CANCER NANOMEDICINE

Making vessels more permeable

Depletion of tumour-associated platelets improves the delivery of anticancer drugs.

Abdullah Muhammad Syed, Shrey Sindhwani and Warren C. W. Chan

The successful treatment of cancer is often hindered by side effects from off-target drug interactions with healthy tissue. This has led researchers to develop strategies to specifically deliver drugs to the tumour. Because tumour blood vessels are more permeable than vessels in healthy tissues, delivering cancer drugs packaged into nanoparticles through such permeable vessels has been used as an approach to improve drug delivery into the tumour. However, this approach has so far achieved limited success in the treatment of solid tumours in human patients[1]. Data from mouse studies have shown that the accumulation of nanoparticles in solid tumours remains low at 0.7% (median value) of the injected dose[1] due to their sequestration by phagocytic cells in organs such as the liver and the spleen. These organs efficiently filter out the nanoparticles from the blood, acting as barriers to the effective delivery of nanoparticles into tumours. In addition, blood-vessel permeability within tumours and between different tumour types varies greatly. Also, the results from one mouse model may not apply to another, which means even greater uncertainty in the translation of nanoparticle-delivery approaches for use in humans. Reporting in *Nature Biomedical Engineering*, Guangjun Nie and co-authors now show that intratumoural drug delivery can be enhanced by functionalizing nanoparticles for the local depletion of tumour-associated platelets that normally repair the leaky vessels[2]. The authors propose that depleting such platelets enhances the permeability of vessels and allows the controlled release of the packaged drugs in the tumour. This approach is part of a growing family of strategies aimed at manipulating the host biology to improve nanoparticle delivery.

Nie and colleagues developed a polymer–lipid–peptide nanoparticle core consisting of a charged amphiphilic polymer in which the hydrophobic part encapsulates the chemotherapeutic agent doxorubicin and the positively charged region adsorbs the antibody R300 (Fig. 1a), which can bind to platelets, causing their microaggregation and removal by macrophages. The nanoparticle shell consists of polyethylene glycol (PEG)-conjugated phospholipids and lecithin, which provide stability and protection, prolonging nanoparticle circulation in vivo. The shell also contains a matrix metalloproteinase 2 (MMP2)-specific cleavable peptide. MMP2 is a matrix enzyme that is overexpressed in a variety of tumours. This design allows the nanoparticles to accumulate and breakdown preferentially in the tumour, first releasing R300 and then allowing the polymer core to slowly release doxorubicin.

In mice, by depleting tumour-associated platelets, Nie and co-authors expected the increased permeability of tumour vessels to facilitate nanoparticle accumulation. Indeed, nanoparticles with the MMP2-cleavable peptide localized preferentially in the tumour, compared with nanoparticles without the peptide, as shown by in vivo fluorescence and photoacoustic imaging (Fig. 1b,c). The platelet-specific R300 antibody further increased intratumoural drug accumulation and retention, compared with nanoparticles without the R300 antibody, although the liver filtered out most of the injected nanoparticles in both cases. The authors did not observe systemic platelet depletion (known as thrombocytopenia), which can be a dangerous side effect of many cancer therapies. The use of the nanoparticles also avoided the cardiac-toxicity profiles associated with the delivery of free doxorubicin, because the nanoparticles degraded over time in the liver. Interestingly, the R300 antibody (either bound to the nanoparticle or in its free form) also led to higher accumulation of Evans Blue (a commonly used dye for testing permeability) in the tumour. The authors' strategy delivered threefold more doxorubicin into tumours compared with when free doxorubicin was used, and translated into a lack of tumour progression in MMP2-expressing tumour models. It also led to a significantly slower growth rate of tumours that expressed low levels of MMP2, and a reduction in metastatic load, compared with a saline injection, as determined by the number of metastatic nodes.

Nie and colleagues attribute these results to their unconventional strategy for delivering nanoparticles, where they manipulated tumour biology rather than relying on existing basal permeability to improve drug delivery. However, the mechanism of action should be explored further. In the absence of nanoparticles, the R300 antibody causes aggregation of platelets in the blood and these micrometre-sized aggregates are then removed by phagocytic cells in the liver and the spleen, which constantly filter the blood. In contrast, the nanoparticles specifically release the antibody only in the tumour matrix, where the diffusion of platelets is expected to be slow. Other unanswered questions remain. Can platelets aggregate inside a tumour matrix and how are they eliminated? Do tumour-associated macrophages within the stroma find and engulf these platelet aggregates? If so, this treatment would also depend on the presence of large numbers of phagocytes in the tumour stroma. How does platelet depletion in the tumour lead to gaps in the blood vessels? How do these gaps, which take hours to form according to the proposed mechanism, lead to enhanced accumulation of nanoparticles that have been mostly cleared from the blood within the first few hours of administration? Addressing these questions will provide valuable information on how to manipulate tumour biology and to enhance drug delivery using nanoparticles.

Manufacturing nanoparticles with complex designs in large-scale, sterile, good manufacturing practice (GMP) facilities is a major challenge hindering the translation of this nanoparticle technology to the clinic. Significant optimization and method development would be required to synthesize these complex structures with consistent and reproducible physicochemical

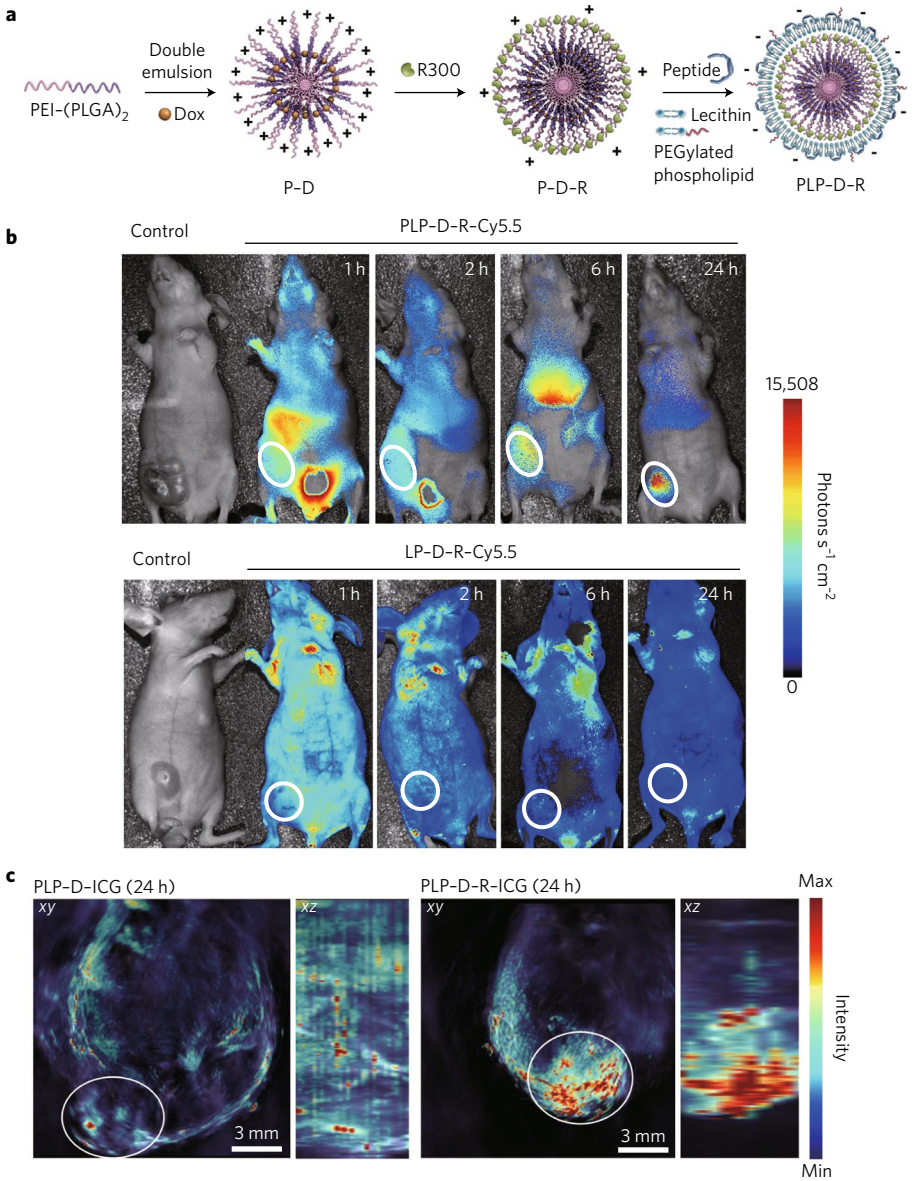

Fig. 1 | A multicomponent nanoparticle system for the dual targeting of a tumour and its associated platelets enhances drug delivery. a, The polymer–lipid nanoparticle consists of a block copolymer poly(etherimide)–poly(lactic-co-glycolic acid)$_2$, PEI-(PLGA)$_2$ (LP), a doxorubicin-loaded core (Dox; D), an R300 antibody (R) that causes microaggregation and subsequent depletion of platelets, and an MMP2-cleavable peptide (P) that causes the nanoparticle to release its contents in the tumour (PLP-D-R). The shell contains lecithins and poly(ethylene glycol) (PEG)ylated lipids. **b**, Time-lapse fluorescence images of nanoparticles (PLP-D-R) with a MMP2-cleavable peptide that lead to higher intratumoural accumulation of the dye (Cy5.5; white ovals) compared with a control formulation that does not contain the peptide (LP-D-R). **c**, 3D photoacoustic images of nanoparticles, containing the dye indocyanine green (ICG) and the platelet-depleting antibody R300 (PLP-D-R-ICG), that also enhance tumour permeability compared with a formulation not containing the antibody (PLP-D-ICG). The white circles represent the location of the tumour. The coordinates *xyz* indicate the orientation of the images, with *xy* corresponding approximately to a plane parallel to the mouse's skin surface. Figure reproduced from ref. [2], Macmillan Publishers Ltd.

and biological properties with low batch-to-batch variability. Also, patient data would be needed to ensure that the mechanism of action for the nanoparticle formulation observed in the mouse model is effective in human patients. At present, the polymer–lipid–peptide nanoparticle requires exquisite control over the timing of peptide degradation and over platelet aggregation to enhance tumour permeability and to increase drug accumulation. Compared with mouse models, differences in tumour pathophysiology in humans could affect nanoparticle efficacy, and therefore the chemistry might need to be modified to obtain the same sequence of events. Finally, the level of MMP2 expression in the tumour may need to be determined for each patient to assess if levels are sufficient for enhanced tumour accumulation of the nanoparticles. The proportion of tumours with significant quantities of platelets in the stroma is also unknown. Tumours may also be insensitive to platelet depletion since thrombocytopenia is a common side effect of chemotherapeutics and was found to affect 61% of patients in a clinical trial for the liposomal formulation of doxorubicin (Doxil)[3]. Like other toxic side effects, thrombocytopenia cannot be accurately predicted from animal models, thus the lack of platelet depletion observed in the study does not guarantee safety and efficacy in humans. Nevertheless, the lessons learned from these early-phase preclinical studies are valuable for the translation of other strategies based on the alteration of tumour biology.

It is also important to point out that improved analysis methods and technologies are urgently needed for evaluating nanoparticle delivery. Currently, the development of nanoparticle drug-delivery strategies aims at either providing new mechanistic insight into the delivery processes, or at translating nanoparticle formulations for clinical use. Mechanistic insights provide the knowledge to guide the design of future nanotechnology, whereas clinical translation impacts patient care. To advance both of these objectives, the drug-delivery processes need to be fully quantified to provide a set of metrics that can be used to benchmark progress. However, the nanomedicine community needs to decide what metrics are the most appropriate and ensure that these are applied across studies to enable meaningful comparisons. Additionally, systematic, comprehensive and mechanistic studies on the delivery processes will require the adaptation of existing and emerging techniques. For example, histopathology analysis and electron microscopy are conventionally used to verify delivery and targeting. But the main problem with using these approaches is that a small fraction of a tumour is imaged and analysed, and that data misinterpretation can occur as a result. High-resolution 3D imaging that maps the accumulation and distribution of nanoparticles in intact tissues is a potential solution to this problem[4]. This would remove biases in data interpretation from a single tissue slice or from a small set

of imaging data. Beyond the development of these analytical techniques, it is also important that datasets are organized and assembled to enable easy data mining. Overcoming the barriers for nanoparticle drug delivery is complex and calls for the community to come together. This is important for the future of many emerging technologies that would benefit from nanoparticle-based delivery systems — such as genome editing, immunotherapy and interfering RNAs — to effectively treat cancer and other diseases. ❐

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