In Silico and Cervical Cancer

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High-Risk (HR) HPV E6 and E7 oncoproteins are considered biomarkers in cervical cancer progression. Several small molecules (plant-derived or synthetic compounds) have been reported as blockers/inhibitors of E6 oncoprotein action, and computational-aided methods have shown of high relevance in their discovery and development. *In silico* approaches have become a powerful tool for reducing the time and cost of the drug development process.

1. Introduction

Cancers are some of the deadliest pathologies, and according to Globocan, the global cancer burden in 2020 increased to 19.3 million cases and 10 million cancer deaths. With these new data, it is estimated that 1 in 5 people will develop cancer during their lifetime, and 1 in 8 men and 1 in 11 women will die from the disease [1]. Thereby, as logical consequence, novel pharmacotherapy approaches have increased in the recent years [2]. In particular, cervical cancer (CC) is considered the fourth cause of death among women worldwide with its establishment being associated with human papillomavirus (HPV) infection [3]. Considering the data available by Globocan regarding this pathology, there were 341,831 deaths in 2020 with a higher incidence in low-income countries (LIC). In fact, in less developed regions such as Africa, Asia, and South America, CC is the primary cancer found in women, which can be due to the lack of screening programs, limited resources and access to health care, or even anti-vaccination movements [4][5]. In developed countries, the incidence of CC cases is lower due to better health services and the high availability of HPV prophylactic vaccines, which constitutes a great step in the prevention of HPV-associated cancers. However, the prophylactic vaccines have only been effective when administered in healthy patients, and they are not able to exert a therapeutic effect or treat an established infection [6].

The current treatments for CC are based on excisional or ablative procedures, surgical resection, radiotherapy, or chemotherapy, which do not specifically target the oncogenic properties of HPV, and therefore lesion recurrence can occur [7]. In addition, most of these procedures can affect normal tissues and can have potential side effects, including bleeding, which cause patient discomfort and can reduce life quality [5]. These constraints highlight the need to improve the current therapeutic approaches by combining strategies or proposing new compounds to offer more specific and less invasive treatments, without affecting healthy tissues. Hence, the scientific community has been focused on different ways to combat CC. A strategy with great potential consists of finding new anticancer agents by targeting the major oncoproteins responsible for HPV-driven carcinogenesis, E6 and E7. In fact, the discovery of the E6 protein X-ray crystal structure, available in protein data bank (PDB ID: 4GIZ and 4XR8, accessed on 20th May 2021), led to an increase in the use of in silico approaches to uncover potential E6 inhibitors [8].

Drug discovery and development is a very expensive and time-consuming process, which can take 10 to 15 years until a drug reaches the market. In the last decades, the pharmaceutical industry has been employing computer-aided drug design (CADD) techniques to accelerate drug development, intending to reduce time, costs, and failures, namely in the final stage [9][10]. This analysis is based on calculated properties and prediction models for drug therapeutic targets and identification of safety liabilities. Typically, CADD can be divided into three categories: structure-based, ligand-based, and hybrid methods [11][12]. Structure-based approaches, including docking and the application of molecular dynamics simulations, use the 3D structure of the target molecule to screen potential ligands. These methods evaluate ligand recognition by the target molecule and the prediction and characterization of binding sites as well as binding affinity [9][12]. For instance, molecular docking is one of the most applied techniques to select promising molecules from large libraries by predicting the orientation of a compound towards the target and characterizing ligand–target interactions. Molecular dynamics techniques entail the motion principles to molecules and are frequently used to perform binding mode studies and to predict the stability of a ligand–target complex, giving a deeper understanding to the researchers on the interaction of a ligand to a biomolecular target [9][11][12][13]. On the other hand, ligand-based methods, including similarity searching,

pharmacophore modeling, and quantitative structure–activity relationship (QSAR) studies, use the information of groups of small molecules with different structures capable of interacting with the target to identify new and powerful compounds [12] [14]. These methods are usually applied when the 3D structure of the target is not available and assume that analogous compounds show similar biological activity and interaction with the target. QSAR modeling allows understanding the effects of structural variables on biological activity to develop compounds with enhanced and optimal pharmacological profiles. Another ligand-based method is similarity searching, which is mostly applied in filtering compounds from big libraries based on the assumption that compounds with structural similarity can have similar bioactivity [9]. When the 3D structure of the target is available, as well as the ligands' structure, it is possible to use hybrid methods. This means combining structure-based and ligand-based methods to perform some types of pharmacophore modeling or to predict the activity considering the biological profile of tested compounds against several targets [12]. In fact, combining CADD methods can be more effective once their advantages complement one another [11][12]. Given the importance of pharmacokinetics and toxicity properties of selected compounds, in silico ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) filters can also be applied to eliminate compounds with potential undesirable physiological qualities [9]. With the improvement of technology and bioinformatic tools, the use of in silico approaches for drug development, mainly in preliminary studies, has increased over the years.

Hence, in this review, we intend to summarize the current treatment used for CC stages induced by HPV persistent infection, present the small molecules that are already being explored as inhibitors/blockers for E6 protein, and retrospectively analyze the studies published in the last years that applied in silico approaches to the design of novel therapeutics for CC treatment. In addition, we will concisely discuss the future perspectives for CC management.

2. HPV, CC Development, and Clinical Treatment

HPV is the etiological agent associated with CC. There are about 200 HPV genotypes of high-risk (HR HPV) and low-risk (LR HPV) identified. HPV16 and 18 are responsible for more than 70% of invasive CC. The HPV genome presents tropism for epithelial cells, and the infections appear mainly in the anogenital tract [3][5][15] and head and neck anatomic sites. While HPV is the hallmark of CC development [15], the classic major risk factors to head and neck cancer are tobacco and alcohol, but in the past few decades, human papillomavirus (HPV) has emerged as a novel risk factor [16][17]. The biology and life cycle of human papillomavirus have been reviewed elsewhere [18]. However, we considered it helpful to readers to give a brief insight into the HPV life cycle that may lead to carcinogenesis.

The HPV genome encodes eight genes; the L1 and L2 structural proteins constitute the capsid that protects the viral genome, and the E1, E2, E4, E5, E6, and E7 proteins are involved in replication, transcription regulation, and oncogenesis. E6 and E7 oncoprotein expression disrupt the cell repair mechanisms by degradation or inhibition of the tumor suppressor proteins p53 and retinoblastoma protein (pRB), respectively, resulting in the immortalization and cellular transformation of infected cells [19][20][21]. The viral life cycle begins with viral particles arriving in the basal layer of the squamous epithelia via micro-abrasions. After reaching the nucleus the viral DNA is replicated, and low quantities of E1, E2, E6, and E7 proteins are produced early in the infection, halting normal keratinocyte development. Then, E2 recruits E1 to increase the number of viral episome copies, which continue to rise as the epithelium differentiates. E6 and E7 proteins are abundantly expressed in the top differentiating epithelial layers, resulting in uncontrolled cell proliferation, and the viral life cycle is completed when L1 and L2 proteins are expressed in the epithelium's highest layer. As a result, the viral genome is encapsulated, and mature virions are released [22][23]. Although many women contract HPV, most infections are eliminated or controlled by the immune system after 1–2 years [24]. The establishment of persistent infection is associated with the appearance of cervical lesions, since the accumulation of DNA damage caused by HR HPV E6 and E7 interactions with tumor suppressors, p53 and pRb, causes apoptosis suppression and uncontrolled proliferation. The chronically infected cells lead to the establishment of cervical intraepithelial neoplasia (CIN), as CIN grade I, which over time can evolve to CIN grade II, III, or invasive carcinoma [23][24].

CC is staged according to different systems. The most widely used is the FIGO system proposed by the International Federation of Gynecology and Obstetrics in collaboration with the International Union Against Cancer (IUCC) [7].

All surgical interventions can cause side effects or complications, including bleeding and damage in the tissue nearby, and simple or radical hysterectomy results in infertility or bladder/bowel dysfunction [25]. With the spread of cancer to other tissues (metastasis), surgery is no longer a viable possibility. Radiation therapy (RT) uses radiation to destroy cancer cells, and it is possible to affect mainly the zones with the tumor in the lower abdomen in CC cases. Some side effects can be infertility, discomfort, and menopause. Chemotherapy involves the administration of cytotoxic drugs to interfere with cell proliferation, killing rapidly dividing cells. The currently approved drugs by the FDA for CC treatment include, among others, bleomycin sulfate, topotecan, pembrolizumab, bevacizumab, cisplatin, paclitaxel, vinorelbine, ifosfamide, fluorouracil, and gemcitabine [26]. Nevertheless, these treatments are unable to effectively distinguish healthy from cancer

cells, affecting several types of dividing cells throughout the entire body. This effect can result, namely, in a higher risk of anemia, bleeding, and infections [3][25]. Hence, the design and development of therapeutic agents that are efficient, non-toxic, and capable of distinguishing cancerous from healthy tissue are essential. In addition, for managing early-stage CC, the employment of less invasive methods to improve quality of life and decrease treatment-related sexual dysfunction is a necessity.

3. Future Perspectives

CC management still needs improvements, as current therapies are mainly surgery, chemotherapy, or radiotherapy. Several drugs have been proposed for treating patients with CC, but most do not overcome clinical trials due to low efficacy [27]. Bearing in mind the role of HR HPV E6 protein in the development and progression of HPV infection to cervical lesion or even invasive carcinoma, the inhibition of E6 function could be useful for treating CC. Results reported in this review support the idea that combining in silico approaches and in vitro studies could lead to a rise in the number of molecules understudy to block/inhibit E6 protein. According to Franconi and co-workers and Duenas-Gonzalez and co-workers, there are no clinical studies using natural or synthetic compounds as E6 or E7 inhibitors yet [28][29], due to low affinity and/or potency on in vitro and in vivo studies. Thus, the employment of in silico methodologies in the drug development process can be a great help to quickly find potential inhibitors and circumvent possible undesirable properties of compounds. Therefore, identification and functional evaluation of proteins associated with E6 could provide an insight into CC carcinogenesis and thus allow the design of specific strategies towards tumor cells. Moreover, it is essential to search and find cost-effective treatment options that could bring better outcomes for the patients. A strategy in this context could explore the potential use of plant-derived compounds, usually associated with lower toxicity and side effects when compared with classic anticancer agents. In addition, considering that drug distribution to the tumor site is low, the use of suitable drug delivery systems (DDS) compatible with the anticancer agents could be explored to achieve better clinical response with lower toxicity, as DDS could be functionalized with ligands that are specifically recognized by cancer cells [30]. Thinking about the fact that HPV infection is localized in the anatomical regions that can be easily reached for topical treatment, the possibility of locally delivering small molecules or natural compounds could be a valid option. Indeed, in low-income countries where women only can reach health facilities a few times in a lifetime, the combination of HR HPV test positivity and treatment in a single visit could be fundamental. A "screen-and-treat" approach allows reducing travel time, minimizing the number of visits, transport, childcare needs, and reducing the cost [31]. In places where is difficult to reach people and a return visit is not an option, self-sampling for HPV screening and mobile treatment for precancer could be applied [32]. Moreover, visual inspection with acetic acid (VIA) can be applied as a triage method for LIC, once it is low-cost and offers the option of treatment immediately or shortly after diagnostic testing. One of the biggest problems of cervical neoplasia is the resistance of tumor cells to chemotherapy and radiotherapy. Thus, the combination of anticancer agents in DDS with conventional chemotherapy or radiotherapy could also be a solution. This represents another line of investigation that needs to be explored in the near future. In terms of CC management, it would be interesting to explore a combinatory approach targeting E6 and E7 oncoproteins. In this case, the aim consists of exploiting molecules able to interact with E6 and E7 proteins, once both have a relevant role on CC progression. From this point forward, the use of in silico methods would be the key to pursue this approach.

Overall, our research group aims to propose a more specific, efficient, and non-toxic/invasive therapeutic approach for cervical cancer management. Thus, in silico approaches, such as those described in the present manuscript, will be used to select promising compounds as E6 potential inhibitors. Additional studies will be conducted with HPV E6 recombinant proteins and the selected compounds to characterize the kinetic magnitude and affinity constants of the compound–protein interaction. Then, the most promising compounds will be applied in in vitro studies with HPV-positive and HPV-negative cell lines to confirm the inhibitor/blocker effects of the E6 oncoprotein. In addition, drug delivery systems can be developed to circumvent a possible high toxicity and low availability of the compounds and, for instance, to combine this approach of E6 inhibitors with gene therapy to supplement p53 content and induce the cancer cell apoptosis.

4. Conclusions

The high impact that CC has in developing countries is undeniable. This review has briefly discussed the role of HPV in CC carcinogenesis as well as its different stages and current treatments. Subsequently, the potential of natural and synthetic small molecules in HR HPV E6 protein inhibition, mainly by targeting the E6/E6AP complex or the E6/E6AP/p53 complex, was discussed. The drug development process is a very expensive and time-consuming process until achieving regulatory agency approval. In silico methods represent a viable solution to these current problems by allowing a fast screening and identification of potential drugs and effective predictions. In terms of impact, in silico methodologies can help to increase the speed of acceptance of potential antiviral drugs with ADMET acceptable profiles for CC management.

Moreover, in silico methods have been applied in the medical field, representing the therapeutic response of drugs on virtual organs and body systems and predicting patients' biological responses to the treatment, and this significantly improves outcomes. Computational-based approaches hold a great promise for improving drug development and revolutionizing clinical research by providing a specific treatment for women diagnosed with cervical cancer. Considering the costs of screening and treatment of CC, and knowing that the highest incidence occurs in developing countries, a more cost-effective treatment is needed. Thus, exploring natural compounds with the ability to impair E6-p53 interaction could be a specific and promising strategy for CC management in a more economical way.

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