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In-vitro Pre Clinical Screening Methods and Treatment Therapies for Cancer: An Overview

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ABSTRACT

Cancer is a major cause of mortality and morbidity world wide . Genetically altered cancer cell gets away from cell cycle and leads towards to cell death and immortal .The global burden of deadly cancer is expected to the 28.4 million 2040. Historical findings of cancer begins from Hippocrates and end with FDA approved chimaric antigen therapy .Over the centuries, pap test was the first cancer screening now it replaced by modern techniques. The various types reported cancers were carcinoma ,sarcoma, tumor , leukaemia, myeloma and malignancy .There are eight most common types of cancer and it can be detected by various methods which includes the physical and lab test. The preclinical *invitro* screening method is ideal for cancer research,screening the new drug and development of drug therapies.Still, there is continues need for better and innovative therapy. Previously the radiation and surgery for most used method to treat the cancer. In this article we aimed to discuss the history including screening,types of cancer,most common types of cancer,*in-vitro* pre clinical screening method and discussed the various therapies used for the cancer treatment.

Keywords: History,Types of cancer,*In-vitro* screening models,Treatment.

INTRODUCTION

Cancer is the uncontrolled growth of cells caused by a wide range of changes in the genome in gene expression. These alterations in gene expression lead to restructuring in the balance of cell proliferation and cell death. The genetically altered cell gets away from the cell cycle and leads towards cell death and becoming immortal. They multiplied uncontrollably and eventually evolve into cancer cells. These cells can occupy nearby tissues and metastasize to distant sites, and bring about significant morbidity and, if untreated, death of the host[1]. Cancer is a major cause of mortality and morbidity worldwide[2], which produced by both external factors (Radiation, Chemicals, and Tobacco)and internal factors (Immune condition, Hormones, and Inherited mutations)[3].Global cancer data indicates that the global cancer burden has risen to 19.3 million with new cancer cases and 10.0 million cancer deaths in 2020 [4](Fig.1).

The most common cases in 2020 such as;

- Breast (2.26 million cases);
- Lung (2.21 million cases);
- Colon and rectum (1.93 million cases);
- Prostate (1.41 million cases);
- Skin (non-melanoma) (1.20 million cases); and
- Stomach (1.09 million cases).

The most common causes of cancer death in 2020 such as;

- Lung (1.80 million deaths);
- Colon and rectum (916 000 deaths);
- Liver (830 000 deaths);
- Stomach (769 000 deaths); and
- Breast (685 000 deaths)[4].

The global cancer burden is expected to be 28.4 million cases in 2040, which is higher than that of 2020 about 47%.[5]

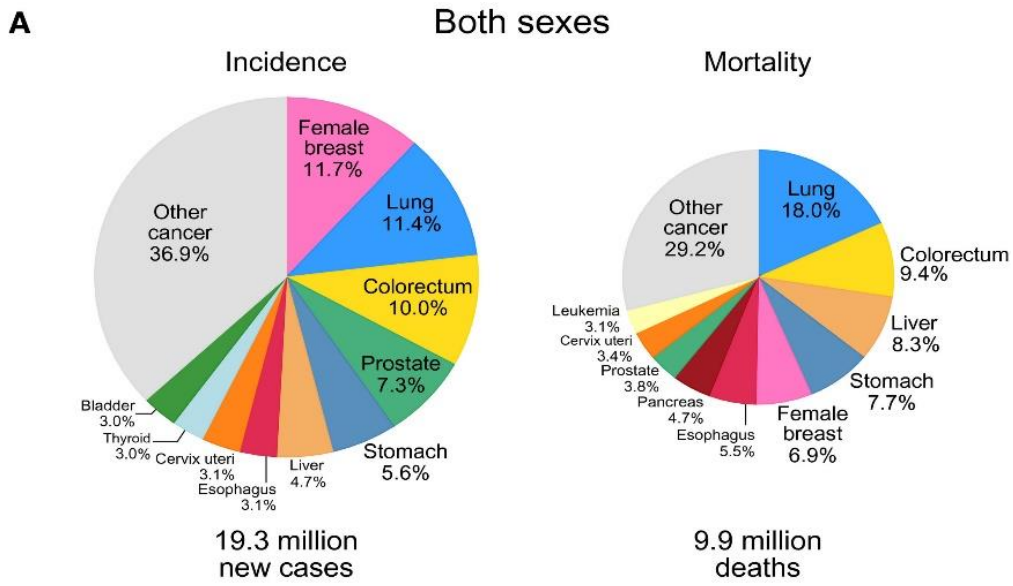

Fig 1: Illustrates the Global burden and cancer deaths[3].

History

The origin of the word cancer is attributed to the Greek physician Hippocrates(460-370BC), who named "Father Of Medicine".He used the words carcinos and carcinoma to characterize non-ulcer forming and ulcer forming tumours[6].

Cancer in the 16th to 18th centuries

Beginning of the 15th-century Scientists developed a greater understanding of the human body, Galileo and Newton began to use the scientific method which later was used to study disease.

1628

Autopsies, performed by Harvey lead to a perception of the circulation of blood through the heart and body.

1761

Giovanni Morgagni of Padua did autopsies to associate the patient's disorder with pathologic results after death. This set foundation for scientific oncology research of cancer.

1728 -1793

Scottish surgeon John Hunter recommended that some cancers might be relieved by surgery. He said "There is no impropriety in removing it "

Cancer in the 19th century

Rudolf Virchow repeatedly named the founder of cellular pathology contributed the scientific explanation for the modern pathologic study of cancer[7].

Cancer in 20th century

1900 -1950

Radiotherapy the use of radiation to kill cancer cells or stop them dividing was developed as a treatment.

1930-1950

Classification of breast cancer initiated, delegating the planning of more rational treatment tailored to the individual.

1939-1945

Us Army discovered that Nitrogen mustard was effective in treating cancer of the lymph nodes (lymphoma) this was the birth of chemotherapy, the use of drugs to treat cancer.

Cancer in 21st century

2000 - The entire human genome is mapped

2004-WHO cancer prevention and control resolution approved by world Health assembly.

2017-The US, FDA approved the first adoptive cell immunotherapy also known as chimeric antigen receptor (CAR) T cell therapy[8].

History of screening

Screening for cancer aid in early detection. Pap test is the first screening method. The test was performed by George Papanicolaou as a research method in understanding the menstrual cycle. As soon as, Papanicolaou evaluate its potential for finding cervical cancer early and introduced his findings in 1923. In that time, cervical cancer incidence and mortality rates have decreased by more than 50% due to screening because it can detect both cervical pre-cancers and cervical cancer at an initial stage.

Modern mammography methods were introduced late in the 1960s and were first officially recommended by the American Cancer Society(ACS) in 1976. A mammogram continues to be the easiest method to screen for breast cancer[9].

Modern Techniques

Colonoscopy, Fecal occult blood, Prostate-specific antigen **(**PSA**)** are the modern techniques of screening.

Colonoscopy

Colonoscopy is otherwise known as coloscopy . It is the endoscopic analysis of the large bowel and the distal part of the small bowel with a fiber optic camera on a flexible tube and it can be passed through the anus. It can remove polyps lesser than one millimeter. It can produce a visual diagnosis and allows for biopsy or removal of detected colorectal cancer lesions[10].

Fecal occult blood

Fecal occult blood testing (FOBT) aims to determine the blood loss in the gastrointestinal tract, from the mouth to the colon. positive stool may lead to either upper gastrointestinal bleeding or lower gastrointestinal bleeding. further, it determines whether it is peptic ulcers or cancer (such as colorectal cancer or gastric cancer). This examination does not directly determine colon cancer but is used in clinical screening for colon cancer. It may also be used to detect the active occult blood loss in anemia[11].

Prostate-specific antigen (PSA)

Prostate-specific antigen **(**PSA**)**is otherwise called gamma-seminoprotein or kallikrein-3 **(**KLK3**).**PSA is a part of the kallikrein-related peptidase family and it is produced by the epithelial cells of the prostate gland. PSA is used for the ejaculate, where it liquefies semen in the seminal coagulum and permits sperm to swimfreely without any inhibition[12]. PSA also involved as instrumental that dissolves the cervical mucus and allows the sperm into the uterus[13]. PSA is not directly an indicator of prostate cancer, but may also determine whether it is prostatitis or benign prostatic hyperplasia[14].

LIST OF CANCER TYPES

Tumor

The new growth of abnormal tissue is often uncontrolled and progressive[15].

Carcinoma

It is a malignancy that begins from epithelial cells. Carcinoma is cancer that develops in a tissue that lines the inner or outer surfaces of the body, and that begins from cells originating in the endodermal, mesodermal, or ectodermal germ layer during embryogenesis[16].

Sarcoma

It is the common term for cancers that develop in the bones and the soft (also called connective) tissue sarcoma forms in the tissues that interrelated, support, and surround other body structures. a lymphoma is a group of blood malignancies that originates from lymphocytes[17].

Myeloma

It is a kind of blood cancer that starts from cells in the bone marrow called plasma cells. Bone marrow is the spongy tissue that occurs inside the inner part of some of the large bones[18].

Malignancy

The term "malignancy" means the presence of cancerous cells that can spread to other sites in the body or to occupy nearby and destroy tissues. Malignant cells tend to have fast, undesired growth and do not die normally because of changes in their genetic makeup.

Malignant cells which resistant to treatment, may return after all detectable traces of them have been removed or destroyed[19].

Leukemia

It is Characterized by abnormal rise in immature WBC type of cancer which already the body's blood forming tissue as well as bone marrow. Unlike other cancer,it does not form into tumor mass.

COMMON TYPES OF CANCER

There are several types of cancer according to their type of tissue where they arise includes,

1. Thyroid cancer
2. Breast cancer
3. Colorectal cancer

4. Non -Hodgkin lymphoma
5. Prostate cancer
6. Renal cell cancer
7. Melanoma
8. Pancreatic cancer.

Thyroid cancer

Thyroid cancer comprises those that arise from follicular cells that emerge from parafollicular cells (c cells). Differentiated thyroid cancer produces from follicular cells contains papillary carcinoma, follicular carcinoma, oncocytic cell carcinoma and anaplastic carcinoma[20].

Indifference, high-resolution neck and thyroid ultrasound can recognize thyroid nodules in about 19% to 68% of randomly selected people, with higher frequencies in women and the elderly[21,22]. The clinical importance of thyroid nodules mostly linked to exclude thyroid malignancy, which arises between 7% and 15% of cases, depending on age, sex, radiation exposure and other factors[23,24].

Breast cancer

It is defined as the proliferation of breast cells and mainly occurs in ducts and lobules[25]. It is the most common type of malignancy in women and it is a heterogeneous disease on the molecular level.

Risk factors such as Economic status, High hormone levels, Age, Race and Iodine deficiency of diet[26].

Colorectal cancer

Colorectal cancer often called bowel cancer takes,which place in the colon, rectum, or appendix. It is the second most common diagnosed cancer in females, third in males, and fourth around the world. It arises from the epithelial lining most commonly resulting in mutation of the Wnt signaling pathway (Wingless and Int-1). This mutation can be either acquired or inherited. They mostly occur in the intestinal gland stem cells[27].

Non - Hodgkin lymphoma

Non -Hodgkin lymphoma is a heterogeneous class of Lympho proliferation malignancies that are much less expected than Hodgkin`s lymphomas and have a far greater idea to propagate an extra nodal location.

Non -Hodgkin's lymphoma on location:

- Oral cavity, Waldeyer`s ring, and pharynx.
- Nasal cavity and par nasal sinuses
- Larynx and trachea[28].

Prostate cancer

Prostate cancer starts when cells in the prostate gland start to grow out of control. It occurs mainly in the male sex glands. As well as seminal vesicles and testicles, the prostate gland secretes the fluid which secretes semen. Prostate cancer is the carcinoma of the prostate gland that may spread to other parts of the body particularly bones and lymph nodes[29].

Renal cell cancer

Renal cell cancer is also known as kidney cancer or renal cell adenocarcinoma. It indicates that cancer arises from the renal epithelium and reports >90% of cancer in the kidney. Renal cell cancer encompasses around >10% histological and molecular subtypes[30].

Melanoma

Melanoma is otherwise known as malignant melanoma. It is a kind of skin cancer that involves pigment-producing cells and is called melanocytes. It is acquired from the neural crest consequently and migrates towards the gastrointestinal tract and brain[31].

Pancreatic cancer

Pancreatic cancer starts when abnormal cells in the pancreas get multiplied divide out of control and form a tumor[32]. It arises from both the exocrine and endocrine parenchyma of the gland. The most common pancreatic cancer is ductal adenocarcinoma which accounts for 80% of all pancreatic cancers[33].

Approaches and diagnosis

Physical exam

- Collection of relevant information under known tumor types.
- Using focused, empathetic communication skills, acquire a relevant cancer-focused history, including a history of the present illness that is concentrated on the current diagnosis and information to date.
- Analysis of a detailed head-to-toe physical exam.
- Organize data for detailed and efficient presentation and documentation[34].

Lab test

These test include

- Complete blood count (CBC)
- Blood protein testing
- Tumor marker tests

Complete blood count (CBC)

It measures the amount of several types of blood cells in a sample of blood. Blood cancers may be determined using this test whether a few types of blood cells or

abnormal cells are found. A bone marrow biopsy helps to confirm a diagnosis of blood cancer.

Blood protein testing

It determines the several types of proteins in the blood that can aid in detecting certain abnormal immune system proteins (immunoglobulins) that are sometimes increased in people with multiple myeloma Other tests, such as a bone marrow biopsy, are used to confirm a suspected diagnosis.

Tumor marker tests

Tumor markers are chemicals made by tumor cells that can be determined in the blood[35].

Biopsy

Cell samples or tissue can be taken from any part of the body[36]. The small piece of it is taken and detected for cancer or other problems which can cause the growth of cancer or look like cancer. The method of taking out a small piece of lump or sample for testing is known as a biopsy[37].

Imaging test

It involves taking pictures of the inside of the body. This experiment shows forms of energy like sound waves, radioactive particles, X-rays, or magnetic fields through the body[38].

PRE-CLINICAL *IN-VITRO* SCREENING MODELS

In vitro is an important tool in cancer research, determining the identification of carcinogens, drug screening, the development of cancer therapies, and providing insight into the molecular mechanisms of tumor growth and metastasis. In this procedure, which comprises the metastatic process, cancer cells migrate or flow through different microenvironments, including the vascular system, stroma, blood vessel endothelium, and the tissue at a secondary site. The potentiality to negotiate these steps is based on the interactions between the cancer cell and the local microenvironment[39].Following are the preclinical *in-vitro* screening models.

1. Cell-based screening assays
    1. MTT
    2. Trypan blue assay (cell viability assay)
    3. Sulphorhodamine B assay
    4. Luciferase assay
    5. Propidum iodide assay
2. Non mammalian model organisms for screening
3. Biochemical screening
4. *In-vitro* tumor Models
    1. Transwell based assays
        1. Migration assay
        2. Invasion assay
        3. Transendothelial migration assay
    2. Spheroids
        1. Hanging drop method
        2. Suspension culture
        3. Non-adherent surface methods
        4. Microfluidac methods
    3. Hybrid Models
        1. Embedder ex vivo tumor section
        2. 3D Invasion models
        3. Avascular microfluidic models
    4.Tumor Microvessel Environmental Models
5. Tumor Clonogenic Assay
6. Hollow fibre assay
7. In-silico method
    1. Structural-based methods
    2. Ligand based methods.

Cell-Based Screening Assays

The human tumor cell lines have been used in screening methods during *in-vitro* cancer research. it plays an important role in determining the cell viability and activity of anticancer activity. Various procedures are performed to determine cell growth or viability. The broadly used growth inhibition assay which was developed by Mosmann and the NCI (National Cancer Institute**)** screening staff is the methylthiazoldiphenyl tetrazolium (MTT)assay[40].

MTT

The tetrazolium-based MTT assay has long been considered as the gold standard of cytotoxicity assay it is most sensitive and has been miniaturized for use as a high throughput screening assay. This assay estimates cell viability with regards to reductive activity as an enzymatic conversion of tetrazolium compound to water-insoluble formazan crystals by dehydrogenase occurring in the mitochondria of living cells. The percentage of growth inhibition was determined, and the concentration of extracts needed to inhibit cell growth by 50% (IC_{50}) was acquired from the dose-response curves[41].

Cell vialibity%=OD of treated cells/OD of control cells*100

Trypan blue assay (Cell viability assay)

It is used to establish the number of viable cells present in a cell suspension. It depends on the principle that living cells possess intact cell membranes that exclude certain dyes, such as Trypan blue and Eosin, whereas dead cells do not. A cell suspension is simply mixed with dye and then visually assessed to determine whether cells take up or exclude dye. The inhibitory effects on tumor cells were performed by using the following formula[42].

% of viable cells= Live cells/ total number of cell X 100.

Sulphorhodamine B Assay

The sulphorhodamine B (SRB) assay is also called as SRB assay.It determines whole-culture protein content, which should be proportional to the cell number. Cell cultures are stained with a protein staining dye, sulphorhodamine B (SRB) assay . SRB is a bright pink anionic dye that binds with the basic amino acids of cells. Unbound dye is then taken out by washing with acetic acid, and protein-bound dye is extracted using unbuffered. Tris base for determination of optical density in a computer-interfaced, 96-well microtiter plate reader. Since dead cells whether it gets lyse or are lost during the procedure, the total number of SRB binding is proportional to the number of live cells left in culture after the drug. This assay can be used to determine the cellular protein content of both adherent and suspension cultures. Screening capacity, reproducibility, and quality control all appear to be enhanced in this assay relative to the tetrazolium salt assays. The assay is time-consuming compared to the MTT assay. Non-replicating and dead cells might contribute to the total protein and interfere with the results[43].

Cytotoxicity(%) $=OD_{DMSO}-OD_{SAMPLE}/OD_{DMSO}* 100$

Propidum Iodide assay

Propidium iodide is flourscent dye pass through dead cell membrane and form intercalate complex with DNA. This is used to characterize the *invitro* growth of cancer cell lines and also test the cytotoxcity of standard. Total cells are incubated with propidum iodide ,the nonviable cells were assessed by first subsequent detection, followed by -20^{o}c freezing for 24hrs,then assess the second measurement of total cell population.Difference between these two measurements gives rise to number of viable cells.This iodide assay is simple,easy and rapid method for detection of 150-500 cells per well for drug testing[44,45].

Luciferase assay

The energy unit of cell is ATP which hydrolysed after cell death.For measuring ATP,bioluminescent based luciferase(luciferin-luciferase) assay was most popular method.The luciferase catalyse the oxidation of luciferin,results luminescence,with conversion of ATP to AMP.The intensity is positively correlated with ATP content and total number of living cells.Luciferase assay was highly sensitivity and reproducible and able to detect 2000 cell/well when compare to MTT assay[46].

Nonmammalian Model Organisms for Screening

Non-mammalian organisms namely yeast-Saccharomyces cerevisiae, the nematode - Caenorhabditis elegans/ fruit fly- Drosophila melanogaster were used for anticancer drug screening in 1990. Because they share signaling and growth regulatory pathways with humans[47].

Biochemical screening

Biochemical screening plays a major role in carrier detection whenever genetic testing has failed or to determine the germline mutations. It determines the affected gene in tumor surveillance[48]. Enzyme-linked immunosorbent assay (ELISA) plays an important role in biochemical screening. It is used to identify and verify new serum markers, namely extracellular Protein Kinase A(ecPKA) and Nicotinamide A-Methyltransferase(NNMT). In this assay, the cancer antigens are evaluated indirectly by determining the presence of auto-antibodies against tumor proteins in human serum. The consequence of the optimization and validation process was done as the result of ecPKA are reproducible and stable assay. NNMT is not sensitive enough[49].

In-vitro Tumor Model

In vitro tumor models have proved that it is important tools for cancer research and provide low-cost screening platforms for drug therapies. *It* varies in convolution and ranges from tumor-obtained cell lines to 3D models of the tumor microenvironment. This Model provides mechanistic insight into tumor growth or proliferation, matrix remodeling, angiogenesis, dormancy, intravasation, extravasation, migration, invasion, and drug delivery[39].

1. Transwell based assays
2. Spheroids
3. Hybrid models
4. Tumor microvessel model.

Transwell based assays

Transwell-based assays are extensively used to evaluate cancer cell migration and invasion.**Types of Transwell**

Based Assays

- Migration Assay
- Invasion Assay
- Trans-endothelial Migration Assay

Migration Assay

It is the movement of cells from one place to another and it is located to the metastatic cascade .cell migration directed by gradients insoluble factors electric field or matrix stiffness[39].

Invasion Assay

Cell Invasion Assays produce a flexible, standardized, high-throughput format for determining the degree to which invasive cells migrate to a barrier

consisting of basement membrane components with chemo attractants or inhibiting compounds[50]. Invasion directs to the migration of cells in 3D ECM(Extracellular matrix). A layer of ECM is settled on the porous membrane to model the basement membrane of the vasculature[39].

Transendothelial migration assay

Transendothelial migration assay assumes plating a concurrent monolayer of endothelial cells onto the porous support. Intravasation and Extravasation can be done by using a transendothelial migration assay. It is utilized to examine brain capillary endothelium which has tight cell-cell junctions.

Spheroids

Spheroids are otherwise called as Multicellular tumor spheroids represent avascular tumor nodules or micro metastases. It is the accumulation of cells grown in suspension or embedded in a 3D matrix using 3D cell culture methods. 3D Spheroids are extensively used for drug screening and studies of tumor growth and proliferation, immune interaction. 3-D cultures - spheroid culture system of *in-vitro* screening – which better reflect the *in-vivo* behavior of cells in tumor tissues and which, recognized as valuable advanced tools for evaluating the efficacy of therapeutic intervention.Spheroids that support necrotic cores mimic poorly vascularized tumors due to the initiation of oxygen and nutrient gradient.

There are 4 general methods of spheroid formation such as[51];

- Hanging drop technique
- None adherent surface
- Cell suspension culture.
- Microfluidic methods

Hanging Drop Technique

This method was proposed in 1907 by Harrison[52]. In this technique, a drop of medium containing suspended cell was covered by coverslip and then it inverted to fall the drop of suspended cell in the concave wall of microscope slide. Due to gravitational forces, the cells will aggregate and forms spheroids.

Non Adherent Surface

In this method of Liquid overlay technique (LOT), the cells on the non adherent surface such as agar and agarose which prevent the attachment to substrate and generate the spheroid formation. This method of non-adherent sufeace is straight forward but does not allow to control over the spheroid size and uniformity.The spheroids growth in microarrays considerably increases th high throughput during the control of spheroid size. The growth
of Spheroid, is directed by using round-bottom non-adherent 96-well plates or by stamped agarose microwells[53].

Cell Suspension Culture

3D cell cultures grow cells into 3D spheroids/ aggregates using a scaffold or matrix. Scaffold or matrix-based 3D cultures can be produced by seeding cells on an a cellular 3D matrix or scaffold or by dispersing cells in a liquid matrix followed by solidification or polymerization by adding carboxymethyl cellulose. Scaffold-free 3D cell spheroids is produced in suspension form by the hanging drop method, or agitation-based approaches and forced floating method[54]. In the scaffold based 3D culture, cells grow in 3D environment naturally and allowing the cells to interact with each other, this interaction in such 3D spatial arrangement affect the range of cellular functions, which includes morphology ,cell proliferation, gene and protein expression and response of the cell due external stimuli.

Microfluidic methods

Microfluidic systems are innovative models for reconstructing the migration, microenvironment, and microcirculation of cells in a metastatic tumor tissue. This model allows to the study of antitumor drugs effects on the inhibition of tumor cell migration.
Microfluidic system was constructed by containing 2 microfluidic channels and a porous membrane which sandwiched between them. The 1st channel represents the vascular equivalent & contains primary endothelial cells . The 2nd channel acts as a reservoir for collecting migratory tumor cells. In this, the endothelial cells acts *in vivo*-like chacteristic under flow conditions. The introduced Green fluorescent protein-labeled tumor cells of epithelial was detected using vital imaging, which showed tightly attached tumor cells to the endothelial membrane[55]. Nowadays, the microfluidic system are becoming prospective model since they allow to precise control of spheroid formation[56]. Microfluidic principle allow the formation, maintenance, & testing of spheroids within a single device.

Hybrid Models

Hybrid models have three types of *in-vitro* tumor models which cannot be categorized as steroids or transwell based it includes

Embedder Ex vivo tumor section

Embedder – it's an Ex vivo tumor section Embedded biopsies or tumor sections conserve the heterogeneity of tumor cell sub population assisting tissue cells and tumor vasculature. This approach is widely used for assuming tumor morphology, growth, and chemosensitivity and has likely a method for screening patient-specific treatment.

3-D invasion models

3D Invasion Models created by seeding cancer cells on ECM materials decrease the complexities of the tumor microenvironment. This helps in imaging live cells to

study cell morphology, cell interactions, and movement.

Avascular microfluidic models

It has been used to study the cancer cell adhesion to endothelial monolayers as a forerunner to extravasation.

Tumor Microvessel Models

The Tumor vasculature is a crucial factor of a tumor microenvironment providing nutrients. Tumor microvessel is used to study interactions between tumor cells and the tumor vasculature are commonly produced by seeding endothelial cells onto predetermined ECM scaffolds or self-assembled through matrix alteration after randomly dispersing endothelial cells within an ECM (Extracellular matrix). The cells of these vessels secrete the factors, which promotes tumor growth[1,39].

Tumor Clonogenic Assay :

Tumor clonogenic assay is also known as soft agar colony-forming assay. tumor clonogenic assay is a coordinated *invitro/invivo* testing procedure. The TCA can be used for predicting clinical response and also with fresh xenograft tissue for choosing the appropriate *in-Vivo* model for screening assays.

It's an *invitro* quantitative method to evaluate the capability of a single cell to grow into a large colony through clonal expansion

Clonogenic activity is a susceptible indicator of Undifferentiated cancer stem cells. The Crystal violet solution is used for this assay to imagine the generated colonies[57].

Hollow Fibre assay :

Small hollow fibers (tubes 1 millimeter in diameter and 2 centimeters long made of a plastic, polyvinylidene fluoride), containing cells from tumor cells are implanted at sites (underneath the skin and in the body cavity of the mouse) at two dosages and the mouse is tested against 12 target tumor cells in different hollow fibers. A total of about 20 compounds per week are screened by this method. Compounds that retard the growth of the cells are proposed for the next level of testing. The average length of this test is four days.

The assays using hollow fiber techniques have been optimized for human cancers arising from the lung, breast, colon, ovary, brain gastric, and hepatocellular cancer mouse cell lines[43].

In-silico Method :

In–silico is also known as the Bioinformatic method. It is used to identify new potential biological targets or to identify targets among a family of related receptors. The in-silico method / computational drug design approaches provide insights into the physicochemical properties associated with anticancer activity at the molecular level.

These are classified mainly two methods

1. Structural based method
2. Ligand-based method

Structure Based Method

Structure-based methods are based on the availability of structural information of the target, that is either detected experimentally / derived by means of homology modeling techniques. This method has obtained successfully identifying and generated novel bioactive compounds.

Ligand-Based Method

Ligand-based drug design is used in the absence of the receptor 3D information and it depends on molecules that bind to the biological target of interest. 3D quantitative structure-activity relationships (3D QSAR) and pharmacophore modeling are used in ligand-based drug design and this can produce predictive models suitable for lead identification and optimization[58].

Treatment of cancer

Approaches for treatment for cancer such as immunotherapy, radiation therapy, cancer vaccinations, stem cell transformation, chemotherapy, surgery of tumor , Gene therapy and (CAR) T cell therapy.

Immunotherapy

The immune system determines and demolishes the abnormal cell and control the growth of cancer. Examples of cancer immunotherapy are

1)The growth of Monoclonal antibodies (mAbs) , which are the product of B lymphocytes. Trantuzumab(Herceptin) is the first FDA-approved mAbs and it targets the cell surface human epidermal growth factor receptor 2 (HER2) which can be demonstrated by cancer cells.

2) The recent advances of the development of mAbs targeting immune regulatory receptors on T-cells, namely Cytotoxic T lymphocyte-associated antigen (CTLA-4) and Programmed cell death protein 1 (PD-1). Such receptors produce negative signals to activated T-cells to inhibit their activity at the termination of their antigenic response. Tumors use this mechanism for immune escape, thereby stopping the antitumor reactivity of Tumor infiltrating lymphocytes (**TILs**). The clinical implementation of inhibitory mAbs that interfere with this tumor immune escape mechanism resulted in the development of long-term survival in melanoma and carcinoma[59].

Radiation therapy

It is defined as irradiation or X-ray therapy. It punctures the DNA of the cancer cells and destroyed the cancer cell by inhibiting their growth of the cells[60]. Even though radiation damages both normal and cancer cells. It aims to increase the radiation dose to abnormal cancer cells and decrease the exposure to normal cells, which are close to cancer cells or in the path of radiation.Normal cells may rapidly repair themselves and perform their normal function estimation than

cancer cells. Cancer cells are not as systematic as normal cells in repairing the damage due to radiation treatment and concluded in differential cancer cell killing[61].

Cancer vaccination

It is an irradiated tumor cells are given along with an adjuvant. This vaccine utilizes tumor cells, it may be possible to induce T cells particular to any antigen demonstrated by the utilized cells. This method is attempted in several tumors, such as lung cancer, melanoma, colorectal cancer, prostate cancer, and renal cell carcinoma.In several cases, tumor cells are genetically modified to add a consequence, namely cytokine production, and costimulation. GVAX is a cancer vaccine that depends on tumor cells genetically modified to produce GM-CSF. It is used after cancer irradiation to inhibit the proliferation of growth of cancer cells[62].

Stem cell transformation

Stem cells are biological cells found in all cellular organisms that can divide and differentiate into diverse specialized cell types and can self-renew to generate more stem cells. Using standard methods brain cancer is difficult to treat due to its rapid spread. Transplanted human neural stem cells into the brain that received intracranial tumors. Within a few days, the cells migrated into the cancerous area and produced cytosine decease, an enzyme that converts a non-toxic pro-drug into a chemotherapeutic agent. As a result, the injected substance was accomplished to reduce the tumors mass by 81 percent. the patient's lymphocytes, and stem cells injected, eventually replacing the immune system of the patient with that of the healthy donor[63].

Chemotherapy

Chemotherapy defines the use of chemicals so that it inhibits the cancer cells or micro-organisms without affecting the host cells[64]. It can be administered in adjuvant, neoadjuvant, combined, and metastatic settings Neoadjuvant therapy is given before primary treatment and adjuvant therapy is initiation therapy which can suppress the proliferation of cancer cells. For breast, lung, ovarian, and colorectal cancer, adjuvant therapy is done[65].

Surgery Of Tumour

It plays a vital role in cancer care and treatment. It can be preventive, diagnostic, therapeutic, supportive, palliative, and remodeling. Preventive surgery is carried out to remove tissue that is possible to become cancer[66].

Surgery is performed by cryosurgery and laser.

Cryosurgery

Cryosurgery is also knowns as cryotherapy. Cryosurgery is a part of treatment in which the highest cold is produced by liquid nitrogen or argon gas and it is used to demolish abnormal tissue. It may be used to treat the initial stage of skin cancer, and precancerous growths on the skin and retinoblastoma.

Lasers

This is a part of treatment in which heavy beams of light are used to slit through tissue. Lasers can accurately target tiny areas, so they can be used for surgeries for accurate results. It may also be used to shrink or demolish tumors or growths that may turn into cancer. Examples such as basal cell carcinoma, cervical, vaginal, and non-small cell lung cancer[67].

Genetherapy

Genetherapyis innovature treatment option, for cancer in order to prevent apoptosis or slow the growth of tumor, gene therapy is one of the promising therapy for different types of cancer,which not in response with traditional treatment.The various approaches of gene therapy, which focusing either on rapidly developing tumour mass to destroy or to prevent their growth, or enhance the healthy cells to fight against cancer. Further more, it also involve in alteration or replacement of cancer gene with normal gene. In gene therapy, gene is delivered to cell therapy carrier, usually the viruses are used as carrier, due to viruses have the ability to identify cancer cells and deliver the gene into them[68]. Recently, research work focused on gene therapy with recombinated cancer vaccines. The techniques meant for improve patient immune's system to recognise the cancer cells, by powerful antigenic and immunostimulatory cellular debris.

Chimeric antigen receptor (CAR) T cell therapy

Over the past decades, scientist believed to rectify the harnessing the strength of immune system to fight against the cancer,this in turn to lead immunotherapy which strengthen the power of patient's immune cell(system) to invade the tumors.one such therapy is CAR –T cell therapy. In Chimeric antigen receptor T therapy -In order to enhance T cell potency and function the T cells are collected from patient via apheresis, with the help of genetic engineering (synthetic biology , targeted specificity of antigens) introducing DNA into those T cells. Interaction with the specific antigen leads to activation Chimeric antigen receptor which recognize an antigen on targeted tumor cells and cytotoxic killing through granzymes and perforins and enhanced immune response through its cytokine release[8]. Some of the US-FDA approved CDR-T cell therapy are Axicabtagene ciloleucel, Brexucabtagene autoleucel , Ciltacabtagene autoleucel , Idecabtagene vicleucel, Lisocabtagene maraleucel, Tisagenlecleucel and Tocilizumab[69].

CONCLUSION

In the past decades , the global cancer burden was increased . Researchers have reported ,the role of genes and production of cancer cells . In which, alteration in gene in cancer was the one of most important discovery . Recently the environmental factors related with alterations in gene expression.New concepts are in need to investigate in order to achieve a increase in survival

for cancer Research.In future , need of comprehensive study an overcoming challenges on promoted cancer treatments. In order to provide an effective treatment strategy future more the available preclinical screening models are needed to ascertain the criteria (data) to be matched to progress into next stage of drug development for new chemical entity.

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