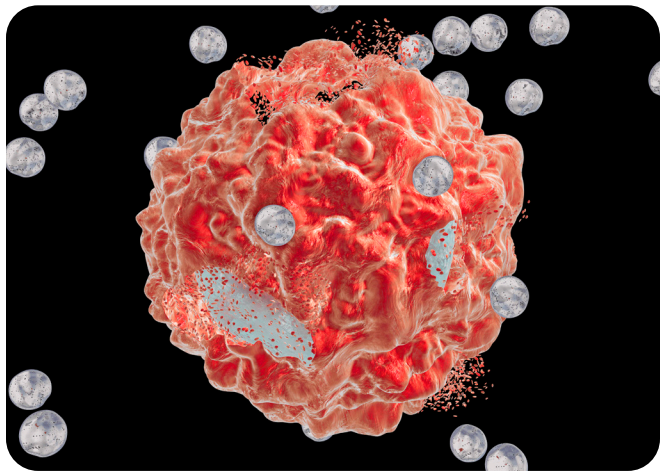
PUBLICATION REVIEW

Tumor-targeting nanoparticles for enhanced PROTAC delivery.

PROteolysis-TArgeting Chimeras (PROTACs) are heterobifunctional small molecules that leverage the ubiquitin-proteasome system (UPS) to selectively degrade disease-relevant proteins. Unlike traditional inhibitors that block protein function, the unique ability of PROTACs to degrade target proteins offers a powerful approach for sustained protein depletion. PROTACS are particularly attractive in oncology, where their mechanism of action presents opportunities to overcome drug resistance and target previously "undruggable" proteins. As a result, several PROTAC-based therapies are under clinical investigation, including notable candidates such as ARV-110 for prostate cancer and ARV-471 for breast cancer.

Despite their promise, PROTACs often suffer from issues such as poor tissue penetration and limited intracellular uptake due to their complex physicochemical properties. Improving targeted delivery therefore remains a significant goal in their development. In light of these challenges, researchers at Peking University have developed tumor-targeted nanoparticles specifically designed for the precise delivery of PROTACs to colorectal cancer cells.[1] This advancement is particularly significant as colorectal cancer is ranked as the third most common cancer worldwide, with current treatments often failing to achieve significant efficacy in the majority of patients. This study therefore presents an innovative strategy combining PROTACs with nanotechnology for enhanced therapeutic efficacy.



Study highlights

- PROTACs demonstrate promise as effective oncologic therapeutics given their improved target selectivity
- The IVIS™ Lumina III optical imaging system and Cellometer™ cell counter played crucial roles in the development and validation of a novel PROTAC therapeutic strategy
- The therapy achieves precise intracellular delivery and could be used in combination with immune checkpoint blockades against diseases like colorectal cancer

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Development of tumor-targeting nanoparticles

Cyclin-dependent kinases 4 and 6 (CDK4/6) are critical regulators of cell cycle progression and are frequently dysregulated in tumors. To improve the delivery of CDK4/6-targeting PROTACs, the researchers engineered pH/cathepsin B sequentially responsive nanoparticles (PSRNs). These nanoparticles were designed to optimize tumor penetration, cellular uptake, and intracellular PROTAC release. Two nanoparticle formulations were developed: a pH-sensitive version (PSRN) and a control, pH-insensitive version (PNRN) to compare efficacy.

The team first investigated how PROTACs were released from the nanoparticles under conditions mimicking the tumor microenvironment. They confirmed that PSRNs required both an acidic environment and the presence of specific proteolytic enzymes for efficient drug release. Under these conditions, PSRNs released 80% of their PROTAC payload within four hours. In contrast, PNRNs released less than 10% even after 24 hours.

Cellular uptake and intracellular localization

Further experiments assessed the uptake efficiency of their nanoparticle formulations in different pH environments. They found that PSRNs exhibited a 1.5-fold higher uptake at pH 6.6 compared to pH 7.4, suggesting that PSRNs would preferentially accumulate in the acidic tumor environment. In contrast, PNRNs showed poor uptake regardless of the pH.

Once inside the cell, PSRNs primarily accumulated in lysosomes. This finding was particularly significant because cathepsin B—which is responsible for triggering PROTAC release—is abundant in lysosomes. LC-MS analysis confirmed the efficiency of PROTAC release, with over 90% of PROTACs released from PSRNs within one hour under acidic conditions.

Enhanced target degradation and tumor cytotoxicity

To evaluate the efficacy of protein degradation, the team measured CDK4/6 levels in various tumor cell lines. Analysis revealed that both PSRNs and free PROTAC efficiently degraded CDK4/6, whereas PNRNs showed limited degradation. Notably, PSRNs exhibited greater degradation in acidic environments, with degradation rates 2.1 to 2.3-fold higher at pH 6.6 than at pH 7.4 and up to 2.4 higher than free PROTAC under the same conditions, confirming their tumor-selective activity.

This degradation efficiency translated into enhanced cytotoxicity against colon carcinoma cells, with PSRNs exhibiting a dose-dependent inhibition of tumor growth. In contrast, PNRNs displayed weak cytotoxicity, consistent with their limited protein degradation capability. To further assess tumor penetration, fluorescently labeled PSRNs were incubated with 3D multicellular tumor spheroids derived from MCF-7, CT26, and PANC-1 cells. After eight hours, PSRNs demonstrated significantly deeper penetration than PNRNs, reaching up to 45 µm into tumor spheroids under acidic conditions.

In vivo pharmacokinetics and biodistribution

In the final stages of their investigation, the researchers conducted *in vivo* studies in BALB/c mice to evaluate the pharmacokinetics and biodistribution properties of the nanoparticles. Pharmacokinetic analysis revealed that PNRNs, due to their strong hydrophobic cores, had a slightly longer elimination half-life than PSRNs (13.7 versus 12.5 hours, respectively).

To investigate biodistribution, the researchers used the IVIS Lumina III *in vivo* imaging system to track fluorescence-labeled nanoparticles in CT26 tumor-bearing mice. Both PSRNs and PNRNs were seen to rapidly accumulate at the tumor site, with amounts increasing significantly over 48 hours (Figure 1). This accumulation was attributed to their prolonged blood circulation and the enhanced permeability and retention (EPR) effect. However, despite similar accumulation levels at the tumor site, PNRNs showed weak penetration into tumor tissues, with fluorescence mostly localized in blood vessels. In contrast, PSRNs diffused deeper into the tumor and the fluorescence intensity remained stable after PBS washing, indicating successful cellular internalization.

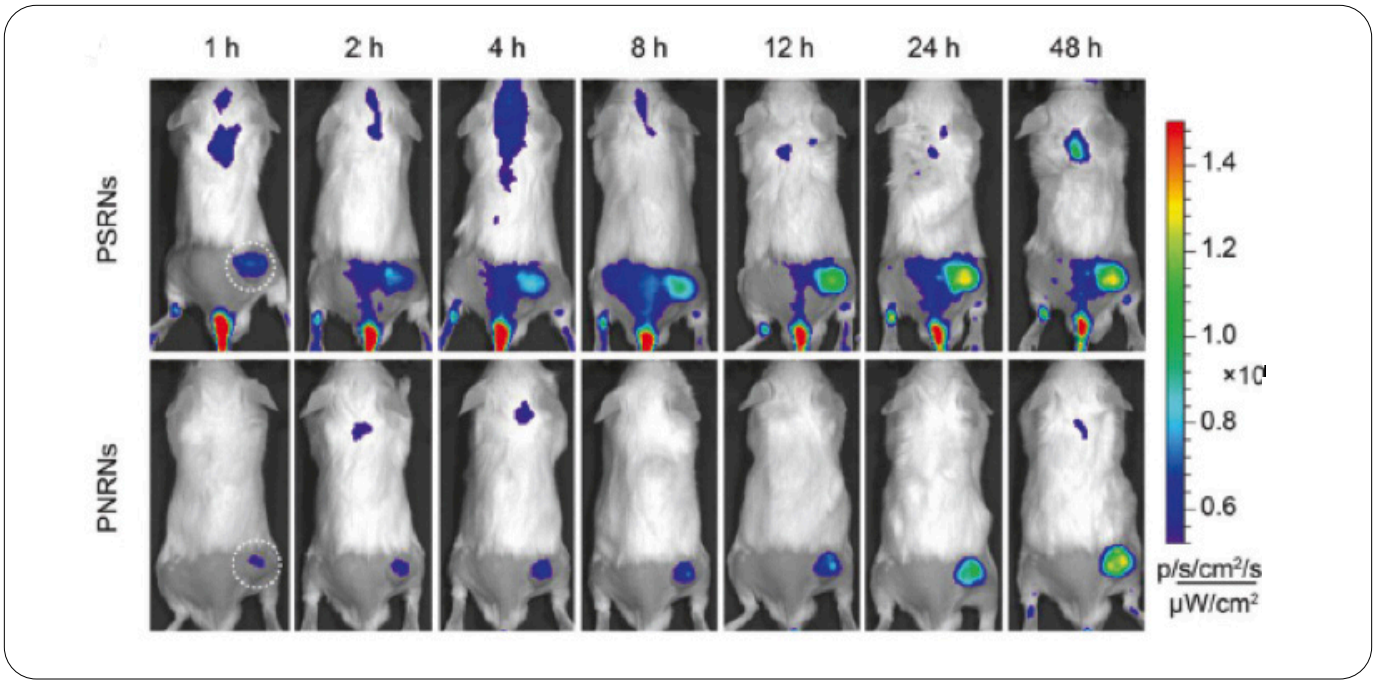

Figure 1: *In vivo* fluorescence images of CT26-tumor-bearing BALB/c mice at different time points after the intravenous injection of PSRNs and PNRNs. Image credit: Yang et al. Used under the Creative Commons CC BY 4.0 license.

Fluorescence imaging of major organs 24 and 48 hours following injection confirmed these biodistribution patterns. The IVIS system revealed substantial tumor accumulation of both PSRNs and PNRNs, with fluorescence intensities measured at 10.2 and 12.7 times higher than surrounding muscle tissue, respectively (Figure 2).

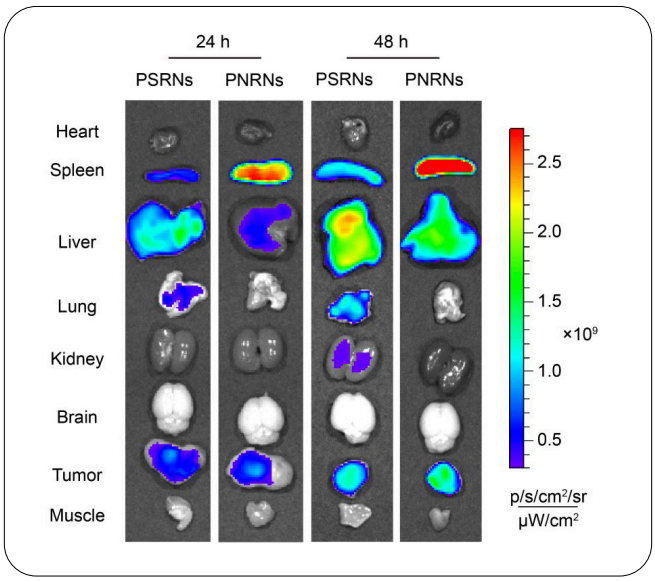

Figure 2: Fluorescence images of the major organs at 24 h and 48 h post-injection of Cy5-labeled preparations. Image credit: Yang et al. Used under the Creative Commons CC BY 4.0 license.

Immune modulation and combination with checkpoint inhibitors

Due to the limited efficacy of current colorectal cancer treatments, the researchers investigated whether PSRNs could enhance the effectiveness of immune checkpoint blockade (ICB) therapy. Flow cytometry analysis revealed that degradation of CDK4/6 led to increased PD-L1 expression in CT26 cells, potentially improving tumor susceptibility to ICBs.

In vivo imaging revealed that PSRNs were more readily internalized by regulatory T cells (Tregs) than PNRNs in tumor-bearing mice. Quantification of immune cell populations using the Cellometer cell counter showed a 1.6-fold higher uptake of PSRNs in Tregs than in CD8+ T cells. This higher uptake led to a reduced Treg/CD8+ T-cell ratio, suggesting that PSRNs could overcome tumor immunosuppression. When combined with ICB therapy, PSRNs extended the median survival time from 22 to 26 days, supporting their potential for combination immunotherapy.

Conclusion

This study demonstrates the potential of tumor-targeting nanoparticles to significantly enhance PROTAC delivery. By improving tumor penetration, cellular uptake, and intracellular release in a sequential manner, this approach addresses some of the key limitations of current PROTAC therapies. In addition, the flexibility of this platform enables the conjugation of different PROTACs, opening up possibilities for targeting of a wide range of tumor types.

Reference

Yang, L., Yang, Y., Zhang, J. et al. Sequential responsive nano-PROTACs for precise intracellular delivery and enhanced degradation efficacy in colorectal cancer therapy. *Sig Transduct Target Ther* 9, 275 (2024). https://doi.org/10.1038/s41392-024-01983-1