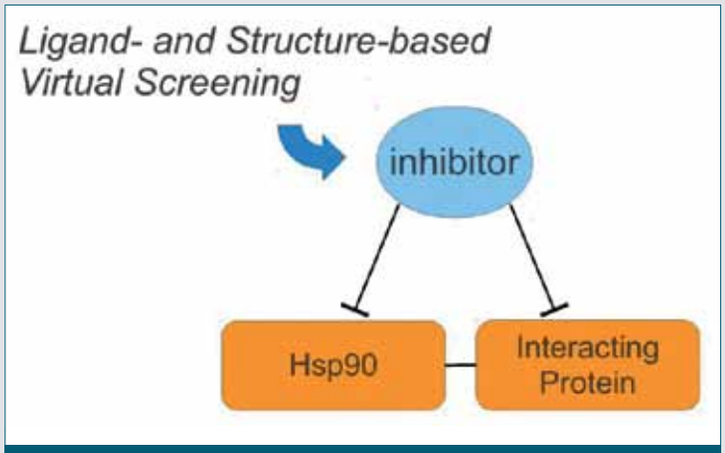

Schematic representation of the aim of the project: use of combined ligand- and structure-based virtual screening techniques to discover potential multi-target inhibitors of the Hsp90 interactome

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RICERCA

TARGETING THE HSP90 INTERACTOME USING IN SILICO POLYPHARMACOLOGY APPROACHES

Hsp90 and its interactome represent an attractive array of targets for polypharmacological drug design strategies in cancer therapy. In this work, we propose a computational protocol aimed at the selection of promising target combinations and potential multi-target active compounds.

Introduction

In recent years, polypharmacology has gained popularity in drug discovery [1]. Especially for complex diseases such as cancer, the ability of a drug to bind to and interfere with multiple targets provides new opportunities for therapeutic intervention. In this article, we focus on Hsp90 and its interactome, whose pivotal role in survival and proliferation of cancer cells renders this array of targets particularly attractive for polypharmacological drug design strategies.

The primary goal of our work is the identification and selection of suitable target proteins from the interactome that might be combined with Hsp90 to explore and exploit a multi-target inhibition approach. This task is accomplished by applying computational methods to mine the structural and biological information associated with potential ligands in public databases and assess the degree of structural similarity between known inhibitors of different targets. Therefore, we propose an integrated ligand- and structure-based approach to select small molecules from databases suitable for consideration as multi-target inhibitors.

Biological background

Cancer is one of the world leading causes of death accounting for 7.6 million deaths in the year 2008. The number of deaths per year is projected to rise to 13.1 million in 2030 [2]. Both genetic and environmental factors may be at the origin of human cancers. In general, cancer cells are characterized by uncontrolled proliferation and survival. Indeed, several cellular pathways might be affected by this pathology contributing to the expression of the transformed phenotype. Heat shock response is often activated in cancer cells, contributing to both initiation and maintenance of the transformed phenotype. Hsp90 is an important component of this process, acting as a molecular chaperone affecting stability and activation of more than 200 client proteins through ATP hydrolysis cycles [3]. Proteins interacting with Hsp90 are often involved in signalling processes, such as transcription factors and kinases.

Polypharmacology and Hsp90

Popular computational methods applied in medicinal chemistry include ligand- and structure-based virtual screening. These techniques aim at finding new small organic molecules with specific activity against a given biological target. Typically, the target is a protein that might act as an enzyme, a receptor, or an ion-channel, whose activity is involved in a pathological mechanism. The interaction of the small molecule with the target is intended to interfere with the protein's activity in a physiologically relevant manner. Following the classical drug design paradigm, the small molecule should be highly selective for the chosen target. Interactions with additional targets might be the cause of the so called off-target effects, which in turn might be responsible for toxicity and thus impair the development of a safe, marketable drug.

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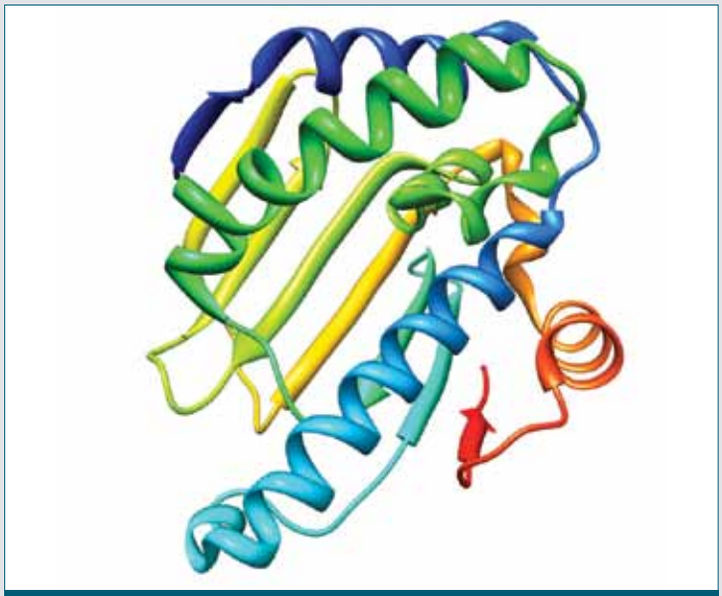

An X-ray crystal structure of Hsp90

The idea of targeting multiple proteins involved in a given pathology was initially applied by prescribing multiple drugs in combination therapies. Nevertheless, some drugs initially designed to be target-selective have been proven later on to exert their therapeutic activity through the simultaneous interaction with multiple targets. Hence, the paradigm of trying to design drugs acting on a single target has recently been extended by the concept of polypharmacology, which aims at rationally develop therapeutic agents that interact simultaneously with multiple targets. In principle, this approach has the advantage that it could cause an improved therapeutic effect and bypass complications associated with the prescription of two or more drugs in combination therapies. Designing compounds acting on multiple targets is a complex task but, if successful, might often lead to more efficient drugs (in therapeutic areas where target specificity is not a stringent requirement). In this context, a key question is which combination(s) of targets should best be considered for a polypharmacology approach to treat a particular disease.

Given the pivotal role on Hsp90 in cancer, the development of Hsp90 inhibitors has long been a major focal point in oncological drug discovery [4, 5]. The most important chemical classes of Hsp90 inhibitors include, among others, ansamycins, resorcinol derivatives, purines, imidazopyridines, oxazoles. Fourteen drug candidates targeting Hsp90 are now undergoing clinical trials, but none of them has as of yet achieved market approval.

Because Hsp90 is a key node in many biological networks and interacts with a very large number of client proteins and co-chaperones [4], this target and its interactome are thought to be highly relevant for polypharmacology approaches. However, polypharmacological approaches to target Hsp90 are still largely unexplored.

Workflow and results

For rational polypharmacological ligand design targeting the the Hsp90 interactome, a computational procedure has been designed that consists of several steps, the first of which is an activity annotation analysis in public compound databases to collect known inhibitors of Hsp90 and its client proteins.

So identified inhibitors have been used as templates for ligand-based virtual screening of the ChEMBL [6] and ZINC [7] databases. Then, the analysis of the top-ranked database compounds taking activity annotations as well as implications of their targets in cancer into account led to the selection of most promising candidates for interference with Hsp90 in combination with secondary targets. For each target combination selected, a focused compound library has been built with the purpose of finding new multi-target active compounds without any known target annotation. Finally, structure-based virtual screening of these focused libraries against Hsp90 and the secondary targets has been carried out to further refine the selection and evaluate more thoroughly the potential affinity of pre-selected compounds.

Conclusions

The analyses conducted so far suggest that public molecular databases contain a wealth of information about compounds active against the Hsp90 interactome. We have analyzed this information comprehensively and utilized it to design a computational polypharmacological approach. Following our approach, suitable target combinations have been identified and integrated ligand- and structure-based virtual screening applied to identify candidate compounds for multi-target inhibition. Importantly, our computational approach to Hsp90 polypharmacology will be complemented by experimental evaluation of candidates and subsequent iterations of integrated virtual screening.

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RIASSUNTO

Approcci polifarmacologici in silico mirati all'interattoma di Hsp90

Hsp90 ed il suo interattoma sono bersagli attraenti per l'applicazione di strategie polifarmacologiche nella terapia antitumorale. In questo studio viene proposto un protocollo computazionale mirato alla selezione di combinazioni promettenti di bersagli e di potenziali composti attivi su bersagli multipli.