Innovative 3D proteome-wide scale identification of ALKBH5 target for MV1035 small molecule able to reduce migration and invasiveness in U87 glioblastoma cell lines by SPILLO-PBSS

Alessio Malacrida,[1] Mirko Rivara,[2] Omar Ben Mariem,[3,4] Alessandro Di Domizio,[3,4] Giacomo Cislaghi,[3] Mariarosaria Miloso,[1] Valentina Zuliani,[2] Gabriella Nicolini[1]

[1]Experimental Neurology Unit and Milan Center for Neuroscience, University of Milano-Bicocca, Monza (MB); [2]Food and Drug Department, University of Parma, Parma; [3]SPILLOproject, Paderno Dugnano (MI, www.spilloproject.com); [4]Department of Pharmacological and Biomolecular Sciences (DiSFeB), University of Milano, Milano (MI), Italy

Abstract

The innovative *in silico* technologies developed at *SPILLOproject*,[1] *e.g.*, the *SPILLO potential binding sites searcher* (SPILLO-PBSS) software,[2,3] allow to identify targets and off-targets of any small molecule on a multiple-organism proteome-wide scale, and to perform an accurate *multilevel cross-organism transferability analysis* (MCOTA) aimed at rationalising animal testing. SPILLO-PBSS has been successfully used in several research projects, such as a study in which a compound (MV1035) was found to reduce migration and invasiveness in U87 glioblastoma (GBM) cell lines: the human structural proteome was analyzed and the RNA demethylase ALKBH5 has been identified as a target responsible for the observed effects (target experimentally validated). Another top-ranked target identified by SPILLO-PBSS, the DNA repair protein AlkB homolog 2 (ALKBH2), abundantly expressed in GBM cell lines, resulted particularly interesting for its pivotal role in the onset of resistance to Temozolomide (TMZ), the standard first-line treatment for GBM.[2]

Introduction

The effect of MV1035 on U87 glioblastoma cells seemed not dependent on its sodium channel blocking capability and alternative off-target interactions have been explored by SPILLO-PBSS on proteome-wide scale. Differently from traditional structure-based approaches (*e.g.*, molecular docking simulations), this software takes into account protein flexibility and recognizes targets and off-targets even when their binding sites are hidden or strongly distorted (*e.g.*, fully closed).[3] Because of this, SPILLO-PBSS performs better than other structure-based software (Table 1), as shown in the section 'Comparison of SPILLO-PBSS with other related available methods' of the reference paper.[3]

In this way, using SPILLO-PBSS made it possible to find alternative off-target interactions, among which the RNA demethylase ALKBH5 and the DNA repair protein ALKBH2[2] (not detectable by traditional structure-based approaches) where the binding site is closed and apparently "inaccessible" to the ligand.

Materials and Methods

Protein database preparation: all human proteins available in the RCSB PDB (14537 3D-structures from X-ray diffraction or solution NMR, September 2017), excluding redundancies. *RBS generation*: reference binding site including 15 amino acid residues directly interacting with MV1035, suitably designed according to molecular modelling techniques and the standard RBS generation protocol described in SPILLO-PBSS paper.[3] *Screening and ranking of the protein database*: systematic and unbiased search for the MV1035 off-targets and identification of the top-ranked (Figure 1).

Correspondence: Omar Ben Mariem, SPILLOproject, Paderno Dugnano (MI, www.spilloproject.com); Department of Pharmacological and Biomolecular Sciences (DiSFeB), University of Milano, Milano, Italy.
E-mail: omar.benmariem@spilloproject.com

Key words: Targets and off-targets identification; ALKBH5; ALKBH2; animal testing reduction; multilevel cross-organism transferability analysis (MCOTA).

Disclosures: The authors declare that they have no known competing financial interests.

Conference presentation: This paper was presented at the Third Centro 3R Annual Meeting - L'era delle 3R: modelli *in silico, in vitro* e *in vivo* per promuovere la ricerca traslazionale - 30 September - 1 October 2021, Evento online organizzato dal Politecnico di Torino.

Received for publication: 9 July 2021.
Accepted for publication: 7 September 2021.

Biomedical Science and Engineering 2021; 4(s1):178
doi:10.4081/bse.2021.178

SEARCH METHOD	BINDING SITE CONFORMATIONS			
	SUITABLY OPEN	WIDE-OPEN	WIDE-OPEN and OCCUPIED	COMPLETELY CLOSED
GEOMETRIC POCKET SEARCH	✓	✗	✓	✗
CHEMICAL POCKET SEARCH	✓	✓	✗	✗
BINDING SITE COMPARISON	✓	✗	✗	✗
BLIND DOCKING	✓	✓	✗	✗
MONTE CARLO SEARCH	✓	✓	✗	✗
SPILLO-PBSS RBS-BASED FLEXIBLE SEARCH	✓	✓	✓	✓

Table 1. SPILLO-PBSS compared to traditional structure-based approaches

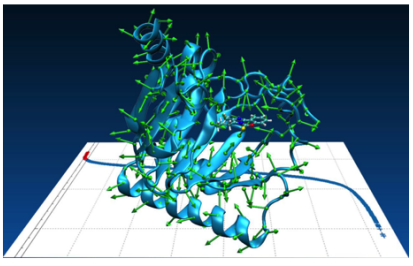

Figure 1. Graphical representation of the SPILLO-PBSS ranked human 3D-structural proteome

Results and Conclusions

SPILLO-PBSS identified on a proteome-wide scale the RNA demethylase ALKBH5 and the DNA repair protein ALKBH2 as targets of MV1035. This brand new molecule was found to reduce migration and invasiveness of U87 glioblastoma cell line and has a great potential in reducing TMZ resistance. The successful experimental validation of the predicted targets confirmed the unique potentialities of this *in silico* approach to optimize various phases of the drug discovery and development processes, including better use of animal testing. Moreover, to evaluate if the described *in vitro* inhibitory effect is also maintained *in vivo*, any further animal test may be rationally designed in compliance with the guidelines provided by the 3Rs principles. In particular, taking advantage of the precise knowledge of the SPILLO-PBSS predicted off-targets of MV1035, along with the structural details provided by this software, an innovative *multilevel cross-organism transferability analysis* (MCOTA) could allow a careful choice of the most suitable model organism and a rational design of highly targeted *in vivo* experiments, as already implemented in other recent similar projects.[4]

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