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Review

Ehrlich ascites carcinoma

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Experimental tumors have great importance in modeling, and Ehrlich ascites carcinoma (EAC) is one of the commonest tumors. EAC is referred to as an undifferentiated carcinoma and is originally hyperdiploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen (TSTA). Frequently, tumor virulence increases via repetitious passages, while the proliferating rate of such tumors increases gradually. However, the differentiation gradually disappears, while the cells get free growth control mechanisms, gain hetero-transplantability and in the end, they are converted to the ascites' form. EAC resembles human tumors which are the most sensitive to chemotherapy due to the fact that they are undifferentiated and that they have a rapid growth rate. The ideal drug being ineffective or minimally effective for normal cells have been focused on, and at this point, the usage of natural sources as an alternative cancer therapy is thought to have a great value for cancer control and programs' destruction.

Key words: carcinoma, transplantability

EHRLICH ASCITES CARCINOMA

The intensive studies on the transplantable tumors were taken into consideration in the last 2 to 3 decades. The planned goal of that research was to improve new techniques especially for experimental tumors in animals that have been underlain at the basis of recent achievements in cancer therapy. Experimental tumors have great importance for the purposes of modeling, and Ehrlich ascites carcinoma (EAC) is one of the commonest. It appeared firstly as a spontaneous breast cancer in a female mouse (Aktaş, 1996; Taşkin, 2002), and then Ehrlich and Apolant (1905) used it as an experimental tumor by transplanting tumor tissues subcutaneously from mouse to mouse. In 1932, Loewenthal and Jahn (1932) obtained the liquid form in the peritoneum of the mouse and named it as "Ehrlich ascites carcinoma" due to the ascites liquid, together with the carcinoma cells. Lettre et al. (1972) had provided not only the seizure of this tumor, but also the conversion of it to the test system which is suitable for qualitative and quantitative cancer researches by their studies during World War II. After 1948, EAC cells had spread rapidly around the research institutes all over the world.

EAC is referred to as an undifferentiated carcinoma, and is originally hyperdiploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen (TSTA) (Kaleoğlu and İşli, 1977). In 1953, Haucscka (Lettre et al. 1972) obtained a sub-clone whose chromosome was tetraploid, while in the following years, such studies about diploid, hypertetraploid (Lennartz et al., 1968) and hypotetraploid (Burns, 1968) sub-clones were performed. However, Lettre et al. (1972) succeeded in obtaining colchicine resistant tumor clone and Sholz, with glycogen (+) and glycogen Ø Ehrlich clones as well (Aktaş, 1996).

The effusion, which contained neoplastic cells that are proliferated after injection of tumor cells into the peritoneal cavity, is referred to as the "ascites". Frequently, tumor virulence increases via repetitious passages, while the proliferating rate of such tumors increases gradually. However, differentiation gradually disappears, while the cells get free growth control mechanisms, gain hetero-transplantability and in the end, are converted to the ascites form (Kaleoğlu and İşli, 1977). Ascites liquid is gray-white, or sometimes has a light bloody viscose liquid

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and contains 10 million neoplastic cells in 0.1 cc (Aktaş, 1996; Kaleoğlu and İşli, 1977).

Following the obtained Ehrlich ascites form, this has been preferred frequently in researches. The reason for its load usage is that the suspension contained homogeneous free tumor cells of the Ehrlich ascites tumor, and in this way, it has a transplantable capacity for certain quantitative tumor cells to another mouse (Klein, 1951). Therefore, it is not only the tumor cell count that is transplanted, but also, the growing tumor size can be determined by common basic counter systems (Ekinci, 2000).

EAC is used as ascites or a solid form due to these purposes, that is, if ascites fluid contains the tumor cell that injects i.p., the ascites form is obtained, but if it contains s.c., a solid form is obtained (Okay, 1998; Zeybek, 1996).

EAC cells grow in suspension in the peritoneal cavity of mice and they do not adhere to the synthetic surface *in vitro* (Aktaş, 1996; Lazebnik et al., 1991; Song et al., 1993; Vinuela et al., 1991). In 4 or 6 days after passage, the ascites fluid is formed and a total of 5 or 12 cc ascites fluid is accumulated (Gümüşhan, 2002).

Following the inoculation into the peritoneal cavity of mice, EAC cells grow in two phases. These two phases are: a proliferating phase, in which the number of cells increases exponentially, and a plateau phase followed by a resting phase, in which a number of cells stay almost constant (Song et al., 1993; Siems et al., 1993; Grune et al., 1992; Skog et al., 1990; Tannock, 1969). Several studies reported that following the 3×10^6 EAC cells transplantation i.p., the number of cells increased exponentially in the 9th day and they were transmitted from the exponential phase to the plateau phase starting from the 9th and 10th day (Bulan, 1990; Altun, 1996; Öner, 1985). In another study, the proliferating rate of EAC cells was characterized in 4 phases. These phases are: a logarithmic phase for 4 or 5 days, following the 10^7 tumor cells transplantation i.p.; a plateau phase, in which the number of cells stayed practically constant on the 5th to 13th day; a transitory proliferating phase on the 13th to 15th day and a second plateau phase on the 15th to 18th day (Szikla et al., 1981).

During the EAC cells transition from the proliferating phase into the plateau phase, morphological and metabolic changes (except the changes in cell kinetics) occur (Aktaş, 1996), such as: structural deterioration (Aktaş, 1996; Siems et al., 1993; Schmidt et al., 1991; Schwendel et al., 1994; Senger et al., 1983; Siems, 1989; Segura et al., 2001; Latha et al., 2000; Haris et al., 1970), decreased number of mitochondria (Siems et al., 1993; Schmidt et al., 1991; Schwendel et al., 1994; Siems, 1989), decreased DNA and RNA biosynthesis (Aktaş, 1996; Siems et al., 1993; Bulan, 1990; Schmidt et al., 1991; Siems, 1989), loss of intracellular purine and pirimidine nucleotides, nucleosides and bases (Siems et al., 1993; Grune et al., 1992; Schmidt et al., 1991; Schwendel et al., 1994; Siems, 1989), a decline of the ATP concentration and turnover (Siems et al., 1993; Skog et al., 1990; Öner, 1985), decreased protein synthe-sis (Burns, 1968, Siems et al., 1993; Skog et al., 1990; Schmidt et al., 1991; Estrela et al., 1992), increased thymidine concentration with a decrease of thymidine kinase activity (Aktaş, 1996; Skog et al., 1990; Szıkla et al.,1981), decreased glutathione (GSH) concentration (Marquez et al., 1989; Lobo et al., 2000; Balint and Holczinger, 1984) and increased triglycerides, cholesterol esters and free fatty acids (Aktaş, 1996; Burns et al., 1983).

The inhibition of NK and T cell responses was dramatically reported to be parallel with an inclination of the repressed macrophages and down-regulator humoral factors (Haris et al., 1970).

EAC cells increased via rapid cell division during the proliferating phase and in the load peritoneal cavity. Ascites fluid accumulation occurred in parallelism with the proliferation of tumor cells. After a given time, the host animal died due to the pressure exerted by the tumor volume and/or the damage that resulted from the tumor (Aktaş, 1996; Altun, 1996; Öner, 1985). During the transition of the EAC cells from the proliferating phase to the plateau phase, the rates of cell viability did not decrease significantly (Schmidt et al., 1991).

For the accumulation of ascites fluid, whether or not the tumor cells secrete a vascular permeability factor that stimulated the accumulation of ascites fluid was investigated, and in conclusion, vessels in the peritoneal cavity of mice with EAC showed that the microvascular permeability increased significantly in comparison with those of the control group. This increased permeability was detected by an effective permeability factor in ascites fluid, but not in the normal plasma and serum (Senger et al., 1983).

Altun (1996) reported that the rate of cell proliferation in the bone marrow was inhibited, depending on the age of the tumor in mice. This showed that inhibitor factors in ascites fluid affected the normal cell population of the host animal.

Contrary to these studies, Altun (1996) in another study investigated the liver regeneration in mice with EAC and reported that tumor growth stimulated the regenerative growth. Gabrilovac et al. (1982) reported that peritoneal fluids, collected in the early phase of tumor growth on the 4th and 6th day after tumor transplantation, were ineffective on the proliferation of EAC cells *in vitro,* but those collected on the 15th day increased DNA synthesis (Gabrilovac et al., 1982). Burns et al. (1968) examined the mitogenic activity of Ehrlich ascites carcinoma factor (EACF) isolated from the cellular ascitic fluid in liver and in other tissues of adult mice, and reported that DNA synthesis was stimulated by this factor's mitogenic activity in liver, submandibular gland, exorbital lachrymal gland and the epithelium of the tongue of adult mice (Yeh et al., 1985).

Donenko et al. (1992) examined the effect of Ehrlich tumor cell's dialysate and ascites fluid on the *in vivo* progression of EAC and teratoma T-36 and concluded that, the ascites fluid, together with the tumor cell's dialysate, protected the tumor cells *in vivo*. In comparison with the control group, EAC dialysate and ascites fluid increased the rates of the tumor cell progression by 195 and 153%, respectively.

ALTERNATIVE APPROACHES IN CANCER THERAPY

In modern medicine, 3 methods are generally used for cancer therapy; chemotherapy, radiotherapy and surgery. Nowadays, chemotherapy has been thought to be the best effective therapy (Kayaalp, 1996).

The main principle of chemotherapy, which serves as a drug treatment in cancer, is to prevent the growth and progression of tumor cells or to destroy them by the effect it has on tumor cells more than the normal cells of the patient without side effect or with minimum side effect (Mycek et al., 1998). In consequence, the aim is to provide a lethal toxic effect of the used drugs to tumor progression. Generally, the prevention of metabolic pathways in cell replication is aimed. Furthermore, this effect is aimed to be specific for only malign cells. However, all the used drugs for cancer therapy are not specific on cancer cells, in that they do not only affect the proliferated cells, but also the normal cells. Therefore, all cancer therapeutics are toxic and their dosage-response curves are upright.

If tumor metastasis occurs and surgical treatment is impossible, chemotherapy is preferably used for the therapy. At the same time, it is applied after surgical and radiation therapies to prevent micro-metastasis.

Although the cancer chemotherapy has a half century clinical story, thousands of chemicals were investigated in this study. However, only a few of these chemicals classified due to different characteristics is used as a drug to treat cancer nowadays (Öner, 1985).

The most sensitive tumors to chemotherapy are poorly differentiated and they grow rapidly (Mycek et al., 1998). Nonetheless, lots of cancer chemotherapeutics affect the normal cells of patients seriously (Mascarenhas, 1994). For instance, cytostatics in cancer therapy focus on the intracellular targets and its effect mechanism, which is a natural cell damage. However, the resistance of some tumor types against this drug group and also hepatoxic, nephrotoxic, cardiotoxic, etc side effects on normal cells make new agents for cancer therapy necessary (Soini et al., 1998).

Scientists' studies about cancer therapy have focused on the ideal drug being ineffective or minimally effective for normal cells (Gümüşhan, 2002). At this point, the usage of natural sources is thought to have a great value for cancer control and programs' destruction (Suffiness and Pezzuto, 1991).

The usage of plant preparations in medicine has a great historical inheritance among people (Duke, 1985). Nature gives a great deal of effective anti-cancer agents such as dactinomycin and doxorubicin derived from microorganisms and vinblastine, irinotecan, topotecan, vincristine and taxanes from plants which are used frequently in recent years. Several plants were reported to stimulate the immune system in different pathways. In addition, they increased specific cellular and humoral immune responses (Bhakuni et al., 1969). Moreover, there is a growing trend for herbal drugs because of low toxicity and high medical effectiveness of the extracts from these plants.

CONCLUSION

EAC has a resemblance with human tumors which are the most sensitive to chemotherapy due to the fact that it is undifferentiated and that it has a rapid growth rate. Due to the resemblance, some researchers reported that some plant extracts were effective against EAC (Ozaslan et al., 2007, 2009a, 2009b; Cragg and Newmann, 1999).

Although there are a lot of floristic studies, approximately 10% of the 250,000 complex plant species only were investigated at their chemical and pharmacological sites. Nonetheless, the search of new toxic agents from natural sources has been conducted in collaboration with scientists, worldwide (Cragg and Newmann, 1999).

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