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CYTOTOXIC EFFECT OF TAMRA ON HEPATIC CELL CANCER CELL LINES - A CRITICAL REVIEW

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ABSRTACT

The *Ayurvedic* system of medicine has great antiquity, dating back to about 5000 years B.C. In the medieval period with the advent of Rasashastra use of different Metals, Minerals in therapeutics was intiated. Tamra (Copper) is one such metal included under shudda loha varga and is recommended in the management of various disorders such as Shwasa, Yakrut Vikara, sthoulya Arbuda etc, Different compounds of Copper I,e Tamra Bhasma, Tamra Sindhura, Tamra garbha Pottali are potential therapeutic agents, As Tamra and its formulations are indicated in Yakrut vikara, they can prove effective in Hepatocellular carcinoma. The Anti-Cancer action or cytotoxic action of Tamra can be screened on Human Cancer cell line studies (Hep G_2, Hep B3) using MTT Assay method. This is a reliable and easily feasible method to scientifically validate and aid in the development of new anticancer agents.

Keywords; Cell Line Studies, Cancer, Tamra

INTRODUCTION

There are over 200 different known cancers that affect human being and is the one among the leading causes of death worldwide. Cancer comprise group of diseases that involves rapid abnormal cell growth with the potential to invade or spread to other parts of the body, later process is called as metastasis. Metastases are the primary cause of death from cancer. The

malignancies are usually named after the organs or tissues where the cancers develop. For example, lung cancer starts in the lung, and Hepatic cancer starts in the Liver. Cancers also may be described by the type of cell that formed them, such as an epithelial cell or a squamous cell.

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 The most common causes of cancer death in 2020 were, lung (1.80 million deaths), colon and rectum (935 000 deaths), liver (830 000 deaths), stomach (769 000 deaths) and breast (685 000 deaths).Now the latest cancer report released by the Indian Council of Medical Research (ICMR) and National Centre for Disease Informatics and Research (NCDIR), Bengaluru confirms the sharp increase in India's cancer cases, estimating that it could further increase by 12% in the next five years.

Currently available treatment protocols are not gold standard and produce critical side effects, hence there is a need to screen the anticancer drugs on human cancer cell lines using Assay methods.

DESCRIPTION

Scientists have identified different stage of cancers, indicating that several gene mutations are involved in cancer pathogenesis. These gene mutations lead to abnormal cell proliferation. Normal cells may become cancer cells. Before cancer cells form in tissues of the body, the cells go through abnormal changes called hyperplasia and dysplasia. In hyperplasia, there is an increase in the number of cells in an organ or tissue that appear normal under a microscope. In dysplasia, the cells look abnormal under a microscope but are not cancer. Hyperplasia and dysplasia may or may not become cancer. (Credit: Terese Winslow). The common and important etiological factors of Cancer are Smoking and Tobacco, Diet and Physical Activity, Sun and Other Types of Radiation. Viruses and Other Infections.

All tumor cells show the six hallmarks of cancer. These characteristics are required to produce a malignant tumor.

- Cell growth and division - proper signals absent
- Continuous growth and division even given contrary signals
- Avoidance of programmed cell death
- Limitless number of cell divisions
- Promoting blood vessel construction
- Invasion of tissue and formation of metastases.

Liver cancer is the sixth common cancer and the second leading cause of death from cancer around the world. Hepatocellular carcinoma (HCC) is the primary malignancy of the liver. It is the third leading cause of cancer death in the world, and the second in China.

The major risk factor for liver cancer is exposure to hepatitis viruses and environmental pathogens. Hepatitis B virus (HBV) infection accounts for 60% of total liver cancer, and hepatitis C virus (HCV) is responsible for 33%. Other risk factors include alcohol-related cirrhosis, nonalcoholic fatty liver disease, and obesity.

Different treatment modalities such as chemotherapy, radiotherapy, cytotoxic agents and Surgery are available but the outcome is not appreciable. Much sever adverse effects of Chemotherapy also reported. In fact, side-effects and toxic effects associated with these therapies are also the major cause of the world. The formulations prepared from Tamra has been indicated in Yakrut Vikara and Rakta Vikruti janya rogas, hence may prove beneficial and potential in the management of Hepatic Cell Carcinoma. Hence there is a need of thorough research in this direction.

Cell Line study

Human cell lines are immortalized cells propagated in vitro from primary explants of human tissue or body fluid. The use of human cell cultures as a model for more complex biological systems is an integral part of molecular biology, and biomedical research. Human cancer-derived cell lines are fundamental models used in laboratories to study the biology of cancer, and to test the therapeutic efficacy of anticancer agents . Hella was the first cultured cancer line. It was derived from cervical cancer cells taken from Henrietta Lacks in 1951

Much progresses in cell culture techniques resulted in establishment of various human tumor cell lines. Currently, we are able to use human tumor lines as well as murine ones for the examination of drug sensitivity.Cell culture is the process by which cells are grown under controlled conditions, generally outside their natural environment. After the cells of interest have been isolated from living tissue, they can subsequently be maintained under carefully controlled conditions and can be used in different research studies.Among the hepatocellular cancer cell lines are Hep G_2, HepB3 cells are widely used in research.The Hep G2 cell line has been isolated from a liver biopsy of a male Caucasian aged 15 years, with a well differentiated

hepatocellular carcinoma. The cells secrete a variety of major plasma proteins e.g. albumin, α2-macroglobulin, α 1-antitrypsin, transferrin and plasminogen

MTT assay

A number of assay methods to study anticancer activity have been developed. Initially growth inhibitory activity was evaluated after drug exposure by counting cell numbers . Then human tumor clonogenic assay (HTCA) was designed to measure proliferative cells. Recently colorimetric MTT assay in 96-well microplates was developed, which is serving as a reliabl tool to establish drug efficacy based on disease-oriented screening (DOS) with different human tumor cell lines.

Small molecule compounds and antibodies can be used to target cancer cells and block tumor growth and progression. The most common strategy for hepatocellular carcinoma is inhibition of angiogenesis signals. Drugs used to target liver cancer include:

- Sorafenib Tosylate (Nexavar)
- Regorafenib (Stivarga)

This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. DMSO, Isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

Tamra

Tamra falls under the category of shudha loha varga. Acharya Charaka describes it as parthiva dravya. The references of Tamra dhatu is found since vedakala but its extensive therapeutic applications were described from 7th century onwards during the development period of Rasashastra, Tamra dhatu is a Copper that rarely occurs in native form in nature. It can be procured from its ores such as Cuprite, Tenorite, chalcopyrite, copper glance etc.

Copper is an important trace element in humans and is tightly regulated in the body. High levels of copper are maintained in the brain for neurotransmitter production, and in the body, copper is involved in metabolic

activity and immune response. Copper is used by various enzymes in the body as a helper for the chemical reaction. The chemical reaction may involve creating energy, decreasing inflammatory response, blood clotting and growth hormone stimulation, as transcription factor in RNA etc.

Two varieties of Tamra are mentioned in which Nepaliya variety is considered as grahya Tamra. It has Vishahara, Lekhana properties having pre-dominantly kashaya, Madhura rasa. Researches on Tamra have proved that it possess Anti microbial, anti oxidant and Immuno Modulator properties. It has got It has wide range of therapeutic uses in the management of liver disorders such as Hepatitis. Hepatomegaly, Liver cirrhosis. Multiple formulations of Tamra are found described in literature of Rasashastra for different pathological conditions such as Hikka, Hrudroga, Shwasa, yakrut Vikara, Raktavaha Sroto Vikara, Arbua, Sthoulya. Some of the formulations of it are Somanathiya Tamra Bhasma, Tamragarbha Pottali, Tamra Sindhura, Tamra Parpati Hridayarnava rasa, Nityananda Rasa,

The Tamra Yogas mentioned above such as Nityananda Rasa. Tamrasindhura and Tamragarbha Pottali can be effectively used in the treatment of different kinds of Arbuda. They have potential to reach to the target site and exhibit desired action by binding to the receptor cells. Their action can be enhanced by processing the yogas with suitable herbal drugs which possess significant cytotoxic action on cancer cells. Bhallataka, Triphala, Sadapushpi, Ashwagandha are some of the drugs having proved efficacy on cancer.

Copper mediated DNA damage is caused by Reactive oxygen species, because Cu+ is a mutagenic. Much of the copper induced damage is a result of copper binding to specific sites on double strand DNA, resulting in strand scission. Copper causes apoptosis that seems to be mediated by DNA damage and subsequent p53 activation. copper level directly concerned with production of more ROS, high exposure of ROS cause apoptosis in malignant cells (cancerous cells) 26 (Gupte, 2009). Copper induces the decrease of mitochondrial membrane potential and occurrence of the mitochondrial permeability transition (MPT) induces apoptosis therefore appears to be dependent on introduction of the MPT, but the prominent contribution of

mitochondria to ROS generation also suggest an important role of mitochondria in cell death31 (Krumschnabel *et al*., 2005).

DISCUSSION

Human cancer-derived cell lines are fundamental models used in laboratories to study the biology of cancer, and to test the therapeutic efficacy of anticancer gents .American Type Culture Collection (ATCC) Cell Biology Collection is an organization which consists of over 3,600 cell lines from over 150 different species. There are some limitations which are to be carefully checked to get a proper results. cell lines in place of primary cells. Precaution to be taken towards secondary Cell lines should display and maintain functional features as close to primary cells as possible.Since cell lines are genetically manipulated this may alter their phenotype, native functions and their responsiveness to stimuli. The other major problems associated with cell lines are contamination with other cell lines and mycoplasma. In the year 1970 it was noticed that there was cross contamination of cell lines with Hella Cells and are distributed by Cell banks.

The MTT assay has been most widely applied in the assessment of cytotoxic e. drug therapy. The advantages of the MTT assay include ease and rapidity of performance, reproducibility of the results and observed clinical correlation between in vitro and in vivo testing

The epidemiological burden of cancer in the world is rising exponentially. The Radiotherapy and chemotherapy drugs used are not able to recover the pathological condition and also the adverse effects developed during therapy are major concern. The traditional medicine, Ayurveda has documented number of herbal and herbo-mineral medicines that are beneficial in the treatment of Cancer. These medicines are to be scientifically validated by adopting Reverse Pharmacology method (I.e from clinics to lab). Ayurveda medicines are time tested and will have minimum or no side effects if administered by Yukti. Tamra is processed extensively by adopting samanya and vishesha shodhana and subjected to further process as per requirement I,e Marana (for Tamra Bhasma preparation) Kupipakwa method (in case of Tamra Sindhura preparation) etc. These process will not only eliminate impurities but improves pharmacological and therapeutic profile

of Tamra Preparations. The failure of apoptosis (Natural Cell Death) contribute to the development of human cell malignancies. To regulate the apoptosis in cancerous cells there are many pathways. The research studies on copper states that the mitochondrial permeability transition pathway is followed by copper.. Reactive oxygen species (ROS) generation, regulation of p53 gene, activation of Bax, Bad protein, Cytoahrome C, Caspase 9, Caspase 7, Caspase 3, FasL, caspase 8, inhibition of Bcl-2 protein (an anti apoptotic protein) induces apoptosis in carcinogenic cells.

(https://www.researchgate.net/publication/341984445_)

Hence Tamra may regulates tumor suppressor protein p53 that plays a role in the molecular response to DNA damage by acting as a DNA-binding transcription factor that regulates specific target genes to arrest the cell cycle, induce repair mechanisms, and initiate apoptotic cell death

CONCLUSION

The MTT assay is most reliable method in the assessment of cytotoxic drug therapy. The application of the MTT protocol is technically feasible in cell cultures grown in suspension. The assays can predict drug resistance with high accuracy and, in some cases, drug sensitivity. The cancer is a more progressive disease with high mortality rate that requires effective anticancer agents to reverse the pathology, The different compounds of Tamra have potential role in the management of cancer. To understand their cytotoxic effect on cancer cells, cell line studies play prominent role and may come up with scientific revolutions in the field of Ayurveda.

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