DRUG DESIGN

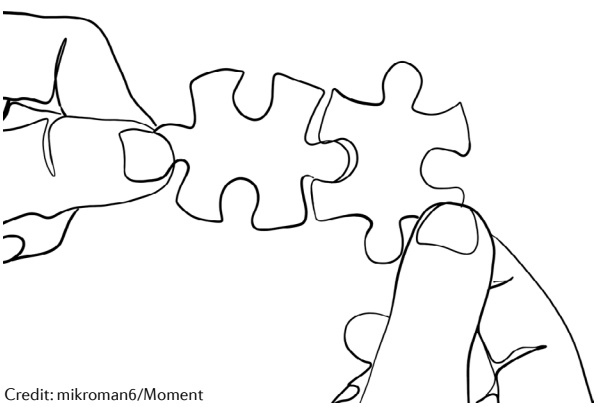
Harnessing the PERx of covalent protein drugs

Small-molecule drugs that bind to their target covalently rather than non-covalently can have desirable features such as greater potency and longer-lasting effects. The therapeutic potential of covalent protein drugs, however, remains unexplored as proteins generally don't bind targets covalently. Now, Li et al. have reported a new biotechnology platform that can generate covalent small-protein biologics by incorporating an unnatural amino acid into a selected protein, which then binds the target protein covalently. They demonstrate its potential by designing an inhibitor of the PD1–PDL1 interaction that has anticancer activity in a mouse model.

> "PD1(FSY) significantly reduced tumour growth compared with PD1(WT) and exhibited greater anti-tumour activity than the same dose of atezolizumab"

Although covalent binding between small-molecule drugs and targets can have benefits, off-target reactivity can be a key disadvantage, and some desirable therapeutic targets such as protein–protein interactions are inherently challenging to modulate with small molecules. These issues could be overcome by using covalent protein drugs, as proteins generally have higher specificity for their targets than small molecules and their larger size could be more effective at blocking protein–protein interactions.

Thus, Li et al. set out to develop a method to modify the reactivity of the protein drugs while maintaining their specificity. In this approach — which they have named 'proximity-enabled reactive therapeutics' (PERx) — they introduce in the protein drug an unnatural amino acid (in this case fluorosulfate-L-tyrosine (FSY)) that has latent chemical reactivity and remains inert inside the protein and in vivo. However, when the protein drug binds to its target, the unnatural amino acid gets close enough to a natural residue in the target for formation of a specific covalent bond to occur.



Credit: mikroman6/Moment

To test the approach, the authors chose to generate an antagonist that could bind the checkpoint ligand PDL1 covalently to block the endogenous PD1–PDL1 interaction. "While immune checkpoint inhibitors such as antibodies to PD1 or PDL1 are effective in a small fraction of solid tumours, there are many limitations of antibody therapy that limit its clinical application," explains Wang, one of the lead authors of the study. Some of these limitations are tumour penetrance or adverse Fc-effector functions. Thus, alternative approaches using molecules with lower molecular masses are actively being investigated.

The authors introduced the latent unnatural amino acid FSY into the ectodomain of human PD1 and showed that the resultant PD1(FSY) covalently bound to only PDL1, in vitro and on the cell surface of several cancer cell lines. In vivo, analysis of the xenografts from mice injected with PD1(FSY) intravenously through the tail or peritumourally, showed that PD1(FSY) bound PDL1 covalently, whereas wild-type PD1 (WT PD1) did not.

The authors then examined whether the covalent binding ability of PD1(FSY) could enhance T cell activation by blocking the endogenous PD1–PDL1 interaction. They saw that injection with PD1(FSY) inhibited tumour growth in a xenograft mouse model reconstituted with human peripheral blood mononuclear cells (given that PD1(FSY) binds human PDL1 but not mouse PDL1) much more efficiently than WT PD1 did.

Next, the authors explored the ability of PD1(FSY) to enhance the antitumour activity of chimeric antigen receptor (CAR) T cells, as approaches to improve the efficacy of CAR T cells in solid tumours are needed. To that end, they engineered human T cells to express a CAR specific for the ephrin type-A receptor 2 (EPHA2) and injected them into immunocompromised mice carrying tumour xenografts from H460 cells, which express EPHA2.

Injection of CAR-EPHA2 T cells slowed down tumour growth compared with no treatment, and additional WT PD1 improved this outcome. However, the combination with PD1(FSY) inhibited tumour growth even further to levels comparable with those achieved by a combination of CAR T cells and atezolizumab, a monoclonal antibody against PDL1.

Finally, the authors assessed the antitumour efficacy of PD1(FSY) in a more physiological, immune system-humanized mouse model, in which tumours develop an immune suppressive microenvironment. PD1(FSY) significantly reduced tumour growth compared with PD1(WT) and exhibited greater antitumour activity than the same dose of atezolizumab. "This study is a proof of concept that this new humanized solid tumour mouse model can provide a powerful preclinical platform to examine the efficacy and safety of immunotherapy," explains Xu, another lead author of the article.

The method reported by Li et al, PERx, provides a general platform technology to convert a wide range of interacting proteins into covalent binders, enabling the possibility to reach proteins that cannot be currently targeted by conventional noncovalent protein drugs. "We will apply this PERx strategy to various interacting protein pairs to generate protein drugs for different diseases, and to develop covalent protein binders to facilitate basic biological research," concludes Wang, another lead author of the study. "I think this work will lead to a new generation of protein therapeutics that work in covalent mode."

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