Models For Screening Anticancer Drugs

Adib Hussain[1], Arabinda Nayak[1]

[1] Gupta College of Technological Sciences, Asansol, West Bengal, India

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Abstract

Cancer remains one of the most challenging diseases to combat, necessitating the development of effective and targeted anticancer drugs. The process of discovering and screening potential compounds for their anticancer properties is both time consuming and expensive. To expedite this process and enhance drug discovery efforts, computational models have emerged as valuable tools for screening and predicting the efficacy of potential anticancer drugs. This study aims to develop and evaluate multiple anticancer screening models using diverse computational techniques. A comprehensive dataset comprising chemical compounds and their associated anticancer activities will be collected from publicly available databases and literature sources. Several machine learning algorithms and neural networks, will be employed to build predictive models. Various molecular descriptors, including physicochemical properties, 2D and 3D molecular fingerprints, and structural features, will be extracted to represent the chemical compounds. Feature selection techniques will be applied to identify the most informative descriptors, enhancing model performance and interpretability. The impact of different algorithms, feature representations and descriptor selection techniques on model performance will be thoroughly investigated. Additionally, the models' interpretability will be assessed to gain mechanistic insights into the structure- activity relationships of the screened compounds. The developed models will be rigorously evaluated using appropriate validation strategies. The results of this study will contribute to the advancement of computational anticancer drug discovery and facilitate the identification of potential lead compounds for further experimental validation. The validated models can serve as valuable tools for prioritizing candidate compounds, reducing the time and cost associated with traditional screening methods, and accelerating the discovery of novel anticancer agents.

Keywords - Cancer, etiology, epidemiology, pathophysiology, screening methods

Author Correspondence

Arabinda Nayak

Gupta College of Technological Sciences, Asansol, Westbengal

Email : arabinda00717@gmail.com, Mob. 98244 52783

1. Introduction:

Cancer is a complex disease marked by abnormal cell growth, capable of affecting any part of the body and occurring at any age, though the risk increases with aging. Despite advancements in early detection and treatment, cancer remains the second leading cause of death globally. In 2012, there were 14.1 million new cancer cases and 8.2 million deaths, with projections indicating 21.7 million cases and 13 million deaths by 2030[1].

Cancer's causes are diverse, involving genetic, environmental, and lifestyle factors, as well as infections like hepatitis B, C, and human papillomavirus. Both genetic predispositions and exposure to carcinogens play roles in cancer development, while differences in incidence and mortality rates between men and women are notable, potentially due to variations in biology, exposure, and treatment response[2].

Current treatments, including surgery, radiation, and chemotherapy, often cause significant side effects, as they may damage healthy cells. Chemotherapy, in particular, faces limitations due to toxicity, drug resistance, and poor selectivity in targeting cancer cells. New strategies, such as prodrugs and drug delivery systems (DDS), aim to address these issues by improving drug selectivity and reducing systemic toxicity. Prodrugs are inactive compounds that become active upon metabolism, offering greater precision in targeting cancerous cells and minimizing damage to healthy tissues. DDS technology further enhances treatment efficacy by delivering drugs directly to tumors, reducing side effects.

Although progress has been made, existing treatments still present challenges, including immune system suppression and damage to vital organs. Research into alternative therapies, such as immunotherapy and targeted drug delivery systems, continues in the hope of developing more effective and less harmful cancer treatments[3].

2. Etiology of Cancer :

The causes of cancer have long been a topic of interest, with environmental exposures playing a significant role, as noted during a 1950 World Health Organization symposium. The discovery that immigrants tend to develop cancers more common in their new countries suggested that environmental factors, rather than genetics, were the primary cause of most cancers. This led to the founding of the International Agency for Research on Cancer (IARC) in 1965 to study cancer's origins.

Early links between occupational exposures and cancer were established, such as scrotal cancer in chimney sweeps and lung cancer in miners. Over time, several professions were linked to higher cancer rates due to exposures to carcinogens, such as β-naphthylamine, which caused bladder cancer in dye workers. Despite these findings, early attempts to induce cancer in animals using irritants failed to fully explain its mechanisms[4-5].

Alcohol has been classified by the IARC as a Group 1 carcinogen, although moderate drinking was associated with reduced risks of thyroid and lung cancers. Studies have also shown that even low concentrations of many chemicals can have carcinogenic effects, and natural substances, including some plant toxins, have been linked to cancer in animals.

Occupational exposures, such as radium in watch dial painters and benzene in chemical workers, were also shown to cause rare cancers like osteosarcomas and leukemia. Dialysis patients were found to have an increased risk of cancer, likely due to chronic kidney disease and immune suppression. Additionally, infectious agents have been identified as significant contributors to cancers, especially in underdeveloped countries. Understanding these links has improved cancer prevention strategies, and the IARC continues to research carcinogens across various domains[6].

3. Pathophysiology of Cancer:

Pathophysiology focuses on abnormal changes in body processes associated with diseases, particularly biological mechanisms related to conditions like cancer and diabetes. Research in this area aims to identify biological markers and mechanisms that explain the etiology and pathogenesis of diseases. The link between diabetes and cancer was first suggested in 1932, but large-scale studies later confirmed a strong association between diabetes and increased risks of cancers such as pancreatic, liver, endometrial, breast, colon, and bladder. In contrast, prostate cancer shows a negative correlation with diabetes.

Diabetes can double the risk of developing certain cancers like hepatocellular and pancreatic, and survivors of some cancers are more prone to develop diabetes. Obesity, a major risk factor for diabetes, has also been linked to cancer due to its inflammatory and endocrine effects. Research shows that obesity and cancer are connected through mechanisms like insulin resistance, hyperglycemia, and hyperinsulinemia[7].

Obesity contributes to cancer risk, with BMI-based measures often used to assess general adiposity, although visceral fat measurements may be better indicators. Obesity is a significant risk factor for postmenopausal breast cancer and other cancers. Bariatric surgery has been shown to lower cancer risk by reducing obesity-related malignancies.

Diabetes patients are at a higher risk of developing cancer soon after diagnosis, possibly due to prolonged hyperinsulinemia. Cancer survivors, especially those treated with radiation or chemotherapy, are also at risk of diabetes. There is a need for improved diabetes screening and management among cancer patients. Research suggests that co-morbid conditions like diabetes significantly affect life expectancy and quality of life more than the original cancer, highlighting the need for integrated care[8].

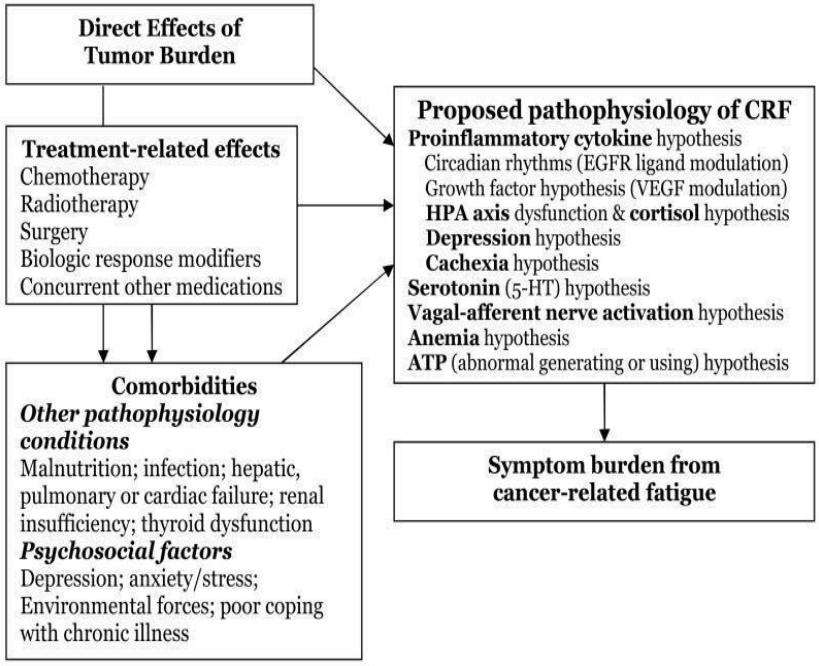

Figure showing various pathophysiological factors for cancer

4. Epidemiology of Cancer:

Pathophysiology studies the abnormal changes in body processes due to diseases, focusing on biological mechanisms linked to conditions like cancer and diabetes. Research aims to identify biological markers and mechanisms to explain disease etiology and pathogenesis. The link between diabetes and cancer was first suggested in 1932, with later studies confirming the strong association between diabetes and cancers such as pancreatic, liver, endometrial, breast, colon, and bladder. In contrast, diabetes is negatively associated with prostate cancer. Diabetes can double the risk of hepatocellular and pancreatic cancers, and cancer survivors are more prone to develop diabetes. Obesity, a key risk factor for diabetes, is also linked to cancer through mechanisms such as insulin resistance, hyperglycemia, and hyperinsulinemia. Obesity is connected to an increased risk of cancers, especially postmenopausal breast cancer, with BMI often used as a measure, although visceral fat assessments may be more accurate. Bariatric surgery has been shown to lower the risk of obesity-related cancers[9].

Diabetes patients are at a higher risk of cancer shortly after diagnosis, likely due to prolonged hyperinsulinemia. Cancer survivors, particularly those treated with radiation or chemotherapy, are also at higher risk of diabetes. Improved screening and management of diabetes in cancer patients are essential, as co-morbid conditions like diabetes can impact life expectancy and quality of life more than the original cancer. Integrated care is crucial for these patients, underscoring the need for further research and attention to shared risk factors[10].

5. Models for Screening Anticancer Drugs:

Drug screening models are crucial in the early stages of drug discovery to predict the activity, efficacy, and safety of potential medications before clinical trials. Various models are utilized in this process, including in silico models, which employ computer-based simulations to predict drug behavior, interactions with target proteins, and pharmacokinetics; in vitro models, involving laboratory experiments on cells or tissues (e.g., cell-based assays) to assess a drug's activity and toxicity; and in vivo models, where animal testing evaluates drug efficacy, safety, and pharmacokinetics using species like mice or rats. Additionally, high-throughput screening (HTS) employs automated systems to rapidly screen large compound libraries for target activity, while 3D organoids use three-dimensional cell cultures to mimic organ functions, providing more accurate predictions of drug responses. Finally, patient-derived models utilize cells from patients to create personalized models that study specific disease mechanisms and drug responses. These models serve as essential tools in drug discovery, requiring further preclinical and clinical validation before a drug can be approved for human use[11-12].

5.1 *In Silico models of screening*:

5.1.1 Quantitative Structure Activity Relationship model:

Introduction:

Quantitative structure-activity relationship (QSAR) is an empirical mathematical model that establishes a statistically significant association between chemical structures and biological features, enabling the prediction of a new chemical's biological or toxicological properties based solely on its structure. This approach enhances efficiency by eliminating the need for time-consuming experimental testing. Including the amino acid (AA) sequence of receptors in QSAR models can further improve prediction accuracy by accounting for receptor structure and activity. The application areas for QSAR have expanded to encompass drug design, drug toxicity prediction, enzyme interaction mechanisms, and biological activity prediction. QSAR primarily

relies on three techniques: molecular description, chemical similarity search, and machine learning[13]. The foundation of QSAR lies in acquiring molecular descriptors, which are information-rich numerical features extracted from chemical structures. These descriptors can be qualitative (e.g., MACCS keys) or quantitative (e.g., molecular field) and can range from 1D to 6D. Various categories of descriptors include composition, molecular property, topological, and geometric descriptors. The QSAR modeling process involves several key steps: molecular coding to represent complex molecules as vectors of attributes; constructing training and testing sets from an appropriate number of compounds; and verifying the model's applicability and predictability through internal and external validation. Iteratively refining feature combinations and algorithm parameters is crucial for optimizing the model's performance[14].

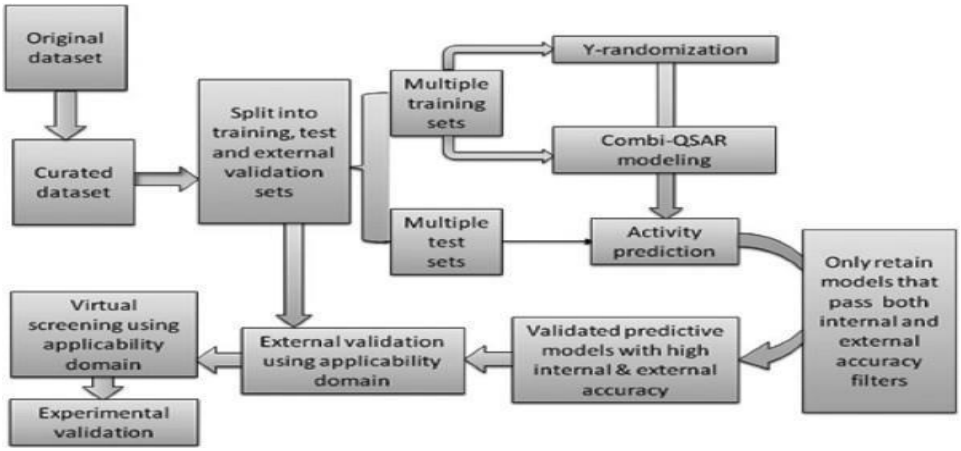

Figure showing QSAR model development and validation
(Pub Chem, http://pubchem.ncbi.nlm.nih.gov/, 2008)

Further Consideration:

The success of QSAR is attributable to the change in how the ligand structure is represented, which accurately captures the basic variance in the ligand's scope. For example, regardless of how complicated the resulting physicochemical and biological interactions are, the structural difference between two molecules alone defines their bioactivity. Maximizing the space of positive and negative samples is the ultimate goal of ligand comparison. If the developed QSAR
models are expected to be useful for drug synthesis as well as drug screening, properties such as synthesizability, hydrophobicity, drug-likeness, the Lipinski rule, and false-positive concerns must be considered[15].

Conclusion:

ML and DL techniques rely on information theory and data fitting theories to analyze experiment data obtained from NMR, X-ray, and cryo-EM. The accuracy of predictions primarily depends on the availability of reliable and sufficient data, as well as the computational power required for processing experiment data through MM/QM simulations. However, many fields in computational biology lack the necessary resources, making ML/DL less effective in those areas. To address this issue, powerful free tools like AlphaFold can be utilized.

The broad applicability of ML in various domains relies on its ability to solve problems in a manner similar to humans, including tasks such as classification, clustering, elucidation, verification, and determining the importance of factors. Currently, iterative thinking is being incorporated system[16].

5.1.2 Molecular Docking Model:

Introduction:

Lung cancer is a leading global cause of mortality and is often diagnosed at advanced stages. While limited studies exist on scopoletin's anti-cancer effects, traditional Chinese medicine (TCM) has gained attention for its therapeutic potential against cancer, prompting researchers to explore its mechanisms and target malignancies[17].

Procedure:

Cells from A549, BEAS-2B, HCT-116, and HepG2 lines were cultured in RPMI1640 media supplemented with 10% fetal bovine serum (FBS), 100 U/mL of penicillin G, and 100 µg/mL of streptomycin, at 37°C with 5% CO2. The cells were seeded at a density of 1 × 10^4 cells per well in 96-well plates and treated with varying concentrations of scopoletin (1, 2, 4, 8, 16, and 32 µg/mL) for 24 hours. Following treatment, 10 µL of MTT was added to each well, and after 4 hours of incubation, the supernatant was removed. The formazan product was dissolved in 150 µL of dimethyl sulfoxide (DMSO), and absorbance was measured at 490 nm using a Spark 10 M microplate reader[18].

Molecular docking was conducted using AutoDockTools-1.5.6, which operates on a semi-flexible principle. Pre-docking of proteins and small molecules was facilitated by PyMOL. Gene targets were retrieved from the PPI network and downloaded in PDB format from the RSCBPDB database. The 3D structures of scopoletin were generated using ChemDraw and loaded into AutoDockTools-1.5.6, where they were hydrogenated, charged, and the number of rotatable bonds calculated. Non-protein molecules were eliminated using PyMOL, and the lowest energy pose was identified through AutoDockTools-1.5.6. The binding affinity is considered stronger when the dock binding free energy is lower than −4 kcal/mol[19].

Results:

MTT assay results indicated that scopoletin significantly reduced cell viability in A549 cells compared to the control ($p < 0.05$ and $p < 0.01$) as the concentration increased, while it showed no cytotoxic or antiproliferative effects on BEAS-2B cells after 24 hours. The half-maximal inhibitory concentration (IC50) of scopoletin for A549 cells was approximately 16 µg/mL, with cell viabilities of 95.06%, 82.09%, 72.78%, 62.84%, 50.38%, and 41.81% at 1, 2, 4, 8, 16, and 32 µg/mL concentrations, respectively. This indicates that scopoletin restricts A549 cell growth in a concentration-dependent manner. Furthermore, scopoletin inhibited the proliferation of HepG2 and HCT-116 cells. Although scopoletin demonstrated anti-cancer effectiveness, its specific mechanism remains unclear. Thus, this study aims to screen suitable cancer cell lines using MTT assays and explore potential targets and pathways through network pharmacology and high-throughput molecular docking.

The findings suggest that scopoletin exhibits anti-non-small cell lung cancer (NSCLC) effects while showing no harmful effects on healthy lung epithelial cells. Notably, EGFR emerged as a significant target during the network pharmacology analysis, and further experimental validation is underway to corroborate these results[20-2]

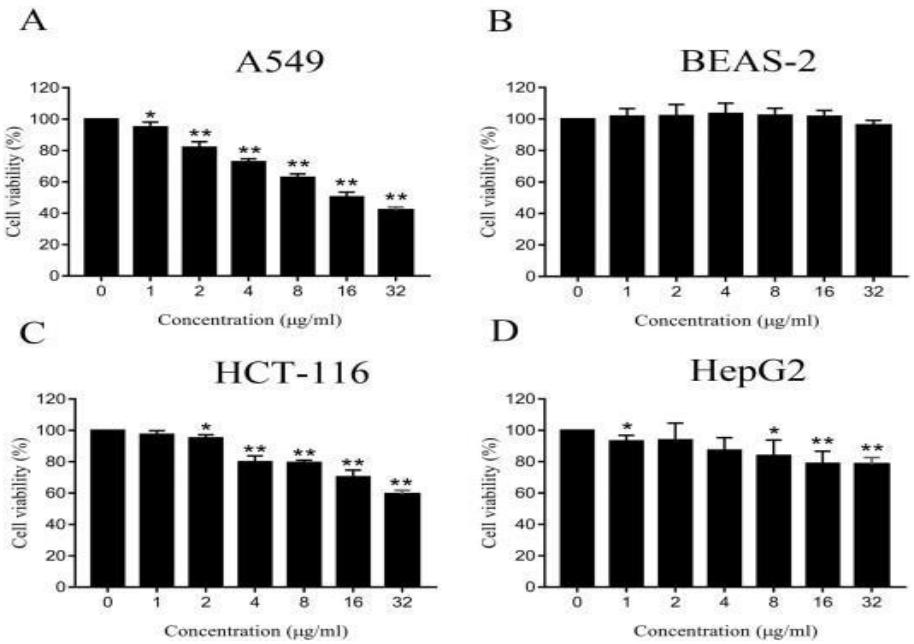

Figure showing the effect of Scopoletin on the viability of A549 (A), BEAS-2B (B), HCT-116 (C) and HepG2 (D) cells.

5.2 In Vivo Models of Screening:

5.2.1 Xenograft Model:

Cell line-derived xenograft (CDX) models, formed by implanting cancer cell lines into immunodeficient mice, have significantly contributed to cancer drug therapy development. In contrast, patient-derived xenograft (PDX) models better mimic human cancer by using tumor tissue, preserving biological features and microenvironments. However, CDX models often lose biological properties after multiple passages and have a high proportion of cancer cells relative to stromal cells. Advances in molecular biology have clarified the signals involved in cancer progression, highlighting the importance of the tumor microenvironment, which includes stromal cells like tumor endothelial cells, cancer-associated fibroblasts, and tumor-associated macrophages, in cancer growth and metastasis[22-23].

Requirements :

In PDX models, several immunodeficient mice are implanted with human cancer tissue. The survey's findings showed that a variety of mouse strains, including naked mice, SCID mice, NOD/SCID mice to SCID/beige mice, NOG or NSG mice, Rag2 knockout (KO) mice, and Rag2 KO/Jak3 KO mice, are utilized in PDX models. The two most popular models are naked mice and NOG or NSG mice. While naked mice are easier to handle because to their lack of fur, NOG or NSG mice are genetically altered, extremely immunodeficient mice with a high rate of tumour transplantation success[24].

Procedure :

Eighteen out of nineteen academic researchers who responded to a survey on "anticancer drugs developed" and "PDX models used" reported utilizing human tumor tissue for their work. Among these researchers, 27.8% indicated they had established their own standard operating procedures (SOPs) for maintaining patient-derived xenograft (PDX) models, while 66.7% stated they followed defined processes but lacked formal SOPs. To encourage the broader adoption of

publicly available PDX models, it is crucial to implement an integrated quality control and assurance mechanism across research teams. Enhancing the quality of PDX models also hinges on comprehensive data accompanying these models. Survey results revealed that the more detailed the PDX model, the more valuable the information, specifying what is essential, recommended, or optional[25-26].

Most researchers reported documenting key patient information when creating PDX models, including age, gender, disease name, treatment history, medical history, infection status, and consent status, alongside tumor characteristics and histopathological features. Additionally, they recorded details about the animal strain used, the animal's sex, PDX tissue passage number, and tumor engraftment rates. The PDX Minimal Information (PDX-MI) framework has been proposed to standardize the essential data collected, ensuring consistency in quality and facilitating drug sensitivity testing using the PDX models[27].

Conclusion :

PDX models should be stored in a negative pressure rack with a HEPA filter for microbial control in SPF conditions. However, a positive pressure rack is acceptable if BSL2 compliance is met. Standard operating procedures (SOPs) must be established for creating PDX models, along with a system to manage essential information. It is recommended to use PDX models with up to nine passages for experiments, while models with ten or more passages should undergo appropriate quality assessments before use[28].

5.2.2 Mouse Model:

Introduction:

Myelosuppression is the most frequent dose-limiting hazard of anticancer medicines observed in humans. Finding drugs that are less myelosuppressive than those that are currently used in clinical practise is a key goal of anticancer drug research programmes and evidently, a reliable experimental test technique is required to determine whether novel medications have a myelosuppressive impact[29].

Materials and Methods :

Male BDF 1 mice weighing 25–291 g were used in these experiments; they were purchased from Charles River Laboratories in Wilmington, Massachusetts. The mice were kept in cages made of wire and had unrestricted access to food and water**.** Based on their myelosuppressive effects in man, the anticancer medicines examined in these investigations were divided into three categories: (a) myelosuppression is dose-limiting; (b) myelosuppression occurs but is not dose-limiting; and (c) little or no myelosuppression occurs. Except for BCNU, which was dissolved in 100% ethanol and diluted with saline before being administered intravenously (IP), other medications were dissolved in saline or water. For each IV or IP injection, the mice got the appropriate dose at a volume of 0.25 ml/mouse or 0.5 ml/mouse due to the drug concentrations in solution[30-31].

Experimental Design: Blood was drawn from the retro-orbital plexus of mice (40–50 per drug, 10/dose) two days before drug administration. Total WBC counts were measured using a Model S Coulter Counter, and absolute neutrophil counts were calculated from Wright-stained blood smears. Mice received a single drug dose (56%, 75%, 100%, or 133% of the LDs0) on day 0. Blood samples were taken on days 4 and 7 post-treatment to measure WBC counts, with day 4 typically showing the lowest neutrophil levels. Drugs' leukopenic and neutropenic effects were classified as significant (>65%), moderate (35%-65%), or minor (<35%) based on WBC and neutrophil counts at maximum tolerated doses[32].

Conclusion :

The ability of the mouse to qualitatively predict the organ toxicities of anticancer medicines in humans has been tested. The current experiments sought to ascertain if the mouse would serve as a useful animal model for testing the myelosuppressive effects of anticancer medications, the neutropenic effects seen in mice were consistent with the myelosuppressive effects seen in people and there are no false positives, which is significant. We used a single-dose therapy and set post-treatment blood sampling time points to assess how these medications affected mice neutrophil levels and to more precisely pinpoint the nadir day and effect, each drug's post-dose bleeding timeline may have been optimized[33].

5.3 In vitro models 0f screening:

5.3.1 Three-dimensional micro physiological model:

Introduction:

Current drug screening methods often rely on 2D systems or animal models to assess toxicity, pharmacokinetics, pharmacodynamics, and organ system effects. While 2D cell cultures lack the complexity of in vivo 3D tissues, animal models fail to fully replicate human-specific drug responses. Replicating 3D arrangements of human cells, with multiple organ systems and a circulatory network, is highly desirable since vasculature links organ systems and supports nutrient and waste exchange. Drug effects on target tissues depend on multiple systems, such as the gastrointestinal, circulatory, and urinary systems in chemotherapy. Anticancer drugs like anthracyclines and methotrexate can cause dose-dependent myelosuppression, though hematopoietic growth agents have improved the management of this side effect. To address the need for better preclinical toxicity models, high-throughput screening systems using 3D human tissues have been developed. These systems incorporate tumor, cardiac, and bone marrow tissue modules, connected by human microvessels, to evaluate drug efficacy and organ-specific toxicity[34-35].

Procedure:

Robust in vitro microphysiological systems have been developed for high-throughput preclinical screening of anticancer drugs to assess potential side effects across multiple tissues and organ systems. These systems must replicate key physiological processes and anatomical features of in vivo tissues, though it is neither necessary nor practical to fully mimic every aspect of tissue architecture. The goal is to reflect the optimal anatomical complexity that impacts drug distribution and response. Correlating in vitro results with in vivo physiology presents unique challenges for each organ system. The following sections discuss key organ characteristics, their interactions with anticancer drugs, and techniques for simulating these traits in microphysiological platforms[36-38].

Results:

The vasculature is crucial for nutrient delivery and waste removal in human tissues, necessitating the presence of perfused vessels with physiological flow in human microphysiological systems to replicate the complex 3D arrangement of cells and extracellular matrix (ECM). While diffusion primarily governs molecular transport across vascular walls into normal tissues, limited convection occurs, particularly in capillaries. Vascular permeability varies across tissues and is influenced by factors like blood flow. Hematopoietic stem cells (HSCs), which can remain dormant, self-renew, or differentiate into progenitors, are often targeted by antineoplastic drugs, leading to immunosuppression, anemia, and thrombocytopenia, increasing infection risk. Therefore, a system must simulate the maintenance

of healthy HSCs, production of lymphoid, erythroid, and myeloid lineage cells, and their release into the circulatory system. Incorporating an immune compartment, particularly a hematopoietic one, into integrated microphysiological systems will significantly enhance anticancer drug screening methodologies[39-42].

Conclusion:

Prior to clinical trials, there is a critical need for innovative techniques to screen anticancer medications. We propose developing a 3D tissue platform that integrates essential components from various organ systems to evaluate the efficacy and potential side effects of anticancer drugs. These human cell-derived tissues mimic the in vivo characteristics of vascular drug transport and tissue response and are perfused by a microvasculature. Housed within a microfluidic device, the system allows for non-invasive monitoring of tissue states and control over factors affecting tissue physiology and drug response. Currently, the device includes vascular, tumor, cardiac, and bone marrow tissues, with the capability to expand by adding new tissue modules. This in vitro approach will facilitate the early identification of potential side effects of anticancer treatments before they manifest in clinical trials[43-45].

5.3.2 Transgenic Model:

The development of cancer therapeutics targeting specific molecular pathways is a key focus in modern biology and chemistry. Understanding tumor biology and the mechanisms of action for targeted molecules, along with their side effects, is essential for drug development[46]. This study explores the use of genetically modified *Arabidopsis thaliana* as a rapid, cost-effective screening method to evaluate the efficacy of human anticancer drugs. Four known inhibitors of human cancer pathways were tested for their effects on the plant's cytoskeletal and endomembrane networks, utilizing GFP-tagged microtubule proteins. The results demonstrate *Arabidopsis* as a viable alternative to traditional in vitro methods for preliminary drug screening[47].

Procedure:

Paclitaxel, known for stabilizing tubulin polymerization in plants, was used to test our strategy. After 24 hours, a 10 µM dose caused minor microtubule (MT) disorganization in some cells, with more significant effects at higher concentrations. At 30 µM, widespread MT disorganization occurred across various cell types, accompanied by plastid detachment and curvilinear morphology due to excessive MT growth. This dose also disrupted the ER and impaired vacuolar transport, as seen by reduced GFPChi fluorescence. In transgenic plants, moderate effects were only observed at 200 µM, with some petiole cells showing MT damage, while others remained unaffected. ER disorganization was minimal, though stomatal turgor was compromised[48-51].

Result:

It has been shown that A. thaliana is susceptible to several clinically significant medications, including cytotoxic medications and kinase inhibitors. When seen as a whole, our findings show the approach's potential value in locating novel anticancer medications that can be further studied against particular human targets. This strategy should be taken into account in the future for a systemic evaluation of various cytotoxic or molecular targeting medicines that is both affordable and effective[52].

6. Discussion:

The screening of anticancer drugs involves various models, each with distinct advantages and limitations. No single model can fully capture the complexity of human tumors. Therefore, a

combination of approaches such as cell-based assays, animal models, computational techniques, and microfluidic systems is essential to gain a comprehensive understanding of drug efficacy, toxicity, and mechanisms of action. Integrating these models improves the likelihood of identifying promising drug candidates for further development and clinical trials. Staying updated on advancements in this field is crucial as new models may have emerged[53-54].

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