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Type	Oral communication
Session	Structural Bioinformatics
Title	Targeting Potential Binding Pockets in HIV-1 Matrix Protein for Novel Anticancer Agents
All Authors	A. Rondina(1), S. Messali(1), A. Zani(1), P. Fossa(3), F. Caccuri(1), A. Caruso(1), P. D'Ursi(2)

Affiliation

(1) Section of Microbiology Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy
(2) Institute for Biomedical Technologies, National Research Council (ITB-CNR), 20054 Segrate (MI), Italy
(3) Department of Pharmacy, Section of Medicinal Chemistry, School of Medical and Pharmaceutical Sciences, University of Genoa, 16132 Genoa, Italy

Motivation

Lymphoma represents the main cause of death among HIV-1 infected patients. HIV-1 matrix protein (refp17), which is known for its fundamental role in viral assembly and maturation, can undergo mutations and insertions along its protein sequence. These variants (vp17s) are responsible for the B-cell clonogenic activity in HIV-1 seropositive patients with lymphoma, suggesting their possible role in lymphomagenesis through activation of the EGFR/PI3K/Akt signaling pathway (Giagulli C. et al., 2017). The functional epitope spanning from amino acid 2 to 21 is responsible for promoting B-cell proliferation and growth. Computational studies carried out so far by our research team have identified key residues (Trp16 and Try29) involved in the clonogenic activity of the vp17s. These residues make hydrophobic interactions with a conserved hydrophobic pocket on the protein, anchoring the epitope and enabling its functionality within the globular domain of p17. Molecular dynamics have shown that in all variants the two residues escape from the hydrophobic pocket; afterwards, the functional epitope moves away from the globular domain, becoming unstable and thus available for interaction with the receptors mentioned above. In this study, we validate these hypotheses in vitro and carry out a drug repositioning strategy aimed at positively helping the drug design of novel anticancer agents in AIDS oncology.

Methods

To validate the computational data in vitro, we produced the recombinant p17 proteins carrying mutation at positions Trp16 and Tyr29, alone or in combination. We obtained three recombinant proteins, two with the single amino acid mutations (W16A or Y29A), and one with the double mutation (W16A and Y29A). Each protein was tested for its ability to promote clonogenic activity by performing the B-cell colony formation assay. Regarding computational studies, given the validation of experimental data, accelerated molecular dynamics simulations (aMDs) were extended to 2 micron to better explore the conformational space of vp17s (NHL-a101, NHL-a104, and vp17c2). The most representative conformations resulted from cluster analysis were subsequently used for a binding pocket analysis, using Fpocket. Based on the found evidence, an exhaustive virtual screening was performed using a ligand library containing AIFA approved drugs. Drug repositioning was performed using the Autodock Vina software with default parameters, except for the exhaustiveness set to 48. From the obtained results, poses were filtered based on an already established drug repositioning pipeline, developed by our research group.

Results

To confirm the findings of previous computational studies, alanine scanning mutagenesis strategy was used to assess the significance and role of key residues involved in the clonogenic activity of vp17s. Three recombinant refp17s (Trp19A, Tyr16A and Trp19A plus Tyr16A) were tested for their capability of modulating B-cell growth by clonogenic assay. Data obtained demonstrated a significant increase of B-cell proliferation by recombinant proteins compared to untreated cells or cells treated with refp17. We have thus been able to demonstrate that introducing specific mutations into the backbone of refp17 could induce clonogenic activity like in vp17s, thus validating the conformational mechanism hypothesized by us. The same test was carried out in the presence of the p17 neutralizing monoclonal antibody MBS-3, with an unrelated antibody (anti-p24) as a control, or with sera collected from vaccinated patients containing neutralizing antibodies. Both tests confirmed that the mutated p17 proteins as well as the vp17s were neutralized. Therefore, the results suggest the need to focus on potential binding pockets in the proximity of residues 10-20 and proceed with drug repositioning studies to inhibit the clonogenic activity of vp17s. Binding pockets analysis was performed on the most representative cluster conformations, followed by a drug repositioning using a home-made ligand library. The analyses identified two potential binding pockets located in the N-terminal region, in a position able to hypothetically interfere with those residues previously identified as being responsible for clonogenic activity (Arg15, Lys18, and Arg20) and thus preventing interactions with possible receptors (He W. et al., 2018). Based on the binding poses obtained from the repositioned drugs, it seems that only one of the two pockets under consideration can accommodate a ligand that can interact with the residues critical for clonogenicity. Moreover, the arrangement of the binding modes suggests the possibility of applying a fragment-based approach or a pharmacophore approach to develop new ligands. Regarding the latter, virtual screening results will be used to create a pharmacophore, which will then be used to filter the

most similar molecules in databases (such as the FDA and natural products) to identify the most promising hits.

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Corresponding Author	
Name, Surname	Alessandro, Rondina
Email	a.rondina001@unibs.it
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