Chapter - I

Introduction
1 Introduction

1.1 Cancer and Origin:

Cancer occurs when normal cells undergo a transformation that causes them to grow and multiply in an uncontrolled fashion. The cells form a mass or tumor that differs from the surrounding tissues from which it arises. The evolution of cancer is a multistep process that initiate with a genetic alteration in a single cell. For a normal cell to progress into a neoplastic state, it needs to consecutively acquire a list of hallmark capabilities that will ultimately transform it into a tumorigenic or malignant state (Hanahan et al, 2000). The various hallmark capabilities of cancer cells include as stated below in Figure 1.

![Image of Figure 1: The hallmarks of cancer (Hanahan and Weinberg, 2011).](image)

1.1.1 Proliferative Signaling:

This is one of the most fundamental traits of cancer cells. Normal cells sustain homeostasis by controlling the entry and progression of cells through the cell cycle by amending the production and release of growth signals. Most of these signals are growth factors that bind cell-surface receptors which comprise intracellular tyrosine kinase domains. These then emanate signals through branched intracellular signaling
pathways that regulate progression through the cell cycle. Cancer cells deregulate these signals in diverse ways: by producing growth factor ligands themselves (autocrine signaling), sending signals to fuel normal cells to produce growth factors, elevating the levels of receptor proteins displayed at the cancer cell surface thereby rendering such cells hyper responsive to growth factor ligands or by achieving growth factor independence (Cheng et al, 2008).

1.1.2 Evading Growth Suppressors:

Tumor supressor genes such as Retinoblastoma (Rb) and TP53 act as gatekeepers of cell cycle progression; in whose deficiency the cell undergoes uncontrolled proliferation. The Rb protein play an imperative role in amalgamating signals from assorted intracellular and extracellular sources and settle on whether the cell should ensue through the cell cycle or not (Burkhart et al, 2008). The p53 protein on the other hand receives signals from the cell’s intracellular circuits in response to cellular stress. p53 can halt progression through the cell cycle until the conditions are normal or it can trigger apoptosis. Most cancers show deficit of tumor supressor gene function which enable the cell to undergo persistent proliferation.

1.1.3 Resisting cell death:

Programmed cell death by apoptosis acts as a innate barrier for cancer growth (Adams et al, 2007; Lowe et al, 2004). Apoptosis is triggered by frequent physiological stresses such as signaling imbalances and DNA damage. The apoptotic machinery is composed of upstream regulators and downstream effector components. The regulators are of two types; one receives and processes extracellular death-inducing signals (for example the Fas ligand/Fas receptor), and the other senses intracellular signals. Each culminates in activation of a normally latent protease (Caspases 8 and 9, respectively), which proceeds to initiate a cascade of effector caspases conscientious for the execution of apoptosis, in which the cell is broken down and consumed by its neighbors and phagocytic cells. The “apoptotic trigger” that conveys signals between the regulators and effectors is controlled by balancing pro- and anti-apoptotic members of the Bcl-2 family of regulatory proteins. Bcl-2 acts by binding and suppressing the activity of two pro-apoptotic triggering proteins (Bax and Bak). Tumor cells have evolved an array of strategies to avert apoptosis. The dearth of
p53 tumor suppressor function exterminates critical damage sensors from the apoptosis-inducing pathway (Junttila et al, 2009). Cancer cells may also increase the expression of anti-apoptotic regulators or down regulate pro-apoptotic factors.

1.1.4 Enabling replicative immortality:

Normal cells in the body are able to replicate only a constrained number of times. Cancer cells on the other hand entail incessant replicative potential to form tumors. There are two main barriers for unlimited replication potential: senescence, which is an irreversible entrance into a non-proliferative but viable state, and crisis/apoptosis, which implicate cell death. Telomeres have been shown to be involved in the capability for unimpeded proliferation (Blasco, 2005; Shay et al, 2000).

Telomeres consist of multiple tandem hexanucleotide repeats which diminish progressively after each cell division, shortly losing the competence to protect the ends of chromosomal DNAs from end-to-end fusions. Such fusions craft unstable dicentric chromosomes which imperil cell viability (Kawai et al, 2007; Hansel et al, 2006). Telomerase, the enzyme which adds telomere repeat segments to the ends of telomeric DNA, is almost deficient in non-immortalized cells but is expressed in functionally considerable levels in human cancer cells. Thus, telomerase is able to defend against the progressive telomere erosion that would otherwise transpire in its absence. Cancer cells thus maintain unlimited replicative potential by up-regulating expression of telomerase or, less recurrently by a recombination-based telomere maintenance mechanism.

1.1.5 Inducing Angiogenesis:

Tumors require nutrients and oxygen to grow as well as an ability to eliminate waste and carbon dioxide. The tumor neo-vasculature, generated by the process of angiogenesis provides these needs. The process of angiogenesis takes place through a series of steps:

1) Formation of new endothelial cells and their assembly into tubes (vasculogenesis)
2) Formation of new vessels from existing ones.

After embryogenesis, the process of angiogenesis becomes largely dormant. In the adult during some physiological processes such as wound healing and female
reproductive cycling, angiogenesis is turned on, but only momentarily. In tumor cells
an ‘‘angiogenic switch’’ is always activated and remains on, causing normally
quiescent vasculature to continually form new vessels (Hanahan et al, 1996). The
angiogenic switch is restricted by factors that either provoke (VEGF-A) or counter
angiogenesis (TSP-1) (Baeriswyl et al, 2009 and Bergers et al, 2003). These angiogenic
regulators are signaling proteins that bind to stimulatory or inhibitory cell surface
receptors displayed by vascular endothelial cells.

1.1.6 Activating invasion and metastasis:

Invasion and metastasis is one of the central hallmarks transforming a neoplasm
into a malignant tumor. This invasion-metastasis cascade entails a series of steps: local
invasion, intravasation by cancer cells into nearby blood and lymphatic vessels, transport of
cancer cells passing through the lymphatic and hematological systems, movement of
cancer cells from the lumina of vessels into the parenchyma of distant tissues
(extravasation), formation of small nodules of cancer cells (micrometastases), and finally
the growth of micrometastatic lesions into macroscopic tumor (colonization) (Talmadge et
al, 2010; Fidler, 2003). Cancer cells develop alterations in their shape as well as in their
attachment to other cells and to the extracellular matrix. Most cancer cells show down-
regulation and mutational inactivation of E-cadherin, a key cell-to-cell adhesion molecule
(Berx et al, 2009; Cavallaro et al, 2004).

1.1.7 Additional hallmarks:

Other distinct attributes of cancer cells have also been proposed which play an
important role in the development and progression of cancer. Such hallmarks of cancer
cells are now referred as emerging hallmarks. Cancer cells are able to reprogram
cellular energy metabolism for supporting incessant cell growth and proliferation
(Warburg, 1930). They are also able to elude attack and elimination by immune cells
(Vajdic et al, 2009). Some additional hallmarks such as DNA damage/replication
stress, proteotoxic stress, mitotic stress, metabolic stress, and oxidative stress have also
been proposed by numerous studies (Luo et al, 2009).
1.2 Classification of cancer

Cancers are classified in two ways: by the type of tissue in which the cancer originates (histological type) and by primary site, or the location in the body where the cancer first developed. This classification is based on International Classification of Diseases for Oncology, Third Edition (ICD-O-3).

1.2.1 Classification by type of tissue (Histological Type)

- **Carcinoma**: This type of cancer originates from the epithelial layer of cells that form the lining of internal organs.
- **Sarcoma**: These cancers originate in connective and supportive tissues including muscles, bones, cartilage and fat.
- **Myeloma**: These originate in the plasma cells of bone marrow.
- **Leukemia**: These cancers affect the bone marrow which is the site for blood cell production.
- **Lymphoma**: These are cancers of the lymphatic system. These may affect lymph nodes at specific sites like stomach, brain, intestines etc.
- **Mixed types**: These have two or more components of the cancer. Some of the examples include mixed mesodermal tumor, carcinosarcoma, adenosquamous carcinoma etc.

1.2.2 Classification by Grade:

- **Grade 1**: well differentiated cells with insignificant abnormality
- **Grade 2**: cells are moderately differentiated and tendency towards abnormality
- **Grade 3**: cells are poorly differentiated and very abnormal
- **Grade 4**: cells are immature and primitive and undifferentiated
1.2.3 Classification by Stage:

The most commonly used method uses classification in terms of tumor size (T), the degree of regional spread or node involvement (N), and distant metastasis (M). This is called the TNM staging.

“T0” signifies no evidence of tumor, “T 1 to 4” signifies increasing tumor size and involvement and “Tx” signifies carcinoma in situ or limited to surface cells.

“N0” signifies no nodal involvement and “N 1 to 4” signifies increasing degrees of lymph node involvement. “Nx” signifies that node involvement cannot be assessed. Metastasis is further classified into two - “M0” signifies no evidence of distant spread while “M1” signifies evidence of distant spread.

1.3 Breast Cancer

Breast cancer is the second most common cancer among women and fifth cause of death from cancer worldwide with approximately 1.7 million new cases every year. Studies have shown that the breast cancer risk doubles each decade. Although breast cancer is considered to be a disease of the developed countries, close to 50% of the incidence and death from breast cancer occur in the under-developed countries. The incidence rate is almost 4 times more in eastern Asia compared to middle African countries. With today’s technology and awareness among women, it is possible to detect breast cancer at an early stage and treat them. Despite these advancements survival rates vary. The highest survival rate is 80% or more in Northern America, Japan, Sweden etc., whereas the poor survival rate (below 40%) have been documented from the low income countries.

The Indian Council of Medical Research (ICMR) indicated in 2016 that the total number of new cancer cases is expected to be 14.5 lakhs and the figure is likely to reach nearly 17.3 lakh new cases in 2020. [Over 17 lakh new cancer cases in India by 2020: ICMR News dated 19 May 2016].

In 2017, an estimated 252,710 new cases of invasive breast cancer were diagnosed among women and 2,470 cases will be diagnosed in men. In addition, 63,410 cases of in
situ breast carcinoma were diagnosed among women. Approximately 40,610 women and 460 men are expected to die from breast cancer in 2017 (American Cancer Society, 2017).

Breast cancer is the major cause of morbidity and mortality among females ranking number one among females in Indian metropolitan cities like Delhi, Kolkatta, Pune and Thiruvanathapuram, Bangalore, Mumbai and in Northeast, whereas in rural areas such as Barshi it still hold a second position.

Factors as marital status, location (urban/rural), BMI, breast feeding, waist to hip ratio, low parity, obesity, alcohol consumption, tobacco chewing, smoking, lack of exercise, diet, environmental factors were major risk factors in India leading to increasing incidence cancer; however, the reason for high incidence of breast cancer in younger women are not well known. Delayed disease presentation due to illiteracy, lack of awareness, financial constrains in some regions of India leads to late diagnosis, which in turn increases mortality rate.

1.4 Risks

Breast cancer risk is related to nulliparity and late first birth, early menarche and late menopause. Current use of oral contraceptives and of postmenopausal hormone replacement therapy is associated with increased breast cancer risk. Alcohol consumption also increases risk. Family history of breast cancer and high mammographic density are among the best recognized breast cancer risk factors, which assist in identifying high risk women for screening purposes.

1.5 Genetics

There is some evidence of an increased risk of breast cancer associated with polymorphisms of genes involved in the biosynthesis of estradiol, particularly the CYP19 gene. In addition, breast cancer risk is greatly increased in carriers of the mutated forms of the genes BRCA1, BRCA2 and p53 (Bernard et al, 2008).

1.6 Breast Cancer Treatment

Treatment options for breast cancer include surgery, chemotherapy, radiotherapy, hormone therapy, and targeted therapy.
1.6.1 Surgery

There are different types of breast cancer surgery. The most suitable surgery is done depending on the tumor's characteristics and whether the tumor has metastasized. Lumpectomy involves the removal of only the cancerous lump and the surrounding margin of normal tissue. Quadrantectomy involves the removal of one quarter of the breast. Total mastectomy is the removal of the entire breast (Cancer Research, UK).

1.6.2 Chemotherapy

Chemotherapy is the treatment of cancer with one or more cytotoxic antineoplastic drugs known as "chemotherapeutic agents" (Table 1). It may be given with a curative intent or it may aim to prolong life or to palliate symptoms. It is often used in conjunction with other cancer treatments, such as radiation therapy or surgery.

Table 1 Classification of chemotherapeutic drugs based on their mechanism of action (National Health Services).

<table>
<thead>
<tr>
<th>Class/Type of Chemotherapeutic Drugs</th>
<th>Mechanism of Action</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkylating Agents</td>
<td>They impair cell function by forming covalent bonds with the amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules.</td>
<td>Cisplatin</td>
</tr>
<tr>
<td>Anti-metabolites</td>
<td>These binds to Purines and Pyrimidines thus Preventing their incorporation into DNA during the &quot;S&quot; phase. This hinder normal development and division of cells. They also affect RNA synthesis.</td>
<td>Fluorouracil</td>
</tr>
<tr>
<td>Plant alkaloids &amp; terpenoids</td>
<td>Derived from plants and block cell division by preventing microtubule function which is vital for cell division.</td>
<td>Vinca alkaloids: Vinblastine, Taxanes: Taxol</td>
</tr>
<tr>
<td>Topoisomerase Inhibitors</td>
<td>Topoisomerases are essential enzymes that maintain the topology of DNA. Inhibition of type I or type II topoisomerases interferes with both transcription and replication of DNA by preventing DNA supercoiling.</td>
<td>Type 1: Camptothecins Type 2: Etoposide, Etoposide phosphate</td>
</tr>
</tbody>
</table>
1.6.3 **Radiation therapy**

Radiation therapy is treatment with high-energy rays or particles that destroy cancer cells. It’s of two types: External beam radiation and Internal beam radiation (brachytherapy). In external beam radiation, the radiation is focused from a machine outside the body on the area affected by the cancer. On the other hand Brachytherapy involves the delivery of radiation from inside via radioactive seeds or pellets that are placed into the breast tissue next to the cancer.

1.6.4 **Hormone Therapy**

Estrogen promotes the growth of about 2 out of 3 of breast cancers. Estrogen and progesterone receptor positive breast cancers can be treated by blocking the effects of estrogen or lowering estrogen levels. Hormone therapy does not help patients whose tumors are both ER and PR negative. Tamoxifen is an anti-estrogen drug that works by temporarily blocking estrogen receptors on breast cancer cells thus preventing estrogen from binding to them.

1.6.5 **Targeted Therapy**

Genetic changes in cells leads to cancer. Targeted therapy works by targeting these specific changes. Trastuzumab, a monoclonal antibody attaches to a growth-promoting protein known as HER2/neu, which is present in larger than normal amounts on the surface of the breast cancer cells in about 1 of 5 patients. Breast cancers with high levels of this protein tend to grow and spread more aggressively. Trastuzumab can help slow this growth and may also stimulate the immune system to more effectively attack the cancer (American Cancer Society).

1.6.6 **Cancer Drug Resistance**

The design of cancer chemotherapy has become increasingly sophisticated, yet there is no cancer treatment that is 100% effective against disseminated cancer. Resistance to treatment with anticancer drugs results from a variety of factors including individual variations in patients and somatic cell genetic differences in tumors, even those from the same tissue of origin. The most common reason for acquisition of resistance to a broad range of anticancer drugs is expression of one or more energy-
dependent transporters that detect and eject anticancer drugs from cells, but other mechanisms of resistance including insensitivity to drug-induced apoptosis and induction of drug-detoxifying mechanisms probably play an important role in acquired anticancer drug resistance (Gottesman, NIH).

Resistance to chemotherapy has been correlated to the presence of at least two important molecular “pumps” in tumor-cell membranes that actively expel chemotherapy drugs from the interior. This allows tumor cells to avoid the toxic effects of the drug or molecular processes within the nucleus or the cytoplasm. The pumps commonly found to confer chemo resistance in cancer are (Cancer Multidrug Resistance, 2000):

- P-glycoprotein coded for by MDR-1 gene
- Multidrug resistance associated protein (MRP)

These proteins belong to a large family of ATP-dependent transporters known as the ATP-binding cassette (ABC) family. ABC proteins can utilize the energy derived from ATP hydrolysis to perform a directed transmembrane movement of their substrates, open or close a specific membrane channel (e.g. ion-channels), or regulate the permeability of multi-protein channel complexes (receptors) (Higgins, 1992).

Despite the documented benefits of ER-targeted therapy in breast cancer, it is known that not all patients who have ER or PR expressing tumors respond to endocrine manipulation (de novo resistance) and a substantial number of patients who do respond will develop disease progression or recurrence while on therapy (acquired resistance). Recent research into the molecular biology of ER signaling has revealed a remarkably complex interactive signaling with other growth factor signaling pathways in breast cancer cells, potentially explaining some of the reasons behind resistance (Massarweh et al, 2006).

1.6.7 Development of newer Drugs

Chemotherapeutic drugs are toxic to normal cells leading to severe side effects. The most common medications affect mainly the fast-dividing cells of the body, such as blood cells and the cells lining the mouth, stomach, and intestines. Common side-effects include, Immunosuppression, myelosuppression, mild to severe anemia,
gastrointestinal distress and alopecia. The adverse impacts on health due to use of chemotherapeutic drugs shift the attention to search for novel drugs among natural compounds with promising bioactivity that lacks such side effects and specifically targets cancer cells. Many natural as well as synthetic agents have demonstrated to elicit apoptosis specifically in cancer cells (Gordaliza, 2007; Wang et al, 2009; Nagy et al, 1996; Sirion et al, 2011). Synthetic derivatives are often found to be more active than parent compounds (Anand et al, 2007).

1.6.8 Role of Natural Products in Drug therapy

Natural products (NPs) or natural product based drugs have received considerable attention in drug therapy due to their chemical diversity, biochemical specificity and biocompatibility. The use of NPs dates back to ancient Mesopotamian days. Even ancient texts on Ayurveda such as Charaka Samhita and Sushruta Samhita have articulated about the use of NPs as early as 900 B.C. and 600 B.C. respectively. Some of the NPs and NP based drugs which possess anticancer potential include Vincristine, Vinblastine, Paclitaxel, Docetaxel, Topotecan, Irinotecan, and Etoposide. Though NPs are widely used in drug therapy, they have some limitations such as difficulties in access and use, high cost of collection of natural product samples, requirement of several separation cycles, concerns about intellectual property rights, etc. Most importantly, safety and toxicity are crucial to determine the effectiveness of natural product based drug research. Therefore these issues have led to a significant decrease in natural product based drug research in the last decade (Torres et al, 2012).

Polyphenols represent one of the most prevalent classes of molecules present in our daily diet. Chalcone is an important member of the polyphenolic family. Structurally chalcones are α,β-unsaturated ketone containing the reactive ketoethylenic group -CO-CH=CH-. Curcumin is a hydrophobic phytopolyphenolic compound isolated from the rhizomes of Indian herb Curcuma Longa along with other curcuminoids like demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). Curcumin and its analogues, derivatives, hybrids, physically modified forms and formulations exhibit wide range of biological activities including antiviral (Ly et al, 2015), antidiabetic (Nabavi et al, 2015), antihypertensive and antihypercholesterolemic (Rachmawati et al, 2016), antiretroviral (Prasad et al, 2015), antiallergic (Zhang et al, 2015), antibacterial (Vetvicka et al, 2016), antifungal (Lee et al, 2014), antioxidant (Gorinova et al, 2016) properties and so
on. Especially there are quite a few reports on anticancer activity of curcumin and related compounds (Quin et al, 2017; Bondi et al, 2017; Mehta et al, 2014). There are several molecular targets and mechanisms of action proposed to account for the anticancer property of curcumin and its counterparts. Curcumin functions as an antiproliferative agent, antioxidant and carcinogen-blocking agent and it targets transcription factors, growth factors, receptors, enzymes, kinases, oncogenes, etc. Either one or combination of survival signal reduction, induction of apoptosis, arrest of cell cycle progression, generation of reactive oxygen species (ROS) are proven to be mechanisms of action for Curcumin against different cancers. Curcumin is proven to interdict the survival and metastasis of prostate cancer cells via the Notch-1 pathway (Yang et al, 2017).

Mangostin is a natural organic compound isolated from various parts of the Mangosteen tree (*Garcinia mangostana*). *Garcinia mangostana* L. (mangosteen; Clusiaceae) is a tropical fruit native to Southeast Asia, that is often referred to as “the queen of fruits. The Mangosteen pericarp comprises an array of polyphenolic acids including Xanthones and Tannins. Among them, Polyphenols are of great interest as chemopreventive agents because of their anti-oxidative and possible anti-cancer activity (Sun et al, 2002).

Xanthones are characterized by the presence of one or more prenyl and hydroxy groups in their tricyclic ring system. Over 200 Xanthones are currently known to exist in nature and approximately 50 of them are found in the mangosteen (46). α-Mangostin (1,3,6-trihydroxy-7-methoxy-2,8-bis (3-methyl-2-butenyl)-9 H-xanthen-9-one), and γ-mangostin (1,3,6,7-tetrahydroxy-2,8-bis(3-methylbut-2-enyl)xanthen-9-one are the main xanthones isolated from G. mangostana. α-Mangostin has been identified as the most abundant xanthone in the mangosteen extract. Xanthones are gaining more interest due to their remarkable pharmacological properties including analgesic, antioxidant, anti-inflammatory, anti-cancer, anti-allergy, antibacterial, anti-tuberculosis, antifungal, antiviral, cardio protective, neuroprotective, and immunomodulatory properties (Cui et al, 2010; Jung et al, 2006; Chen et al, 2008; Akae et al, 2008).

The anti-cancer properties of *G. mangostana* extracts or pure Xanthones have been extensively studied both *in-vitro* and *in-vivo*: 
Yukihiro et al reported growth inhibitory effects of various xanthones extracted from the pericarp of mangosteen. Four structurally similar prenylated xanthones [α-mangostin (αM), β-mangostin (βM), γ-mangostin (γM), and methoxy-β-mangostin (βM−ME)] exhibited growth inhibition of human colon cancer DLD-1 cells with methoxy-β-mangostin showing the least activity and γ-mangostin showing the maximum activity. The IC$_{50}$ value of α-Mangostin was found to be 7.5μM which is close to that of 5-FU. α-Mangostin was found to induce apoptosis mediated cell death (intrinsic pathway).

Anti-colon cancer effects including cytotoxicity, apoptosis, anti-tumorigenicity of alpha and gamma mangostin was investigated on HCT 116 human colorectal cancer cell line. The In-vivo anti-colon cancer activity was also investigated on subcutaneous tumors established in nude mice. α-Mangostin and γ-Mangostin showed a dose dependent cytotoxicity showing IC$_{50}$ values of 6.5 ± 1.0 μg/ml and 5.1 ± 0.2 μg/ml, respectively due to induction of the mitochondrial pathway of apoptosis. Three key steps in tumor metastasis including the cell migration, cell invasion and clonogenicity, were also inhibited. The treatment with the α-Mangostin extract caused significant reduction in the tumor size compared to untreated group (Aisha et al, 2012).

Apoptosis is the main mode of cell death induced by Mangostin and its derivatives. The mechanisms of apoptosis are highly complex, involving multiple molecular events. There are two major apoptotic pathways: the intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway. Caspases 3 and 7 are effector / Caspases 8 and 9 are initiator caspases. Matsumoto et al demonstrated that α-Mangostin activated caspase-9 and -3 but not -8 in HL60 cells, indicating that α-Mangostin mediate the mitochondrial pathway in the apoptotic process (Masutomo et al, 2004). Parameters of mitochondrial dysfunctions such as swelling, loss of membrane potential, decrease in intracellular ATP, ROS accumulation, and cytochrome c/AIF release, were observed within 1 or 2 h after the treatment, indicating that α-mangostin targets mitochondria in the early phase. Mangostin-induced apoptosis was found to be mediated by a caspase-independent pathway via mitochondria with the release of Endo-G (Nakagawa et al, 2007). Endo-G, a known 30-kD nuclease residing in mitochondria, is able to induce nucleosomal DNA fragmentation (Nakagawa et al, 2007).
Graviola, an extract from the tropical tree *Annona Muricata* was found to induce necrosis of pancreatic cancer cells. Studies show that the compounds that are naturally present in a Graviola extract inhibited multiple signaling pathways that regulate metabolism, cell cycle, survival, and metastatic properties in pancreatic cancer cells (Torres et al, 2012).

Digalloylresveratrol (DIG) a recently synthesized substance combines the natural polyphenolic compounds gallic acid and resveratrol. Studies indicate that DIG is an excellent free radical scavenger and strongly inhibit cell cycle progression and colony formation in pancreatic cancer cell lines (Arora et al, 2011).

Honokiol, a biologically active constituent of oriental medicinal herb *Magnolia officinalis/grandiflora* was found to exert growth inhibitory effects on pancreatic cancer cell lines by causing cell cycle arrest at G1 phase and induction of apoptosis (Gescher et al, 2012).

Resveratrol is the most important stilbene related to cancer. It possesses a natural anti-proliferative activity due to its role as a phytoalexin (plant antibiotic). It is believed to have also multiple bioactivities including anti-cancer, anti-carcinogenesis and anti-inflammatory effects. The mechanisms by which resveratrol might produce these effects are not completely understood, but the main molecular mechanism seems to be the activation of sirtuin proteins. There is considerable interest in developing resveratrol for cancer prevention and treatment. The plasma pharmacokinetics of resveratrol in humans is now reasonably well defined, and studies have shown that repeated daily doses are safe and well tolerated (Kuppuswamy et al, 2014).

Apart from plant-derived phytochemicals, marine organisms contain structurally diverse bioactive compounds which are gaining importance in food and health care. Marine organisms are known to produce novel and pharmacological compounds with fewer adverse effects. The secondary metabolites from these marine-derived bioactive compounds are of current interest to cure several ailments including cancers (Pandey et al, 2013).

In continuum, a secondary metabolite from a marine organism known to possess numerous pharmacological properties (Pandey et al, 2013) viz. anti-oxidant, anti-inflammatory, and potential anti-tumor properties is phycocyanin from Spirulina.
Phycocyanin potently inhibits proliferation of TNBC cells. In addition to proliferation inhibition, treatment with phycocyanin affected other hallmarks of TNBCs as well, viz. angiogenesis and migration and was non-toxic to normal cells (Ravi et al, 2015).

Nutraceuticals, mostly phytochemicals mediate their positive health benefits directly, by affecting specific molecular targets such as genes, or indirectly as stabilized conjugates affecting metabolic pathways. They have the ability to control the DNA damaging factors in cancer cells and regulate DNA transcription in tumors. The aim of phytochemistry research is to understand and formulate mechanistic pathways by which these naturally derived chemicals can alter the fate of a cell. For tumor cells to survive, they should be able to proliferate, obtain energy, and establish angiogenic pathways, in a tumor mass. Altering genes that affect these pathways can serve as suitable tools to decrease tumor mass and also allow for tumor regression. Various studies have shown that these molecules can induce apoptosis, inhibit cellular proliferation, affect angiogenesis, and affect cancer metabolism in various cancers, all of which are hindrances to tumor growth. Thus an effective nutraceutical is one that will have a low nontoxic dose to create the magnitude of death inducing changes in the tumor dynamics (Saldanha et al, 2012).

1.6.9 **Molecular targets of phytochemicals**

It is quite evident from the literature that nutraceuticals are multi-targeting agents. They modulate an array of signaling pathways, as well as individual molecular targets. Available literature reveals that natural anticancer agents have been shown to touch upon virtually every single molecular target. To simply point out a few major signaling pathways/targets affected by nutraceuticals, we can identify EGFR family receptors, Ras/Raf signaling, MAPK/ERK pathway, PI3K/Akt/mTOR pathway, Notch family, Wnt/β-catenin signaling, Sonic hedgehog signaling, hormone receptors (such as ER/progesterone receptor), TGF-β signaling, insulin-like growth factor signaling, cAMP signaling, the STAT3 signaling pathway, etc. In addition to these classical targets, nutraceuticals are also being realized to efficiently modulate emerging targets, such as cancer stem cells (Saldanha et al, 2012; Kim et al, 2013; Dandawate et al, 2013), microRNAs (miRNAs) (Bao et al, 2012; Li et al, 2010; Saini et al, 2010)
epithelial-mesenchymal transition (EMT) (Chiyomaru et al, 2013) and the causes of epigenetic modifications (Baribeau et al, 2014; Hirata et al, 2014).

Through their action against these molecular targets, nutraceuticals kill the cancer cells at many different levels: they inhibit cancer cells’ proliferation, induce apoptosis/cell cycle arrest and suppress invasion/metastasis/angiogenesis. These cytotoxic effects are mediated through the action of nutraceuticals against factors, such as bcl2, survivin, vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), urokinase-like plasminogen activator (uPA) cyclooxygenase-2 (COX-2), etc. In addition to numerous reports on the In-vitro effects of nutraceuticals, there are many In-vivo reports that document the beneficial anticancer effects of nutraceuticals in animal model systems; however, control and rationally-designed phase II/III clinical trials are awaited (Kim et al, 2013; Hirata et al, 2014).

1.6.10 Main phytochemicals studied for cancer care

1.6.10.1 Polyphenols

Polyphenols are plant secondary metabolites that contain one or more hydroxyl group attached to a benzene ring in their structure. More than 8000 different polyphenols found in food (mainly in wine, tea, coffee, cocoa, vegetables and cereals) are present in the human diet. They may be classified into different groups according to their number of phenol rings and the structure that links these rings. In this context, the groups of phenolic acids, flavonoid, stilbenes and curcuminoids are the most important for their capacity for impeding initiation of carcinogenic process and to restrain cancer progression (Kim et al, 2013).

1.6.10.2 Epigallocatechin-3-gallate

EGCG (epigallocatechin-3-gallate) is the foremost catechin found in green tea (Camellia sinensis). The regular consumption of green tea in Asian countries has been related to several health benefits and it is recognized as the most effective cancer-preventive beverage. The antitumor properties of EGCG has been recognized on multiple cancer cell lines, In-vitro. The clinical applications of green tea have the limitation of low bioavailability and conversion into inactive methylated metabolites. Moreover, the biotransformation of the green tea polyphenols is different in human
from rat and mouse, which may explain the inter-species difference in anti-carcinogenic properties (Clifford et al, 2013). Furthermore, the genetic polymorphisms in gene responsible for the biotransformation of EGCG, such as catechol-O-methyltransferase (COMT), need to be considered when designing green tea efficacy studies. However, the wide range of antitumor effects exerted by EGCG suggests that this compound is a potential tool for cancer prevention and therapy, both alone and in combination with antitumor drugs or other phytochemicals (Moyers et al, 2004).

1.6.10.3 Quercetin

Quercetin, a representative member of the flavonoid class, is a plant-derived compound obtained from various fruits and vegetables that can reach levels in the human diet as high as 16-25 mg/day (Hertog et al, 1993). The effects of quercetin are considered to be related to the induction of cell apoptosis through multiple mechanisms. In-vivo studies of the anticancer effects of quercetin have demonstrated that oral administration can prevent induced carcinogenesis, particularly in the colon and furthermore, inhibit melanoma growth, invasion, and metastatic potential (Zhang et al, 2012).

1.6.10.4 Resveratrol

Resveratrol is the most important stilbene related to cancer. It possesses a natural anti-proliferative activity due to its role as a phytoalexin (plant antibiotic). It is believed to have also multiple bioactivities including anti-cancer, anti-carcinogenesis and anti-inflammatory effects. The mechanisms by which resveratrol might produce these effects are not completely understood, but the main molecular mechanism seems to be the activation of sirtuin proteins. There is considerable interest in developing resveratrol for cancer prevention and treatment. The plasma pharmacokinetics of resveratrol in humans is now reasonably well defined, and studies have shown that repeated daily doses are safe and well tolerated (Gescher et al, 2012).

1.6.10.5 Turmeric

Turmeric (Curcuma longa) the underground rhizome of the turmeric plant has been an essential part of the Indian pantheon of spices for thousands of years. Curcumin is under intensive investigation because of its known anti-inflammatory activity that could impact on risk for cancer. Research has shown that Curcumin has an
effect in modulating the effects of TNF-α and for its ability to suppress a constellation of molecular signals mediated by the nuclear transcription factor; NFκβ. Inhibition of NFκβ can have profound consequences for a tumor cell. Few proteins targets in the cell modulated by Curcumin are: COX-2, VEGF, the chemokines MCP-1 and MCP-4, the interleukins IL-1 and IL-6, and IGF (Wargovich et al, 2012). Phycocyanin (PC) is a major light-harvesting or photosynthetic pigment protein present in the phycobilisomes of Spirulina platensis. The medicinal and pharmacological properties of PC have been reported in several studies. Studies have demonstrated the hepatoprotective, antioxidation, anti-inflammatory, and radical scavenging properties. One of the most important recent health benefits of phycocyanin that has received much attention is for its antitumor activity.

Various mechanisms of its antitumor activity studied in different tumor cell lines have been proposed ranging from the interference of DNA synthesis in the tumor cells, activation of caspase-dependent programmed cell death pathways, inhibition of tumor cell growth by pathways other than apoptosis like a membrane destruction, leading to increased leakage of cell constituent, or stimulation of expression level of the proto-oncogene c-myc, to the improvement of host immune functions (Gardeva et al, 2014).

The effect of phycocyanin was tested on growth and multiplication of human chronic myeloid leukemia cell line (K562). Results indicate significant decrease (49%) in the proliferation of K562 cells treated with 50 µM phycocyanin up to 48h. It was shown that phycocyanin induced apoptosis in K562 cells by Cytochrome c release from the mitochondria into the Cytosol, down regulation of Bcl-2 and PARP cleavage (Subhashini et al, 2004).

The effect of phycocyanin was tested on the growth and multiplication of human hepatoma cell lines (HepG2). Observations recorded show decreased cellular viability, loss of nuclear entities, fragmentation as a result of programmed cell death. These properties of phycocyanin attribute to its impending ability as an anti-cancer drug for therapy of human hepatoma (Basha et al, 2008).

The effect of purified phycocyanin was studied on the growth and proliferation of human uterine cervical cancer HeLa cells. The results indicated a significant decline
in the number of treated cells when weigh against control/untreated cells. Electron-microscopic observations presented apoptotic features, including chromatin condensation, membrane blebbing, and cell shrinkage and microvilli loss. Molecular evidence corroborates the activation of pro-apoptotic gene and downregulation of anti-apoptotic gene expression which facilitate apoptosis (Li et al, 2006).

Bio-informatics is playing an important role in drug discovery, assessment and development, especially with respect to the absorption, distribution, metabolism, excretion (ADME) and toxicity (T). Phosphatidyl Inositol-3 kinase (PI3kinase) is one of the most important regulatory protein that is involved in controlling cell proliferation and functions by modulating different cellular targets [Michael et al, 2016]