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A Review on *in-vitro* Methods for Screening of Anticancer Drugs

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Abstract
Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Nowadays research is being carried out for the development of new anticancer agents which selectively interfere with and inhibit tumour growth. Though there are a number of anticancer agents available in the market only few drugs are effective in the treatment of cancer. So, a proper selection procedure is required for the development of a lead compound as anticancer agent. Thus, Invitro drug screening is the starting point for the development of new antitumor agents. Through in – vitro screening it is possible to screen a number of compounds each day. This review summarizes the most popular in-vitro cell-based screening methods, which are used for evaluating the anti-cancer activity of newly evolved anti-cancer drugs.

Keywords
In-Vitro Screening Methods, Anti-Cancer Agent, MTT assay, Clonogenic assay, DNA fragmentation assay.

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INTRODUCTION
Cancer refers to cells that grow out-of-control and invade other tissues. Cells may become cancerous due to the accumulation of defects, or mutations, in their DNA. Certain inherited genetic defects (for example, BRCA1 and BRCA2 mutations) and infections can increase the risk of cancer. Environmental factors (for example, air pollution) and poor lifestyle choices (e.g. smoking) can also damage DNA and lead to cancer.[1]
Most of the time, cells are able to detect and repair DNA damage. If a cell is severely damaged and cannot repair itself, it usually undergoes programmed cell death or apoptosis. Cancer occurs when damaged cells grow, divide, and spread abnormally instead of undergoing apoptosis.[2]
For rapid screening of new anticancer compounds, murine models of rapidly growing cancer were developed, for example, sarcoma 180, carcinoma 755, and L1210 mouse leukemia model which were later replaced by the P388 murine leukemia model. Several clinically important anticancer agents such as methotrexate, actinomycin D, 6-mercaptopurine, 5-fluorouracil were identified using these murine models; however, success were achieved mainly in the case of rapidly growing cancers, e.g. lymphomas, childhood leukemia, and germ line tumors while relatively limited successes were seen in the treatment of the slow-growing common solid tumors

of the adults, e.g. lung, breast, and colorectal cancers.[3]

Public institutions, the pharmaceutical industries, and biotech companies invent lots of compounds with desired anti-cancer activity. Only assertive number of drugs and concepts of actions evaluated clinically because of cost and other ethical considerations. Therefore, a pre-selection, called the screening process is required to identify products that will be able to perform anti-tumor effects. Various types of treatments are available for cancer patients like surgery, chemotherapy, radiations etc. But there are lots of side effects related to these treatments, hence now-a-days scientists are focusing towards development of new anticancer drugs. The biological evaluation of these newly synthesized compounds include various in-vitro or in-vivo techniques. Direct screening by in-vivo technique requires lot of expenses and approval from animal ethics committee hence it is always better to screen the synthesized drugs by various in-vitro techniques which are cheaper. After screening by in-vitro technique, the promising compounds can be screened further by in-vivo technique.

IN-VIVO AND *IN-VITRO* SCREENING

The traditional anticancer drug screening models includes animal experiments and cell-based screening assays.

Large scale screening using animal systems is highly unethical and strictly regulated. High throughput screening (HTS) plays an essential role in contemporary drug delivery processes. Prescreening enable the identification of carcinogens, the development of cancer therapies, drug screening and providing insight into the molecular mechanisms of tumor growth and metastasis. Because of ethical, medical and economical limitations and constraints on the number of patients eligible for clinical trials, most of the research has been done in experimental system.

Most pre-clinical data on new anticancer drugs were obtained using transplanted tumors in mice. For practical reasons, scientists mainly use ectopically-implanted, subcutaneously growing tumor models, frequently as xenografts of human origin. An enormous variety of different tumor systems for in vivo evaluation of new anticancer agents is available.[4] Mostly murine host systems are used for experimental tumor therapy because of the availability of in-bred lines at relatively low costs.

SIGNIFICANCE OF *IN-VITRO* SCREENING

The ultimate goal of the development of molecular targeted drugs is to improve the efficacy and selectivity of cancer treatment. Commonly used cytotoxic anti-cancer drugs were discovered through random high-throughput screening (HTS) of synthetic and natural products in cell-based cytotoxic assays. Despite the chemical diversity of these agents, the mechanisms of action are limited and most compounds are DNA-damaging agents with a low therapeutic index. With these screening approaches, the mechanism of action is not a primary determinant for selecting a drug for further processes, and as a result, none of the drugs is directly targeting the molecular lesions which are responsible for malignant transformation.[5]

METHODS FOR *IN-VITRO* SCREENING

1. MTT Assay
2. SRB Assay
3. Clonogenic assay
4. Thymidine incorporation Assay
5. Alamar Blue Assay
6. LDH Assay
7. Comet Assay
8. DNA Fragmentation Assay
9. Trypan Blue Exclusion Assay
10. Potato Disc Tumor Induction Assay

- **MTT Assay: (Colorimetric Assay Based Upon Tetrazolium Salt)**

MTT Assay is a colorimetric assay for assessing cell metabolic activity.[6] The amount of colour produced by a specific reagent in the assay can be measured by colourimetry. The method determines the concentration of a chemical element or chemical compound in a solution with the aid of a colour reagent. NAD (P) H-dependent cellular oxidoreductase enzymes, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide to its insoluble formazan, which has a purple color. (Figure 1)

Types of Tetrazolium Salts

1. MTT:

It is a yellow colored tetrazole, which was converted into purple formazan in the cell. A solubilizing solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a coloured solution. The quantification is done by taking absorbance of this solution at specific wavelength, normally it is taken at 500 and 600 nm by the use of spectrophotometer. The degree of light absorption is dependent on the degree of formazan concentration accumulated inside the cell and on the cell surface.

Greater the formazan concentration the deeper the purple colour and thus the lower the absorbance. [7]

2. XTT:

(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5carboxanilide) has been processed to replace MTT, to yield more sensitive and greater effective range. (Figure 2) The advantage of this assay is, obtained formazan product is water-soluble, which will avoid final addition of solubilizing solvent.

3. MTS:

(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4 sulfophenyl)-2H-tetrazolium), by the addition of phenazine methosulfate (PMS) produces formazan product that has a maximum absorbance at 490-500 nm in phosphate-buffered saline. The assay has usually known as a 'single-step' MTT assay, which will offer us for straight addition of reagent to cell culture without intermittent steps, which is essential in the MTT assay. However, this convenience makes the MTS assay sensitive to colourimetric interference. (Figure 3)

4. WSTs (Water-soluble Tetrazolium salts):

8(2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium), this assay has been developed to obtain different absorption spectra of the formed purple colored formazan. Water-soluble tetrazolium salts are more recent alternatives to MTT.

Significance

Tetrazolium dye reduction is generally assumed to be dependent on NAD(P)H-dependent oxidoreductase enzymes largely in the cytosolic compartment of the cell. Therefore, reduction of MTT and other tetrazolium dyes depends on the cellular metabolic activity due to NAD(P)H flux. Cells with a low metabolism such as thymocytes and splenocytes reduce very little MTT. In contrast, rapidly dividing cells exhibit high rates of MTT reduction. It is important to keep in mind that assay conditions can alter metabolic activity and thus tetrazolium dye reduction without affecting cell viability. In addition, the mechanism of reduction of tetrazolium dyes, *i.e.* intracellular (MTT, MTS) *vs.* extracellular (WST-1), will also determine the amount of product. [8] (Figure 4)

- **SRB assay (sulforhodamine-B assay)**

Sulforhodamine B is a fluorescent dye with uses spanning from laser-induced fluorescence (LIF) to the quantification of cellular proteins of cultured cells. This red solid dye is very water-soluble.9 The dye has maximal absorbance at 565 nm and maximal fluorescence emission at 586 nm. It does not exhibit pH-dependent absorption or fluorescence over the range of 3 to 10. (Figure 5)

SRB is an anionic dye and it is aminoxanthene, which can react with basic amino acid residues of protein to form an electrostatic complex under moderately acidic conditions. The cells get prepared by thoroughly washing it, fixed and stained it with the dye. The formation of colour is quick and stable and the absorbance can be readily measured at wavelength between 560 and 580nm.The concomitant change in the amount of dye which is incorporated in the culture contribute to the increase or decrease in the total number of cells. These changes will show the degree of cytotoxicity or cell viability caused by the test compound. This evaluation depends on the uptake or incorporation of the pink amino xanthine (negatively charged) dye by amino acids (basic) in the cells. The greater amount of dye is taken up by the cell if adequate amount of cell walls present and after washing and fixing, the released dye will give a more acute color and greater absorbance when get lysed. It is rapid, susceptible, sensitive, and inexpensive method for measuring the cellular protein content of the cell.

Significance

Sulforhodamine B is often used as a membrane-impermeable polar tracer or used for cell density determination via determination of cellular proteins (cytotoxicity assay).

- **Clonogenic Assay**

A clonogenic assay is a cell biology technique for studying the effectiveness of specific agents on the survival and proliferation of cells. The clonogenic assay determines cell proliferation. It is an in-vitro type of cell survival assay.[10] The ability of a cell to proliferate indefinitely is said to be clonogenic. The principle need of the assay is the ability of a cell to rise into a colony. The set of at least 50 cells is defined as colony. The ability of a single cell to grow into a large colony that can be visualized with the naked eye is proof that it has retained its capacity to reproduce. Initially the clonogenic assay have played an essential role in radiobiology, by studying the effects of radiation on cells. Now they are widely used to examine the effect of ionizing radiation on cell or chemotherapeutic agents such as etoposide, etc., or anti-angiogenic agents such as endostatin, and cytokines and their receptors, either alone or in combination therapy. (Figure 6)

- **Thymidine Incorporation Assay**

This assay is the most common assay which utilizes a strategy by the use of radioactive nucleoside, 3H-thymidine. Cell proliferation assays measure the incorporation of a radiolabeled DNA precursor, ^{3}H- or ^{14}C-thymidine, into the replication strands of DNA produced during cell division. Cultures are typically set up in micro plates. The labeled DNA is usually

captured with a cell harvester on glass fiber filter discs, which are then placed in liquid scintillation counting vials or directly harvested into a filter plate for counting on a scintillation beta-counter. The assay can also be performed using scintillating Cytostar-T plate, which requires no filtration step .[11](Figure 7)

Significance

The colony-forming assay is an accurate measure of viability because it measures a cell's ability to undergo multiple cell divisions after treatment. 3H-thymidine incorporation appears to be highly predictive of colony formation in a variety of cell types. The 3H-thymidine assay is simple and easy to perform, is applicable to both adherent and non-adherent cells, is relatively high throughput, and, most importantly, is highly predictive of colony-forming ability. This assay is widely applicable, highly predictive, and can be used as a high-throughput test for drug discovery.

- **Alamar Blue Assay:**

Resazurin (7-Hydroxy-3*H*-phenoxazin-3-one-10-oxide) is a phenoxazine dye, non-toxic, cell permeable agent that is blue in colour (blue dye), which itself weakly fluorescent and redox sensitive. [12] For different types of cell conditions, like bacterial and mammalian cell the alamar blue agent is used as redox indicator. It is used in microbiological, cellular, and enzymatic assays because it can be irreversibly reduced to the pink-colored and highly fluorescent Resorufin (7-hydroxy-3*H*-phenoxazin-3one). Commercially it is available as the sodium salt. The agent is termed under alamar blue which is also available under another name like Vybrant and UptiBlue.

Resazurin is reduced to resorufin by aerobic respiration of metabolically active cells, and it can be used as an indicator of cell viability. It can be used to detect the presence of viable cells in mammalian cell cultures. The indicator is water soluble therefore assay is simple to perform, thus eliminating the washing/fixing or extraction process. Once the indicator gets enter into the cell, it gets reduced to resorufin, which is highly florescent and red in colour. Viable cell will increase the colour intensity of the media containing the cell by making it highly fluorescence. (Figure 8)

Significance

Resazurin is effectively reduced in mitochondria, making it useful also to assess mitochondrial metabolic activity. The alamar blue Assay is designed to measure quantitatively the proliferation of various human and animal cell lines, bacteria and fungi. The bioassay may also be used to establish relative cytotoxicity of agents within various chemical classes.

- **LDH assay:**

Lactate dehydrogenase (LDH), which is a soluble cytosolic enzyme present in most eukaryotic cells, which releases into culture medium upon cell death due to damage of plasma membrane. The increase of the LDH activity in culture supernatant is proportional to the number of lysed cells. LDH cytotoxicity assay kit provides a colourimetric method to measure LDH activity. LDH catalyzes the reduction of NAD^+ to NADH in the presence of L-lactate, while the formation of NADH can be measured in a coupled reaction in which the tetrazolium salt INT is reduced to a red formazan product. The amount of the highly colored and soluble formazan can be measured at 490nm spectrophotometrically. There are two type of cell death programe which is perceived in both disease pathologies and normal, one is apoptosis and other one is necrosis. The necrosis cell death depends on the permeabilization of plasma membrane. Because of this permeabilization, the inflammatory responses may come which may be reflected towards the inflammatory diseases. [13] The damage to the plasma membrane will indicate the necrosis of cell or necrotic cell death. The enzyme such as alkaline and acid phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase etc. leads to the release during cell death by apoptosis. LDH is a stable cytoplasmic enzyme which is released into the medium in an adequate amount by following loss of membrane integrity on the conclusion of apoptosis. Therefore, LDH assay can be used to determine the effect of cytotoxic agent or any chemical compound and has been used to evaluate the cell membrane purity. (Figure 9)

Different chemical compounds such as Anti-cancer drugs cause release of LDH (cytoplasm). The secreted LDH is allowed to react with the solution containing lactate, NAD+ and a coloring agent or dye. LDH act as an enzyme and catalyzes the reaction of conversion of lactate to pyruvate by reducing NAD+ to NADH. Calculating the amount of LDH released during the reaction helps us to study necrosis. A tetrazolium salt can be used in this assay to detect the leakage of LDH into culture medium. The reaction of conversion of lactate to pyruvate and release of LDH goes in several steps; first LDH yields nicotinamide adenine Di-nucleotide (NADH), which catalyzes the reaction of oxidation of lactate to pyruvate. In the next step, a tetrazolium salt which is added in the beginning of the reaction used this NADH for the formation of colour compound called formazan in the presence of electron acceptor. Colourimetrically the formation of formazan can be quantified at wavelength of 490-529nm. The amount of formation of colour

compound formazan is proportional to amount of cell lysed. [14] The percentage of necrotic cells present in the cell sample can be evaluated by taking linearity of the assay. Different types of commercial kits are available for performing different type of conditions.

Significance

Due to the inherent linearity of the assay, it can be easily used to accurately determine the percentage of damaged or injured cells in a sample. In addition, its simplicity, reliability, and accuracy make it an excellent alternative to ^{51}Cr release cytotoxicity assays, a highly sensitive method that measures low levels of cytotoxicity by using radioactive isotopes.

- **COMET Assay:**

The comet assay single cell gel electrophoresis assay SCGE is an uncomplicated and sensitive technique for the detection of DNA damage at the level of the individual eukaryotic cell. It involves the encapsulation of cells in a low-melting-point agarose suspension, lysis of the cells in neutral or alkaline (pH>13) conditions, and electrophoresis of the suspended lysed cells. The term "comet" refers to the pattern of DNA migration through the electrophoresis gel, which often resembles a comet. The comet assay (single-cell gel electrophoresis) is a simple method for measuring deoxyribonucleic acid (DNA) strand breaks in eukaryotic cells. Cells embedded in agarose on a microscope slide are lysed with detergent and high salt to form nucleoids containing supercoiled loops of DNA linked to the nuclear matrix.[15] Electrophoresis at high pH results in structures resembling comets, observed by fluorescence microscopy; the intensity of the comet tail relative to the head reflects the number of DNA breaks. The likely basis for this is that loops containing a break lose their supercoiling and become free to extend toward the anode. This is followed by visual analysis with staining of DNA and calculating fluorescence to determine the extent of DNA damage. This can be performed by manual scoring or automatically by imaging software. Comet assay is therefore beneficial technique for predicting responses to the drug that are affected by DNA structure. (Figure 10)

Significance

The comet assay is an extremely sensitive DNA damage assay. This sensitivity needs to be handled carefully as it is also vulnerable to physical changes which can affect the reproducibility of results. Due to its simple and inexpensive setup, it can be used in conditions where more complex assays are not available. The applications include genotoxicity testing, human biomonitoring and molecular epidemiology, ecogenotoxicology, as well as fundamental research in DNA damage and repair.

- **DNA fragmentation assay**

Different types of anti-cancer drugs induce apoptosis in susceptible cells, which is included in caspase mediated cell death. Various biochemical events lead to change in the characteristics of the cell (morphology) and cause death. These changes include loss of cell membrane asymmetry and attachment, nuclear and chromosomal DNA fragmentation, chromatin condensation, etc. One of the most important steps in apoptosis is DNA fragmentation, a process which leads into degraded DNA endonucleases during the apoptotic program by the activation of magnesium and calcium dependent nucleases. These enzymes degrade the higher order chromatin structure into smaller DNA piece (50bp in length) or into fragments (300 kb) subsequently. Extreme apoptosis causes atrophy, also called as ischemic damage, where as an inadequacy cause uncontrolled cell propagation or proliferation, which lead to cancer.[16]

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is a method for detecting DNA fragmentation by labeling the 3'- hydroxyl terminal in the double-strand DNA breaks generated during apoptosis. TUNEL is a method for detecting apoptotic DNA fragmentation, widely used to identify and quantify apoptotic cells, or to detect excessive DNA breakage in individual cells. The assay relies on the use of terminal deoxynucleotidyltransferase (TdT),an enzyme that ca talyzes attachment of deoxynucleotides, tagged with a fluorochrome or another marker, to 3'-hydroxyl termini of DNA double strand breaks. It may also label cells having DNA damaged by other means than in the course of apoptosis. This assay is also called as nick translation because the DNA which is to be processed is treated with DNAse to produce single-stranded "nicks", This is then followed by replacement in nicked sites by DNA Polymerase-I, which will elongate the 3' hydroxyl terminus, removing the nucleotides by 5'-3' exonuclease activity, replacing them with dNTPs. The radioactive label DNA fragment used as a probe in the blotting procedures, which would act as an incorporated nucleotide provided in the reaction and it is radio labeled in the alpha phosphate position. Similarly, a fluorophore can also be attached as a fluorescent labeling or an antigen for immunodetection. When DNA polymerase I eventually detaches from the DNA strand, it will leave another nick in the phosphate backbone. The nick has "translated" some distance depending on the processivity of the polymerase. This nick could be sealed by DNA-ligase, or its 3' hydroxyl group could serve as the template for further DNA polymerase I activity. Proprietary

enzymes are available commercially to perform all steps in the procedure. (Figure 11)

Significance

Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay has been designed to detect apoptotic cells that undergo extensive DNA degradation during the late stages of apoptosis. The method is based on the ability of TdT to label blunt ends of double-stranded DNA breaks independent of a template.[17]

- **Trypan blue exclusion assay**

The most primitive and common methods for determining the cell viability is the trypan blue (TB) exclusion assay. Trypan blue is a dye that is cell membrane impermeable (960 Dalton) and they are able to enter into compromised cell membranes. (Figure 12)

Once the trypan blue gets enter into the cell, it will bind to intracellular proteins thereby rendering the cells a bluish colour. The trypan blue exclusion assay allows for a direct identification and enumeration of live (unstained) or dead (blue) cells in a given population. Although TB has been used to determine cell viability for many years, it is considered to be carcinogenic and must be handled with care and disposed of properly. [18]Over time TB actually forms crystal of dye or aggregates of it, for this reason it is suggested that TB should pass through the filter using a 0.2-micron filter before performing assays. (Figure 13)

Significance

The Trypan Blue dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, Eosin, or propidium, whereas dead cells do not. When a cell suspension is simply mixed with the dye and then visually examined to determine whether cells take up or exclude dye. A viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. Periodic cell viability assessment provides an early indicator of the quality of your fresh cells prior to freezing. Viabilities of greater than or equal to 95% are excellent.

- **Potato disc tumor induction assay**

Bioassay methods are used over the years, to determine the anti-tumor activity or tumor inhibition specificity of plant extracts. These bioassay methods have already yielded important discoveries from plant origin or by plant extract including vincristine, the podophyllotoxin etc. The potato disc tumor induction assay was set to be useful for evaluating unknown and novel anti-tumor properties. The potato disc induction assay is based on *Agrobacterium tumefacien (*Rhizobium radiobacter), the bacterium is used to infect for the formation of tumor or crown gall in the plant. [19] The mechanism to produce tumor in plant tissue or to make tumorogenic plant by *A. tumefaciens* is as similar to the animal which shows the validity of such bioassay for evaluation of anti-tumor activity. Different type of studies had been already find numerous and prospective areas of similarity in the mechanism bacteria pathogen of plant and humans.

A. tumefaciens is a Gram-negative soil bacterium, which is rod shaped and virulent that is the causative agent for tumor induction or for crown gall disease. Crown gall is a tumefaction disease in which a mass or lump of tissue protruding from roots and stems of woody and herbaceous plant by which tumor is produced. The masses of tumor could be soft or hard, with or without adulterous effect on the plant. By the infection of plant septum with *A.tumenefaciens,* a tumor-causing plasmid which is also called Ti-plasmid, found in the bacterial DNA, which is incorporated or inject into the plant's DNA. When tissue is damaged, it releases phenol or other chemicals, which will activate the Ti-plasmid. This segment cause plant cell to multiply or develop rapidly without going into the apoptosis, resulting in tumor formation which is similar in the histology to the human and animal cancer or related to nucleic acid contents etc. The resulting tumor is able to proliferate and also susceptible to block the apoptosis like in animal cancer. It has been proven that ability of *A. tumefaciens* for the formation of crown gall on potato discs and subsequent growth of the tumor was in good correlation with compounds their extracts, which are statistically much more informative for *in-vivo* anti-leukemic activity. (Figure 14)

Significance

The crown gall induced in potato discs by *Agrobacterium tumefaciens* is becoming largely utilized in screening anti-tumor agents. The antitumor potato disc assay was shown to be sensitive for variable chemicals that interfere with cell cycle and have different Modes of action.[20]

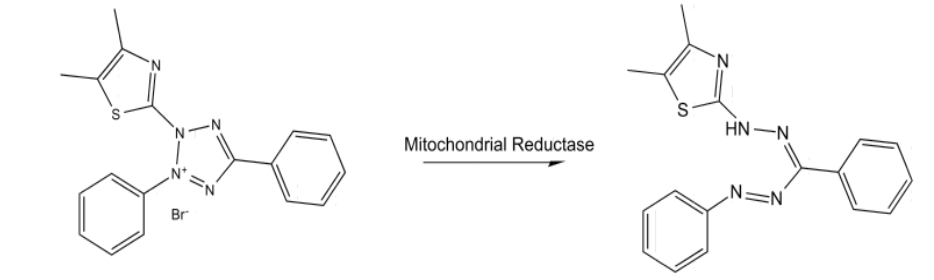

Figure 1: Reduction of MTT salt to Formazan

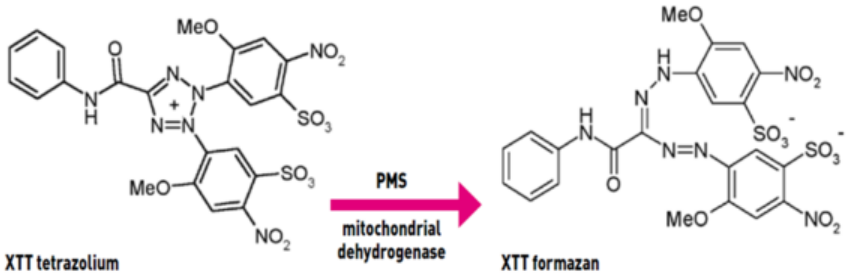

Figure 2: The reduction of XTT to form the colored formazan derivative

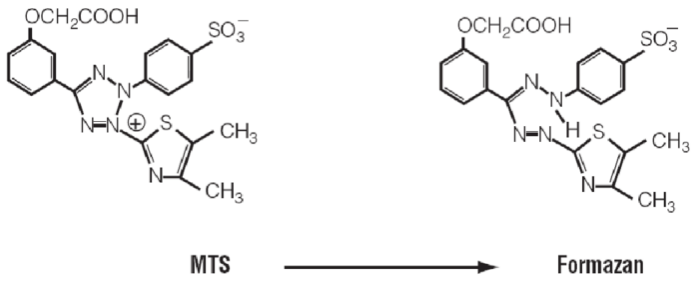

Figure 3: Reduction of MTS salt to Formazan

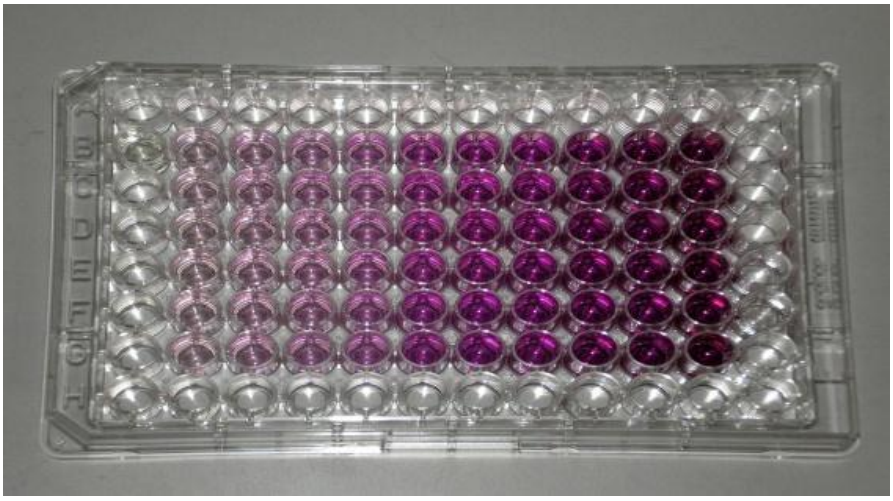

Figure 4: A microtiter plate after an MTT assay. Increasing amounts of cells resulted in increased purple colouring

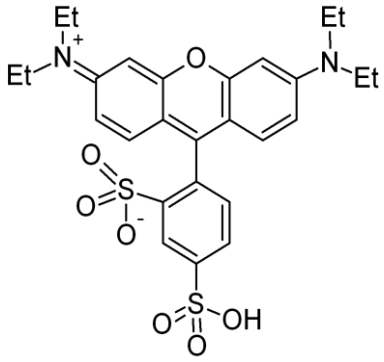

Figure 5: Sulphorhodamine B

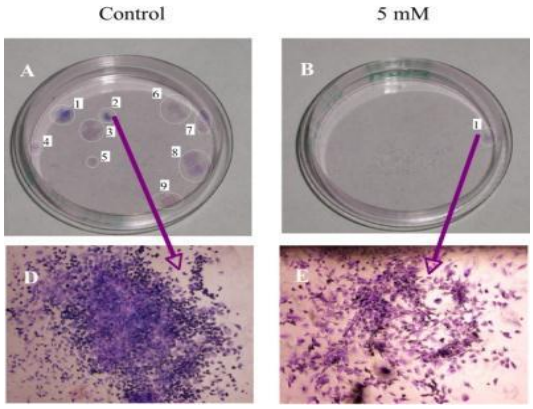

Figure 6: Clonogenic assay of cells *in vitro*

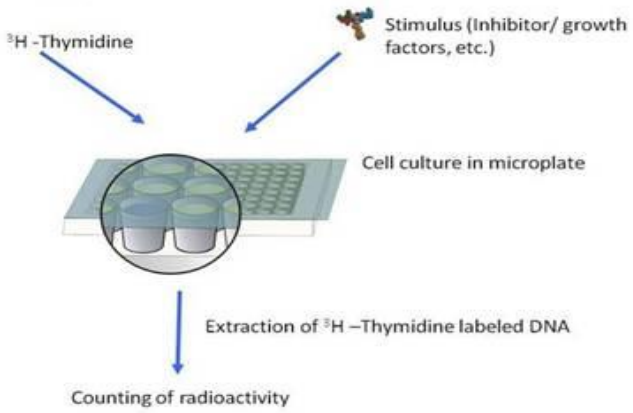

Figure 7: Thymidine uptake assay

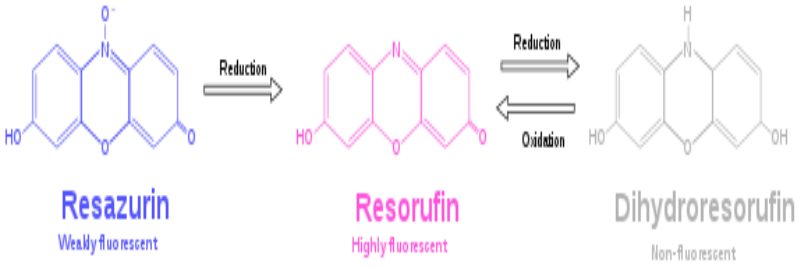

Figure 8: Reduction of Resazurin to resorufin and reversible reduction of resorufin to dihydroresorufin

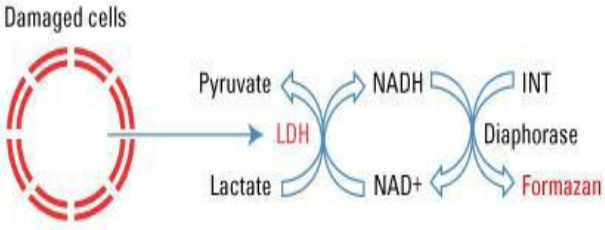

Figure 9: Reduction of LDH to Formazan

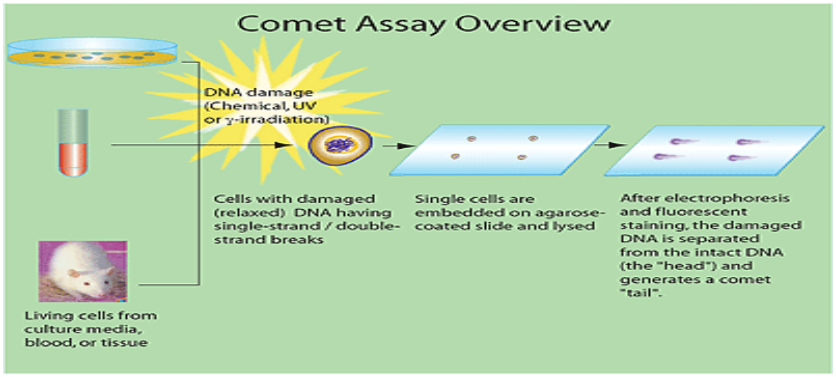

Figure 10: Comet assay (Single cell gel electrophoresis)

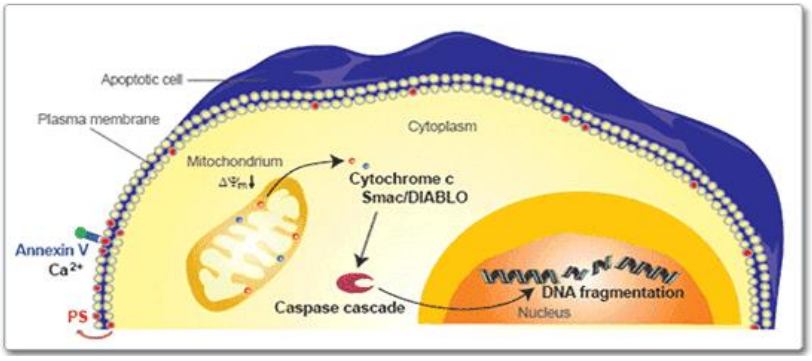

Figure 11: DNA fragmentation

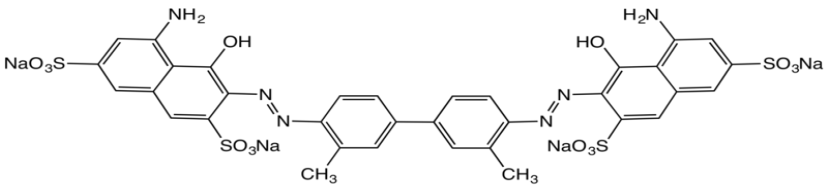

Fig12: Trypan blue salt

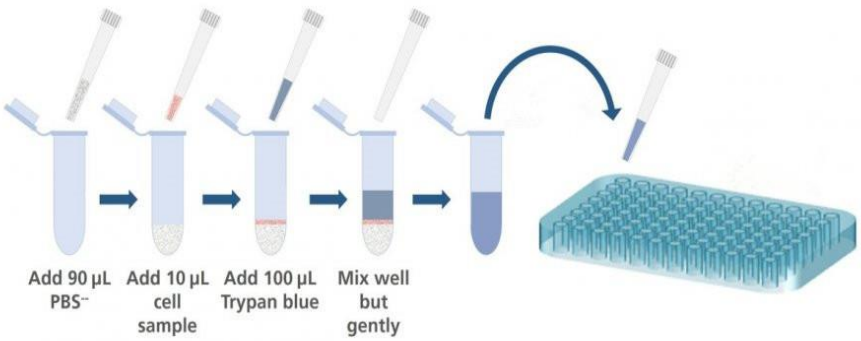

Figure 13: The routine assay process

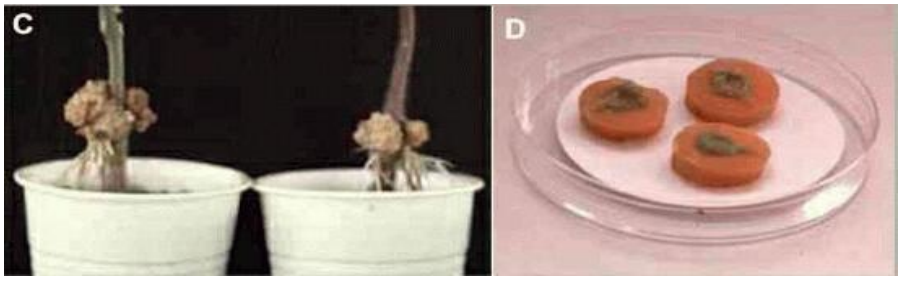

Figure 14: Crown gall tumors on potato disc

CONCLUSION

The official, well-established or Standardized *in-vitro* screening methods are suitable for experimental determination of new anti-cancer agents. Through screening it is possible to overcome the large quantity of potential drugs to a few or most promising agents for further clinical testing by using *in*-vitro to in-vivo procedures. The clinical specification for which the drug is aimed, needs to be considered in the experimental evaluation of the drug. Pre-clinical screening or in-vitro assay is necessary to optimize the compounds for further procedure of development of new anti-cancer agent. A number of *in vitro* techniques are available to determine the metabolic markers to evaluate the anticancer potential of a drug. Each cell viability assay has its own advantages and disadvantages. In the scenario of target-oriented cancer therapeutics, screening of compounds are made to measure towards the desired mechanism of tumor inhibition. In the past decade the empirical screening procedure are designed to evaluate highly potent or cytotoxic agents which produced low selectivity and efficacy in solid tumors when clinically using it. However, empirical screening procedure combined with novel or new ethical knowledge, arising from genome and proteome research as well as bio-information technologies might be the most beneficial for the determination or designing of new anti-cancer agent.

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