Introduction
Cancer of the uterine cervix represents the second most common cancer in females worldwide and is a major cause of morbidity and mortality (Moore, 2006). In developing countries, cervical cancer is often the most common cancer in women and may constitute up to 25% of all female cancers (Burd, 2003, Waggoner, 2003). In 2006, 9,710 women were diagnosed with cervical cancer and 3,700 women died from the disease in U.S.A. (American Cancer Society, 2006). Worldwide, morbidity and mortality rates are far higher with an incidence of 4,90,000 new cases and 273,000 deaths occurring in 2005, according to the World Health Organization. About 80 percent of these cases occur in developing countries (Cox, 2006; WHO data, 2005).

The association between Human papillomavirus (HPV) infection and cervical neoplasm was first demonstrated in the early 1980s by Harold zur Hausen (Gissmann and zur Hausen, 1980; de Villiers et al., 1981). HPVs are members of the Papillomaviridae family and are in the genus Papillomavirus which contains more than 100 types of the virus classified by genotype (de Villiers et al., 2004). Papillomaviruses infect the basal keratinocytes of squamous epithelium and may cause benign (noncancerous) tumors called warts, or papillomas, although some infections never produce clinical symptoms. All HPVs trigger proliferation of the cells they infect, but only a few types are associated with the risk of developing cancer. Based on their association with cervical cancer and precursor lesions, HPVs can be divided into high-risk, intermediate-risk, and low-risk subtypes. Low risk subtypes are associated with venereal warts (condyloma acuminata), whereas intermediate and high-risk subtypes are associated with cervical dysplasia and invasive carcinoma. High-risk HPV infection is the primary risk factor for the development of cervical carcinoma (zur Hausen, 1999). Over 90 percent of cancers of the uterine cervix contain high risk HPV DNA (zur Hausen, 2001). However, infection with high-risk HPV does not guarantee a patient will develop cervical cancer because most infections clear within 12-18 months, and there have been very rare instances where women have developed cervical cancers that are HPV-negative (Avrich et al., 2006). Cervical tumors can be resistant to traditional courses of chemotherapy and those patients who do respond positively to chemotherapy have tumors that can recur (Alexander et al., 2005; Dreyer et al., 2005; Lindeque, 2005; Tewari and Monk, 2005).
Human Papilloma Virus (HPV) Genome Structure

HPV is a relatively small (55nm diameter) non-enveloped virus. The virus contains a double stranded, circular DNA genome containing ~8000 base pairs (Fig. 1). It has an icosahedral capsid composed of 72 capsomers, which contain at least two capsid proteins, L1 and L2. The HPV genome can be divided into three regions, the noncoding long control region (LCR), or the upper regulatory region (URR), and the early (E) and late (L) gene region (protein encoding) (Zheng and Baker, 2006). The long control region of 400 to 1,000 bp contains overlapping binding sites for many different transcriptional activators and repressors, including activating protein-1 (AP-1), and nuclear factor-1 (NF-1). The LCR regulates transcription from the early and late regions, and therefore controls the production of viral proteins and particles (Bernard, 2002). The early region is downstream of the LCR and contains six open reading frames, E1, E2 and E4–E7, and is involved in viral replication and oncogenesis. These encode all viral proteins except for the viral capsid proteins, which are encoded in the late region. The L1 and L2 genes in the late region encode the major and minor capsid proteins, both of which are required late in the viral life cycle to encapsulate the virus.

Life Cycle of Human Papilloma Virus (HPV)

Papillomaviruses’ primary host tissue is squamous epithelial tissues such as skin or the mucosal surfaces of the genital tract, anus, mouth, or upper respiratory tract (Doorbar, 2005). Initial HPV infection requires access to cells in the basal layer by infectious particles, which for some HPV types are thought to require a mild abrasion or microtrauma in stratified epithelium (Fig. 2). For high-risk mucosal viruses, such as HPV16, the formation of cervical lesions may be facilitated by the infection of columnar cells, which can subsequently form a basal layer of transformed stratified epithelium. The nature of the cell surface receptor used for viral attachment is not known, although heparin sulphate and stabilizing proteoglycans have been suggested to be epithelial cell receptors for HPV (Giroglou et al, 2001). Once in a host cell, the life cycle of HPV can be separated into two stages, i.e., nonproductive and productive. In the nonproductive stage, the virus maintains its genome as a low copy number episome by using the host’s DNA replication machinery to synthesize its DNA in basal layer of the epithelium (Flores et al, 1997).
Figure 1: Circular diagram of HPV-16 genome and open reading frames. The three separate regions of the HPV genome along with early genes E1-E7 and late genes L1 and L2 are shown (Adapted from zur Hausen, 1999).
Figure 2: The course of events that, over time, can lead to the development of precancerous and cancerous cervical tumors. Induction of cancerous tumors takes decades and is preceded by the development of early and late precancerous lesions called cervical intraepithelial neoplasias (CIN), which may regress. Persistent HPV infection is required for progression to carcinoma (Adapted from zur Hausen, 2002).
The pattern of viral gene expression in these cells is not well defined, but it is generally believed that the viral E1 and E2 proteins are expressed in order to maintain the viral DNA as an episome (Wilson et al., 2002) and to facilitate the correct segregation of genomes during cell division (You et al., 2004).

The productive stage of the viral life cycle occurs in the terminally differentiating suprabasal layers of the epithelium. In these cells, the virus switches to a rolling circle mode of DNA replication and amplifies its genome to higher copy number, expresses late genes encoding capsid proteins, and produces viral progeny (Flores et al., 1999). As a rule, in benign warts and pre-neoplastic lesions, the HPV genome is not integrated into the host cell genome (it is maintained in episomal form). However, in true neoplasia, it is wholly integrated into the host genome, although some authors have shown the coexistence of episomal and integrated forms in cervical cancer (Kristiansen et al., 1994). The site at which the viral DNA is opened during this process of integration is fairly constant, and occurs within the E1/E2 open reading frame of the viral genome. The E2 protein can function as either an activator or repressor of viral gene transcription depending upon the location of the E2-binding sites within the promoter region of the viral genome (Bernard et al., 1989; Phelps et al., 1987). However, as the E2 region of viral DNA normally represses the transcription of the E6 and E7 early viral genes, this interruption causes E6 and E7 protein overexpression (Finzer et al., 2002). After all, while HPV integration means the end of the viral life cycle due to the functional inactivation of large parts of the viral genome, it leads to de-regulated expression of the viral oncogenes E6 and E7.

Many experimental studies have investigated genomic HPV integration sites, and although integrated HPV genomes have been observed to show preferences for relatively few loci, no general integration hot spot has been identified. A recent review of integration sites confirmed that HPV integration sites are randomly distributed over the whole genome with a clear predilection for ‘fragile’ sites. No evidence supporting the targeted disruption or functional alteration of critical cellular genes by the integrated viral sequences has been unearthed.
Molecular Pathophysiology of Cervical Cancer

In normal epithelium, basal cells are sequestrated from the cell cycle following migration into the suprabasal cell layers and are committed to terminal differentiation. During HPV infection, E7 and E6 are expressed in these cells, and abolish cell cycle progression restraints and delay normal terminal differentiation (Sherman et al, 1997) (Fig. 3). The effects of E6 and E7 on p53 and pRB and on many other cellular proteins have been extensively investigated, and significant alterations in cell cycle regulation can be attributed to the biochemical interactions between these two viral oncogenes and their respective cellular binding partners (Munger et al, 2001) (Fig. 4). Moreover, recently it was demonstrated that the E6 and E7 cooperatively disturb the mechanisms of chromosome duplication and segregation during mitosis, and thereby induce severe chromosomal instability (Duensing and Munger, 2001).

HPV E7 Oncoprotein

The critical role of HPV E7 protein in the malignant transformation of cervical epithelial cells is attributed to its effects on pRb, a member of the ‘pocket protein’ family, which also includes p107 and p130 (Vogelstein et al, 1993). The proliferation of normal human cells follows an orderly progression through the cell cycle under the influence of cyclin/cyclin dependent kinase (cdk) complexes. Each cyclin/cdk complex controls a specific cell cycle transition, the key downstream targets of the G1 phase cyclin/cdk complexes are members of the pRb family, i.e., Rb, p107, and p130. During G1 progression, Rb is sequentially phosphorylated by cyclin D1/cdk4 and 6 and cyclin E/cdk2 complexes, whereas hypophosphorylated pRB represents the active form and inhibits S phase entry, thus the sequential phosphorylation of Rb inhibits the repressor activity of pRb. The repressor activities of pRB and of the related pocket proteins p107 and p130 are mediated by members of the E2F family of transcription factors. In G0/G1 hypophosphorylated pRB is bound to E2F. Since pRB encodes a transcriptional repressor domain, pRB/E2F complexes function as transcriptional repressors. Following phosphorylation by cdk in G1, pRB/E2F complexes dissociate and E2F acts as a transcriptional activator, which results in S phase entry (Weinberg, 1995).
Figure 3: Processes involved in HPV-induced carcinogenesis
Uninfected Epithelium

B High Risk HPV Infection

Figure 4: Stimulation of cell-cycle progression by high-risk HPV types

HPV infection leads to deregulation of the cell cycle. Regulation of protein expression in uninfected epithelium is shown in (A). In the presence of high-risk HPV (B), the regulation of proteins necessary for cell proliferation is altered, allowing HPV to stimulate S-phase entry in the upper epithelial layers.

(A) Uninfected epithelium. The expression of proteins necessary for cell-cycle progression is controlled by pRB, which in non-cycling cells associates with members of the E2F transcription factor family (centre). In the presence of growth factors, cyclin D/Cdk4/6 is activated, which leads to Rb phosphorylation and the release of the transcription factor E2F, which drives the expression of proteins involved in S-phase progression. p16 regulates the levels of active cyclin D/Cdk in the cell, providing a feedback mechanism that regulates the levels of MCM, PCNA (proliferating-cell nuclear antigen) and cyclin E. p14ARF, whose expression is directly linked to that of p16, regulates the activity of the MDM (murine double minute) ubiquitin ligase, which maintains p53 at a level below that required for cell cycle arrest and/or apoptosis. (B) HPV-infected epithelium. In cervical epithelium infected by high-risk HPV types, progression through the cell cycle is not dependent on external growth factors, but is stimulated by the E7 protein, which binds and degrades pRB and facilitates E2F-mediated expression of cellular proteins necessary for S-phase entry. Although p16 levels rise, normal feedback is by-passed, as HPV-mediated cell proliferation is not dependent on cyclin D/Cdk4/6. The rise in the level of p14ARF, which occurs in the absence of p16-mediated feedback, leads to the inhibition of MDM function and an increase in the level of p53. This is countered by E6, which associates with the E6AP ubiquitin ligase in order to stimulate the degradation of the p53 protein and prevent growth arrest and/or apoptosis. In low-grade cervical disease, where E7 levels are carefully regulated, it is thought that E7-mediated cell proliferation can sometimes be inhibited as a result of association with p21 and cyclin E/Cdk. The high levels of E7 found in cervical cancer cells are thought to overcome this block by binding and inactivating the Cdk inhibitor p21 (Adapted from Doorbar, 2006).
However, the virally encoded protein, E7, binds to hypophosphorylated pRb and displaces the E2F transcription factor from pRb. Thus, binding to Rb by E7 is essentially for E7-induced cells transformation, because E7 proteins, which are associated with HPV strains with a low cervical cancer risk, show little or no affinity for pRb. As for the E7/pRB interaction, E7 targets pRb for ubiquitin-mediated degradation by the proteasome (Boyer et al., 1996).

Moreover, it was suggested that the cellular transformation activity of E7 tightly correlates with its ability to degrade pRB (Jones and Münger, 1997). It appears that pRB degradation, not solely binding, is important for the E7-induced inactivation of pRb (Gonzalez et al., 2001).

In addition to regulation by phosphorylation and dephosphorylation, cdks are regulated by a group of functionally related proteins called cdk inhibitors. In differentiating epithelial cells, high levels of cdk inhibitors (p21\(^{casp}\) and p27\(^{kip1}\)) can lead to the formation of inactive complexes consists of E7, cyclinE/cdk2 and either p21 or p27. As a result, it appears that during HPV infection, the ability of E7 to stimulate S-phase entry is limited to a subset of cells with low levels of p21/p27, or to cells which express high enough levels of E7 to subvert the block to S-phase entry. However, recent studies suggest that E7 can also interfere with the activity of the cyclin dependent kinase inhibitors p21\(^{casp}\) and p27\(^{kip1}\) and thus override normal G1 checkpoint control (Jones et al., 1997; Zerfass-Thome et al., 1996). In addition, to cyclin/cdk complexes and cdk inhibitors, E7 can also associate with other proteins involved in cell proliferation, including histone deacetylases (Antinore et al., 1996) and components of the AP-1 transcription complex (Longworth and Laimins, 2004), through p21 upregulation.

**HPV E6 Oncoprotein**

Although, E7 protein can independently immortalize various human cell types in tissue culture, efficiency is increased when E7 and E6 are coexpressed (Munger et al., 1989). Thus, E6 protein is believed to complement the role of E7 protein, and prevent apoptotic induction in response to unscheduled S-phase entry mediated by E7. However, the importance of HPV E6 in cancer appears to be primarily due to its effects on the cellular tumor suppressor gene, p53. The most commonly found alterations to p53 in cancers, such as, colon, breast, and lung cancer, are deletion, insertion, and point mutation (Bartek
et al, 1990; Rodrigues et al, 1990; Takahashi et al, 1991). The p53 gene negatively regulates the cell cycle and “loss of function” mutation in p53 is required for tumor formation (Levine et al, 1991). Up to 99% of invasive cervical carcinomas have been found to contain HPV 16 or 18 DNA and in these few are found without evidence of HPV p53 mutations (Crook et al, 1992).

Normally p53 is transiently upregulated after DNA damage, which leads to cell cycle arrest in the G1 phase and apoptosis. This arrest allows time for DNA repair, and if repair is not possible, cells are committed to apoptotic death. P53 acts through downstream regulators, such as p21, which leads to cdk inhibition and the eventual blockade of Rb gene phosphorylation, thus preventing cell cycle progression. However, virally encoded E6 binds to a cellular ubiquitin/protein ligase, E6-AP, and simultaneously to p53, which results in the ubiquitination of p53 and its subsequent proteolytic degradation (Ferenczy et al, 2002). Other consequences are presently emerging from the E6/E6-AP interaction; potentially important is the recent observation of E6-AP-mediated ubiquitination and degradation of the Src family tyrosine kinase Blk (Oda et al, 1999). It is conceivable that the presence of E6 partially blocks this degradation and thereby stabilizes the respective kinase and stimulates mitotic activity (Oda et al, 1999). This could explain, in part, growth-stimulatory functions of the E6 protein of high-risk HPVs.

E6 proteins of high oncogenic risk HPVs, i.e., HPV 16 and 18, have a higher affinity for p53 than lower oncogenic risk types (HPV 6 and 11) (Crook et al, 1991; Lechner et al, 1994). Moreover, although the association between E6 and p53, and the inactivation of p53-mediated growth suppression and/or apoptosis have been well documented, this association can also lead to apoptosis via changes in the expressional levels of Bax and Bcl-2 family members (Selvakumaran et al, 1994). Consequently, the presence of E6 is considered to predispose the development of HPV associated cancers, by allowing the accumulation of chance errors in host cell DNA to go unchecked. Moreover, the E6 protein of high-risk HPV types can also stimulate cell proliferation independently of E7 through its C-terminal PDZ-ligand domain (Thomas et al, 2002). Both the p53 and Rb proteins interact with the double minute 2 gene (MDM2). P53 acts as a transcriptional activator of MDM2, whereas MDM2 acts in an autoregulatory fashion by providing
negative feedback to p53 transcription. MDM2 can also interact with Rb and restrain its action.

**Other Oncogenes Involved in Pathogenesis of Cervical Cancer**

Although, the over-expressions of E6 and E7 appear pivotal in the development of cancer, their expressing is not enough either for the immortalization of cultured human cells, or for malignant conversion. Rather, other specific genetic abnormalities are important during cervical carcinogenesis and the aggressiveness of cervical tumors. In addition to p53 and Rb, the ras family genes (K-ras, H-ras and N-ras) and c-myc oncogene might also have a role in the pathogenesis of cervical cancer (Baker et al., 1998; Bourhis et al., 1990; Dokianakis et al., 1998; Garzetti et al., 1998; Riou et al., 1987; Brenna et al., 2002). Each of these genes has been reported to be overexpressed in cervical cancer and several of them have been associated with a poor prognosis.

**Epigenetic Changes**

In recent years the importance of epigenetic changes in inactivation of a tumor suppressor gene and the establishment of the malignant phenotype has been illuminated. In malignancies, some tumor suppressor genes are not transcribed because their promoter regions are methylated. Of the hypermethylation events studied in association with carcinogenesis, promoter CpG island hypermethylation has been frequently investigated in many human cancers, including cervical cancer (Esteller et al., 2001; Muller et al., 1998; Virmani et al., 2001; Yang et al., 2004; Kang et al., 2005). Dong et al., (2001) showed that promoter hypermethylation of at least one of the genes p16, DAPK, MGMT, APC, HIC-1, and E-cadherin occurred in 79% of cervical cancer tissues and in none of normal cervical tissues from several hysterectomy specimens. Virmani et al., (2001) detected aberrant methylation of at least one of the genes p16, RARβ, FHIT, GSTP1, MGMT, and hMLH1 in many cervical cancer tissue samples.

**Co-Factors of Cervical Cancer**

It has been well established that a persistent infection in combination with high-risk HPV is the main risk factor of cervical cancer. Subclinical, clinical, and latent HPV infections are considered the most common sexually transmitted infections. The prevalence of HPV infection among sexually active young women is in the range of 5-40% (Ho et al., 1998; IARC Working Group, 1995; Melkert et al., 1991). Further, most HPV infections are
transient and it is estimated that newly diagnosed HPV infections cleared within 12–18 months in approximately 80% of women, as the humoral immune system is brought to bear on the virus. Even low-grade precancerous lesions do not always progress into high grade lesions. Instead, a cytotoxic T cell response is elicited against HPV-infected keratinocytes in the majority of cases. This suggests that other factors are involved in the carcinogenesis of cervical cancer. Smoking, high parity, and the long-term use of oral contraceptives are considered proven co-factors.

Smoking

Smoking has long been associated with cervical cancer risk (Castellsague and Munoz, 2003). A recent review of the relation between smoking and cervical cancer found consistent associations (Szarewski and Cuzick, 1998; Deacon et al., 2000; Hildesheim et al., 2001; Plummer et al., 2003). Interestingly, a multi-center case–control study of cervical adenocarcinoma reported a negative association between smoking and cervical adenocarcinoma and a positive association between smoking and the risk of squamous cell carcinoma (Lacey Jr. et al., 2001).

Sexually Transmitted Diseases

Herpes Simplex Virus -2 (HSV-2) has been found to be carcinogenic in both in vitro and in vivo studies. It was hypothesized that HSV-2 and HPV may act synergistically with HSV-2 to initiate mutations and carcinogenesis in HPV-infected cervical cells (de Sanjose, 1994; Zur Hausen, 1982). A consistent but modest association between the presence of serum IgG antibodies to Chlamydia trachomatis and cervical cancer has been reported in epidemiological studies (de Sanjose et al., 1994; Dillner et al., 1994). In a IACR multicenter study, C. trachomatis seropositivity increased the risk for cervical cancer among HPV-positive women by 2.1-fold (Smith et al., 2002). Numerous studies have addressed the association between HIV and cervical neoplasia (Boyle and Smith, 1999; Jay et al., 2000; Conley et al., 2002; Ellerbrock et al., 2000; Moscicki et al., 2000; Ferenczy et al., 2003), and the Center for Disease Control and Prevention included invasive cervical cancer in its definition of AIDS.

Oral Contraceptives

Steroid contraceptive hormones have been identified to be a cofactor of HPV-related cervical carcinogenesis in many epidemiological studies. However, although some
epidemiologic studies have produced inconsistent results, the majority of studies have found that prolonged use of these agents increases the risk of cervical cancer (Moreno et al., 2002; Castellsague and Munoz, 2003; Smith et al., 2003; Moodley et al., 2003).

**Evasion of Cell-Mediated Immunity by HPV**

Human papilloma viruses have evolved several mechanisms to evade effective cellular immune responses (Frazer et al., 1999). First, they have evolved mechanisms to limit the extent to which viral antigens are exposed to immunologic recognition. They delay expression of abundant viral proteins (the capsid proteins encoded by the late viral genes) until the terminal differentiation stage of squamous epithelium, an anatomically superficial location where immunocompetent cells would have less access to them. In contrast, in the basal epithelium, where the early genes (such as E6 and E7) are expressed, the level of protein expression is low and confined to a nuclear location, thus potentially limiting an effective immune response against the cells in which the virus is actively replicating. Second, viral proteins such as E7 may modulate immune responses and the over expression of E7 protein may inhibit the antigen presenting function of dendritic cells in the epithelium (Barnard and McMillan, 1999; Rudolf et al., 2000; Garrido et al., 2000). Third, keratinocytes may be relatively less susceptible to cytotoxic T lymphocyte-mediated lysis than other infected cells and may even present antigen in a tolerogenic manner (Brady et al., 2000). Fourth, Keratinocytes infected by HPV can modify the immune response in several ways: through the secretion of various cytokines (IL-1α, IL-6, IL-8, TNF-α, TGF-β) and soluble receptors for TNF-α, or through the production of Th2 cytokines (IL-4 and 10) in squamous carcinomas, configuring an evasion mechanism against the T-cell mediated immune response (Woodworth and Simpson, 1993; Malejczyk et al., 1996; Kim et al., 1995).

**Role of Proinflammatory Cytokines in Cervical Cancer**

The anti-tumor immune response has been reported to be regulated by several factors, which includes cytokines produced by tumor and other cells of tumor stroma. It seems likely that the local cytokine microenvironment, acting on tumor cell or on the adjacent cells, can either block or facilitate tumor growth, and that proinflammatory cytokines strongly influence the immunologic state. Various cytokines have been implicated in the pathogenesis of cervical cancer, among which interleukin-6 (IL-6) has received particular
attention because it is a central proinflammatory cytokine involved in female genital infection and is abundant in the microenvironment of cervical cancer (Tartour et al., 1994; Richter et al., 1999). Viral infection and exposure to proinflammatory cytokines such as IL-1α and tumor necrosis factor-α can stimulate IL-6 gene expression by the keratinocytes (Iglesias et al., 1995). IL-6 expression in tumor tissues is high and correlates with the severity of cervical cancer (Tartour et al., 1994; Wei et al., 2001). Also, IL-6 may contribute to a local immunosuppressive effect that protects the tumor cells from the host immune system (Tilg et al., 1997). Previous studies have shown that several proinflammatory cytokines (IL-1α, IL-6, or TNF-α) stimulate proliferation of carcinoma cell lines derived from several different tissues including cervix (Ito et al., 1993; Wu et al., 1993; Sugarman et al., 1986; Lewis et al., 1987, Wei et al., 2003). Thus, these proinflammatory cytokines might act as paracrine or autocrine growth factors in promoting malignant progression of cervical cancer.

**Current Treatments for Cervical Cancer**

Treatment options for HPV positive uterine cervical tumors vary with the stage of disease at diagnosis, the health of the patient, and desire for bearing children, but in all cases involves surgical removal of the malignant tumor (Moore, 2006). The standard first line treatment for invasive cervical cancer is usually cisplatin (Platinol) and 5-fluorouracil (5-FU, Adrucil, Efudex) used in combination and in addition to radiation. Recurrent and late stage cervical cancers are often treated with a combination of platinum, bleomycin, methotrexate, and 5-FU (DuPont and Monk, 2006). Chemotherapy is administered intravenously, through injection, or in pill form. Side effects often accompany treatment and may be severe; they can include nausea, vomiting, diarrhea, and leukopenia (low white blood cell count). Most of these side effects are a result of drug toxicity to healthy tissues such as skin, hair follicles, and epithelial cells that line the digestive tract (Stewart and Viswanathan, 2006). Patient tumors can develop resistance to these therapies as well (Muggia et al., 2004). Hence, there is the need to develop alternate, safer, less toxic, and more specific therapies for uterine cervical cancer.

**Garlic (*Allium sativum*) as an Anticancer Agent**

Experimental and epidemiological studies over the past few decades have provided ample evidences in support of associations between plant food intake and reduced cancer risk.
Many phytochemicals are proven to have anticancer activities and many are in use for cancer therapeutics. Among them garlic (Allium sativum) has been of much interest, mostly due to the epidemiological reports which linked increased garlic consumption with reduced prevalence of many human diseases. Health benefits of Allium vegetables including garlic have been noted throughout recorded history, dating back to 1400 BC (Rivlin, 2001). The known medicinal benefits of garlic and other Allium vegetables and their constituent organosulfur compounds (OSCs) include lowering of serum cholesterol level, inhibition of platelet aggregation and increased fibrinolysis (Agarwal, 1996: Rahman, 2001), stimulation of immune function through activation of macrophages and induction of T-cell proliferation (Lau et al., 1991; Lamm and Riggs, 2000), reduction of blood glucose level (Sheela et al., 1995; Augusti and Sheela, 1996), radioprotection (Singh et al., 1995), improvement of memory and learning deficit (Moriguchi et al., 1996; Nishiyama et al., 1997), protection against microbial, viral and fungal infections (Cellini et al., 1996; Avato et al., 1997; Guo et al., 1993), and anticancer effects (Milner, 2001; Thomson and Ali, 2003). Initial evidence for the anticancer effect of Allium vegetables was provided by population-based observational studies (You et al., 1989; Steinmetz et al., 1994; Hsing et al., 2002).

Preclinical animal studies have indicated that OSC analogues are highly effective in affording protection against cancer induced by a variety of chemical carcinogens (Belman, 1983; Sparnins et al., 1986; Wargovich et al., 1988; Reddy et al., 1993; Suzui et al., 1997). Elucidation of the mechanisms by which OSCs may offer protection against cancer has been a passionate subject of research for the past 20 years.

**Chemical Constituents of Garlic**

Chemical analyses have indicated that garlic bulbs, leaf, shoot, flower and roots are the source of many compounds having medicinal properties with varied pharmacological functions (Ross, 1999). Fresh garlic contains water (65%), carbohydrates (28% mainly fructans), proteins (2% mainly allinase), fiber (1.5%), and fat, as well as essential amino acids (1.2%) (arginine, alanine, cystine, glutamic acid, aspartic acid, glycine, histidine, isoleucine, leucine, phenylalanine, proline, serine, threonine, tryptophan, valine, tyrosine etc.), vitamins (β-carotene, ascorbic acid, biotin, thiamin, riboflavin, niacin, nicotinic acid etc), minerals (Al, Fe, Mg, Co, Cu, Mn, Ni, P, K, Na, Sn, Zn, Sc, Cr etc.), organosulfur
compounds (Alliin, Allicin, Z-ajoene, diallylsulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), methylallylsulfide (MADS), methylallylsulfide (MAS), methylallyl disulfide (MATS), methy lallyl disulfide (MPDS), dimethyltrisulfide (DMTS), dimethyldisulfide (DMD S), allylpropyl disulfide (APDS), S-(2-carboxy-propyl)-glutathione, S-alllylmercaptocysteine (SAMC), S-allylcysteine (SAC) and many other chemical compounds (ferulic acid, caffeic acid, p-coumaric acid, quercetin, sativosides, scordines, scordinines, degalactotigonin, gitonin, dithiins, dithiane thione, thiazole) etc.) (Abdullah et al., 1988; Ejaz et al., 2003; Li, 2000). The sulfur chemistry of garlic is fairly well understood (Block, 1985). The main sulfur compound in intact garlic is γ-glutamyl-S-alk(en)yl-L-cysteine, which is hydrolyzed and oxidized to yield Alliin (Block, 1985) (Fig. 5). Alliin accumulates naturally during storage of the bulbs at cool temperature and is the odorless precursor of the organosulfur compounds (OSC) believed to be responsible for the anticancer effect of garlic (Belman, 1983; Sparnins et al., 1986; Wargovich et al., 1988; Reddy et al., 1993; Suzui et al., 1997). Processing of garlic bulbs (crushing, cutting or chewing) releases a vacuolar enzyme alliinase that acts on alliin to give rise to extremely unstable and odoriferous compounds, including Allicin (Fig. 6). Allicin and other thiosulfinates decompose to oil-soluble OSC, including DAS, DADS, DATS, di thiins and ajoene (4,5,9-trithiadodeca-1,6,11-triene-9-oxide) (Block, 1985) (Fig. 6, 7). Further transformation of organosulfur compounds can occur after interaction with free sulfhydryl groups, including those present in cysteine, glutathione, or proteins, producing S-allylcysteine, S allylmercaptocysteine, S-(2-carboxy-propyl)-glutathione etc. (Weisbe rger and Pensky, 1958; Gilbert, 1990) (Fig. 7).

**Mechanism of Cancer Prevention**

Several mechanisms have been proposed to explain the cancer-preventive effects of Allium vegetables and related OSCs. These include inhibition of mutagenesis by inhibiting the metabolism, inhibition of DNA adduct formation, free-radical scavenging, and effects on cell proliferation and tumor growth. Although there are evidences supporting these mechanisms for OSCs, they are still speculative, and further research is needed to support causality between such properties and the cancer preventive activity in experimental animals.
Figure 5: Bioconversion pathway of Garlic Organosulfur Compounds
Figure 6: Chemical reactions in processed *Allium* vegetables and generation of organosulfur compounds
Figure 7: Chemical structures of widely studied natural organosulfur compounds
Modulation of Phase I Detoxification Enzymes

Carcinogenic activity of many environmental pollutants is often dependent on their activation by cytochrome P450-dependent monooxygenases. OSCs derived from garlic have been studied for the ability to inhibit experimental cancer in various animal models, primarily through alteration of expressions of cytochrome P450 (CYP) enzymes (Phase I detoxification enzymes). The suppression of vinyl carbamate (VC) and N-nitrosodimethylamine (NDMA)-induced mutagenesis in Salmonella typhimurium TA100 by dialyl sulfide (DAS) has been reported to be correlated with their inhibition of CYP2E1-mediated p-nitrophenol hydroxylation and NDMA N-demethylation (Surh et al., 1995). DAS also acts as a competitive inhibitor of N-dimethylnitrosamine demethylase activity (Brady et al., 1988). It also decreased the activity of CYP2E1 in a time- and dose-dependent manner and induced the activities of CYP2B1 and pentoxy- and ethoxyresorufin dealkylases in hepatic microsomes (Brady et al., 1991). DAS, DADS, and AMS also decreased p-nitrophenol hydroxylase activity and CYP2E1 protein concentration in rat liver (Reicks and Crankshaw, 1996). DADS increases the activities of several monooxygenases and transferases in intestine and liver and also the protein levels of epoxide hydrolase and CYP2B1/2 (Haber et al., 1995). Garlic oil induced the expression of CYP2B1 and decreased the expression of CYP2E1 (Sheen et al., 1999).

The modulation of hepatic drug-metabolizing enzymes in rats treated with dimethyl sulfide (DMS), methylpropyl disulfide (MPDS), dipropyl sulfide (DPS), dipropyl disulfide (DPDS) and DADS induced ethoxyresorufin O-deethylase, methoxyresorufin O-demethylase and mostly pentoxyresorufin O-depentylase and decreased nitrosodimethylamine N-demethylase and erythromycin N-demethylase. These modifications of enzyme activities were accompanied by an increase of CYP2B1, 2 and a decrease of CYP2E1 (Siess et al., 1997). Collectively, modulation of carcinogen activation may be one of the mechanisms by which garlic constituents may offer protection against chemically induced cancers.

Induction of Phase II Detoxification Enzymes

Experimental evidence exists to suggest that garlic constituents may function as a double-edge sword in the prevention of chemically induced cancers by inhibiting carcinogen activation and enhancing detoxification of activated carcinogenic intermediates through
the induction of Phase II enzymes, including glutathione S-transferases (GST) and quinone reductase (Sparnins et al., 1988; Hu et al., 1996; Hu and Singh, 1997; Hu et al., 1997). Wattenberg and colleagues showed that prevention of BP-induced forestomach and lung cancer in mice by garlic OSCs was correlated with elevation of hepatic and target organ total GST activity (Sparnins et al., 1988). The activity of mammary and liver GST was increased by the addition of garlic powder to the diet of rats (Liu et al., 1992). Previous studies have shown that DAS, DADS and DATS administration to A/J mice results in induced expression of Alpha (mGSTA3-3, mGSTA1-2, mGSTA4-4), Mu (mGSTM1-1) and Pi class GST (mGSTP1-1) in the liver, lung and forestomach (Hu et al., 1996; Hu and Singh, 1997; Hu et al., 1997). However, OSC-mediated prevention of BP induced forestomach tumorigenesis, but not lung neoplasia, in A/J mice is most closely correlated with the induction of mGSTP1-1 (Hu et al., 1996; Hu and Singh, 1997).

Garlic organosulfides DAS, DADS, DPS and DPDS induced the expression of NAD(P)H: quinone oxidoreductase (NQO) enzyme, implicated in the detoxification of activated quinone metabolite of BP, in the forestomach and/or lung of A/J mice (Singh et al., 1998). Subsequently, Kong and colleagues showed a positive correlation between OSC-mediated induction of Phase II enzymes, activation of antioxidant response element and accumulation of transcription factor nuclear factor E2-related factor 2 in HepG2 hepatoma cells (Chen et al., 2004). DADS increased the activities of phase II detoxification enzymes quinone reductase (QR), glutathione transferase (GST), and uridine diphosphate (UDP)-glucuronosyl transferase in forestomach, glandular stomach, duodenum, jejunum, ileum, caecum, colon, liver, kidney, spleen, heart, lungs and urinary bladder of rats and suggested that such enzyme induction contributed to the protective activity of garlic against gastrointestinal tract (GIT) cancers (Munday and Munday, 1999). Thus, it is reasonable to conclude that the induction of Phase II enzymes, especially GST, represents another potential mechanism to explain OSC-mediated prevention of chemically induced cancers.

**Inhibition of DNA Adduct Formation**

DNA adducts are believed to be an initial step in carcinogenesis. The anticarcinogenic action of garlic can be attributed to its role in preventing DNA-carcinogen adduct
formation and activation of carcinogen (Liu et al., 1992). In rat mammary gland, different garlic preparations decreased the occurrence of dimethylbenz[a]anthracene (DMBA)-DNA adducts in vivo and the amounts of total and individual adducts correlated positively with mammary tumor incidence (Amagase and Milner, 1993). DNA adducts induced by incubation of human bladder tumor cells with N-acetyl-2-aminofluorene (2-AF) were inhibited by DAS and DADS (Chung et al., 2004). Further, a water extract of raw garlic and SAC, but not DAS, significantly inhibited benzo(a)pyrene [B(a)P]-DNA adduct formation in simulated human peripheral blood lymphocytes in vitro (Hageman et al., 1997). Shenoy and Choughuley showed that onion and garlic juices inhibited the nitrosation reactions in vitro in a dose-dependent manner (Shenoy and Choughuley, 1992). Production of N-nitrosomorpholine (NMOR), a known liver carcinogen, has been reported to be reduced by water extracts of garlic and deodorized garlic powder (Dion et al., 1997). The occurrence of 7-methyldeoxyguanosine (7-MedG) and 06-methyldeoxyguanosine (06-MedG) was decreased in rat liver when garlic powder was added to their diet containing amino pyrine and sodium nitrite (Lin et al., 1994).

Free Radical Scavenging

Free radicals have been related to several age-related diseases, including cancer (Weisburger, 2001). Garlic possesses strong antioxidant activity and it is due to its organosulfur compounds (Shobana and Naidu, 2000). Alliin metabolite Allicin has been shown to be responsible for the oxygen radical scavenging property of garlic (Siegers et al., 1999). It was also shown to prevent lipid peroxidation in liver homogenates (Prasad et al., 1995). The protective effect of pretreatment with garlic and tomato against N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced genotoxicity and oxidative stress was observed in Swiss mice (Kumaraguruparan et al., 2005). Garlic and onion oils stimulated the activity of glutathione peroxidase (GPx) and inhibited the ratio of reduced/oxidized glutathione produced by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) in epidermal cells (Perchellet et al., 1986). GPx activity was also increased in animal tissues by DAS administration against B(a)P-induced genotoxicity in mouse forestomach. DAS and DADS also increased the activity of glutathione reductase, and garlic oil increased the activity of superoxide dismutase (SOD) (Gudi and Singh, 1991). Similarly, DAS and garlic homogenates decreased catalase (CAT) in the livers of rats and mice.
Aged garlic extract (AGE), SAC, and SAMC exhibited radical scavenging activity in both chemiluminescence and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays against t-butyl hydroperoxide-induced ROS in liver microsomal fraction (Imai et al., 1994). DAS, DADS, and AMS showed selective actions on different markers in tests for their ability to react with carbon tetrachloride (CCl₄) generated free radicals (Fanelli et al., 1998). Aged garlic extract containing both water and lipid soluble organosulfur components including S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) is reported to have antioxidant action by enhancing cellular antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Borek, 2001). SAC and SAMC also increased the synthesis of glutathione (GSH) in human prostate cancer cells (Pinto et al., 1997).

Inhibition of Cell Cycle Progression

Cell cycle consists of a series of events involving growth stimulus, replication and division of a eukaryotic cell (Molinari, 2000; Murray, 2004). Cellular stresses may activate signal transduction pathways, referred to as checkpoints, which lead to cell cycle arrest (Molinari, 2000; Murray, 2004). The cell cycle checkpoints ensure completion of phase specific events and protect against genomic instability or, in cases where the damage is too severe, switch the cell fate to programmed cell death (Molinari, 2000; Murray, 2004). Many anticancer treatments initially cause perturbations in cell cycle progression and the interrupted phase depends on the genetic background of the cell as well as the mode of action of a given treatment. Studies have shown that garlic-derived OSC can suppress growth of cancer cells of different anatomical locations in association with cell cycle arrest, mainly in the G₂/M phase of the cell cycle.

Milner and colleagues were the first to show that DADS treatment caused dose-dependent and time dependent accumulation of human colon cancer cells in the G₂/M phase of the cell cycle (Knowles and Milner, 1998; Knowles and Milner, 2000). The DADS-mediated G₂/M phase cell cycle arrest in human colon cancer cells was accompanied by a decrease in the kinase activity of the Cdk1/ cyclin B1 complex, reduction in complex formation between Cdk1 and cyclin B1, and a decrease in Cdc25C protein level (Knowles and Milner, 2000). Similar effects have been reported in other cellular systems with other organosulfur compounds (OSCs) (Tan et al., 2004;
Arunkumar et al., 2006; Wu et al., 2004; Xiao et al., 2005; Herman-Antosiewicz and Singh, 2005; Herman-Antosiewicz, 2007; Antosiewicz, 2006). For instance, DADS (20 μmol/L, 12 h) caused inactivating phosphorylation of Cdk1 in HL-60 cells (Tan et al., 2004) or decreased Cdk1 level in PC-3 human prostate cancer cells in a dose-dependent manner (Arunkumar et al., 2006). DATS was much more effective than either DADS or DAS in causing G2/M phase cell cycle arrest (Xiao et al., 2005). These results further support the notion that even a subtle change in OSC structure (the oligosulfide chain length) could have a significant impact on its biological activity. Interestingly, a normal prostate epithelial cell line PrEC was resistant to growth inhibition and cell cycle arrest by DATS (Xiao et al., 2005). The DATS-induced G2/M phase cell cycle arrest in PC-3 cells was associated with increased Tyr15 phosphorylation of Cdk1, inhibition of Cdk1/cyclin B1 activity, increased inhibitory phosphorylation of Cdc25C at Ser216, and downregulation of total Cdc25C protein level (Xiao et al., 2005). The DATS-mediated hyperphosphorylation and decline in protein level of Cdc25C were abrogated in the presence of anti-oxidants, suggesting a redox-sensitive mechanism for these effects (Xiao et al., 2005). Recent studies have revealed that DATS-mediated cell cycle arrest, at least in human prostate cancer cells, is linked to c-Jun N-terminal kinase (JNK)-dependent generation of reactive oxygen species (ROS) (Antosiewicz, 2006).

Several studies show that OSCs affect the microtubule network in cancer cells that might initiate mitotic block or apoptosis. For example, treatment of SW480 human colon cancer cells or NIH3T3 mouse fibroblasts with 150 μmol/L water-soluble SAMC caused rapid microtubule depolymerization and cytoskeleton disruption in interphase cells (Xiao et al., 2003). DATS, but not DADS or DAS, has been shown to induce mitotic arrest in HCT-15 and DLD-1 human colon cancer cells in association with disruption of the microtubule network in interphase cells and inhibition of spindle formation in mitotic cells (Hosono et al., 2005). This study further revealed DATS-mediated oxidative modification of tubulin-β at residues Cys12 and Cys354 (Hosono et al., 2005). Another oil-soluble garlic compound, Z-ajoene, caused G2/M phase cell cycle arrest and disruption of the microtubule network in normal marsupial kidney cells and inhibited tubulin polymerization in vitro (Li et al., 2002). A few reports have also shown that garlic-derived OSCs arrest cancer cells in phases other than G2/M. The DADS mediated
suppression of human nosopharyngeal carcinoma cell growth correlated with S phase arrest (Zhang et al., 2006). Allitridi, synthetic DATS, was shown to arrest human gastric cancer BGC823 cells in the G\textsubscript{i} phase and was accompanied by a decrease in cyclin D1 level and an increase in p27 protein level (Lan et al., 2003). Nevertheless, inhibition of cell cycle progression appears to be a common cellular response to many structurally diverse OSCs.

**Induction of Programmed Cell Death (Apoptosis)**

Apoptosis (also known as programmed cell death) is a tightly controlled and evolutionarily conserved process of cellular suicide critical to normal embryonic development and maintenance of tissue homeostasis. Dysregulation of programmed cell death underlies numerous pathological conditions including cancer and, therefore, apoptosis is a valid target in cancer therapy and prevention (Kaufmann, 2000; Ghobrial et al., 2005). Garlic-derived OSCs have been shown to modulate a number of key elements in cellular signal transduction pathways linked to the apoptotic process. The majority of garlic-derived compounds activate the so called intrinsic or mitochondria-mediated pathway in the execution of apoptosis, which involves loss of mitochondrial membrane potential and release of apoptogenic molecules from the mitochondria to the cytosol (Hengartner, 2000; Thornberry and Lazebnick, 1998). Activation of the intrinsic apoptotic pathway is regulated by the Bcl-2 family of anti-apoptotic (eg. Bcl-2 and Bcl-xL) and proapoptotic (eg. Bax and Bak) proteins (Chao and Korsmeyer, 1998). Garlic-derived OSCs are believed to trigger apoptosis by modulating the levels of Bcl-2 proteins. For example, DAS or DADS treatment increased the ratio of Bax/Bcl-2 in SH-SY5Y neuroblastoma cells, as well as in H460 and H1299 lung cancer cells compared with untreated controls (Karmakar et al., 2007; Hong et al., 2000). A time-dependent upregulation of Bax protein level and concomitant downregulation of Bcl-xL protein level was observed in DADS treated MDA-MB-231 breast cancer cell line (Nakagawa et al., 2001). The Z-ajoene-induced apoptosis in HL-60 cells was associated with caspase-mediated cleavage of Bcl-2 (Li et al., 2002).

A previous study has shown that DATS is a more potent inducer of apoptosis in PC-3 and DU145 prostate cancer cells than DAS or DADS (Xiao et al., 2004). The DATS induced apoptosis in prostate cancer cells correlates with a decrease in Bcl-2 level as well as with
hyperphosphorylation of this protein, which reduces Bcl-2/Bax interaction and activates the mitochondrial pathway of apoptosis (Xiao et al., 2004). The DATS-induced apoptosis in LNCaP cells correlated with a modest increase in protein levels of pro-apoptotic Bcl-2 family members Bax and Bak (Kim et al., 2007). The DATS-mediated hyperphosphorylation of Bcl-2 in PC-3 and DU145 cells is caused by activation of JNK and, to a lesser extent, extracellular signal-regulated kinase \( \frac{1}{2} \) (ERK1/2) (Xiao et al., 2004). Overexpression of Bcl-2 in PC-3 cells conferred statistically significant protection against DATS-induced apoptosis (Xiao et al., 2004). On the other hand, ectopic expression of Bcl-2 failed to protect against DATS-mediated cell death in LNCaP human prostate cancer cells (Kim et al., 2007). Furthermore, a recent study showed that DATS-mediated inhibition of PC-3 xenograft growth in nude mice correlated not only with increased apoptosis but also with induction of Bax and Bak proteins in the tumor tissue (Xiao et al., 2006).

A previous study has shown that the DATS-induced apoptosis in human prostate cancer cells was, at least in part, regulated by the Akt-Bad pathway (Xiao et al., 2006). One of the pro-survival functions of Akt (also known as protein kinase B) is to phosphorylate Bad, which causes cytoplasmic sequestration of Bad and consequently protection against interaction with anti-apoptotic Bcl-2 family members. DATS treatment markedly reduced Akt activity in PC-3 and DU145 cells and consequently lowered the phosphorylation of Bad at Ser155 and Ser136, which diminished complex formation between Bad and cytosolic 14-3-3β (Xiao et al., 2006). Overexpression of constitutively active Akt in PC-3 cells conferred significant protection against DATS-induced apoptosis (Xiao et al., 2006).

Experimental evidence exists to support a critical role of ROS as an intermediary of OSC-induced apoptosis. For instance, DADS-induced apoptosis in HL-60 cells is correlated with ROS generation (Kwon et al., 2002). The DADS-induced ROS formation in SH-SY5Y neuroblastoma cells is evident as early as 15 min after treatment and is accompanied by oxidation of cellular lipids and proteins (Filomeni et al., 2003). ROS generation in DADS treated cells was associated with activation of JNK (Filomeni et al., 2003). Overexpression of Cu, Zn-superoxide dismutase or pretreatment with spin trapping molecule 5,5'-dimethyl-1-pyrroline N oxide offered protection against DADS-
induced ROS generation, oxidative damage of cellular macromolecules and apoptosis in SH-SY5Y cells (Filomeni et al., 2003).

A few studies have suggested that apoptosis induction by OSC might result from an increase in free intracellular calcium (Karmakar et al., 2007; Sundaram and Milner, 1996; Sundaram and Milner, 1996; Park et al., 2002; Sakamoto et al., 1997). Park et al. reported DADS-mediated increase in intracellular calcium level which was followed by an increase in hydrogen peroxide level and caspase-3 activation (Park et al., 2002). Recently, it has been shown that both DAS and DADS cause an increase in calcium level in SH-SY5Y cells, which leads to activation of a non-caspase cysteine protease calpain which can contribute to cell death by inducing mitochondria-mediated apoptosis independently of caspases (Karmakar et al., 2007).

Some of the studies cited above have compared apoptotic responses to OSC in cancer cells versus normal cells. Strikingly, malignant cells appear to be more sensitive to OSC-mediated apoptosis than normal non-transformed cells. For example, viability of primary neurons was minimally affected by treatment with 50 or 100 µmol/l DAS or DADS, whereas the neuroblastoma of SH-SY5Y cells treated with these concentrations of DAS or DADS exhibited a marked reduction in cell viability (Karmakar et al., 2007). Similarly, the viability of a normal prostate epithelial line PrEC was not affected by DATS treatment even at concentrations that are highly cytotoxic to prostate cancer cells (Xiao et al., 2005; Kim et al., 2007). Finally, Z-ajoene has been shown to cause apoptosis in human leukemia cells, but not in peripheral mononuclear blood cells of healthy donors (Dirsch et al., 1998). The mechanism behind the differential sensitivity of cancer cells and normal cells to apoptosis induction by OSCs remains to be elucidated.

**Histone Modification**

OSC may affect cancer cell proliferation through modification of histone acetylation and, thus, regulation of gene expression. It has been reported that treatment of DS19 mouse erythroleukemia and K562 human leukemia cells with DADS increases acetylation of histones H4 and H3 (Lea et al., 1999). DADS and its metabolite, allyl mercaptan, inhibited histone deacetylases in rat hepatoma and human breast cancer cells and it has been suggested that histone acetylation may mediate the differentiation process of erythroleukemia cells (Lea et al., 1999). Growth inhibitory effects of Allicin. SAMC
and SAC on DS19 cells and SAMC on Caco-2 human colon and T47D human breast cancer cells are correlated with increased histone acetylation (Lea et al., 2002). The DADS-induced accumulation of Caco-2 and HT-29 colon tumor cells in the G2/M phase of the cell cycle is correlated with inhibition of histone deacetylase, hyperacetylation of H3 and H4 histones, and upregulation of p21 mRNA and protein level (Druesne et al., 2004; Druesne et al., 2004).

**Inhibition of Angiogenesis and Metastasis by Garlic Constituents**

Recent studies using cellular and animal models indicate that garlic extract and its components are able to affect tumor angiogenesis and metastasis. The formation of new blood vessels is necessary for the growth of solid tumors because evidence exists to suggest that tumor growth beyond 1 mm in diameter is restricted by angiogenesis (Folkman, 2003). A study by Matsuura et al. showed that aged garlic extract (AGE) suppressed proliferation of transformed human and rat endothelial cell lines and reduced the invasiveness of the endothelial cells by about 20%-30% (Matsuura et al., 2006). Additional tests indicated that AGE increased the adhesion of the endothelial cells to collagen and fibronectin in a dose-dependent manner; thus, reducing their motility (Matsuura et al., 2006). In another study, Xiao et al. examined the effects of DAS, DADS and DATS on human umbilical vein endothelial cell (HUVEC) viability and have shown that DATS is the most potent of the three analogs in reducing the viability of HUVEC (Xiao et al., 2006). Alliin was shown to significantly reduce VEGF and fibroblast growth factor-2 (FGF-2)-induced tube formation and angiogenesis in HUVEC (Mousa and Mousa, 2005). A recent study showed that DADS and DAS not only inhibited endothelial cell proliferation and migration, but also reduced matrix metalloproteinases 2 and 9 (Thejass and Kuttan, 2007). Based on the reviewed studies it can be concluded that components of garlic extract (in combination or alone) present a great potential as anti-angiogenic and anti-metastatic agents.

**Modulation of P-glycoprotein Mediated Multidrug Resistance**

Multidrug resistance (MDR) mediated by the over-expression of drug efflux protein P-glycoprotein (P-gp) is one of the major obstacles to successful cancer chemotherapy. Garlic OSCs have been shown recently to modulate the P-gp mediated multidrug resistance. DAS (8.75x10^-3 M) decreased the induced levels of P-gp in K562 leukemic
resistant cells back to the normal levels (Arora et al., 2004). In another study, it was shown that OSCs present in a garlic-rich diet might alter chemotherapeutic treatments using P-gp substrates in renal brush-border membranes (Demeule et al., 2004). In a recent study, effects of dietary phytochemicals on P-gp function were investigated using human MDR carcinoma KB-C2 cells and the fluorescent P-gp substrates daunorubicin and rhodamine 123. DAS and DATS were shown to be capable of modulating the effect of P-gp overexpression (Nabekura et al., 2005).

In summary, research over the past 20 years has revealed that garlic derived OSCs can not only inhibit chemically induced cancers but can also suppress growth of cancer cells in culture and in vivo. The garlic compounds appear to target multiple pathways, including the cell cycle machinery, the intrinsic pathway for apoptotic cell death and angiogenic pathway, which may all contribute to their anticancer activities.

Apoptosis (Programmed Cell Death)

Apoptosis, or programmed cell death (PCD), is an evolutionarily conserved mechanism for the selective removal of aging, damaged or otherwise unwanted cells (Thorburn. 2004; Lawen, 2003; Abe et al., 2000; Strasser et al., 2000; Peter and Krammer, 1998). It is an essential component of many normal physiological processes such as embryogenesis, normal tissue development, and the immune response (Vaux and Korsmeyer, 1999). Thus, regulation of apoptosis is critical for tissue homeostasis and its deregulation can lead to a variety of pathological conditions including carcinogenesis and chemoresistance (Sheikh and Huang, 2004; Ozoren and El-Deiry, 2003; Green and Evan. 2002; Zornig et al., 2001; Daniel et al., 2001; Thompson, 1995).

Apoptosis is mediated primarily through the activation of specific proteases called caspases (cysteiny1, aspartate-specific proteases) (Algeciras-Schimnich et al., 2002; Ozoren and El-Deiry, 2003; Salvesen and Dixit, 1997; Stegh and Peter, 2001; Thorburn. 2004). Caspases are effectors of cell suicide and cleave multiple substrates, leading to biochemical and morphological changes that are characteristic of apoptotic cells (Abe et al., 2000; Strasser et al., 2000). These alterations include: mitochondrial outer membrane permeabilization; cell membrane remodeling and blebbing; exposure of phosphatidylserine (PS) at the external surface of the cell; cell shrinkage with cytoskeletal rearrangements; nuclear condensation; and DNA fragmentation (Ashkenazi
and Dixit, 1999; Green and Evan, 2002; Lawen, 2003; Peter and Krammer, 2003; Schulze-Osthoff et al., 1998; Thorburn, 2004). These morphological changes culminate in the formation of apoptotic bodies that are normally eliminated by phagocytosis (Geske and Gerschenson, 2001; Wallach, 1997).

In mammalian systems, the extrinsic death receptor pathway and the intrinsic mitochondrial pathway are the two major signaling systems that result in the activation of the executioner/effector caspases and the consequent demise of the cell (Abe et al., 2000; Ozoren and El-Deiry, 2003; Peter and Krammer, 2003; Strasser et al., 2000; Thorburn, 2004) (Fig. 8). In many cell types, including cancer cells, activation of the extrinsic pathway also engages the mitochondrial pathway for full execution of cell death (Jaattela, 2004; Kroemer, 2003; Thorburn, 2004). Thus, many apoptotic signals merge at the mitochondria, and thus mitochondria have been termed “gatekeepers” of the apoptotic machinery (Jaattela, 2004; Kroemer, 2003; Thorburn, 2004). As gatekeepers, the proteins comprising the intrinsic mitochondrial pathway are the major mediators of the cytotoxic effects of many chemotherapeutic agents and radiation therapy (Brenner et al., 2003; Costantini et al., 2000; Debatin et al., 2002; Hersey and Zhang, 2003). Cancer cells often evade this apoptosis and develop chemoresistance and radioresistance. Indeed, disruption of the mitochondrial apoptotic machinery has been observed in many tumors (Daniel et al., 2001; Morisaki and Katano, 2003). It is also likely that disruption of the mitochondrial machinery or mutations in the mitochondrial DNA could play a role in cancer initiation. Because of the central role of mitochondria in these processes, various components of the mitochondrial machinery can be targets for novel therapeutic strategies.

The Mitochondrial Pathway of Apoptosis

Mitochondria are thought to be the primary organelles involved in mediating most apoptotic pathways in mammalian cells (Green and Kroemer, 2004; Kroemer, 2003; Ravagnan et al., 2002; Sorice et al., 2004; Zamzami and Kroemer, 2001). Mitochondria are engaged via the intrinsic pathway of cell death, which can be initiated by a variety of stress stimuli, including ultraviolet (UV) radiation, γ-irradiation, heat, DNA damage, the actions of some oncoproteins and tumor suppressor genes (i.e. p53), viral virulence factors, and most chemotherapeutic agents (Fig. 8) (Kroemer, 2003). These diverse forms
of stress are sensed by multiple cytosolic or intraorganellar molecules. Transduction of
these signals to the mitochondria ultimately results in alterations of the outer
mitochondrial membrane (OM) (Esposti et al., 2003; Green and Kroemer, 2004; Kuwana
et al., 2002; Zamzami and Kroemer, 2001). These changes in the OM then lead to
increased permeability to proteins that normally reside between the OM and the inner
mitochondrial membrane (IM), enabling these proteins to escape the mitochondria and
diffuse into the cytosol.

The mitochondrial pathway of apoptosis can also be activated in response to death
ligands. In a majority of cells (type II cells), including tumor cells, extracellular death
signals engage the mitochondria in a way that is equivalent to the intrinsic pathway (Abe
et al., 2000; Algeciras-Schimnich et al., 2002; Ozoren and El-Deiry, 2002; Peter and
Krammer, 1998). In these cells, signals originating from the death ligand-induced
activation of caspase-8 and caspase-10 bifurcate into two arms, one of which directly
engages mitochondria via a sequence of events causing activation of the effector caspases
(i.e. caspase-3). The second arm promotes the cleavage of non-caspase substrates, such as
Bid, inducing changes in the mitochondrial OM and the release of apoptogenic factors
and activation of caspase-9, which then cooperates with the less-efficient activation of
caspase-8 in these cells.

The Release of Proapoptotic Factors

Mitochondria contain and release many soluble proteins that are involved in the apoptotic
cascade (Fig. 9) (Daniel et al., 2001; Debatin et al., 2002; Green and Kroemer, 2004;
Reed, 2004). The variety of mitochondrial proteins participating in this pathway indicates
the pivotal role of these organelles in determining cellular fates. Bcl-2 family members
control apoptosis by regulating the permeabilization of the mitochondrial membrane
(Chao and Korsmeyer, 1998; Cory et al., 2003; Daniel et al., 2001). The release of
mitochondrial proteins, including cytochrome c, apoptosis-inducing factor (AIF), second
mitochondria-derived activator of caspases (Smac/Diablo), high-temperature requirement
A2 (HtrA2/Omi), and endonuclease G, is believed to play a pivotal role in inducing
programmed cell death (PCD) (Martinou and Green, 2001; Zamzami and Kroemer,
2001).
Figure 8: Schematic representation of the intrinsic and extrinsic apoptotic pathways
Mitochondrial membrane permeabilization is regulated by an elegant balance of opposing actions of proapoptotic and antiapoptotic Bcl-2 family members. Bax, Bad, and Bak promote the release of cytochrome c and AIF through the formation of transmembrane channels across the mitochondrial outer membrane, while Bcl-2 and Bcl-XL delay this release and abort the apoptotic response, leading to cell survival. Besides the release of mitochondrial proapoptotic components, the loss of mitochondrial membrane integrity results in the loss of many essential biochemical cellular functions such as ATP synthesis and results in the generation of reactive oxygen species (ROS). The increased levels of ROS are directly linked to the oxidation of lipids, proteins, and nucleic acids.
Cytochrome C

Cytochrome c (Cyt c), a small (13 kDa) nuclear encoded mitochondrial protein, was the first protein identified as being released from mitochondria upon apoptosis. It is considered a key regulator of apoptosis because once it is released from the mitochondrial intermembrane space (IMS), the cell is irreversibly committed to death (Green and Evan, 2002; Kluck et al., 1997; Zhivotovsky et al., 1998a; Zhivotovsky et al., 1998b). The release of cytochrome c to the cytosol is considered among the major steps in the intrinsic death pathway (Kluck et al., 1997; Newmeyer and Ferguson-Miller, 2003; Zhivotovsky et al., 1998a). Once it escapes to the cytosol, it is captured by the apoptosis protease activating factor (APAF-1), a 130 kDa adaptor protein (Soengas et al., 1999; Zou et al., 1999). Prior to binding Cyt c, APAF-1 is virtually inactive. Once bound to Cyt c, the APAF-1 monomer goes through a cytochrome c-induced conformational change that promotes its activation. Further oligomerization occurs, resulting in a cartwheel-shaped heptameric structure containing seven Cyt c/APAF-1 complexes. This larger multiprotein complex is termed the apoptosome (Acehan et al., 2002; Adrain et al., 2001; Adrain et al., 1999; Srinivasula et al., 1999). Pro-caspase-9 is recruited to the apoptosome through its CARD domain, promoting its cleavage and converting it to an active protease (Adrain et al., 1999). Consequently, caspase-9 dissociates from the complex and goes on to activate effector caspases (3, 6, and 7) which collectively orchestrate the execution of apoptosis (Slee et al., 1999; Srinivasula et al., 1999; Zou et al., 1999).

Apoptosis-Inducing Factor

The precursor of the protein AIF is synthesized in the cytosol and imported into mitochondria (Susin et al., 1999). It contains an N-terminal mitochondrial localization sequence (MLS) which is cleaved upon its mitochondrial translocation to form the mature 57 kDa AIF (Susin et al., 1999). Under apoptosis-inducing conditions, AIF translocates through the permeabilized mitochondrial outer membrane to the cytosol (Cande et al., 2002; Susin et al., 1999). Subsequently, AIF is transported to the nucleus where it induces ATP-independent nuclear chromatin condensation, as well as large-scale DNA fragmentation (Cande et al., 2002; Susin et al., 1999).
Smac/Diablo

Second mitochondria-derived activator of caspases (Smac) is a 22 kDa mitochondrial protein also known as direct IAP-associated binding protein with low pI (Diablo). Inhibitors of apoptosis (IAP) family members have the ability to interact and inhibit the enzymatic activity of caspases through their baculovirus inhibitor repeat (BIR) functional motif (Deveraux and Reed, 1999; Miller, 1999). The Smac/Diablo precursor is synthesized in the cytosol, then imported to the mitochondria where it is cleaved and activated. A mature form of Smac/Diablo is released to the cytosol under apoptotic conditions. Unlike cytochrome c, which directly activates APAF-1 and caspase-9, Smac/Diablo binds to the BIR domains of multiple IAP members, antagonizing them and promoting indirect caspase activation (Ekert et al., 2001; Srinivasula et al., 1999; Verhagen and Vaux, 2002).

HtrA2/Omi

HtrA2, also referred to as Omi, is a mitochondrial protein that belongs to the family of serine proteases. This proapoptotic protein is expressed as a 50 kDa precursor that is cleaved at the N-terminal, upon translocation to the mitochondria, to generate the active 36 kDa protein (Hegde et al., 2002; Martins et al., 2002; Suzuki et al., 2001; Verhagen and Vaux, 2002). Similar to cytochrome c and Smac/Diablo, mature HtrA2/Omi localizes to the IMS (Hegde et al., 2002; Suzuki et al., 2004). Its release to the cytosol is stimulated by apoptotic triggers. Upon its release, HtrA2/Omi binds directly to the BIR domain of IAPs and inhibits their caspase-inhibitory activity (Suzuki et al., 2001).

Endonuclease G

As with most mitochondrial proteins, Endonuclease G is expressed as a precursor in the cytosol. Upon its translocation to the mitochondria, the 33 kDa protein is cleaved to a 28 kDa mature form (Cote and Ruiz-Carrillo, 1993). During apoptosis, endonuclease G is released from the mitochondrial IMS and translocates to the nucleus, where it causes oligonucleosomal DNA fragmentation (Li et al., 2001; van Loo et al., 2001). Endonuclease G release appears to be dependent on caspase activation downstream of mitochondria (Arnoult et al., 2003).
Mitochondrial Proteins and Caspase Activation

Among the various proteins that leak out of mitochondria, a few, such as cytochrome c, play a major role in promoting caspase activation. (Kluck et al., 1999; Saeiens et al., 2004) These apoptogenic factors are released in a hierarchical manner during cell death. Upon activation of the intrinsic pathway, cytochrome c, Htr2A/Omi and Smac/Diablo are released first, with similar kinetics (Saeiens et al., 2004). The subsequent release of AIF and endonuclease G (Arnoult et al., 2003; Penninger and Kroemer, 2003) is associated with more severe damage to both the outer and inner membranes. Notably, cytochrome c has been shown to be directly involved in the mediation of cell death, as it is indispensable for the activation of Apaf-1 and subsequent formation of the apoptosome (Arnoult et al., 2003).

The apoptosome itself is a platform for recruiting and facilitating the autocatalytic activation of pro-caspase-9, the apical caspase of the intrinsic pathway of apoptosis (Adams and Cory, 2002; Baliga and Kumar, 2003; Cain et al., 2002; Chinnaiyan, 1999; Hill et al., 2003; Salvesen and Renatus, 2002; Shi, 2002). The activation of caspase-9 leads to the local accumulation of zymogens, promoting an autocatalytic process of downstream caspase activation (Adams and Cory, 2002; Baliga and Kumar, 2003; Cain et al., 2002; Chinnaiyan, 1999; Hill et al., 2003; Salvesen and Renatus, 2002; Shi, 2002). However, the apoptosome requires additional regulatory factors, including Smac/Diablo, for full activation of the caspase cascade. Smac/Diablo interacts with several IAPs to release them from their inhibitory interaction with pro-caspase-9 and other caspases (Adams and Cory, 2002; Baliga and Kumar, 2003; Cain et al., 2002; Shi, 2002). Smac/Diablo is also present in the mitochondria, where it is directly attached to the OM and is released upon alterations in the OM permeability (Cain et al., 2002; Saeiens et al., 2004).

The Bcl-2 Family of Proteins and Regulation of the Mitochondrial Pathway to Cell Death

The process of mitochondrial release of proapoptotic factors such as cytochrome c is elegantly regulated through members of the Bcl-2 family (Antonsson et al., 1997; Cory et al., 2003; Danial and Korsmeyer, 2004; Green and Kroemer, 2004; Schendel et al., 1997) (Fig. 9). In mammals, the antiapoptotic members of this family include Bcl-2, Bcl-XL.
and Bcl-W, while the proapoptotic members include Bax, Bak, Bad, Bik, Bim, and Bid. The proapoptotic family members are further classified based on domain sequence homology into two groups: one that contains multiple BH domains and one that contains only the BH3 domain (Cheng et al., 2001; Fiers et al., 1999; Kuwana and Newmeyer, 2003; Wei et al., 2001). The fate of the cell depends to a great degree on the precious balance of function between these proapoptotic and antiapoptotic Bcl-2 proteins. Studies have shown that Bax, Bad, and Bak promote the release of AIF and cytochrome c, while Bcl-2 and Bcl-XL delay the release and abort the apoptotic response, promoting cell survival (Cory and Adams, 2002; Yang et al., 1997).

It is believed that Bcl-2 family members regulate the apoptotic response by controlling mitochondrial membrane permeabilization (MMP) (Green and Kroemer, 2004). The proapoptotic proteins Bax and Bak have been shown to contribute to the formation of transmembrane channels across the mitochondrial OM, leading to the escape of AIFs (Dejean et al., 2005; Korsmeyer et al., 2000; Kuwana et al., 2002; Nechushtan et al., 2001; Wei et al., 2001). Bcl-2, Bcl-W, and Bcl-XL are, on the other hand, believed to prevent pore formation and to inhibit the release of cytochrome c from the mitochondria (Kluck et al., 1997; Yang et al., 1997). Moreover, heterodimerization of Bax or Bad with Bcl-2 or Bcl-XL is thought to inhibit their protective effect.

Bid is a potent proapoptotic protein that is normally located in the cytosol, but also shuttles through the surfaces of intracellular membranes due to its lipid-interacting capacity. Bid plays an important role in the mitochondrial pathway to apoptosis as it has been identified as the link between the death receptor signal and the release of cytochrome c. Activated caspase-8 engages the intrinsic apoptotic pathway through the truncation of Bid (Li et al., 1998; Luo et al., 1998). Upon death signaling, activated caspase-8 cleaves Bid (26 kDa) into two fragments: a C-terminus fragment (15 kDa) and an N-terminus fragment (11 kDa) (Luo et al., 1998). The 15 kDa fragment, which contains the BH3 domain, is termed truncated Bid or tBid. This functional fragment translocates to the mitochondria where it interacts with several proteins through its BH3 domain (Wang et al., 1996). There are two modes of Bid proapoptotic action. (1) In the BH3-dependent mode, Bid interacts with the antiapoptotic Bcl-XL through its BH3 domain and prevents the formation of the Bcl-XL/Apaf1 antiapoptotic complex. (2) In
the BH3-independent mode, after truncation, Bid is proposed to form selective channels similar to BAX through its structural motifs (Chou et al., 1999; McDonnell et al., 1999). Moreover, tBid has been shown to induce the oligomerization of Bax and Bak, resulting in MAC formation and the subsequent release of proapoptotic cytochrome c (Eskes et al., 2000; Wei et al., 2000).

The mitochondrial receptor for caspase-cleaved Bid is thought to be cardiolipin (CL), a mitochondrial lipid (Esposti et al., 2003; Kuwana et al., 2002; Newmeyer and Ferguson-Miller, 2003; Sorice et al., 2004). CL is a glycerophospholipid that is synthesized and localized in the inner membrane of the mitochondria, making it one of its major constituents (Khosravi-Far and Esposti, 2004; McMillin and Dowhan, 2002; Schlame et al., 2000; Wright et al., 2004). This dimeric molecule apparently plays a significant role in controlling the mitochondrial membrane structure and function. It has been proposed that upon apoptotic stimulation, CL contributes to the apoptotic signal through the recruitment of cytosolic proteins such as tBid to the mitochondrial membrane. Additionally, it is thought that CL is involved in altering MMP, leading to the subsequent release of proapoptotic factors (Lutter et al., 2000).

**Targeting Mitochondria in Cancer Therapy**

As mitochondria are gatekeepers of apoptotic signals, targeting mitochondria to induce apoptosis of malignant cells is an important therapeutic strategy. In the past several years, extensive research has focused on screening for chemical compounds, small molecules and peptides that could target the mitochondria. Therapeutic tactics have included strategies that involve the Bcl-2 family proteins, activation of PTPs, the respiratory chain, mitochondrial DNA depletion, and selective targeting of ROS-stressed malignant cells, as well as targeting inhibitors of apoptosis such as IAPs (Dias and Bailly, 2005). Targeting the antiapoptotic members of the Bcl-2 family, namely Bcl-2 and Bcl-XL, and targeting the PTP are among the most studied mechanisms (Dias and Bailly, 2005; O’Neill et al., 2004; Shangary and Johnson, 2003; Walensky, 2006). The induction of proapoptotic protein release through increased PTP formation and opening has been explored in the recent years as a possible mechanism for cancer treatment. As a chemotherapeutic approach, this method involves perturbation of the mitochondrial membrane through direct targeting of the components of the membrane permeability transition pore complex.
(PTPC) (Brenner et al., 2003; Costantini et al., 2000; Debatin et al., 2002; Fantin and Leder, 2006; Galluzzi et al., 2006; Khosravi-Far and Espositi, 2004; Morisaki and Katano, 2003; Reed, 2004).

In summary, the intrinsic and extrinsic death pathways leading to changes in mitochondrial permeability, the components of the PTPC, including members of the Bcl-2 family, apoptogenic factors and their regulators, and mutations in mtDNA have been studied extensively in the past for their contributions to cancer progression or resistance to therapy. These constitute an extensive list of targets that could induce apoptosis, some with possible specificity for cancer cells. Therapeutic agents against many of these targets, including Bcl-2 family members and components of the PTP, are currently at various stages in the development pipeline. The ultimate goal of these studies is to generate novel mitotoxic agents that can selectively induce apoptosis of cancer cells and reduce the possibility of resistance.

Role of Glutathione (GSH) in Maintaining Cellular Redox Environment

Glutathione (GSH) is a tripeptide produced naturally in the body, with an intracellular concentration of 1–10 mM in mammalian cells. It is the most abundant non-protein molecule in the cell and has a number of physiological roles. GSH is synthesized intracellularly from its constituent amino acids: glutamic acid, cysteine and glycine via two sequential ATP-consuming steps, which are catalysed by γ-glutamylcysteine synthetase (γ-GCS) and GSH synthetase (Fig. 10).

Under normal conditions, the majority of GSH exists in reduced form (0.5 to 10 mM) (Kosower and Kosower, 1978). Oxidation of the reduced GSH to form oxidized glutathione (GSSG) is carried out either by direct interaction with free radicals or, more often, when GSH acts as a cofactor for antioxidant enzymes such as glutathione peroxidases (GPx) during the reduction of H2O2 and phospholipid hydroperoxide glutathione peroxidases. Also, GSSG is formed when glutathione transhydrogenase (glutaredoxin reductase) uses GSH to reduce the disulfide bond in glutaredoxin, the downstream action of which leads to the formation of deoxyribonucleotides (via the activation of ribonucleotide reductase) (Holmgren, 1989). However, highly efficient NADPH-dependent reduction of GSSG by glutathione reductase is able to maintain the intracellular concentration of GSSG at very low levels of around 5 to 50 mM (Kosower
The ratio of the GSSG/GSH couple can serve as an important indicator of the cellular redox environment (Schafer and Buettner, 2001). Glutathione S-transferases (GST) catalyse the conjugation of GSH with many exogenous as well as endogenous electrophilic substrates. GSTs are a family of phase II detoxification enzymes, which have broad substrate specificity. The human GSTs consist of six classes, namely alpha (α), mu (μ), pi (π), theta (θ), zeta (ζ) and omega (ω), each of which is divided into one or more isoforms. The expression levels of different isoforms of GSTs are reported to be tissue specific (Salinas and Wong, 1999). GSTs are present predominantly in hepatic cells and other mammalian cells and tissues, such as erythrocytes and in the intestine. GST catalyses the conjugation of GSH with endogenous compounds such as the lipid peroxidation product, 4-hydroxy-2-nonenal, estrogens and cholesterol-5,6-oxide and with xenobiotics including environmental carcinogens such as benzo(a)pyrene 7,8-dihydrodiol-9,10-epoxide (BPDE) and acrolein, pesticides such as DDT and a number of drugs which include cisplatin and acetaminophen (Eaton and Bammler, 1999).

GSH, which is transported to the cell membrane, becomes a substrate for membrane-bound γ-glutamyl transpeptidase (Griffith et al., 1978). γ-glutamyl transpeptidase and dipeptidase catalyse the extracellular breakdown of GSH (Fig. 10). The γ-glutamyl transpeptidase catalyses cleavage of the gamma linkage between glutamate and cysteine in an ATP-dependent manner, which generates cysteinylglycine. Cysteinylglycine is subsequently cleaved by dipeptidase to yield free cysteine and glycine (Fig. 10).

The transportation of GSH is a very important aspect of GSH metabolism. Intracellular GSH concentrations vary between 0.5 and 10 mM; however, only micromolar concentrations of GSH are found in blood plasma. The ability of many cells to transport GSH into the extracellular compartment may help in the transfer of cysteine, a limiting amino acid in glutathione synthesis de novo, between cells via the γ-glutamyl cycle (Dickinson and Forman, 2002). In addition, GSH may protect the cell membrane by reducing harmful compounds such as highly reactive oxygen species in the cellular environment and facilitating the transport of molecules such as disulfides (Denecke and Fanburg, 1989).
Figure 10: Glutathione biochemistry and intracellular pools. Some molecular mechanisms where GSH plays a key regulatory role are indicated within mitochondria, nucleus, and the endoplasmic reticulum. aa, amino acids; x, molecules that bind GSH forming conjugates; FRs, free radicals; (1) GGT; (2) γ-glutamyl amino acid (γ-Glu-aa) transporter; (3) dipeptidases; (4) Cyst(e)ine transporters; (5) γ-Glu-cyclotransferase; (6) 5-oxoprolinase; (7) GCS; (8) GSHS; (9) GPx; (10) GR; (11) transhydrogenases; (12) GSTs.
Functions of Glutathione

GSH has several different, yet vital, physiological roles, highlighted by its abundance within cells. It is the major cellular antioxidant and is crucial in maintaining the balance between oxidation and antioxidation. GSH is also important in cellular detoxification, and is required in many aspects of the immune response.

Antioxidation

Oxygen radical stress is caused as a byproduct of aerobic metabolism within all aerobic organisms. Highly active intermediates are formed, namely hydrogen peroxide and superoxide, which promote oxygen radical production, which in turn leads to cellular damage (Halliwell, 1994). GSH is the major endogenous soluble antioxidant in mammalian cells. It provides protection against oxidative stress through serving as a substrate for the antioxidant enzymes GSH peroxidase and phospholipid hydroperoxide GSH peroxidase (Takebe et al., 2002) that convert peroxides into less harmful fatty acids, water and GSH disulfide. It is also able to protect cells against oxidative stress by non-enzymatic scavenging of free radicals. Reduced GSH can directly scavenge radicals and peroxides via mixed disulfide formation, or upon oxidization to GSSG. This is able to prevent harmful effects on tissues associated with peroxidation of cell membrane lipids (Toborek and Hennig, 1994). There is evidence that a variety of oxygen radical stresses can result in GSSG formation and depletion of GSH in the short term (Deneke and Fanburg, 1989).

Cells are very sensitive to signals via changes in their environment. A vast number of cellular processes are affected by the redox state of the cell, in which GSH has a pivotal role, with many signaling molecules being activated by GSH redox status, either directly, or indirectly through interaction with free radicals. Molecules activated in this way include activator protein-1 (AP-1), responsible for the expression of a number of genes including those for many cytokines, TGF-β and collagenase, activator protein-2 (AP-2), c-Jun N-terminal kinase (JNK), stress-activated protein kinase (SAPK), protein kinase C (PKC) and tyrosine kinase (Janssen et al., 1993; Hayes and McLillen, 1999). These molecules are involved in a wide range of cellular pathways such as cellular proliferation, differentiation and morphogenesis. A decrease in GSH level is also able to stimulate NF-κB activation. In this way, GSH is able to regulate indirectly the expression of genes that
contain the NF-kB binding site in their promoter including numerous cytokines, receptors for cytokines and cell adhesion molecules. This activation is blocked by antioxidants such as the GSH precursor N-acetyl L-cysteine and other thiol compounds (Fernandez et al., 1999).

**Immunomodulation**

GSH is required in many stages of the immune response. Intracellular GSH has been shown to modulate not only T-cell function, including the binding, internalization, and degradation of interleukin-2 (Liang et al., 1989), but also DNA synthesis (Suthanthiran et al., 1990). GSH and other sulfhydryl compounds can also enhance cytotoxic T-cell activation, proliferation and differentiation (Multhoff et al., 1995). Studies have shown not only that in vivo administration of GSH can activate cytotoxic T-cells, but also that depletion of intracellular GSH can inhibit the activation of lymphocytes, suppressing their cytotoxic functions and increasing susceptibility of the cell to radiation damage (Droge et al., 1994).

**Detoxification**

Cells are constantly being exposed to damaging chemical substances (toxins) including environmental pollutants, smoke, heavy metals and drug metabolites. There are many mechanisms for detoxification, including phase I reactions, which are primarily catalysed by cytochrome P-450, hydrolysis or reduction, and phase II reactions, which involve the conjugation of toxins to an endogenous chemical. Conjugation to GSH is the major Phase II reaction in mammalian species, and is particularly important in the detoxification of electrophilic substances such as epoxides, alkenes, halides and heavy metals (Kaplowitz et al., 1985). The liver, kidney and lungs have the highest exposure to toxins and are therefore richest in GSH. GST is able to catalyse nucleophilic substitution to form GSH conjugates (Salinas and Wong, 1999). GSH conjugates can then be eliminated via an ATP-dependent GS-X pump (Ishikawa, 1992).

**Prooxidation**

In an interesting paradox, the interaction of GSH with metal ions can also lead to an overall ‘oxidizing’ effect, by the generation of superoxide anions from the transfer of electrons from the metal ions to molecular oxygen (Pompella et al., 2003). Cleavage of the $\gamma$-glutamyl bond in GSH by membrane-bound $\gamma$-glutamyl tranferase (GGT) increases
its ability to reduce metals, and it has been shown that GGT/GSH-mediated prooxidant reactions can lead to oxidative modification of a number of molecular targets. This may represent a novel mechanism for modulation of cellular signal transduction (Paolicchi et al., 2002).

Role of Glutathione in Cancer

GSH metabolism has a complicated role in both cancer and antineoplastic therapy. While GSH is important in the detoxification of carcinogens, its elevated state in many types of tumours may also increase resistance to chemo- and radiotherapy (Midander et al., 1982; Nguyen et al., 2001; Vukovic et al., 2000; Vukovic et al., 2001). GSH levels have been shown to be elevated in a number of different human cancer tissues including, bone marrow (Joncourt et al., 1995), breast (Perry et al., 1993), colon (Redmond et al., 1991; Berger et al., 1994), larynx (Mulder et al., 1995) and lungs (Cook et al., 1991; Oberli-Schrammli et al., 1994). Elevated levels of γ-GCS have also been identified in different human cancer tissues including colon (Tatebe et al., 2002), lung (Soini et al., 2001), breast (Seven et al., 1998), liver (Huang et al., 2001) and other types of cancers (iida et al., 1999). Many GSH-based therapeutic strategies have focused on lowering GSH levels in order to increase sensitivity of cells to ionizing radiation, and to decrease the resistance to many chemotherapeutic drugs (Chen et al., 1998). The development of inhibitors for specific molecules in the GSH synthesis/GST detoxification pathway may help to elucidate the complex role of GSH in cancer, provide better strategies in cancer prevention and enhance the effectiveness of current chemotherapeutic approaches.

Aim of this Study

The possibility that Allicin can suppress the proliferation of cervical cancer cells by inducing cell cycle arrest or apoptosis makes it a potential chemotherapeutic agent against cancer of the uterine cervix. The central hypothesis of this study is that Allicin suppresses the proliferation of cervical cancer cells by inducing cell cycle arrest, restores the function of p53 by upregulating its expression in HPV positive cervical carcinoma cells resulting in the activation of pro-apoptotic genes and apoptotic cascade and abrogates the functions of HPV E6 and E7 viral proteins by downregulating their expressions. The specific aims described below were investigated to test this hypothesis.
(1) The efficacy of the Allicin to induce cell cycle arrest and apoptosis in cervical cancer cell line was investigated.

(2) The ability of Allicin to upregulate the expression of p53 in cervical cancer cell line was determined.

(3) The possibility that Allicin could activate the pro-apoptotic genes and apoptotic cascade was also investigated.

(4) The potential of Allicin to suppress or abrogate E6 and E7 protein function was also examined.

(5) The effect of Allicin on the expressions of different proinflammatory cytokines in monocytes from cervical cancer patients was also investigated.