the delivery of proteins to the intestine is complicated by their degradation in the digestive tract and subsequent loss of therapeutic activity. Therefore, the search of new systems for effective delivery of therapeutic proteins is an actual task. This study is aimed to the investigation of antitumor effect of binase immobilized on natural halloysite nanotubes (HNTs). Here, we have developed the method of binase immobilization on HNTs and optimized the conditions for the enzyme loading and release (i); we have found the non-toxic concentration of pure HNTs which allows to distinguish HNTs- and binase-induced cytotoxic effects (ii); using dark-field and fluorescent microscopy we have proved the absorption of binase-loaded HNTs on the cell surface (iii) and demonstrated that binase-halloysite nanoformulations possessed twice enhanced cytotoxicity toward tumor colon cells as compared to the cytotoxicity of binase itself (iv). The enhanced antitumor activity of biocompatible binase-HNTs complex confirms the advisability of its future development for clinical practice.

http://dx.doi.org/10.3389/fphar.2017.00631

Keywords

Bacillus pumilus, Binase, Colon adenocarcinoma, Cytotoxicity, Halloysite, Nanotubes, RNase

References

[1] Ahmed, F., Shoaib, M., Azharc, M., Um, S. H., Yousuf, R. I., Hashmi, S., et al. (2015). In-vitro assessment of cytotoxicity of halloysite nanotubes against HepG2, HCT116 and human peripheral blood lymphocytes. Colloids Surf. B Biointerfaces 135, 50-55. doi: 10.1016/j.colsurfb.2015.07.021

[2] Alshehri, R., Ilyas, A. M., Hasan, A., Arnaout, A., Ahmed, F., and Memic, A. (2016). Carbon nanotubes in biomedical applications: factors, mechanisms, and remedies of toxicity. J. Med. Chem. 59, 8149-8167. doi: 10.1021/acs.jmedchem.5b01770

[3] Ariga, K., McShane, M., Lvov, Y., Ji, Q., and Hill, J. (2011). Layer-by-layer assembly for drug delivery and related applications. Expert Opin. Drug Deliv. 8, 633-644. doi: 10.1517/17425247.2011.566268

[4] Cabrera-Fuentes, H. A., Aslam, M., Saffarzadeh, M., Kolpakov, A., Zelenikhin, P., Preissner, K. T., et al. (2013). Internalization of Bacillus intermedius ribonuclease (BINASE) induces human alveolar adenocarcinoma cell death. Toxicon 69, 219-226. doi: 10.1016/j.toxicon.2013.03.015

[5] Cao, L., Hettiarachchi, G., Briken, V., and Isaacs, L. (2013). Cucurbit[7]uril containers for targeted delivery of oxaliplatin to cancer cells. Angew. Chem. Int. Ed. Engl. 52, 12033-12037. doi: 10.1002/anie.201305061

[6] Davies, C., Lundstrom, L. M., Frengen, J., Eikenes, L., Bruland, S., Kaalhus, O., et al. (2004). Radiation improves the distribution and uptake of liposomal doxorubicin (caelyx) in human osteosarcoma xenografts. Cancer Res. 64, 547-553. doi: 10.1158/0008-5472.CAN-03-0576

[7] Deng, Z. J., Morton, S. W., Ben-Akiva, E., Dreaden, E. C., Shopsowitz, K. E., and Hammond, P. T. (2013). Layer-by-layer nanoparticles for systemic codelivery of an anticancer drug and siRNA for potential triple-negative breast cancer treatment. ACS Nano 26, 9571-9584. doi: 10.1021/nn4047925

[8] Du, M., Guo, B., and Jia, D. (2010). Newly emerging applications of halloysite nanotubes: a review. Polym. Int. 59, 574-588. doi: 10.1002/pi.2754

[9] Dudkina, E., Ulyanova, V., Shah Mahmud, R., Khodzhaeva, V., Dao, L., Vershinina, V., et al. (2016). Three-step procedure for preparation of pure Bacillus altitudinis ribonuclease. FEBS Open Bio 6, 24-32. doi: 10.1002/2211-5463.12023

[10] Dzamukova, M., Naumenko, E., Lvov, Y., and Fakhrullin, R. (2015). Enzyme-activated intracellular drug delivery with tubule clay nanoformulation. Sci. Rep. 5:10560. doi: 10.1038/srep10560

[11] Fakhrullina, G. I., Akhatova, F. S., Lvov, Y. M., and Fakhrullin, R. F. (2015). Toxicity of halloysite clay nanotubes in vivo: a Caenorhabditis elegans study. Environ. Sci. Nano 2, 54-59. doi: 10.1039/C4EN00135D

[12] Fan, X., Jiao, G., Zhao, W., Jin, P., and Li, X. (2013). Magnetic Fe3o4-graphene composites as targeted drug nanocarriers for ph-activated release. Nanoscale 5, 1143-1152. doi: 10.1039/c2nr33158f

[13] Farvadi, F., Tamaddon, A., Sobhani, Z., and Abolmaali, S. (2016). Polyionic complex of single-walled carbon nanotubes and PEG-grafted-hyperbranched polyethyleneimine (PEG-PEI-SWNT) for an improved doxorubicin loading and delivery: development and in vitro characterization. Artif. Cells Nanomed. Biotechnol. 45, 855-863. doi: 10.1080/21691401.2016.1181642

[14] Garipov, A. R., Nesmelov, A. A., Cabrera-Fuentes, H. A., and Ilinskaya, O. N. (2014). Bacillus intermedius ribonuclease (BINASE) induces apoptosis in human ovarian cancer cells. Toxicon 92, 54-59. doi: 10.1016/j.toxicon.2014.09.014

[15] Ilinskaya, O., Decker, K., and Koschinski, A. (2001). Bacillus intermedius ribonuclease as inhibitor of cell proliferation and membrane current. Toxicology 156, 101-107. doi: 10.1016/S0300-483X(00)00335-8

[16] Ilinskaya, O., Singh, I., Dudkina, E., Ulyanova, V., Kayumov, A., and Barreto, G. (2016). Direct inhibition of oncogenic KRAS by Bacillus pumilus ribonuclease (binase). Biochim. Biophys. Acta 1863(7 Pt A), 1559-1567. doi: 10.1016/j.bbamcr.2016.04.005

[17] Johnson, D. B., and Puzanov, I. (2015). Treatment of NRAS-mutant melanoma. Curr. Treat. Options Oncol. 16, 15. doi: 10.1007/s11864-015-0330-z

[18] Joo, K. I., Xiao, L., Liu, S., Liu, Y., Lee, C. L., Conti, P. S., et al. (2013). Crosslinked multilamellar liposomes for controlled delivery of anticancer drugs. Biomaterials 34, 3098-3109. doi: 10.1016/j.biomaterials.2013.01.039

[19] Joussein, E., Petit, S., Churchman, J., Theng, B., Righi, D., and Delvaux, B. (2005). Halloysite clay minerals - a review. Clay Miner. 40, 383-426. doi: 10.1180/0009855054040180

[20] Kamble, R., Ghag, M., Gaikawad, S., and Panda, B. K. (2012). Review article halloysite nanotubes and applications: a review. J. Adv. Sci. Res. 3, 25-29.

[21] Kolpakov, A. I., and Il'inskaia, O. N. (1999). The optimization of a method for determining RNAse activity by using high-polymer RNA. Klin. Lab. Diagn. 5, 14-16. doi: 10.1590/1414-431X20154734

[22] Lai, X., Agarwal, M., Lvov, Y., Pachpande, C., Varahramyan, K., and Witzmann, F. (2013). Proteomic profiling of halloysite clay nanotube exposure in intestinal cell co-culture. J. Appl. Toxic. 33, 1316-1329. doi: 10.1002/jat.2858

[23] Lee, J. H., Chen, K. J., Noh, S. H., Garcia, M. A., Wang, H., Lin, W. Y., et al. (2013). On-demand drug release system for in vivo cancer treatment through self-assembled magnetic nanoparticles. Angew. Chem. Int. Ed. Engl. 52, 4384-4388. doi: 10.1002/anie.201207721

[24] Liu, M., Wu, C., Jiao, Y., Xiong, S., and Zhou, C. (2013). Chitosan-halloysite nanotubes nanocomposite scaffolds for tissue engineering. J. Mater. Chem. 1, 2078-2089. doi: 10.1016/j.ijbiomac.2012.06.022

[25] Lundqvist, M., Sethson, I., and Jonsson, B. (2004). Protein adsorption onto silica nanoparticles: conformational changes depend on the particles' curvature and the protein stability. Langmuir 20, 10639-10647. doi: 10.1021/la0484725

[26] Lvov, Y., Aerov, A., and Fakhrullin, R. (2014). Clay nanotube encapsulation for functional biocomposites. Adv. Colloid Interface Sci. 207, 189-198. doi: 10.1016/j.cis.2013.10.006

[27] Lvov, Y., DeVilliers, M., and Fakhrullin, R. (2016). The potential of halloysite tubule clay in drug delivery applications. Expert Opin. Drug Deliv. 13, 977-988. doi: 10.1517/17425247.2016.1169271

[28] Makarov, A. A., Kolchinsky, A., and Ilinskaya, O. N. (2008). Binase and other microbial RNases as potential anticancer agents. Bioessays 30, 781-790. doi: 10.1002/bies.20789

[29] Massaro, M., Amorati, R., Cavallaro, G., Guernelli, S., Lazzara, G., Milioto, S., et al. (2016a). Direct chemical grafted curcumin on halloysite nanotubes as dual-responsive prodrug for pharmacological applications. Colloids Surf. B Biointerfaces 140, 505-513. doi: 10.1016/j.colsurfb.2016.01.025

[30] Massaro, M., Riela, S., Baiamonte, C., Blanco, J. L. J., Giordano, C., Meo, P. L., et al. (2016b). Dual drug-loaded halloysite hybrid-based glycocluster for sustained release of hydrophobic molecules. J. Mater. Chem. 4, 2229-2241.

[31] Mironova, N. L., Petrushanko, I. Y., Patutina, O. A., Sen'kova, A. V., Simonenko, O. V., Mitkevich, V. A., et al. (2013). Ribonuclease binase inhibits primary tumor growth and metastases via apoptosis induction in tumor cells. Cell Cycle 12, 2120-2131. doi: 10.4161/cc.25164

[32] Mitkevich, V., Tchurikov, N., Zelenikhin, P., Petrushanko, I., Makarov, A., and Ilinskaya, O. (2010). Binase cleaves cellular noncoding RNAs and affects coding mRNAs. FEBS J. 277, 186-196. doi: 10.1111/j.1742-4658.2009.07471.x

[33] Mitkevich, V. A., Kretova, O. V., Petrushanko, I. Y., Burnysheva, K. M., Sosin, D. V., Simonenko, O. V., et al. (2013a). Ribonuclease binase apoptotic signature in leukemic Kasumi-1 cells. Biochimie 95, 1344-1349. doi: 10.1016/j.biochi.2013.02.016

[34] Mitkevich, V. A., Makarov, A. A., and Il'inskaia, O. N. (2014). Cellular targets of antitumor ribonucleases. Mol. Biol. 48, 214-222. doi: 10.1134/S0026893314020137

[35] Mitkevich, V. A., Orlova, N. N., Petrushanko, I., Simonenko, O. V., Spirin, P. V., Prokofieva, M. M., et al. (2013b). Expression of FLT3-ITD oncogene confers mice progenitor B-cells BAF3 sensitivity to the ribonuclease binase cytotoxic action. Mol. Biol. 47, 249-252. doi: 10.1134/S002689331302009X

[36] Mitkevich, V. A., Petrushanko, I. Y., Spirin, P. V., Fedorova, T. V., Kretova, O. V., Tchurikov, N. A., et al. (2011). Sensitivity of acute myeloid leukemia Kasumi-1 cells to binase toxic action depends on the expression of KIT and AML1-ETO oncogenes. Cell Cycle 10, 4090-4097. doi: 10.4161/cc.10.23.18210

[37] Olombrada, M., Lázaro-Gorines, R., López-Rodríguez, J. C., Martínez-Del-Pozo, Á., Oñaderra, M., Maestro-López, M., et al. (2017). Fungal ribotoxins: a review of potential biotechnological applications. Toxins 9:E71. doi: 10.3390/toxins9020071

[38] Safari, S., Emtiazi, G., and Shariatmadari, H. (2005). Sorption and immobilization of cellulase on silicate clay minerals. J. Colloid Interface Sci. 290, 39-44. doi: 10.1016/j.jcis.2005.04.030

[39] Shen, R., Li, J., Ye, D., Wang, Q., and Fei, J. (2016). Combination of onconase and dihydroartemisinin synergistically suppresses growth and angiogenesis of non-small-cell lung carcinoma and malignant mesothelioma. Acta Biochim. Biophys. Sin. 48, 894-901. doi: 10.1093/abbs/gmw082

[40] Shirshikov, F., Cherepnev, V., Ilinskaya, O., and Kalacheva, N. (2013). A hydrophobic segment of some cytotoxic ribonucleases. Med. Hypotheses 81, 328-334. doi: 10.1016/j.mehy.2013.04.006

[41] Shutava, T. G., Fakhrullin, R. F., and Lvov, Y. M. (2014). Spherical and tubule nanocarriers for sustained drug release. Curr. Opin. Pharmacol. 18, 141-148. doi: 10.1016/j.coph.2014.10.001

[42] Soares, P., Sousaa, A., Silvab, J., Ferreiraa, I., Novoc, C., and Borges, J. (2016). Chitosan-based nanoparticles as drug delivery systems for doxorubicin: optimization and modeling. Carbohydr. Polym. 147, 304-312. doi: 10.1016/j.carbpol.2016.03.028

[43] Sun, J., Yendluri, R., Kai, L., Lvov, Y., and Yan, X. (2017). Enzyme-immobilized clay nanotube - chitosan membranes with sustainable biocatalytic activity. Phys. Chem. 19, 562-567. doi: 10.1039/c6cp07450b

[44] Tully, J., Yendluri, R., and Lvov, Y. (2016). Halloysite clay nanotubes for enzyme immobilization. Biomacromolecules 17, 615-621. doi: 10.1021/acs.biomac.5b01542

[45] Ulyanova, V., Shah Mahmud, R., Dudkina, E., Vershinina, V., Domann, E., and Ilinskaya, O. (2016). Phylogenetic distribution of extracellular guanyl-preferring ribonucleases renews taxonomic status of two Bacillus strains. J. Gen. Appl. Microbiol. 62, 181-188. doi: 10.2323/jgam.2016.02.005

[46] Ulyanova, V., Vershinina, V., and Ilinskaya, O. (2011). Barnase and binase: twins with distinct fates. FEBS J. 278, 3633-3643. doi: 10.1111/j.1742-4658.2011.08294.x

[47] Vergaro, V., Abdullayev, E., Lvov, Y., Zeitoun, A., Cingolani, R., Rinaldi, R., et al. (2010). Cytocompatibility and uptake of halloysite clay nanotubes. Biomacromolecules 11, 820-826. doi: 10.1021/bm9014446

[48] Vert, A., Castro, J., Ribó, M., Benito, A., and Vilanova, M. (2017). Activating transcription factor 3 is crucial for antitumor activity and to strengthen the antiviral properties of onconase. Oncotarget 8, 11692-11707. doi: 10.18632/oncotarget.14302

[49] Vogelzang, N. J., Aklilu, M., Stadler, M., Dumas, M. C., and Mikulski, S. M. (2001). A phase II trial of weekly intravenous ranpirnase (onconase), a novel ribonuclease in patients with metastatic kidney cancer. Invest. New Drugs 19, 255-260. doi: 10.1023/A:1010633004157

[50] Wei, W., Minullina, R., Abdullayev, E., Fakhrullin, R., Millsa, D., and Lvov, Y. (2014). Enhanced efficiency of antiseptics with sustained release from clay nanotubes. RSC Adv. 4, 488-494. doi: 10.1039/C3RA45011B

[51] Yendluri, R., Lvov, Y., DeVilliers, M., Vinokurov, V., Naumenko, E., Tarasova, E., et al. (2017). Paclitaxel encapsulated in halloysite clay nanotubes for intestinal and intracellular delivery. J. Pharm. Sci. doi: 10.1016/j.xphs.2017.05.034 [Epub ahead of print].

[52] Zelenikchin, P., Pukhovskaya, V., Garipov, A., Makeeva, A., Sokolova, E., and Ilinskaya, O. (2016). Obvious and hidden reasons of breast cancer cell sensitivity to antitumor RNase. BioNanoScience 6, 528-533. doi: 10.1007/s12668-016-0269-y

[53] Zhang, Y., Gao, R., Liu, M., Shi, B., Shan, A., and Cheng, B. (2014). Use of modified halloysite nanotubes in the feed reduces the toxic effects of zearalenone on sow reproduction and piglet development. Theriogenology 83, 932-41. doi: 10.1016/j.theriogenology.2014.11.027