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Cover story

Just getting into cells is not enough

Gemcitabine (2',2'-difluorodeoxycytidine, dFdC) is a deoxycytidine analog that is used for chemotherapy of a wide spectrum of cancers, including pancreatic, breast, lung, and ovarian cancers. Unfortunately, gemcitabine is easily deactivated through deamination catalyzed by deoxycytidine deaminase (dCDA), and tumor cells often acquire resistance to the drug, significantly limiting its clinical efficacy. Gemcitabine enters tumor cells by nucleoside transporters, such as hENT1, and intracellular enzymes, e.g., deoxycytidine kinase (dCK), converts it to its active metabolites, gemcitabine diphosphate (dFdCDP) and triphosphate (dFdCTP). dFdCTP incorporates into DNA, terminates DNA synthesis, and causes tumor cell death, while dFdCDP inhibits ribonucleotide reductase (RR). RR, having RRM1 and RRM2 subunits, is an enzyme required for the synthesis of deoxynucleotides (dNTPs). Thus, tumor cells with decreased expression of hENT1 and dCK, or increased expression of RR, are resistant to gemcitabine. There have been significant efforts to improve the efficacy of gemcitabine against tumor cells by developing gemcitabine prodrugs, such as 4-(*N*)-stearoyl gemcitabine (4-(*N*)-GemC18). 4-(*N*)-GemC18 is not sensitive to deamination and is not dependent on nucleoside transporters to enter cells. Formulations of 4-(*N*)-GemC18 into solid lipid nanoparticles (SLNs) [1] and lectin-modified PLGA microparticles [2] are known to further improve their antitumor activity and overcome gemcitabine resistance.

In this issue, Professor Zhengrong Cui and his group present the mechanisms underlying the ability of 4-(*N*)-GemC18-SLNs to overcome gemcitabine resistance [3]. Initially, they made two interesting observations. First, in the RRM1-overexpressing, gemcitabine resistant TC-1-GR cells, 4-(*N*)-GemC18 dissolved in aqueous solution was not as cytotoxic as 4-(*N*)-GemC18-SLNs, although the uptake of 4-(*N*)-GemC18 by TC-1-GR cells was significantly higher and faster than the 4-(*N*)-GemC18-SLNs. Second, cells absorbed 4-(*N*)-GemC18-SLNs by clathrin-mediated endocytosis, whereas the 4-(*N*)-GemC18 entered cells by passive diffusion. These findings led to a hypothesis that the way 4-(*N*)-GemC18 enters tumor cells is critical to overcome the gemcitabine resistance. To test the hypothesis, the Cui team studied the intracellular location and degradation of 4-(*N*)-GemC18 and 4-(*N*)-GemC18-SLNs.

Their data showed that the delivery of 4-(*N*)-GemC18 into lysosomes using SLNs allowed gemcitabine to be efficiently hydrolyzed from 4-(*N*)-GemC18 and converted to its active metabolites, resulting in overcoming the resistance caused by RRM1 overexpression. It is speculated that 4-(*N*)-GemC18-SLNs happen to 'channel' the 4-(*N*)-GemC18 into a 'nature's' pathway that has evolved for cells to efficiently recycle nucleic acids from within or outside cells to undergo 'nucleotide salvage synthesis'. Once the 4-(*N*)-GemC18-SLNs are internalized into lysosomes, enzymes in lysosomes digest the SLNs and hydrolyze 4-(*N*)-GemC18 to release gemcitabine, which is then

exported out of lysosomes by specific nucleoside transporters, e.g., hENT3. Due to compartmentalization of enzymes responsible for nucleotide salvage synthesis, gemcitabine that is exported out of lysosomes may have been directly presented to enzymes such as dCK and efficiently converted to dFdCDP and dFdCTP, with minimal deamination. In contrast, when 4-(*N*)-GemC18 in solution diffuses into tumor cells, gemcitabine hydrolyzed from the 4-(*N*)-GemC18 outside of lysosomes may not be efficiently presented to enzymes such as dCK for activation, while subjected to deamination by dCDA. Gemcitabine alone can enter tumor cells with the help of nucleoside transporters, but again may not be efficiently converted to its active metabolites, dFdCDP and dFdCTP, especially in tumor cells that overexpress RRM1.

While the mechanism proposed by the Cui group is reasonable and can certainly explain why 4-(*N*)-GemC18-SLNs are more cytotoxic than gemcitabine or 4-(*N*)-GemC18 to tumor cells, more studies have to be carried out to confirm the mechanism and provide more insights. In clinics, overcoming gemcitabine resistance caused by the alterations in the expressions of genes critical to gemcitabine uptake and intracellular metabolism is important, but it is not sufficient for effective treatment. In many tumors such as advanced pancreatic ductal adenocarcinoma, for which gemcitabine is the first line treatment, the physical barrier caused by tumor desmoplastic stroma severely limits the perfusion of tumors by gemcitabine, let alone gemcitabine carried by nanoparticles. Nonetheless, understanding the biological mechanisms of gemcitabine resistance provides rational new approaches to developing improved gemcitabine delivery systems. The lessons learned by gemcitabine can, of course, be applied to other antitumor agents.

References

- [1] W.-G. Chung, M.A. Sandoval, B.R. Sloat, D.S.P. Lansakara-P., Z. Cui, Stearoyl gemcitabine nanoparticles overcome resistance related to the over-expression of ribonucleotide reductase subunit M1, *J. Control. Release* 157 (2012) 132–140.
- [2] L. Neutsch, E.-M. Wirth, S. Spijker, C. Pichl, H. Kählig, F. Gabor, M. Wirth, Synergistic targeting/prodrug strategies for intravesical drug delivery—lecithin-modified PLGA microparticles enhance cytotoxicity of stearoyl gemcitabine by contact-dependent transfer, *J. Control. Release* 169 (2013) 62–72.
- [3] P. Wonganan, D.S.P. Lansakara-P., S. Zhu, M. Holzer, M.A. Sandoval, M. Warthaka, Z. Cui, Just getting into cells is not enough: mechanisms underlying 4-(*N*)-stearoyl gemcitabine solid lipid nanoparticle's ability to overcome gemcitabine resistance caused by RRM1 overexpression, *J. Control. Release* 169 (2013) 17–27.

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