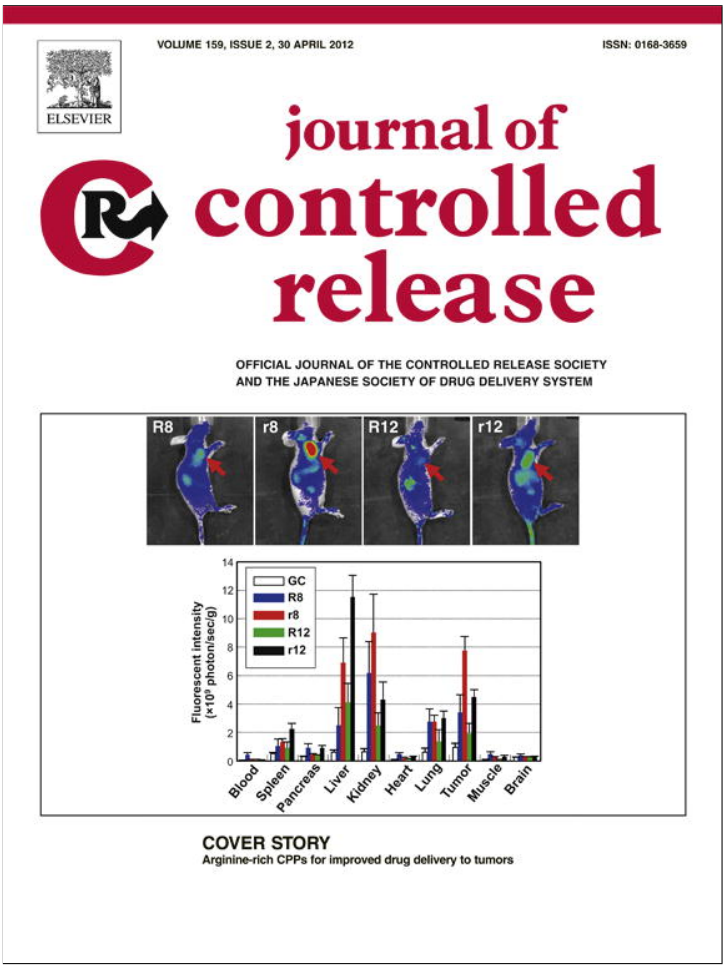

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Cover Story

Arginine-rich CPPs for improved drug delivery to tumors

Cell-penetrating peptides (CPPs) have been used as a powerful tool for intracellular delivery of various bioactive molecules. A number of pharmaceutical molecules with low membrane permeability (ranging from small molecular weight compounds to proteins and nucleic acids) have been efficiently delivered into cells by conjugation or complex formation with CPPs [1]. Although CPPs have been successfully used for delivery of various agents into numerous types of cells, CPPs do not have specificity toward any particular cells. Naturally, it is common to observe that CPPs and their conjugates accumulate in the liver, kidney, lung, and spleen presumably due to the abundance of blood capillaries and/or macrophage-like cells in these organs [2]. To avoid non-specific accumulation of CPPs to non-target organs, local administration or combination of other targeting molecules such as RGD has been employed for *in vivo* administration using CPPs to delivery to tumors [3].

In this issue, Professor Futaki and his group reported the more efficient accumulation of oligoarginine, especially octaarginine that is comprised of D-arginines (r8), in tumor xenografts than other CPPs when intravenously injected in mice [4]. There were several reports on biodistribution of arginine-rich CPPs, but they were studied in animals without bearing tumor tissues. For this reason, Professor Futaki and his colleagues examined the biodistribution of typical arginine-rich CPPs, including HIV-1 Tat (48–60), penetratin, and the L- and D-forms of the oligoarginines (8-, 12-, and 16-mers) in tumor-xenografted nude mice after intravenous injection. Alexa660-labeled peptides were used for *in vivo* and *ex vivo* fluorescence imaging. When the accumulation to tumor xenografts was compared, octaarginine comprised of L-arginines (R8) was about twice more efficient than Tat (48–60) and penetratin. The effects of oligoarginines were studied in more detail by examining the differences in the biodistribution between the L- and D-forms (*i.e.*, R8 and r8) of the 8-, 12-, and 16-mers. For both isomers, octaarginine was more effective *in vivo* than the other longer oligomers. It is interesting to note that the *in vivo* result was different from the *in vitro* cellular uptake of oligoarginines in serum free conditions, where the longer oligomers were more efficient. The higher efficiency of the longer oligoarginines is expected due to their superior binding when the cells are available in the absence of any serum proteins. The Futaki group also showed that *in vivo* tumor accumulation of the D-form of octaarginine (r8) was twice better than the L-form (R8). Based on this result, the authors conjugated doxorubicin to r8 to examine its *in vivo* efficacy. Free doxorubicin showed the tumor growth inhibition activity at 6 mg/kg, but r8-doxorubicin at 4 mg/kg. While doxorubicin 6 mg/kg was accompanied with considerable weight loss of the mice, no significant decrease in the body weight was observed for r8-doxorubicin 4 mg/kg, suggesting that the r8 conjugation may reduce the side effects on the treatment of the anticancer agent.

Professor Futaki and his team provide interesting explanations on the unique feature of r8. They speculated that the balance in the affinity of the peptides to serum proteins and tumor tissues and the stability of the peptides against proteolytic degradation are important factors for tumor accumulation of oligoarginines. Since oligoarginines are known to bind to serum proteins [5], the serum/peptide complexes may leak through blood vessels into tumor tissues. The leaked serum/peptide complexes can interact with proteoglycans or other acidic cell-associated molecules with high affinity [5], resulting in efficient trap by tumor tissues. The Futaki team further provided an explanation for the higher accumulation of D-isomer (r8) than L-isomer (R8) at tumor. Degradation of the L-form of peptides during blood circulation is expected to result in decreased binding affinity for serum proteins, and thus less accumulation in tumor tissues. This may explain that the L-form of oligoarginines showed less accumulation at tumor tissues. The r12 has a higher affinity for serum proteins and this may prevent effective transfer of the peptide to tumor tissues, and this makes r8 retained better in tumor tissues.

While r8 shows the best performance in accumulation in tumors, it should be pointed out that the percentage of the CPPs accumulated in the tumor xenografts is still very low. Like any other drug delivery systems, the majority of the administered CPPs are accumulated in the kidney, lung and liver. While the accumulation of CPPs in those organs cannot be fully avoided by any delivery systems, the unique feature of r8 is that the relatively less amount is cumulated in the non-target organs, while relatively more is found at the target tumor. The recent article by Elsadek and Kratz [6] has clearly shown that albumin is one of the most effective delivery systems for tumor targeting, and the interaction of r8 with albumin, with just the right affinity as described above, may have contributed to the superior property of r8. Although using r8 itself is not going to solve the problem of targeted drug delivery, the increased accumulation at tumors with decrease in other organs by r8 deserves our attention. This is because cumulative advances of many small improvements will eventually make big differences in targeted drug delivery.

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