Development of Cyclic RGD-functionalized Maleimide-*closo*-dodecaborate Albumin Conjugate (cRGD-MID-AC) as a Tumor Targeting Boron Carrier

Hiroyuki Nakamura[1], Kazuki Kawai[1,2], Takushi Takata[3], Minoru Suzuki[3]

[1] *Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan*

[2] *School of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan*

[3] *Institute for Integrated Radiation and Nuclear Science, Kyoto University, Osaka, Japan*

E-mail: hiro@res.titech.ac.jp

Introduction: Serum albumin is a native protein and a major component of blood plasma. Serum albumin behaves as a compound carrier in blood, and the compound bound to serum albumin can circulate in blood without draining. Moreover serum albumin is accumulated in tumor tissue due to enhanced permeability and retention (EPR) effect. Therefore, we have focused on a serum albumin as an attractive boron carrier. We previously reported maleimide-containing *closo*-dodecaborate albumin conjugate (MID-AC) [1]. MID was found to conjugate to free SH of cysteine and lysine residues in bovine serum albumin (BSA) under physiological conditions [2,3]. MID-AC showed high and selective accumulation in tumor and significant tumor growth inhibition in colon 26 tumor-bearing mice subjected to thermal neutron irradiation. In this paper, we designed the cyclic RGD-functionalized MID-AC (cRGD-MID-AC) as tumor active targeting boron carriers and evaluated their biological activity. Cyclic RGD is known to strongly bind to $\alpha_v\beta_3$ integrin which is overexpressing on many cancer cells and neovascularities.

Materials and Methods: Cyclic RGD-functionalized MID-AC was prepared by the double modification method. BSA was first reacted with cyclic RGD-maleimide in PBS (pH7.4), subsequently reacted with MID. After ultrafiltration, the double-modified cRGD-MID-AC was obtained. The cell-uptake experiments of cRGD-MID-AC were carried out with U-87MG (human brain cancer, $\alpha_v\beta_3$ positive) and PC-3 (human prostate cancer, $\alpha_v\beta_3$ negative) cells and the boron accumulation in cells was determined by immunostaining using anti-MID antibody. Furthermore, we examined the *in vivo* biodistribution of cRGD-MID-AC and MID-AC using U-87MG xenograft model mice. cRGD-MID-AC or MID-AC were injected via the tail vein and the boron concentration in tumor tissue were determined by ICP-OES.

Results: cRGD-MID-AC was significantly accumulated into U-87MG cells. Interestingly, the accumulation of cRGD-MID-AC into PC-3 cells was lower than that into U-87MG cells, indicating that cRGD-MID-AC interacts with $\alpha_v\beta_3$ integrin and accumulate into $\alpha_v\beta_3$ positive cells, selectively. Furthermore, significant accumulation of cRGD-MID-AC in tumor was observed in tumor-bearing mice.

Conclusion: We succeeded in the preparation of cRGD-MID-AC using the double modification method. cRGD-MID-AC has a function of targeting $\alpha_v\beta_3$ positive tumors, thus it is considered to be a possible candidate as an active targeting boron carrier to tumor for BNCT.

References

1. S. Kikuchi, D. Kanoh, S. Sato, Y. Sakurai, M. Suzuki, H. Nakamura, J. Control. Release, *237*, 160–167 (2016)
2. H. Nakamura, S. Kikuchi, K. Kawai, S. Ishii, S. Sato, Pure Appl. Chem. *90*, 745-753 (2018)
3. S. Ishii, S. Sato, H. Asami, T. Hasegawa, J. Kohno, H. Nakamura, Org. Biomol. Chem. *17*, 5496–5499 (2019).