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Original article

Biochemical evaluation of virtual screening methods reveals a cell-active inhibitor of the cancer-promoting phosphatases of regenerating liver

Birgit Hoeger [a], Maren Diether [a], Pedro J. Ballester [b, c, **], Maja Köhn [a, *]

[a] European Molecular Biology Laboratory, Genome Biology Unit, Meyerhofstr. 1, 69117 Heidelberg, Germany
[b] European Molecular Biology Laboratory – European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton CB10 1SD, United Kingdom
[c] Inserm U1068, Centre de Recherche en Cancérologie de Marseille, France

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ABSTRACT

Computationally supported development of small molecule inhibitors has successfully been applied to protein tyrosine phosphatases in the past, revealing a number of cell-active compounds. Similar approaches have also been used to screen for small molecule inhibitors for the cancer-related phosphatases of regenerating liver (PRL) family. Still, selective and cell-active compounds are of limited availability. Since especially PRL-3 remains an attractive drug target due to its clear role in cancer metastasis, such compounds are highly demanded. In this study, we investigated various virtual screening approaches for their applicability to identify novel small molecule entities for PRL-3 as target. Biochemical evaluation of purchasable compounds revealed ligand-based approaches as well suited for this target, compared to docking-based techniques that did not perform well in this context. The best hit of this study, a 2-cyano-2-ene-ester and hence a novel chemotype targeting the PRLs, was further optimized by a structure–activity-relationship (SAR) study, leading to a low micromolar PRL inhibitor with acceptable selectivity over other protein tyrosine phosphatases. The compound is active in cells, as shown by its ability to specifically revert PRL-3 induced cell migration, and exhibits similar effects on PRL-1 and PRL-2. It is furthermore suitable for fluorescence microscopy applications, and it is commercially available. These features make it the only purchasable, cell-active and acceptably selective PRL inhibitor to date that can be used in various cellular applications.

1. Introduction

Cellular signaling processes are tightly regulated through phosphorylation and dephosphorylation of proteins and second messenger molecules by the action of kinases and phosphatases, respectively, thereby maintaining the correct function of cellular life. A perturbation in these highly controlled regulation processes can lead to the formation and progression of various diseases [1]. It is therefore critical to identify small organic molecules that inhibit unwanted hyperfunction of disease-promoting kinases and phosphatases; on the one hand to diminish disease progression through drug development, on the other hand to gain a better understanding of the biological mechanisms behind these processes [1].

The phosphatases of regenerating liver (PRL)-1, PRL-2 and PRL-3 are of particular interest as drug targets, because these putative oncogenes are strongly involved in cancer formation and progression [2]. They were shown to influence angiogenesis and metastasis

Abbreviations: DIFMUP, 6,8-difluoro-4-methylumbelliferyl phosphate; DSP, dual specificity phosphatase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PRL, phosphatases of regenerating liver; PTP, protein tyrosine phosphatase; PTP1B, protein tyrosine phosphatase 1B; ROCS, rapid overlay of chemical structures; SAR, structure–activity relationship; TCPTP, T-cell protein tyrosine phosphatase; UFSRAT, ultrafast shape recognition with atom types; UPLC-MS, ultra performance liquid chromatography–mass spectrometry; USR, ultrafast shape recognition; VHR, *vaccinia* H1-related phosphatase; VS, virtual screening.

* Corresponding author.
** Corresponding author. Inserm U1068, Centre de Recherche en Cancérologie de Marseille, France.
E-mail addresses: pedro.ballester@inserm.fr (P.J. Ballester), koehn@embl.de (M. Köhn).

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[3,4], each of them representing hallmarks of cancer [5,6]. PRL-1, PRL-2 and PRL-3 are highly overexpressed in various types of cancer [3]. PRL-3 is especially found in liver metastasis rather than the primary tumors [7], making it an important drug target as cancer metastasis is the major cause for poor prognosis in cancer due to the impossibility of surgery at this late state of disease [8,9].

Few normal functions of the PRL phosphatases are known: PRL-1 is an immediate-early response gene in regenerating liver and involved in cell differentiation, and the latter also appears to be the case for PRL-3. Additionally, all three PRLs have been suggested to regulate cell cycle progression [3]. The PRL phosphatases are members of the cysteine-based dual-specificity group of phosphatases (DSPs), which belongs to the protein tyrosine phosphatase (PTP) superfamily. Members of the DSPs not only are able to dephosphorylate phosphotyrosines like the classical PTPs but also to hydrolyze phosphomonoesters of serines, threonines, and non-protein substrates [10]. The sequence identities of PRLs compared to other phosphatases are low, but very high among the members of the PRL family. The PRLs are small (21 kDa), single domain proteins. Uncommon compared to other DSPs, their active site is both shallow and hydrophobic, allowing for the dephosphorylation of phosphotyrosines, phosphoserines and phosphothreonines as well as phosphoinositides (in the case of PRL-3) [3,8,11].

The development of inhibitors of the PRLs, and DSPs in general, is difficult. It is therefore not surprising that, despite their attractive features as drug targets, a drug targeting a DSP has yet to be approved. The challenge in developing phosphatase inhibitors as drug leads lays in often-found drawbacks of limited selectivity and poor pharmacokinetics [1]. This is due to the conserved active site geometry of many DSPs, and their preference to bind negatively charged entities [12]. In particular, finding selective active site inhibitors for one of the members of the PRL-family of phosphatases is a delicate task, since although their active site architecture is quite unique among phosphatases, they all share the shallow and hydrophobic binding pocket, making it difficult to find small molecules being able to only bind to one of the three PRL members [3]. So far, the developed small molecule PRL-inhibitors are either not selective when compared to other phosphatases, they do not show selectivity amongst the PRL-family, or they simply were tested for only one of the PRLs [13–19]. Selectivity within the PRL family may not be necessary for a drug lead as they are all putative oncogenes, but it is desirable to study their co-expression in cancers and potential redundancy in their roles as these aspects still need to be investigated in detail. The most potent cell-active inhibitor identified to date is thienopyridone, with a reported IC_{50} of 173 nM for PRL-1, 277 nM for PRL-2 and 128 nM for PRL-3 [17]. This compound, however, is not commercially available for applications in PRL biology research. Thienopyridone was found through high-throughput screening. Other experimental approaches to discover inhibitors included manual testing of single compounds based on their known cellular effect [19].

Virtual screening is an attractive alternative to search for PRL inhibitors. When a known ligand of the target is available, molecular shape similarity [20] is often used to find molecules with similar shape, but different chemical structure, to an active template (scaffold hopping). USR is a very fast shape similarity technique that has been successfully applied to the prospective identification of new inhibitors of a range of molecular and cell targets [21–26]. A recent pharmacophoric extension of USR termed UFSRAT [27] has also identified novel cell-active inhibitors of a type II diabetes target in prospective virtual screens [28]. Other popular ligand-based techniques include ROCS [29] for shape similarity and MACCS fingerprints [30] for chemical structure similarity. On the other hand, when a known structural model of the target is available, docking techniques can also be used for structure-based virtual screening. Here we use AutoDock Vina [31], which is one of the most accurate docking techniques. A molecular docking approach using a PRL-3 homology model was already applied previously leading to a rhodanine-based micromolar inhibitor of PRL-3. The selectivity of this compound was, however, not examined [13].

In this study, we aimed at elucidating which prospective virtual screening protocols are more suitable for this target while searching for new cell-active PRL inhibitors. Due to its role in cancer metastasis, we focused our computational screens on PRL-3.

Here we applied ligand-based USR, ROCS and MACCS fingerprints to search the same chemical library for similar molecules to thienopyridone. In addition, molecules with a similar shape to thienopyridone were docked into two structural models of PRL-3 using Vina and the resulting scores were used to re-rank these hits. Furthermore, a webserver implementing UFSRAT searches over a second molecular library (see Section 4 for further details) was also used to identify molecules with similar shape and pharmacophoric properties as thienopyridone.

Using a biochemical screen we determined prospective hit rates of the different methods for PRL-3, which suggest that ligand-based screens are more suitable for PRL-3 hit identification than structure-based screens. In particular, USR led to the discovery of a novel inhibitor chemotype for PRL-3, a 2-cyano-2-ene-ester. A following structure–activity-relationship (SAR) study based on this compound revealed a low micromolar, noncompetitive inhibitor of the PRLs with acceptable selectivity over other PTPs. The compound is non-cytotoxic, active inside cells as indicated by its selective reversion of the enhanced cell migration phenotype induced by the PRLs, and commercially available. In contrast to thienopyridone that showed to block cell proliferation independently of PRL-3, our inhibitor can be applied to long-term cellular studies as it does not show an effect on cell proliferation. Furthermore, fluorescence microscopy reveals its localization to the plasma membrane, which can greatly reduce possible off-target effects due to its co-localization with PRL-3. These properties make it currently the only molecule for a broad use in PRL research.

2. Results and discussion

2.1. In silico screens and their biochemical evaluation

Ligand-based virtual screening requires at least one molecule with affinity for the target of interest. Here we used thienopyridone as a search template in all ligand-based runs because this molecule is the most potent PRL-3 inhibitor to date [17] and has a rigid chemical structure, the latter meaning that its lowest energy conformer must be similar to its bioactive conformation. USR was run to search a database with 641,485,760 conformers generated from 3,472,461 lead-like molecules (see the Experimental section for further details), which was completed in just 57 s using a single computing core. ROCS required 128 h in screening the same conformers using the same computer. Unlike USR and ROCS, MACCS fingerprints is a 2D technique and thus operates directly on the chemical structures and therefore it does not require conformational expansion. Thus, searching the 3,472,461 chemical structures, encoded as the same number of MACCS fingerprints, for those with similar structure to thienopyridone took 24 s. At a second stage, we also used the UFSRAT webserver with thienopyridone as search template, which returned the top 200 molecules in a couple of seconds.

In parallel, we explored the use of structure-based virtual screens, although we were aware of the challenges that PRL-3 presents to this approach: the active site is extremely flexible, solvent-exposed and there are no X-ray structures available. We