Chemosensitivity Test

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ABSTRACT
Cancer treatment is a fundamental issue that today’s scientists deal with. The use of appropriate chemotherapeutic agents in cancer treatment is still limited by the trial and error methods. Chemosensitivity test is a method that allows choosing the most appropriate treatment for each patient, and its purpose is to directly measure the drug sensitivity of the cells. It is based on the application of various chemotherapeutic agents to the tumor cells in vitro and their cell reproduction. In this way, the most appropriate agent can be selected from the test analyzes including ATP-TCA, MTT, CD-DST, B (SRB). Based on these results, ultimately, most appropriate treatment can be applied. The overexpression of tumor suppressor genes and the silencing of oncogenes are also other parameters that increase chemosensitivity. Here we reviewed briefly the chemosensitivity methods that are used in the medical field as well as the effect of tumor suppressor genes such as P53 and livin on chemosensitivity.

Keywords: Chemosensitivity, chemotherapy, oncogen, tumor suppressor gene.

The MTT experiment is based on the colorimetric measurement of the insoluble salt formed in metabolically active cells.[5] Another method is to measure cell ATP by luminescence. In this method, the metabolic activity of cells is measured. Cell number is directly related to the amount of ATP.[6] The other method that measures cell mass using sulforhodamine B (SRB), which binds to proteins in fixed cells. The disadvantage of this method is that it does not measure the live cells and therefore, it assumes that proteins from dead cells disappear from the culture.[6,7]

SENsitiviTY MEASUREMENT METHODS IN CANCER DRUGS

The measurement methods of sensitivity include ATP-TCA (ATP Based Chemosensitivity) test, MTT assay, Culture Drug Sensitivity Test (CD-DST) Embedded in Collagen Gel Droplet, B (SRB).[8]
ATP Based Chemosensitivity Assay (ATP-TCA)

This method was developed to solve the problems encountered in other test types lacking drug sensitivity in the early 1990s. It is an *in vitro* drug susceptibility test method widely used in recent years to determine drug sensitivity rates of tumors. ATP-TCA can be used to guide the selection of chemotherapy drugs and potentially improve patients’ clinical responsiveness and survival.

The ATP-based tumor chemosensitivity assay (ATP-TCA) is a standard system that can be adapted to a variety of uses with both cell lines and primary cell cultures. It aids in drug development, mechanical studies to measure drug resistance, and clinical decision making.

This test can be done in two ways and is highly standardized. First, ATP can be measured with recombinant luciferase. Although choices are made regarding dynamics and sensitivity when the reaction is formed, the accumulation of ATP to a point is linear with a maximum of 108 cells. Second, this assay uses a serum-free medium. The growth of lymphoid cells is not supported, it is found in large numbers in tumor tissue.

The ATP-based tumor chemosensitivity assay has proven to be a useful tool for cell-based research and drug sensitivity. Several studies have developed new chemotherapy combinations, particularly mitoxantrone + paclitaxel, therosulfan + gemcitabine, and vinorelbine liposomal doxorubicin. However, in theory, ATP-TCA does not determine the vital activity of cells but is important in evaluating their cytotoxic effects. This method is preferred not only to investigate drug resistance but also to measure the effects of targeted chemical agents.

MTT Test

This test is a simple analysis based on enzyme activity described by Mossman. In this assay, a tetrazolium salt is reduced to [3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl-tetrazolium bromide, MTT] a colored formazone. The product of this reaction only reduces the enzymes found in live active cells.

The MTT test carries 96-well microtiter plates so large scale experiments examining a range of variables can be easily performed. In this way, drug concentration, drug exposure time, and density of the cell can be tested.

The evidences have shown that 30 minutes cell lysis application does not reduce MTT in dead cells. Therefore, a color change was observed in the cells displaying vital activity. In addition, tetrazolium salts have been used to improve the detection of drug cytotoxicity in soft agar colonies.

Culture Drug Susceptibility Test (CD-DST) Embedded in Collagen Gel Droplet

Collagen gel droplet embedded culture test (CD-DST) is another method that measures the sensitivity of drugs used in cancer treatments. The collagen gel droplet culture method, three-dimensional CD-DST, that enables testing of very few clinical samples in a serum-free environment and their quantitative measurement by image analysis.

CD-DST has little effect on non-cancerous cells and it only provides accurate measurement in cancerous cells. This method has been used primarily in digestive system tumor cells. CD-DST offers many advantages, as it can quantify the original growth characteristics of cultured cells and easy anti-cancer effects without contamination with fibroblasts.

Among cancers types, oral cancers usually have a smaller tumor volume. Therefore, CD-DST application is a suitable method for testing the sensitivity of anti-cancer drugs in oral cancers. A successful analysis of anticancer drug sensitivity using CD-DST on cervical lymph node tissue was reported in a 55-year-old patient with advanced hard palate cancer.

The CD-DST method has been reported to be successful in patients by many researchers. This method has an advantage of simplicity with a good correlation between *in vitro* and *in vivo* results. Therefore, it has been thought that CD-DST can be used to predict response to anti-cancer drugs and it will provide an important information by contributing to the development of new chemotherapy regimens and personalized treatment.

Sulforhodamine B (SRB) Test in Cell Culture

Sulforhodamine B assay (SRB) is a method that is used to conduct various screening analyzes to investigate cytotoxicity in cell-based studies.

This method is based on the property of SRB which binds to proteins cytochromatically under mildly acidic conditions and can then be extracted using basic conditions. Thus, the amount of dye can be used as a tool for cell mass and can then be estimated to measure cell proliferation. It consists of four stages: preparation of the treatment, incubation of cells with the treatment of choice, cell fixation, SRB staining and absorbance measurement. This test is limited to manual or semi-automatic screening and can be used effectively and precisely to test chemotherapeutic
drugs or small molecules in adherent cells. At the same time, the effects of miRNA replacement on cell proliferation can be examined, as well as evaluating the effects of gene expression modulation (fragmentation, gene expression upregulation).[25] The SRB assay is suitable for measuring drug-induced cytotoxicity and it is a sensitive assay that can be used even in large-scale applications.[25]

**CHEMOSENSITIVITY AND GENE RELATIONSHIP**

**Overexpression of the P53 Gene**

P53, also known as tumor protein 53, is the tumor suppressor gene that regulates the cell cycle. It has many functions and being anti-cancer is one of its properties.[26,27] When DNA is damaged, it activates the proteins that will repair it, if DNA is not repaired, it initiates apoptosis. In addition, it regulates cell growth, aging, and arrest. Recent findings have shown that p53 plays an important role in drug chemosensitivity and drug resistance.[26,27] Many chemotherapeutic drugs cause DNA damage and induce apoptosis in tumor cells. The effectiveness of cancer therapy is related to its ability to induce a p53-dependent apoptotic response.[28,29]

**Over Expression of P53 in Multiple Drug Resistant Osteosarcoma Cell Lines and Hasmosensitivity**

Osteosarcoma is the most common malignant primary bone tumor, characterized by osteoid and osteoblastic differentiation.[30] It is common in children, adolescents and young adults.[30] Currently, effective chemotherapeutic agents including high-dose methotrexate (MTX), cisplatin (DDP), and doxorubicin (ADM) combined with advanced surgical technology are routinely used in the main therapeutic method of osteosarcoma.[30,31] The use of chemotherapy has increased the patient’s 5-year survival rate from 20% to 70% in the last 30 years.[30,31] The remaining of 30 % patients have developed multidrug resistance (MDR).[30,31] MDR is one of the mechanisms that increase resistance not only to the relevant drugs which the patient is exposed, but also to several other drugs that are not structurally and functionally related.[32] The development of MDR is one of the most important reasons for the failure of chemotherapy in cancer treatment.[31,33] The MDR phenotype has been associated with several irregular genes such as MDR1, MRP, and LRP.[33] Apoptosis deficiency is considered a major cause of MDR in human osteosarcoma. It has been confirmed that

![Figure 1. Overview of collagen gel droplet embedded culture drug susceptibility testing method.](image)
DNA damage and cellular stress factors leads to the activation of tumor suppressor p53. The term 'multi-drug resistance' was first used in the field of oncology/hematology to identify tumor cell colonies that had a significantly reduced response to a chemotherapeutic agent in vitro. The term “multi-drug resistant” has been used to abbreviate “intensive drug resistant” “XDR” term has been used to abbreviate “intensive drug resistant” Mycobacterium tuberculosis, characterized by resistance to both rifampin and isoniazid, as well as fluoroquinolones plus an injectable substance in the field of infectious diseases. Human osteosarcoma cell lines U2OSR2 and KHOSR2 are the preferable cell lines in the regarding study. These cell lines were placed in 6-well plates or 96-well plates. Cell proliferation (the condition of cells to proliferate rapidly under appropriate conditions by completing the cell cycle) was determined by MTT assay. The resulting formazan product was read in a microplate spectrophotometer. Taxol, cisplotin, and doxorubicin drug cytotoxicity was evaluated in vitro using MTT assays. On the 2nd day after transfection, cytotoxic drugs were added. Plates were incubated for a further 5 or 6 days after chemotherapy drug was added. Drugs at the concentrations used in the MTT assay were carried out in the absence of the cells to confirm no change. After culturing 7 days, MTT was added to each well and incubated for 4 hours. When the results are read in a microscopy, the concentration of drug that causes 50% cell death was determined from the percent survival plot against cytotoxic drug concentration.

As a result, it was observed that transfection of wild type p53 inhibits cell proliferation and induces apoptosis. To determine the effect of overexpression of the wild-type p53 gene on cellular proliferation and apoptosis, drug-sensitive and resistant osteosarcoma cell lines were transfected with a p53 expression vector. Different time points (24, 48, 72 and 96 hours) were selected to determine the effect of p53 transfection on osteosarcoma cells, and the results showed that after transfection with the wild type p53 gene, four of the different osteosarcoma cell lines (KHOS, KHOSR2, U-2OS and U-2OSR2) showed a growth, inhibition and apoptosis. Despite the different p53 states, p53 transfection resulted in significant cell growth arrest and induced apoptosis in both KHOS/ KHOSR2 and U-2OS/U-2OSR2 paired osteosarcoma drug sensitive and resistant cell lines.

**SILENCING LIVIN GENE EXPRESSION TO INCREASE CHEMOSensitivity IN TUMOR CELLS**

Livin is a member of the IAP family that has two splice variants containing open reading frames of 298 and 280 amino acids. It plays an important role in tumor progression and formation by inhibiting cell apoptosis. Livin is also called melanoma, IAP, or kidney IAP. It is most commonly expressed from human neoplasms and appears to play a role in tumor cell resistance with chemotherapeutic agents. Apoptosis protein (IAP) inhibitors consist of a group of structurally related proteins with antiapoptotic properties that have been shown to interact with specific cysteine proteases or caspases necessary for the breakdown of certain proteins involved in cell disruption during apoptosis. Currently, there are eight human IAP members identified, including, C-IAP1, C-IAP2, NAIP, Survivin, X-linked IAP (XIAP), Bruce, ILP-2, and Livin. The two splice variants are called Livin-a and Livin-β as the longer and shorter variants, respectively. Livin-a is a longer splice variant than the Livin-splice variant at the junction site and consists of 18 amino acids. It has been shown that livin cannot be detected in most normal differentiated tissues, but in a variety of human cancers such as colon cancer, stomach cancer, breast carcinoma and lung cancer. It is expressed at high levels. It has been reported that Livin may play a role in the progression of tumors and that high expression of Livin is associated with tumor progression. Livin’s overexpression makes malignant cells resistant to chemotherapy, so livin inhibitors are considered as potential additions to chemotherapy in the treatment of malignant cancers. In vitro and in vivo chemosensitivity of livin down regulation to 5-FU (fluorouracil is a drug used to treat cancer) has been described that livin overexpression is associated with apoptosis-resistant phenotype in tumor cells, and livin can provide resistance to some chemotherapeutic drugs. Previous studies have shown that livin is low or not expressed in normal tissues but, it is regulated in various human cancers. The expression of high levels of livin provides a rapid progression of the tumor and decreases the response to chemotherapeutic agents, thus decreasing the survival time.

**Conclusion**

A chemosensitivity test is a promising approach for cancer patients. Since how much drugs given to the patients receiving chemotherapy affect
other healthy tissues and cells, normal cell damage may occur unintentionally in these structures. The chemosensitivity test will allow us to determine which drug will be appropriate for cancer treatment. Ultimately, chemotherapeutic progress can be achieved by increasing the survival rate. Considering the cost of the chemosensitivity test, which has been used for about 10 years, it has been thought that it is more economically profitable than the standard chemotherapy treatment methods for each patient.

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