

# CHAPTER 1

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## *Introduction*

## 1.1 Background

Human race have been stalking by several hardships since the dawn of civilization. In the second epidemiological transition, human has been burdened with several chronic non-infectious diseases. <sup>[1]</sup> Cancer is one such disease that has emerged as a public health burden throughout the world. The disease cancer is characterized by uncontrolled growth of cells of any part of the body and spread of abnormal cells from the original site to adjacent or distant sites. It is a group of disease affecting lives of humans (other animal too), developed as a result of complex interplay between genetic and environmental factors.

Cancer stands as one of the most aggressive and lethal diseases in the present scenario of disease burden throughout the world. In fact, it the second most common cause of death accounting 8.2 million deaths around the world in 2012. The prevalence of cancer in developing countries is estimated to be around 15.6 million, with over 8.0 million new cases and 5.3 million deaths in 2012 and a similar trend has also emerged in the developed countries. <sup>[2]</sup> Recent estimation indicates around 1.0 million incidences, 0.7 million deaths and 1.8 million people living with cancer in India. About 6% of all deaths in India are due to cancers which contribute to 8% of global cancer mortality. More than 70% of the cases in India report for diagnostic and treatment services in the advanced stages of the disease, which has led to the poor survival and high mortality rate. <sup>[2]</sup>

Recent report of Indian Council of Medical Research (ICMR) states that the north-eastern region of India contributes highest incidence of cancer to the country. The age-adjusted incidence rate of cancer indicated that Aizawl (Mizoram), East Khasi Hills (Meghalaya) positioned in top in men and Aizawl (Mizoram), Kamrup (Assam) in women. <sup>[3]</sup> In this region prevalence of head and neck cancer is highest irrespective of sex. In 2011-12, the maximum number of cancer cases in the hospital of Assam has been reported from Kamrup, Nagaon and Dhubri districts. Even though rate of cancer cases are increasing at an alarming rate, the level of awareness remains very low and more than 80% patients from this region come to hospital at a very late stage and have limited chance of recovery. <sup>[4]</sup>

## 1.2 Factors associated with cancer

The etiology of cancer is multifactorial with genetic/epigenetic and environmental factors interacting in complex cascade to produce a particular cancer. Cancer is characterized by deregulated proliferation of cells of any parts of the body. The abnormalities in proliferative potentiality arise due to change(s) in cell's DNA *i.e.* mutations. Some of these change(s) are inherited and others are driven by exposure to certain cancer causing agents, broadly referred to carcinogens.

Physical carcinogens such as electromagnetic radiations such as corpuscular (alpha and beta) radiation, low and high temperatures, mechanical traumas, solid and gel materials etc. alone or in combination produces cancer by physical or chemical mechanisms either by directly damaging DNA or effecting cellular electrochemistry. <sup>[5]</sup> Chemical substances such as polyaromatic hydrocarbons (PAHs), N-nitroso compounds, aromatic amines etc. cause cancer by inducing genetic and epigenetic alterations. <sup>[6]</sup> Besides physical and chemical carcinogens, certain bacteria e.g. *Helicobacter pylori*, viruses e.g. Human Papilloma virus (HPV), Epstein Barr virus and parasitic worms e.g. *Schistosoma haematobium* also cause cancer in human by affecting host cell's genome or through production of toxins. <sup>[7]</sup>

The development of cancer involves multistep processes which includes alterations in genetic and epigenetic components of the cell. Earlier the genetic alteration such as loss of gene function particularly tumor suppressor genes (*BRCA1*, *BRCA2*, *p53*), DNA repair genes (*XRCC1*, *C2*, and *C3*), gain of function of oncogene (*HER2*) were only considered as sole contributor of cancer. <sup>[8-10]</sup> The paradigm has been extended by incorporating alterations in the regulatory component of gene expression *i.e.* epigenome. The epigenetic alteration such as DNA methylation, histone modification, chromatin remodeling etc. prominently regulates the expression of particular gene by regulating promoter proteins, transcription factors etc. and chromatin modifying enzymes. Normal cells maintain a well-balanced transcriptome and proteome by tightly regulating expression of genome. The disruption of the critical balance leads to cancer. <sup>[11]</sup>

### 1.3 Cancer therapeutics

Despite substantial medical advances, cancer still occupies top position in terms of causing morbidity and mortality. <sup>[12]</sup> Over the past 40 years, surgery, radiation, and chemotherapy have been the mainstay of treatment for human malignancies. Each of these treatment modules are restricted by its own set of limitations owing to the factors which includes, location, size, stage of malignancy present, along with the age and medical condition of the patient. Chemotherapy and radiotherapy are conventional treatment options available for cancer patients at both early and late stage of the malignancy; unfortunately, the patients with cancer are generally diagnosed at an advanced stage of the disease. Also dose associated side effects are main safety concern for chemotherapy and radiation therapy. The overall survival rate has not been improved significantly due to frequent presentation with advanced stage disease and the development of secondary primary tumors (SPTs) that remains challenging to control. <sup>[13,14]</sup> Patients with the advanced stage disease may expect only a 30% to 60% cure rate, and survival among patients with head and neck cancer has marginally improved. <sup>[15]</sup> The reasons for the continued high rates of recurrence in several forms of cancer are unclear till date.

Even though number of anticancer agents are known, these have limited efficacy against SPTs and are completely ineffective against the advanced stage cancer that have developed the resistance. The limitations associated with cancer treatment and control shifted the research on anticancer drug development towards a more realistic and holistic strategy using naturally occurring non-toxic chemical entities for the prevention/intervention of both primary tumors and the recurrence of secondary primary tumors post-anticancer therapy. The novel approaches are expected to alter the present survival rates for cancer patients.

### 1.4 Chemoprevention

Chemoprevention is one such novel approach that have gained impetus in the recent past. The term chemoprevention describes pharmacological intervention at early stage of carcinogenesis, before invasive phenotype appears by using non-toxic naturally occurring or synthetic or biological entities. <sup>[16,17]</sup> Cancer chemoprevention basically

involves use of naturally occurring chemical compounds in order to inhibit or reverse and/or delay the carcinogenesis process. <sup>[18]</sup> Though the concept of chemoprevention was developed in 70s, but not much progress were made until 90s, when the World Health Organization (WHO) launched a campaign to prevent cancer. The campaigning adopted three-fold strategies *viz.* prevent all the preventable cancers, cure all that can be cured, and reduce pain and discomfort where cure is not possible. The preventive approaches such as avoidance of cancer causing agents and increased consumption of diet rich in phytochemical with cancer preventive potentials can effectively bring down cancer risk. <sup>[19, 20]</sup> However, former approaches is not possible as common population are unaware about the detrimental effects of many of the cancer causing agents. Therefore, the later approaches appears to be fruitful for prevention of cancer.

### 1.5 Phytochemicals as therapeutic agent

The phytochemicals isolated from the plants are reported to inhibit the action of various carcinogens through activating detoxification process or inducing antioxidant enzymes or reducing inflammatory response or may decrease tumor cell growth by inducing apoptosis and /or cell cycle arrest or as suppressing agents to restrain tumor cells from promotion and progression by destroying one or more cell signaling pathways. These agents also have the potential to reverse the process of carcinogenesis before the development of invasive stage. Importantly these phytochemicals of plant origin are less toxic or not toxic, have high efficiency, capability of oral administration and low cost. The novel phytochemicals proved to not only target a single pathway but multiple pathways can be modulated simultaneously.

Understanding the importance of plant derived compounds in cancer therapy, several phytoconstituents were isolated from the plants and many of them exhibit promising activities. First achievement in curing human cancer was achieved with Vinca alkaloid vincristine or oncovin isolated from *Catharanthus roseus*. <sup>[21]</sup> Isolation of taxanes from the bark of *Taxus baccata*, *T. brevifolia*, *T. Canadensis* and camptothecins from the bark and wood of *Camptotheca acuminata* stands as a landmark in research on anticancer drug development as both of them exhibit significant anti-solid tumor efficacy. <sup>[22,23]</sup>

Paclitaxel sold under the trademark Taxol is used to treat ovarian, breast, lung, pancreatic, non-small cell lung and other cancers. <sup>[24]</sup> More recently, curcumin isolated from the *Curcuma longa* and silibinin from milk thistle plant *Silybum marianum* showed strong chemopreventive and anticancer activity against wide range of cancer such as skin, breast, lung, pancreatic, colon, cervical, prostate, bladder, kidney etc. in various *in vitro/in vivo* and pre-clinical studies. <sup>[25,26]</sup> The resveratrol, a phytoalexin present in many commonly consumed plants such as tea, coffee, grapes, peanuts, berries etc. possess strong chemopreventive and anticancer efficacy against breast, colorectal, liver, pancreatic, and prostate cancers. <sup>[27]</sup>

Besides, phytochemicals, their derivatives were also known to exert potent chemopreventive and anticancer activity in various experimental models. Derivatives of Silibinin such as 2,3-dehydrosilybin, 7-O-methylsilybin, 7-O-galloylsilybin demonstrated better growth inhibitory effects than their parent compound in bladder, colon and prostate cancer cell lines. <sup>[28]</sup> Topotecan and Irinotecan, two analogues derived from camptothecin are active against carcinoma of colorectal, lung, cervix and ovary. <sup>[29,30]</sup>

Though good amount of chemopreventive and anticancer agents have been isolated from plants, a safe, cost effective and site specific chemopreventive and anticancer drug is still a challenge. So, there is urgent need for exploration of novel chemopreventive and anticancer agent that will provide desired degree of selectivity for cancer cells. With the above background and considering the importance of phytochemicals for therapeutic point of view for effective management of cancer, the present research is focused on identification of medicinal plants with chemopreventive and anticancer potentials from the plethora of traditional and indigenous knowledge of north eastern region of India and to validate the efficacy in experimental cancer models. The study also aims to understand the precise biochemical and molecular mechanism of chemoprevention and anticancer potentials along with identification of the active phytoconstituents responsible thereof.

### 1.6 Aims and objectives of the present study

The work embodied in this thesis proceeded the following objectives which are identified against broad perspectives of urgent necessity to forward new molecules as potential drugs to cure cancer described in the preceding introduction. The specific objectives of the present study are as follows:

- Selection and identification of medicinal plant(s) based on traditional knowledge and preparation of extract of the selected parts.
- Study of modulatory effects of extract of selected plant(s) on phase I & II drug metabolizing enzymes and antioxidant status using mice model system.
- Study of chemopreventive potential of the plant extract against chemically induced papillomagenesis using mice model system.
- Fractionation and characterization of plant extract for identification of active molecule/fraction(s).
- Study of molecular events in response to the fractionated extract/active molecule to understand the chemo-preventive action using *in vitro* cell culture system.

### 1.7 Expected Outcome

The systematic evaluation of medicinal plant selected on the basis of traditional and indigenous knowledge may likely yield entirely novel chemopreventive and anticancer drugs and might provide insight into molecular mechanism of action and also lead to the identification of novel molecular targets. Current drugs available in the market are expensive and of low efficacy. The drugs obtained from natural sources through the traditional and indigenous knowledge will be of low cost and the systematic study will lead to identification of highly specific molecules with increased efficacy and specificity for effective management of cancer.