The occurrence of various forms of cancer has been recorded since prehistoric era, modern lifestyle has only aggravated its incidence to gargantuan levels. The types and complexity of cancer are mind-boggling. Despite worldwide efforts being made to find ways to stop this scourge, an effective cure of cancer remains an uphill task. One in every twelve persons up to 64 years of age is likely to be afflicted by one or other form of cancer in his/her lifetime. Global efforts continue to give rise to a wide range of anti-cancer agents with desirable therapeutic potential. However, many of these agents have solubility, stability or toxicity issues that retard or prevent their development into viable treatment strategies. In many cases, targeted delivery has been found to successfully tackle many of such issues. To date, many nano-sized systems such as liposomes, micelles, and nanoparticles have been explored for systemic delivery of anti-cancer agents. Among these technologies, liposomes have become the most well characterized and well established. With clinical implementations of liposomes as drug delivery systems against cancer treatment, a series of strategies have been developed and modified to enhance the therapeutic efficacy of liposomised chemotherapeutic agents. A number of obstacles such as in vivo instability, short half-life in the blood circulation, and lack of target specificity have impeded the clinical application of liposomes as anticancer drug delivery systems. These obstacles have been partially resolved by significant efforts of researchers that ultimately resulted in development of clinically approved liposomal formulations. However, there is ample scope of further development, particularly in the enhancement of target specificity to improve the therapeutic efficacy of liposome-based drugs. The major clinical challenge for cancer therapy remains the eradication (or prevention) of metastatic disease. A principal barrier to destruction of disseminated cancer is its heterogeneous nature as most of the tumors contain subpopulations of cells that are able to subvert host defenses and recruit infiltrating cells that supply needed growth factors and blood
supply. Moreover, metastatic lesions can become autonomous with respect to homeostatic mechanisms of normal tissue architecture. This not only poses main obstacle to most of available chemotherapeutic agents but also affect the success of immunotherapy or the use of biological response modifiers in cancer treatment.

The foreignness/nonself nature of cancer tissue can evoke immune system of the host. As first line of defense against cancer, the macrophages infiltrate the lesions. In general, presence of inflammatory macrophages in growing tumors is maintained through recruitment of circulating monocytes, and in certain tumors, these are developed by the proliferation of mononuclear phagocytes. In regressing murine sarcomas, tumor-associated macrophages (TAMs) are found throughout the tumors, whereas in progressing sarcomas, TAMs are confined to the periphery of the tumor. The presence of noncytotoxic (nonactivated) macrophages in neoplasms could actually enhance tumor growth. Macrophages (and lymphocytes) produce many diffusible growth, angiogenic, and cytotoxic factors. According to the type and level of such mediators, TAMs, therefore, may enhance or inhibit the growth of neoplasms.

Macrophages recognize numerous molecules via a wide variety of their plasma membrane receptors that interact with a specific ligand, an extracellular protein as well as with adhesion molecules such as the integrins. Although carbohydrates and proteins have long been known to play an important role in cell-cell interactions, or as antigenic structures on cell surface, an increasing body of evidence suggests that phospholipids which comprise the cell membrane bilayer are also involved in macrophage mediated recognition of target cells. The mechanism for this recognition is nonimmunologic and requires cell-to-cell contact. Because activated macrophages can destroy phenotypically diverse tumor cell populations including those resistant to killing by
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other host-defense mechanisms and by anticancer drugs. Macrophage-directed therapy represents a potential strategy for elimination of biologically heterogeneous metastatic cells. New therapies based upon stimulation of natural immune mechanisms must be able to circumvent tumor heterogeneity, killing of cells that are resistant to conventional cytotoxic therapies, and ability to provoke host-mediated defenses that favor eradication of metastatic tumor cells. Besides, there is an intense effort to develop newer specific cancer therapies that target altered genetic pathways in tumors. The benefit of such therapies lies in the exploitation of these alterations to achieve an improved therapeutic ratio. The ability to differentiate between normal and tumor cells is contingent upon the identification of an appropriate molecular target, development of modalities that address this target, and most importantly targeted delivery of the potent anticancer agent to the tumor cells. The tumor microenvironment is a critical factor in such development since it acts as a barrier for the delivery of effective treatment. Besides it can also influence the tumor cell phenotype, and thus the appropriateness of a given molecular target, and act as a barrier for the delivery of effective treatment.

It can be speculated that targeted delivery of chemotherapeutic agents in combination with immunomodulators may also offer a promising strategy to eliminate cancer. The activation of immune system could be made possible by certain chemical agents (mostly originated from natural sources) that can modulate immune function nonspecifically. Among various immunomodulators, tuftsin, a tetrapeptide fragment from IgG (289-292 residue of the Fc region) has been found to be effective against cancer as well as infectious diseases. In the present work, its liposomised form has been used extensively for prophylaxis and chemotherapy against polycyclic aromatic hydrocarbons-induced hepatic alterations and fibrosarcoma in model
animals. We have evaluated augmentation of antitumor potential of liposomal etoposide (ETP) by tuftsin against benzo (a) pyrene induced fibrosarcoma in Swiss albino mice. The efficacy of the free form of ETP, liposomised ETP (Lip-ETP) as well as tuftsin-bearing liposomised ETP (Tuft-Lip-ETP) formulations was evaluated on the basis of tumor regression, effect on expression level of p53wt and p53mut as well as survival of the treated animals. Tuft-Lip-ETP (ETP dose 10 mg/kg body weight/day) when administered for 5 days, significantly reduced tumor volume, delayed tumor growth and also up-regulated the expression of p53wt. In contrast, though Lip-ETP delayed tumor growth, it could not decrease tumor size. We also tried to understand the multiple signaling pathways involved in the regression of tumor by liposomal etoposide associated tuftsin against fibrosarcoma by monitoring changes at mRNA as well as protein level. The treatment with liposomised tuftsin was found to modulate both p53 and p21/waf1 in dose dependent manner. A single-dose of tuftsin-bearing liposomal etoposide (Tuft-Lip-ETP) was found to normalize level of p53wt, p53mut and p21/waf-1 in the animals that were preexposed to benzo (a) pyrene treated animals. On the other hand, the effect of tuftsin-bearing liposomal etoposide on expression of bcl-2, bax and caspase-3 was same as observed in animals treated with tuftsin-free liposomal etoposide (Lip-ETP) but significantly distinct from that induced by free etoposide as well as sham tuftsin-liposomes. The results suggest that tuftsin-bearing liposomal etoposide, a novel chemotherapeutic formulation, is capable of favorably regulating the tumor suppressor p53 along with its downstream effective molecule, p21/waf1 in the treatment of various forms of cancer.

In the next phase of study, we tried to evaluate the hepatoprotective properties of tuftsin against 7,12 dimethylbenz (a) anthracene (DMBA) induced alteration in liver of Swiss albino mice. Administration of tuftsin effectively alleviates DMBA induced oxidative
stress, characterized by reduction in apoptotic cell population in hypodiploid region. The inhibition of apoptosis was preceded by decrease in reactive oxygen species (ROS) level and restoration of mitochondrial transmembrane potential. To further elucidate the mechanism of anti-apoptotic effect of tuftsin, we tried to understand its effect on signaling pathways. In DMBA-treated animals down-regulation of anti-apoptotic Bcl-2 and up-regulation of proapoptotic Bax and Caspase 3 was observed in mouse liver. The reduced expression level of phosphatidylinositol 3-kinase (PI3-K) followed by the activation of Akt were also observed. These alterations were restored by tuftsin, indicating Akt-mediated inhibition of apoptosis by tuftsin. The data of the present study demonstrate that tuftsin is effective in combating oxidative stress induced cellular injury of liver cells by modulating Akt, a cell-growth regulator.

We also tried to understand the chemopreventive effect of various liposomal formulation of a dietary constituent diallyl sulphide (DAS) isolated from garlic. The use of chemicals as an alternative to the conventional medicines in the form of food and food products is an effective approach for the treatment of chronic diseases, including cancer. The intervention of chemopreventive strategies for controlling genetic diseases using dietary constituents provides a strong rationale to arrest or reverse the process of carcinogenesis before metastasis occur. During the past few years, cancer chemoprevention by dietary constituents has received a great deal of attention and as a means of effective control over cancer. Studies on the tumor inhibitory compounds of plant origin have yielded an impressive array of novel structures. Besides, epidemiological studies suggest that consumption of diets including fruits and vegetables may reduce the risk of developing cancer. Garlic (*Allium sativum*) has been shown to possess potential health benefits (*cf*. lipid lowering, antimicrobial, chemo-preventive and anticarcinogenic properties) since beginning of recorded history and is
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probably one of the most widely studied medicinal plants. The chemotherapeutic and antitumor activity associated with garlic has been attributed to the presence of various organosulfide based active compounds including DAS. Of the various options available for administration of medicaments, topical application is the most promising approach for treating skin tumors as it leads to localized release of drug at desired site with minimal side effects. However, retention of drugs administered by this mode is low because of extensive diffusion that is more apparent in case of small sized molecules such as DAS. This warrants development of formulations that can modulate pharmacokinetics as well as pharmacodynamic properties of DAS thereby making it more efficacious.

Among various novel drug delivery systems, micro-particulate-based carrier systems viz. micro-emulsion, nano-emulsion, nanoparticles, liposomes, etc. have been reported to improve delivery of drug to the skin. Interestingly, liposome-based formulations, when employed for topical delivery, have been shown to be extremely promising for enhancement of drug penetration, improved pharmacological effects, decreased side effects, controlled drug release and above all their own biodegradable nature. In this regard, considerable attention has been focused on the use of natural as well as tailor-made phospholipid vesicles or liposomes to enhance the therapeutic potential of anticancer agents.

In the present study, we have evaluated chemo-preventive effects of liposomised-DAS (conventional egg PC, pH-sensitive liposomes and escheriosomes) against DMBA-induced skin papilloma. Various liposome-based novel formulations of DAS (250 µg/mouse) were applied topically to the animals after 1 hour of exposure to DMBA (52 µg/mouse/dose). The animals were treated thrice weekly for a total
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period of 12 weeks. The efficacy of the various liposomal formulations of DAS was evaluated on the basis of parameters such as incidence of tumorogenesis, total numbers and sizes of induced tumor nodules. The liposomised DAS formulations were also assessed for their effect on the expression of p53wt, p53mut as well as p21/Waf1. The results of the present study showed that liposomised DAS could effectively delay the onset of tumorogenesis and reduce the cumulative numbers and size of tumor papillomas in treated mice. Treatment of DMBA exposed animals with liposomal formulation of DAS ensued in up-regulation of p53wt and p21/Waf1, while level of p53mut expression was reduced. The promising chemo-preventive nature of liposomal DAS may form the basis for establishing effective means of controlling various forms of cancer including skin papilloma.

The cytosol of cells is an important but relatively inaccessible compartment for many therapeutic macromolecules. Due to unfavorable physicochemical characteristics, in particular large size, or preferential solubility, molecules often show restricted ability to pass through plasma membranes. There is a strong need for a carrier that can deliver membrane-impermeable molecules into the cytosol of target cells. Although liposomes have been widely used for the delivery of therapeutic compounds, their ability to deliver entrapped molecules into cytosol of the target cell remains inefficient. This problem was circumvent was using pH-sensitive liposomes, which release their contents into cytoplasm of the target cells. This approach relies on their selective destabilization in the endolysosomal compartment under acidic pH of the surrounding medium. In fact, pH sensitive liposomes undergo phosphatidyl-ethanolamine (main constituent of these liposomes) mediated phase transition at acidic pH, thereby delivering their content to the cytosol of the tumor cells. The polar head group of PE gets less hydrated as compared to repulsive hydration layer associated with the
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head group of PC. Thus PE provides a more hydrophobic bilayer surface that is susceptible to energetically more favorable interbilayer interactions. The phospholipids facilitate not only the close approach of bilayers, there may also be directly involved in the merging process. In this context PE can form the hexagonal H\textsubscript{11} phase, the formation of which involves the development of non-lamellar structure, an intermediate in membrane fusion. The operative mechanism seems to form the basis of the observed higher efficacy of pH sensitive liposomes over egg PC neutral liposomes.

Keeping into consideration, significance of cytosolic delivery of drugs to effectively control various forms of cancer, we have also developed fusogenic liposomes using lipids isolated from Eschericia coli. The lipids were shown to induce strong membrane-membrane fusion as evident from Resonance Energy Transfer and Content Mixing Assays. Further, the fusion of these liposomes with model living cells (J774 A.1) was demonstrated to result in effective transfer of fluorescent lipid probe to the plasma membrane of the cells. Moreover, these liposomes effectively delivered encapsulated ricin A molecule to the cytosol of the J774A.1 cells, which ultimately resulted in the inhibition of cellular protein synthesis. Interestingly, the liposomes were also found to be efficient vehicles for the in vivo delivery of the drugs to cytosol of the target cells resulting in the destruction of tumor cells more efficiently. We entrapped DAS in escheriosomes (EC-Lip-DAS) and evaluate the efficacy of EC-Lip-DAS in chemoprevention against skin papilloma in model animals. These results imply usage of liposome based drug formulation in prophylaxis as well as chemotherapy against various forms of cancer.