Short Communication

Developing Novel Anticancer Drug Candidates Regarding the Integration of Three Main Knowledge Fields: Computer-Aided Drug Design, Chemical Synthesis, and Pharmacological Evaluation

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Abstract

New small drug like compounds are developed based on the selection of more specific targets considering the interrelationship of three knowledge fields: rational drug design/computer-aided drug design (CADD), chemical synthesis and biological evaluation. It is well-known that the use of CADD strategies in the early stage of drug development can avoid the synthesis of thousands of compounds, driving the efforts to more promising compounds (having suitable pharmacodynamics and pharmacokinetic features as well as low toxicity), reducing the number of biological assays to be performed and, consequently, decreasing the use of animal experimentation. Also, the time and costs involved in the entire radical innovation process can also be reduced, and the chances of success may significantly increase to reach the final product. Herein, we have pointed out some aspects related to the early development phase of anticancer drug candidates concerning the integration of those three main knowledge fields.

INTRODUCTION

Aspects to be considered in the development of antineoplastic chemotherapy

There is a very difficult task to accurately date the onset of cancer treatment, which was initially based on preparations using herbs and other natural sources [1]. The clinical chemotherapy treatment, however, began in 1940 with the discovery of the nitrogen mustard antitumor properties. Its therapeutic effect was evaluated in the animal model using mice transplanted with lymphoid tumors [2].

The revolution in cancer treatment, post nitrogen mustard era, has begun since the development of antimetabolites (methotrexate), though, reaching the present moment with the use of multi-kinases inhibitors, such as sunitinib, and antiproliferative agents, such as those derived from taxol

[3]. Despite the compounds arsenal currently available, the chemotherapeutic treatments have been still considered as highly inadequate and not selective, causing serious side effects [4,5]. In this regard, novel approaches have been adopted in the search for new antitumor drug candidates, emphasizing the selectivity on tumor cells [6]. Novel agents capable of inducing apoptosis cell death, for instance, have shown promising antitumor activity in preclinical models and lung cancer [7]. In fact, such agents, acting on modulation of the anti-apoptotic protein Bcl-2 combined with agents acting on the death receptor (TRAIL, TNF-related apoptosis-inducing ligand, TNF, tumor necrosis factor), currently represent the main interest regarding the therapeutic innovation process in lung cancer [8,9].

The identification and definition of potential new targets for rational designing new drug candidates, more specific and selective, require knowledge on molecular, cellular, and

structural biology fields [10,11]. It is noteworthy to mention that rational strategies for drug development have also considered the complementary findings provided by molecular imaging probes, reinforcing the need for developing novel diagnostic techniques for imaging to innovate in the drug discovery field. In general, molecules such as growth factors or cytokine receptors are expressed during carcinogenesis and may contribute to the cancer cell growth. Therefore, these molecules tend to be reasonable targets for molecular imaging of cancer, for instance [12]. Furthermore, other issues need to be addressed regarding the designing and development of novel chemotherapeutic compounds, such as the antitumor activity intensity, the toxicity level, and the ability to manage drug resistance which is caused by the inherent genomic instability of tumors [13,14].

Unfortunately, concerning the therapeutic approaches available for cancer, the not much promising trajectory has been observed regarding "innovation" over the last decades [15]. Regarding pharmaceutical formulations, targeted delivery systems of therapeutic drugs and diagnostic agents to cancer sites have been reported as potentially significant to improve the therapeutic outcome of treatment while minimizing severe side effects. Since cancer cells exhibit a variety of overexpressed cell surface receptors, target candidates have been provided for selective drug delivery systems. Therefore, the addition to drug delivery systems of targeting ligands, which bind specifically to the receptors on the cancer cells, has been considered as a promising strategy for enhancing substantially the anticancer agents' accumulation in the tumors, for instance [16].

In order to improve the innovation scenario in drug development field, the use of integrative approaches should be considered not only to identify lead compounds more specific to potential molecular targets but also to define and validate novel promising molecular targets in neoplastic cells. Moreover, natural products, such as animal toxins, and small synthetic molecules should be employed side by side as part of the investigation process [17,18].

Interactive research cycles in the anticancer drug candidates' development

In the last 20 years, there has been a substantial change in the investigation of novel molecular targets for treating cancer, combining new and traditional methods or strategies. The main issue, however, relies on seeking the integration of experts in different scientific fields aiming at studying particularly potential targets and related signaling pathways in tumor cells, concerning several aspects, such as molecular and cellular biology, structural information, clinical features, and so on [19]. This interdisciplinary raises optimism about the likelihood of finding new selective antitumor drug candidates having minor cytotoxic side effects, which are commonly associated with the conventional chemotherapy. As in other therapeutic areas, the success of discovering innovative compounds depends on a creative interaction, primarily, among medicinal chemistry, organic chemistry, and biology/pharmacology fields. Also, the advances in genomics and proteomics have increased the chances of identifying novel and significant molecular targets related to signaling pathways of interest, allowing the designing

of more specific compounds based on the structural information available.

Thus, novel antitumor compounds can be discovered throughout the integration of interactive research cycles, starting mainly with the definition of a potential molecular target or related signaling pathway. Then, considering the structural information available, prototypes or ligands can be rationally designed by applying computer-aided drug design (CADD) strategies to drive the chemical synthesis of those compounds which would be more promising. Those compounds, after being synthesized, will be experimentally assayed, allowing the validation of the biological/antitumor effects using *in vitro* and *in vivo* models (proofs of concept, POC).

CADD strategies driving chemical synthesis in the development of new antitumor drug candidates

Since the period when empirical screening was used to discover new drugs, medicinal chemistry, and organic chemistry fields have contributed to the development of many synthetic cytotoxic compounds, as the class of nitrogen mustards. The application of medicinal chemistry to sulfur mustard gas also led to the development of mechlorethamine and its analogues, chlorambucil, and cyclophosphamide, which are still clinically useful in the treatment of malignant solid tumors and leukemia [10,20,21].

Furthermore, the screening of natural products had an important role in the introduction of novel antitumor compounds, such as anthracyclines, Vinca alkaloids, epipodophyllotoxins, and taxanes, as well. Nowadays, however, the research in this area is more focused on the discovery of new evidence regarding the compounds' mechanisms of action, instead of just identifying novel bioactive agents [22]. One reason for that, at least partially, relies on the need to better understand the antineoplastics' physicochemical properties which are directly responsible for the compounds' pharmacokinetic and pharmacodynamics profiles. Another reason relies on the fact that natural products are not the result of an evolutionary selection process to exert a desired specific function, in this case, the antitumor activity [23]. Of note, natural products presenting biological activities of interest would certainly suit well as an inspiration source for the designing and chemical synthesis of new antitumor drug candidates, though. The goal would be finding hit compounds to progress them to the lead and development candidate stage concerning the modification of disease states.

The rational drug design approach, however, is based on the knowledge of the molecular target (enzyme, receptor, ion channel, signaling protein, transport protein, DNA) mostly implicated in the biochemical/signaling pathways involved in the disease process which one intends to fight against, supporting the discovery and development of more specific and efficient compounds [10,24].

The progress in molecular and structural biology have allowed the identification and characterization of several hundreds of novel molecular targets making possible to envisage the design of novel drug candidates at a more scientific level. In addition, different CADD strategies can be considered depending

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on the structural information available. For instance, when the three-dimensional (3D) structure (X-ray diffraction or NMR) of the complex ligand-target is available the structure-based drug design (SBDD) strategy can be applied, and that would be considered the ideal situation for designing new drug candidates. Otherwise, when only the ligands' 3D structural features are available, the ligand-based drug design (LBDD) strategy can be performed. The *de novo* strategy, however, considers the construction of novel ligands based only upon the molecular target 3D structural information [10,25,26].

In all CADD strategies, different molecular modeling and computational chemistry methods as well as chemometrics and quantitative structure-activity relationship (QSAR)/quantitative structure-property relationship (QSPR) approaches can be applied to drive the chemical synthesis for more promising designed compounds. However, *in silico* methods and approaches have limitations, which should be taken into account by the users to avoid false conclusions. Despite to have a great potential, one should not rely on computational techniques in a black box manner and beware of the "garbage in-garbage out" phenomenon. The *cerebral* element is still an essential and critical part of the process.

Molecular modeling and computational chemistry methods, which also include classical mechanics and quantum chemistry [27], have provided a better understanding of molecular systems allowing, for instance, the calculation of molecular properties of distinct natures, such as electronic, hydrophobic, structural/conformational, topologic, geometric, steric, and so on [27-29]. Of note, molecular properties are directly dependent on the compound's chemical structure, and are also responsible for the ligand-target recognition process, at the molecular level, generating the biological response. Then, be aware of the molecular properties related to a molecular system will provide the establishment of qualitative structure-activity or structure-property relationships (SAR or SPR), allowing the definition of the essential structural requirements (chemical framework) for generating the desired biological activity, contributing significantly to drive the designing and synthesis of more promising compounds.

Quantitative approaches (QSAR or QSPR), however, which use various statistical and mathematical tools, are even more powerful to guide the novel compounds designing process. Those formalisms involve the construction and statistical validation of robust predictive QSAR models, which allow the biological activity calculation for novel designed compounds even not yet synthesized [30-33] driving, then, the selection of those more promising to follow the chemical synthesis step to be experimentally tested.

Furthermore, when the 3D molecular structure of the target is available, another approach to reinforce the selection of potentially bioactive compounds would be the molecular docking. This approach can be used in combination with the QSAR formalism, for instance, to have also the calculated binding affinity data regarding those compounds considered more promising to be synthesized [31,33].

Thus, the *in silico* methods/approaches have played an important role in the modern era for discovering new drug candidates, including antitumor agents. They have contributed to establish hypothesis regarding the compounds' mechanisms of action as well as to predict the compounds' pharmacokinetic and pharmacodynamics profiles, through mathematical models [34,35].

FINAL REMARKS

The advances in the development of antitumor drug candidates by integrating the rational CADD strategies, chemical synthesis, and experimental biology, represent a great hope to innovate especially regarding the cancer therapeutic alternatives, providing novel chemical entities, more target-specific and less toxic, improving the patients' prognosis still suffering from the disease.

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