Bioinformatics analysis of andrographolide as an anti-squamous cell carcinoma of the cervix

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Introduction

Cancer represents a complex disease characterized by uncontrolled cellular division and abnormal cell growth patterns. Globally, cancer constitutes the leading cause of mortality, with an estimated 662,301 new cervical cancer cases and 348,874 deaths occurring in 2022 [1]. Indonesia recorded 408,661 new cancer cases in 2022, with a mortality rate of 242,099 deaths [2], representing more than 50% of total cases, reflecting the substantial burden of this disease on public health systems.

Cervical cancer represents the fourth most common cancer in women worldwide, and remains a significant public health challenge in Indonesia. Among the five most common cancer types affecting both men and women in Indonesia are breast cancer, lung cancer, cervical cancer, colorectal cancer, and liver cancer. Indonesia is ranked number 2 among Asian countries in terms of mortality and incidence of cervical cancer, with an age-standardized incidence rate (ASIR) of 23.4 per 100,000 women and age-standardized mortality rate (ASMR) of 13.9 per 100,000 women. Squamous cell carcinoma accounts for approximately 80% of all cervical cancer cases, while adenocarcinoma comprises the remaining cases [3]. Currently, about 70% of cervical cancers in Indonesia are diagnosed at an advanced stage, when treatment is less effective.

Current cervical cancer treatment modalities present significant limitations that compromise patient outcomes and quality of life. Despite recent advances in cervical cancer treatment, therapeutic and surgical procedures for cancer management remain inadequate. Chemotherapy protocols frequently result in drug resistance development and systemic toxicity, while radiotherapy demonstrates insufficient specificity for malignant tissues. Standard treatments including surgery, radiation, and chemotherapy often fail to cure the cancer and cause severe systemic toxicity in patients. Immunotherapy approaches, though promising, remain limited by poor accessibility and affordability, particularly in developing healthcare systems. These therapeutic challenges necessitate the identification and development of novel treatment strategies with improved efficacy and reduced adverse effects.

The pathogenesis of cervical cancer involves complex interactions between human papillomavirus (HPV) infection and genetic factors that regulate cellular proliferation and apoptosis. Genetic analysis has revealed dysregulation of key molecular pathways, including overexpression of matrix metalloproteinases (MMPs) that directly correlate with tumor invasiveness and metastatic potential. Additionally, aberrant expression patterns of cell cycle regulators such as p16, Ki-67, and Bcl-2 demonstrate strong associations with tumor progression and clinical outcomes [4]. Understanding these genetic mechanisms provides valuable insights for therapeutic target identification and drug discovery processes.

Bioinformatics approaches have emerged as powerful tools for investigating cancer genetics and facilitating drug development programs. These computational methodologies enable systematic analysis of large-scale genomic datasets, identification of potential therapeutic targets, and prediction of drug-target interactions. The integration of multiple bioinformatics platforms allows researchers to construct comprehensive molecular networks that reveal critical pathways involved in cancer progression and treatment response.

Natural compounds represent promising candidates for cancer prevention and treatment due to their ability to modulate tumor microenvironments and regulate multiple cellular pathways simultaneously [5]. These small molecules often demonstrate favorable safety profiles compared to synthetic drugs while maintaining significant biological activity against cancer cells. The systematic evaluation of natural compounds through computational approaches provides an efficient strategy for identifying potential therapeutic agents before costly experimental validation.

Andrographolide, a bioactive diterpene lactone isolated from the medicinal plant *Andrographis paniculata*, has demonstrated significant anticancer properties across multiple cancer types in recent investigations. Recent studies have documented its multitarget therapeutic efficacy that andrographolide inhibits NF-κB and COX-2 expression while promoting PTEN expression and suppressing PI3K/AKT signaling pathways in cervical cancer [6]. Additional preclinical research using a patient-derived xenograft model showed that andrographolide significantly reduced tumor size and suppressed cervical squamous cell carcinoma growth by targeting angiogenesis and inducing apoptosis. The compound's natural origin and lack of adverse side effects make it particularly attractive as a therapeutic agent, with comprehensive reviews highlighting its ability to inhibit multiple signaling pathways and contribute to anti-proliferative, anti-metastatic, and apoptotic effects in various cancers [7].

Current investigations have identified several therapeutic targets for andrographolide in cervical cancer subtypes. Recent proteomic analysis demonstrated that andrographolide restores p53 expression via regulation of ubiquitin-mediated proteolysis pathways, promoting intrinsic apoptosis in HPV-16 infected cervical cancer cells [8]. In cervical adenocarcinoma, andrographolide appears to exert its effects through inducible nitric oxide synthase (iNOS) modulation, while separate studies have implicated additional molecular targets in squamous cell carcinoma. However, comprehensive systematic analysis of andrographolide's molecular targets specifically in cervical squamous cell carcinoma remains limited, particularly regarding the integration of network-based approaches with molecular docking validation.

This research aims to identify and characterize the therapeutic targets of andrographolide in squamous cell carcinoma of the cervix through comprehensive bioinformatics analysis. The study employs systematic computational approaches to intersect genes dysregulated in cervical cancer with genes regulated by andrographolide treatment, followed by molecular docking analysis to evaluate binding interactions between andrographolide and identified target proteins. The findings will provide valuable insights for understanding andrographolide's mechanism of action and support future experimental validation studies addressing the urgent need for novel therapeutic approaches in cervical cancer management.

Methods

Data sources and computational resources

This bioinformatics analysis employed multiple databases and computational platforms to identify and characterize andrographolide's therapeutic targets in cervical squamous cell carcinoma. Genes dysregulated in cervical cancer were obtained from the GeneCards database (version 5.16) [9], while andrographolide target proteins were retrieved from STITCH (version 5.0) [10], SwissTargetPrediction [11], and STRING (version 11.5) [12]. Chemical structure data for andrographolide and reference compounds were accessed through the PubChem database [13], and protein structural information was obtained from the Protein Data Bank [14].

The computational analysis was performed using a desktop system equipped with an Intel Core i5-3337U processor, 4GB RAM, and Windows 10 Home operating system. Key software platforms included Venny 2.1 [15] for intersection analysis, WebGestalt 2019 [16] and Metascape v3.5.20230501 [17] for functional enrichment analysis, and Cytoscape 3.9.1 [18] with the CytoHubba plugin [19] for network analysis. Molecular modeling and docking studies utilized Avogadro 1.2.0 [20], AutoDockTools 1.5.7 [21], AutoDock Vina 1.2.3 [22], PyMOL 2.5.5 [23], and BIOVIA Discovery Studio 2.5 [24].

Identification of cervical cancer-associated genes

The methodology was adapted from established bioinformatics protocols (Shivanika et al., 2022) with modifications to address the specific research objectives of molecular target identification and docking validation. Genes deregulated in cervical squamous cell carcinoma were systematically identified through comprehensive database searches using GeneCards (Stelzer et al., 2016). The search strategy employed the primary keyword "cervical squamous cell carcinoma" to ensure comprehensive coverage of relevant genetic alterations associated with this cancer subtype.

Andrographolide target protein identification

Target protein identification followed a systematic two-tier approach to capture both direct and indirect molecular interactions. Direct target proteins were identified through complementary database searches to ensure comprehensive coverage and cross-validation of results.

Using the STITCH database (Szklarczyk et al., 2016), andrographolide was searched as the primary query term with Homo sapiens specified as the target organism. To ensure high-confidence interactions, the minimum required interaction score was set to 0.700, representing high-confidence predictions, with results limited to the top 20 interactors to focus on the most

relevant targets. Parallel searches were conducted using SwissTargetPrediction (Daina et al., 2019), where the SMILES notation of andrographolide was submitted with Homo sapiens as the target organism to predict potential protein targets based on structural similarity approaches.

Indirect target proteins were identified through protein-protein interaction network expansion using the STRING database (Szklarczyk et al., 2021). The previously identified direct target proteins served as seed nodes for network expansion. Multiple protein queries were submitted simultaneously with Homo sapiens as the target organism. Network parameters were configured with a minimum required interaction score of 0.700 to maintain high confidence in predicted interactions, and results were limited to 20 interactors per query to ensure computational tractability while capturing essential network connectivity.

Potential therapeutic target gene determination

Potential therapeutic target genes were identified through systematic intersection analysis of two gene sets: genes dysregulated in cervical cancer and genes regulated by andrographolide treatment. The Venny 2.1 platform (Oliveros, 2007-2015) was employed to perform this intersection analysis and generate comprehensive Venn diagrams for visual representation of overlapping gene sets.

Hub gene identification was performed using network topology analysis through the STRING database (Szklarczyk et al., 2021) and Cytoscape platform (Shannon et al., 2003). The complete set of potential therapeutic target genes was submitted to STRING using the multiple protein search function. The resulting protein-protein interaction network was downloaded in tab-separated values format and imported into Cytoscape for advanced network analysis. The CytoHubba plugin (Chin et al., 2014) was utilized to rank genes based on their degree of connectivity, representing their importance within the protein interaction network. The top 10 hub genes were identified and selected for further analysis based on their network centrality measures.

Functional enrichment analysis

Comprehensive functional annotation was performed to elucidate the biological significance of identified potential therapeutic target genes. Gene Ontology analysis was conducted using WebGestalt 2019 (Liao et al., 2019) with Homo sapiens specified as the target organism. The

analysis employed over-representation analysis methodology with Gene Ontology as the primary annotation system. The complete list of potential therapeutic target genes was submitted for analysis across three GO categories: biological processes, cellular components, and molecular functions.

Pathway enrichment analysis was performed using Metascape v3.5.20230501 (Zhou et al., 2019) to identify relevant biological pathways associated with the target genes. The analysis focused on Kyoto Encyclopedia of Genes and Genomes pathway annotation (Kanehisa et al., 2023) with Homo sapiens as the target organism. Custom analysis parameters were employed to optimize pathway detection sensitivity and specificity for cancer-related processes.

Target protein selection and validation criteria

Target proteins for molecular docking studies were selected through a systematic multi-criteria evaluation process. The selection methodology integrated network topology analysis, biological relevance assessment, and druggability evaluation to identify optimal targets for computational validation.

Primary selection criteria included ranking within the top 10 hub genes based on network connectivity analysis, demonstrated involvement in pro-cancer biological activities through literature validation, and confirmed susceptibility to inhibition by small molecule compounds based on published experimental evidence. Additional validation criteria encompassed the availability of high-resolution crystal structures in the Protein Data Bank and successful validation of docking protocols through native ligand redocking experiments.

Ligand preparation and optimization

Andrographolide structural preparation employed rigorous computational chemistry approaches to ensure accurate molecular representation for docking studies. The initial chemical structure was obtained from PubChem (Kim et al., 2021) and converted to three-dimensional coordinates using Avogadro 1.2.0 (Hanwell et al., 2012). Molecular geometry optimization was performed using Density Functional Theory calculations implemented in Gaussian 16 (Frisch et al., 2016) at the B3LYP/6-31G(d,p) level of theory (Becke, 1993). This optimization protocol ensured accurate molecular geometry and electronic properties essential for reliable docking predictions.

Following DFT optimization, molecular flexibility was incorporated through torsional degree of freedom assignment using AutoDockTools 1.5.7 (Morris et al., 2009). The optimized structure was converted to PDBQT format with appropriate atomic charges and flexibility parameters configured for molecular docking simulations.

Molecular docking protocol and validation

Molecular docking validation was performed through native ligand redocking experiments to establish protocol reliability and accuracy. For each target protein, the co-crystallized native ligand was extracted and redocked to its binding site using AutoDock Vina 1.2.3. Grid box parameters were centered on the native ligand position with appropriate dimensions to encompass the complete binding site. Root-mean-square deviation values were calculated using PyMOL to assess docking accuracy, with RMSD values below 2.0 Å considered acceptable for protocol validation according to established benchmarks [].

Andrographolide docking simulations were conducted using the validated grid box parameters for each target protein. Binding energy calculations were performed using the AutoDock Vina scoring function, which incorporates multiple energy terms including van der Waals interactions, hydrogen bonding, desolvation effects, and conformational entropy changes. Control compound docking was performed in parallel using established inhibitors specific for each target protein to provide comparative binding energy assessments.

Molecular interaction analysis and visualization

Comprehensive analysis of protein-ligand interactions was conducted through both two-dimensional and three-dimensional visualization approaches. Two-dimensional interaction diagrams were generated using BIOVIA Discovery Studio 2.5 to identify specific amino acid residues involved in binding and characterize interaction types including hydrogen bonds, hydrophobic contacts, and electrostatic interactions.

Three-dimensional visualization and analysis were performed using PyMOL 2.5.5 to provide spatial context for binding interactions and assess geometric complementarity between andrographolide and target proteins. Binding site analysis included evaluation of pocket volume, surface area, and electrostatic properties to understand the molecular basis of binding specificity and affinity.

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