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AUTHOR'S VIEWS

Designing and repurposing drugs to target intrinsically disordered proteins for cancer treatment: using NUPR1 as a paradigm

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ABSTRACT

Intrinsically disordered proteins (IDPs) do not have a well-defined structure, but they have key biological tasks in cancer development. By using the disordered cancer-related protein NUPR1 as a proof-of-concept, we have developed a new multidisciplinary approach to tackle drug-design against IDPs, using it to repurpose drugs for treating pancreatic adenocarcinoma (PDAC).

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Intrinsically disordered proteins lack of stable secondary and tertiary structures. However, they carry out key biological functions, mainly associated with transcription modulation, signaling, cellular differentiation and cell division.[1] These processes are critical in the development of many diseases, and therefore intrinsically disordered proteins (IDPs) could be considered as potential drug targets.[2,3] Currently, only ~2% of all human proteins are targeted by approved drugs, and all of them are folded.[4] These proteins catalyze a chemical reaction, bind small molecules, or transport chemical species through a membrane. Interface regions in protein-protein interactions (PPIs) involving well-folded target proteins are relatively large (1000–6000 Å^2).[5] Conversely, IDPs function involves conveying a signal or modulating enzyme activity, through transient, non-catalytic complex formation, generally with a rather weak affinity.[1,6] As in the case of some well-folded proteins, drug-targeting an IDP would involve disruption of PPIs by a small, designed molecule. However, for IDPs, the protein-protein interfaces involved lack a clear pocket for ligand binding.[7] Therefore, designing drugs against an IDP will face the same challenges affecting inhibition of PPIs in well-folded proteins but, in addition, we shall have a larger proportion of potentially dynamic cavities, with drugs having a weaker affinity than in well-folded proteins, and moreover, we do not know whether the partner biomolecule will be folded or unfolded. Therefore, we need new strategies to tackle drug-design for IDPs, or a fresh re-thinking of the approaches we currently have.

We have recently faced these challenges when we have undertaken the effort to develop an inhibitor for NUPR1 (formerly known as p8, and now best known as NUPR1, UniProtKB O60356), an 82-residue-long (8 kDa), monomeric, basic IDP over-expressed in response to some, if not all, cellular stresses, and intervening in development of pancreatic ductal adenocarcinoma (PDAC). This protein plays key roles in apoptosis by interacting with other IDPs, and it is implicated in DNA binding and repair.[8]

By using NUPR1 as a proof-of-concept in designing drugs against an IDP, we followed a bottom-up approach as shown in Figure 1.[9] This multi-disciplinary endeavor implies the combined use of *in silico*, *in vitro*, *in cellulo* and *in vivo* techniques already at hand. In contrast to other procedures for designing drugs against IDPs,[2] our method does not rely on a complete experimental determination of the conformational ensemble of the IDP. Rather, it depends on the particular features of a screened compound, which is chemically modified, and studied, in complementary ways (*in vitro* and *in vivo*) to provide a full view of its mode of action (Figure 1).

In a first stage, we characterized the interactions between NUPR1 and several potential ligands by screening a collection

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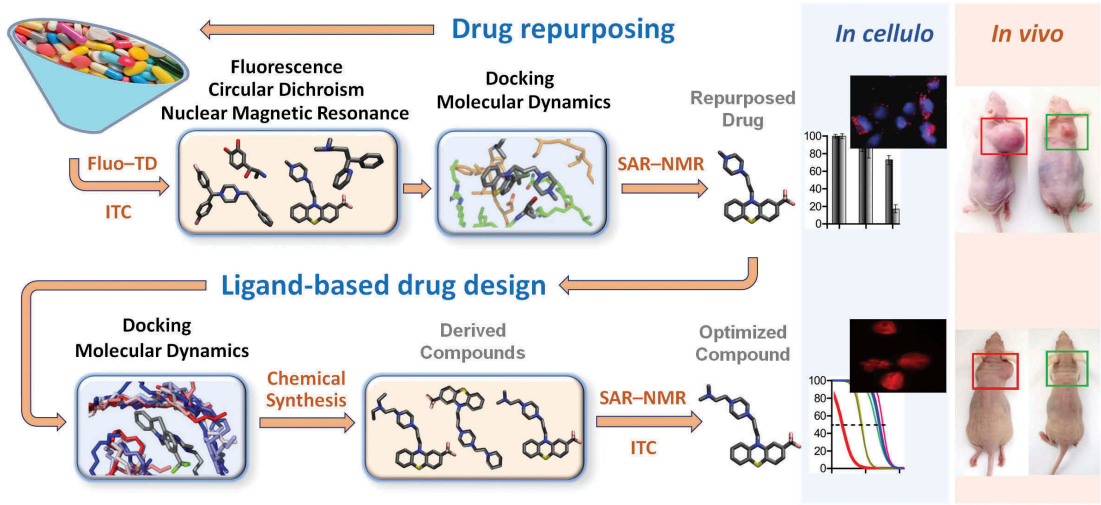

Figure 1. Multi-disciplinary approach to drug discovery. A complete workflow for drug selection and design: fluorescence thermal denaturation (Fluo-TD), isothermal titration calorimetry (ITC) and structure-activity relationships by NMR (SAR-NMR) can be used as screening methods for drug selection and design, and other spectroscopic techniques to guide the molecular simulations and modeling. The molecules selected (in our case, TFP), either repurposed drugs or optimized compounds, are tested using a variety of additional techniques *in cellulo* and *in vivo*.

of 1120 compounds approved by Food Drug Administration (FDA) (the Prestwick Chemical Library, http://www.prestwickchemical.com/libraries-screening-lib-pcl.html) using fluorescence thermal denaturation, on the basis of the largest shifts in thermal curves. Next, in parallel and blindly, we carried out a four-part strategy based on experimental and computational methods: (i) we determined the thermodynamic parameters of the binding reaction of the compounds to NUPR1 by using isothermal titration calorimetry (ITC); (ii) on the basis of spectroscopic restraints (mainly from fluorescence, circular dichroism and NMR), we performed molecular dynamics (MD) simulations to obtain an ensemble of NUPR1 conformations; (iii) we used this ensemble to dock the screened compounds; and (iv) we determined structure-activity relationships (SAR) by NMR with the complexes consisting in NUPR1 and the screened compounds (i.e. detecting residues affected in NUPR1 by the presence of the compound). The blind strategy combining SAR-NMR and MD simulations confirmed the validity of our approach, as we essentially observed a close analogy in the protein residues whose signals were affected by binding. The dissociation constants for the compounds measured by ITC were in the same order (micromolar range) as those found for the natural binding partners of NUPR1.[8] At the end of the procedure, we identified Trifluoperazine (TFP) as the compound with the largest affinity for NUPR1, and showing selective binding to the predicted protein residues. The use of TFP *in vivo* on human pancreatic cancer cells-derived xenografts implanted into immune-compromised mice showed that the tumor growth almost completely stopped in the animals treated with the drug.[9] Overall, in principle, this was a successful case of drug repurposing, and a notable pioneer attempt for an IDP. Unfortunately, the doses of TFP needed also led to neuronal side-effects: strong lethargy and hunched posture of the mice.

More recently, we tried to improve the anticancer effect and reduce the side-effects of TFP.[10] Thus, we extended our previous study by performing an *in silico* ligand-based design relying on a combination of MD and docking, which guided the organic synthesis of TFP-derived compounds. The new synthesized compounds were analyzed by ITC and SAR-NMR, and that with the smallest dissociation constant was selected. Moreover, the experiments *in cellulo* showed an evident increase of the anticancer activity on PDAC-derived cells and, *in vivo*, we found a total regression of the xenografted pancreatic tumor in mice, without side-effects. In addition, the modified TFP was also efficient against other cancer types, inducing concomitantly cell death by necroptotic and apoptotic mechanisms.

Our work[9,10] gives a proof-of-concept that drug-targeting IDPs are feasible. We started from a commercially available compound library and, then, we repurposed a drug; therefore, the approach may be equally applicable from scratch to identify new drugs. The key point in our workflow is that the lead compound must roughly comply with the particular biophysical targeted-protein features, and any modifications or redesign should still comply with those protein characteristics.

Disclosure of Potential Conflicts of Interest

The authors have filed a patent entitled "NUPR1 inhibition for treating cancer" on May 31, 2018 (European Patent Application n. EP18305672.0).

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