CHAPTER 1

General Introduction

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1.1. Cancer: A general view

Cancer is a group of diseases which is caused due to uncontrolled growth of cells [1]. They form lumps of tissues called tumors which can be either cancerous (malignant) or non-cancerous (benign). The cancerous tumors can invade and spread into nearby tissues as well as travel to distant parts of the body where they can form new tumors, a process known as metastasis. However, benign tumors do not form tumors in nearby cells although they may grow to large size, and if removed, do not usually regrow. On the other hand, cancerous tumors may sometimes regrow even after being removed [2]. Extensive or multiple-organ metastasis is the main cause of mortality due to cancer [3]. The classification of cancers has been discussed below.

1.1.1 Types of cancer

Till date, more than 250 types of cancers have been discovered and are generally named after the organs or tissues which they affect [4]. Sometimes cancer can also be named after the cell type from which they originate, for example, cancer originating from epithelial cells are called carcinoma and those arising from mesenchymal cells are called sarcoma [2]. Based on the tissue of origin or cell types, cancers are broadly divided into three types namely solid cancer, blood cancer and mixed cancer [1,2] which have been discussed below:

i. ***Solid cancers:*** These types of cancers are the most common of all, encompassing 80-90% of all cases. The common types of solid cancers are briefly mentioned below:

   a. ***Carcinoma:*** These are formed by the epithelial cells which line the outer and inner surfaces of the body. There are various types of carcinomas depending of the tissue or organ which they affect. Adenocarcinoma forms in the mucus-secreting glands, such as, prostrate, breast, lung and colon. Squamous cell carcinoma occurs in the squamous epithelium, such as, the skin or lining of the esophagus. Basal cell carcinoma, which is prevalent in skin cancer, develops from the epidermal basal cells.

b. ***Sarcoma****:* These are cancers that arise from the mesenchymal cells, which form connective tissues like bones, muscles, cartilage, blood vessels and fibrous tissues (ligaments and tendons). Osteosarcoma is the most frequently occurring type of bone cancer. Similarly, liposarcoma arises from fat cells in soft tissues, such as, those in limbs or abdomen.

c. ***Melanoma****:* Cancer in cells which form the melanocytes are called melanomas. The melanocytes form melanin which impacts on the skin color and other pigmented tissues such as the pupil.

ii. ***Blood cancers****:* Cancers that arise in the blood cells or in the lymphatic system is called blood cancer. Some of the common types of blood cancers include:

a. ***Leukemia***: Cancers that originate in the blood-producing cells of the bone marrow. Unlike solid tumors, they form large numbers of leukemia cells and leukemic blast cells, which are abnormal white blood cells (WBC). These abnormal cells lower the population of normal cells due to which normal functions of blood cells such as providing oxygen to tissues or fighting infections get negatively affected.

b. ***Lymphoma***: It is a type of cancer which begins in the B or T lymphocytes, which are WBCs and form an important part of the immune system. In this type of cancer, the lymph nodes and lymph vessels are affected by the presence of abnormal lymphocytes.

c. ***Multiple myeloma***: In this type of cancer the plasma cells, which are also a type of immune cells, get affected and are called as myeloma cells. These myeloma cells multiply in the bone marrow and disperse to different organs of the body forming bone tumors.

iii. ***Mixed cancers****:* This type of cancer involves more than one type of classification or subtypes. Some of the mixed types of cancers include adenosquamous carcinoma and carcinosarcoma.

Cancer arises due to genetic changes which leads to uncontrolled growth of cells and eventually formation of tumor which have been discussed below.

1.1.2 Causes of Cancer

Replication and growth of cells is under tight genetic control. The DNA of the cell contains numerous genes which regulate the cells in performing necessary functions such as growth and replication. Any changes in the DNA, called mutations, may result in errors in these instructions resulting in breakdown of the cell machinery and uncontrolled proliferation of the cell [5]. These mutations called gene mutations can instruct a normal healthy cell to perform the following functions:

i. ***Rapid growth:*** A gene mutation may result in the cell growing and proliferating rapidly which results in the formation of new cancerous cells having the same mutations in them.

ii. ***Failure to arrest uncontrolled cell proliferation:*** Normal cells contain genetic instruction which regulates the cell growth. However, mutations in tumor suppressor genes present in the DNA allow cancer cells to grow rapidly and accumulate in tissues.

iii. ***Errors in DNA repair mechanism:*** In normal cells, the DNA repair mechanism check for errors in a cell's DNA and correct them. Mutations in the DNA repair genes would lead to non-correction of errors incorporated in the DNA, which may result in the cells becoming cancerous.

However, along with gene mutations various several other risk factors are also responsible for cancer development which are discussed below.

1.1.3 Risk factors of Cancer

A number of factors may typically increase the risk of cancer development in healthy persons. However, people are also diagnosed with cancer without any of the risk factors being associated with cancer progression [3,5].

i. ***Age:*** Although cancer can be detected at any age, more often it is diagnosed in adults, specifically older adults, since it may take years or even decades to develop. For instance, in the USA in 2009 ≥50% of the cancer incidences are of patients ≥65 years of age [6].

ii. ***Habits and lifestyle choices:*** Various lifestyle choices such as smoking, alcohol consumption, tobacco use, sunburn due to over exposure to the sunlight, being obese are some of the habits which further enhances the risk of developing cancer. For instance, smoking is responsible for almost 30% of all the cancers and causes about 90% of lung cancer cases in the developed countries [6].

iii. ***Family history:*** A small fraction of cancers is inherited, if cancer is common in the family, there may be a possibility that the mutations are inherited generation after generation. For instance, about 5-10% of pancreatic cancer is implicated with a known inherited cancer syndrome associated with a known genetic mutation [7]. Such persons may undergo genetic testing to evaluate their likelihood of developing cancer, however, presence of mutations does not necessarily mean that cancer is imminent.

iv. ***Health conditions:*** Certain chronic infections may increase the risk of developing cancer. For example, approximately 13% of cancers in 2018 globally were associated with Helicobacter pylori, Human papillomavirus, Hepatitis B, Hepatitis C and Epstein-Barr viruses [8]. Other examples include chronic inflammatory bowel disease such as Ulcerative colitis, which primarily affects the lining of colon and rectum through continuous inflammation and colon injury, may enhance the probability of developing colorectal cancer. Moreover, obesity may be associated with 20% of cancer deaths in women and 14% of cancer deaths in men [6].

v. ***Environmental factors:*** The environment may contain harmful cancer-causing agents (carcinogens) which may contribute to cancer development. For instance, exposure of a person to regular secondhand smoke (chemical carcinogen) from colleagues at workplace may increase the risk for developing cancer. Other chemical carcinogens include asbestos, aflatoxin and arsenic. Similarly, a person may be exposed to physical carcinogens (ionizing or ultraviolet radiation) or biological carcinogens (viruses, bacteria, or parasites) which may also lead to developing cancer. For instance, according to a model used by the World Health Organization (WHO), around 5% of the European population get exposed to asbestos (carcinogen) which

could result in mesothelioma in 425 men and 56 in women, and lung cancer in 771 men and 206 women [9].

1.2 Cancer: Global burden

The earliest traces of cancer were identified among fossilized bone tumors, ancient Egyptian mummies and historical texts. The oldest account, dating back to about 1600 BC, was discovered in Egypt where the Edwin Smith Papyrus describes 8 cases of breast tumors which were surgically removed by cauterization [10]. Today, cancer is the second leading cause of mortality worldwide and owing to current trends it may surpass cardiovascular diseases as the leading cause of mortality by the end of this century [11]. The rising global cancer burden exerts considerable physical, emotional, and financial pressure on individuals, families, communities, and healthcare systems. Particularly in low- and middle-income countries where healthcare systems are inadequately equipped to cope with this challenge, causing a significant number of cancer patients worldwide to lack access to timely and high-quality diagnostic and treatment services (World Health Organization). According to the Global Cancer Observatory (GLOBOCAN) 2022 estimates, there were 20 million incidences diagnosed and 9.7 million deaths reported. Lung cancer ranks first both in terms of incidence with 2.5 million new cases and mortality with 1.8 million deaths, whereas, breast cancer ranks second in terms of incidence with 2.3 million cases and fourth in terms mortality with 0.66 million deaths among cancer patients worldwide [12] (Figure 1.1). However, in India, breast cancer has the highest incidence (13.6%) and mortality (10.7%) rate, whereas lung cancer is ranked fourth in incidence (5.8%) and mortality (8.2%) in 2022 [13].

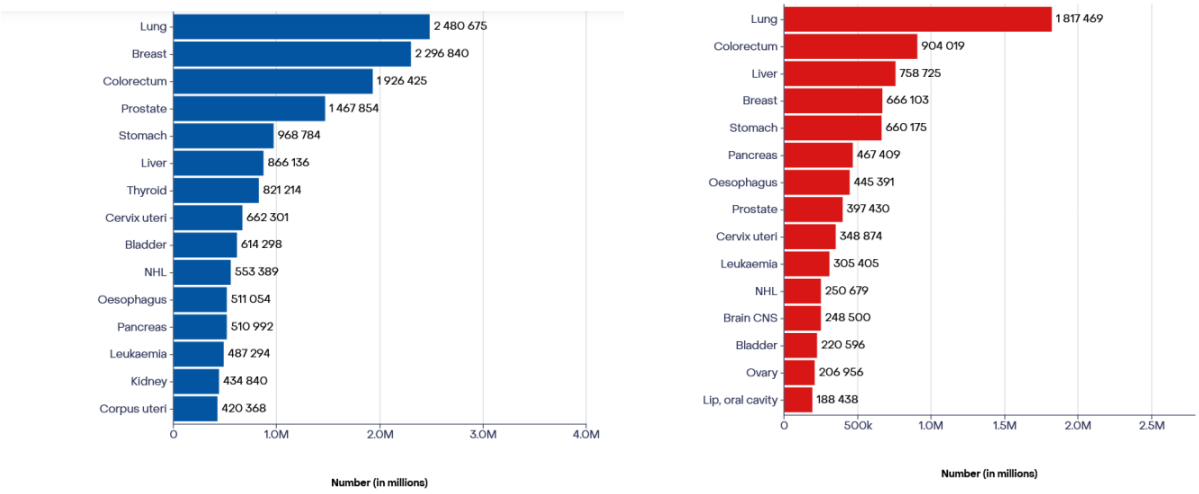

Figure 1.1: Global Burden of different Cancer types in terms of Incidence (Blue color) and Mortality (Red color) (Adopted from GLOBOCAN 2022).

The incidence and mortality of different cancers varies among both the sexes. In the United States, prostate cancer has the highest incidence (17.9%) in males followed by lung cancer (8.8%), whereas, in females, breast cancer has the highest incidence (25.0%) followed by lung cancer (10.4%). However, in India, lip and oral cavity cancer is the most predominant type of cancer in males (15.6%) followed by lung cancer (8.5%), whereas, in females, breast cancer has the highest incidence (26.6%) followed by Cervix uterine cancer (17.7%). Cancer may progress through multiple stages over a long duration of time and involve various genetic factors and signaling pathways or mediators [14] (Figure 1.2).

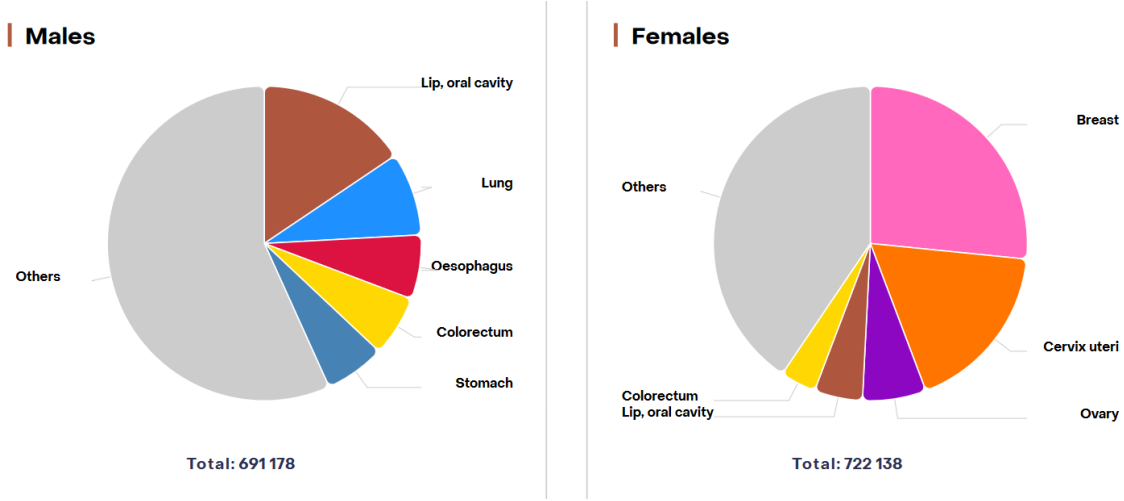

Figure 1.2: Incidence of different cancer types among males and females in India

(Adopted from GLOBOCAN 2022)

Thus, the immense burden of cancer demands therapeutic strategies that will allow early diagnosis, better grading of tumors to guide treatment, and for developing efficient prophylaxis. This would hold great promise for overcoming the complex challenges related to manage the disease.

1.3 Hallmarks of cancer

The hallmarks of cancer comprises a set of molecular, biochemical and cellular capabilities or traits acquired by a human cell during the multistep transition to neoplastic growth or tumor development [15]. It establishes an organizing principle for rationalizing the complexities of the disease. The core hallmarks of cancer which have been highlighted in light blue background in Figure 1.3 have been discussed below.

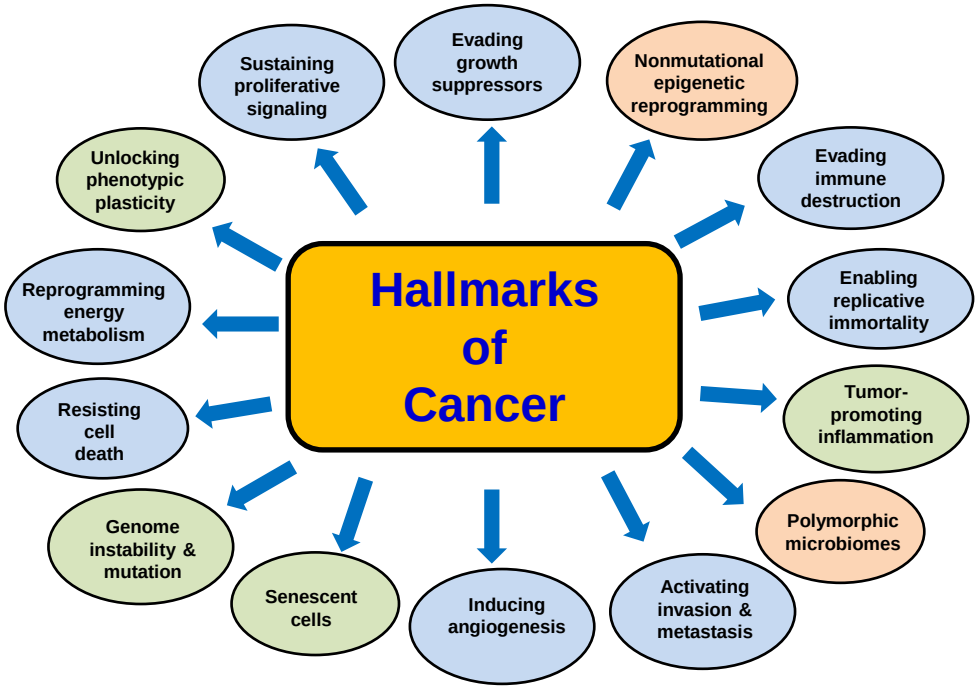

Figure 1.3: Hallmarks of Cancer. The 'Core hallmarks' are depicted in light blue background, the 'Emerging hallmarks' are in light green background, and the 'Enabling characteristics' are in light red background.

i. ***Sustaining proliferative signaling***

The ability to maintain continuous proliferation remains the fundamental characteristic of cancer cells. Normal tissues maintain the balance of cell number and normal tissue architecture and function by keeping a check on the growth-stimulating signals that maintain continuum through the cell division cycle and cell growth. However, cancer cells alter these signals and sustain proliferative signaling in different ways. Cancer cells may release growth factor ligands on their own which can act by the expression of cognate receptors, thus, producing stimulation for autocrine proliferation. Conversely, they may transmit signals to induce normal cells present within the tumor microenvironment for supply of various growth factors [16,17]. Cancer cells may alter structures in the receptor molecules by increasing the amount of cell surface receptor proteins, as a result, it greatly enhances the responsiveness to otherwise insufficient amounts of growth factor ligand. Activation of components of signaling pathways that function downstream of these receptors also promote the independence of growth factors.

ii. ***Evading growth suppressors***

The cancer cells must also bypass strong mechanisms that negatively regulate cell cycle progression, which is mostly controlled by the tumor suppressor genes. Two canonical tumor suppressors namely, Retinoblastoma (RB) and Tumor protein 53 (TP53) encode the RB-associated and p53 proteins respectively, which control the cellular regulatory circuits that decides whether the cells should undergo proliferation or alternatively, activate cell cycle arrest and apoptosis. The RB protein regulates growth-inhibitory signals that originate from various external and internal sources [18] [19,20], while p53 receives intracellular signals *i.e.*, stress and genomic damage. p53 can halt cell cycle progression in case of extensive genome damage or during inadequate amounts of nucleotide reserves, growth-promoting signals, glucose or oxygen supply. Alternatively, it may trigger apoptosis if the damage is irreparable. Therefore, mutations in these tumor suppressor genes leads to inappropriate replication of cells and development of tumors and eventual evasion of growth suppressors by cancer cells.

iii. ***Resisting cell death/evading apoptosis***

Apoptosis is a critical hallmark of cell death and serves as a barrier to cancer development [21,22]. The apoptotic machinery comprises both upstream regulators and downstream effector components [21]. The upstream regulators receive and process signals and initiate apoptosis through either of the two pathways: the extrinsic apoptotic pathway which receives extracellular death-inducing signals and involves transmembrane receptor-mediated interactions (e.g. assembly and binding of receptors and ligands: Fas ligand/Fas receptor and TNF α ligand/TNFR receptor); and the intrinsic pathway which includes a sequence of non-receptor-mediated events that produce intracellular signals (e.g. insufficient growth factors, cytokines, hormones, radiation, toxins, hypoxia, viral infections, free radical, etc.) and directly modulate the intracellular targets and involves mitochondrial-initiated processes. Activation of proteases (caspase 8 in extrinsic pathway) and (caspase 9 in intrinsic pathway) further initiates a series of proteolytic activities incorporating effector caspases which play a key role during the execution of apoptosis. Bcl-2 family of regulatory proteins consists of members which act as either pro- or anti-apoptotic

regulators. Tumor cells however, evolve strategies to escape apoptosis in various ways including evasion of TP53 tumor suppressor activity, increased antiapoptotic regulators (Bcl-2, Bcl-xL) expression or survival signals (Igf1/2) and downregulation of proapoptotic proteins (Bax, Bim, Puma,etc.) [15].

iv. ***Enabling replicative immortality***

Non-cancerous cell lineages traverse through only a limited number of successive cell divisions. This restriction exists due to senescence and crisis (cell death). However, cancer cells exhibit unlimited replicative potential by achieving immortalization. Telomeres which protect the chromosome ends are associated with the ability for uncontrolled proliferation [23,24]. The multiple tandem hexanucleotide repeats present in telomeres gradually shorten in normal cells, thus failing to protect the terminal ends of chromosomal DNAs by generating unstable dicentric chromosomes resulting in a scrambling of karyotype eventually leading to apoptosis. The number of generations a cell's progeny can sustain depends on the length of telomeric DNA of the cell. Telomerase which helps in the addition of repeat sequences of the telomeric end of the DNA, is highly expressed in majority of immortalized cells like cancer cells. Thus, upregulation of telomerase expression in cancer cells proceed to form tumors where they are able to maintain the telomeric DNA length sufficient enough to avoid senescence and apoptosis. Hence, telomere shortening acts a tool which determines the limited replicative potential of normal cells which the cancer cells must overcome.

v. ***Inducing angiogenesis***

Angiogenesis is the process by which new blood vessels are formed from the pre-existing one. Tumors require nutrients and oxygen along with regulation of metabolic wastes and carbon dioxide for sustenance which is achieved by neovascularization obtained by angiogenesis. As the tumor progresses, angiogenesis is continuously triggered which causes otherwise resting blood vessels to continuously sprout new vessels for sustaining the growing neoplastic tumors [25]. Several regulators of angiogenesis mostly signaling peptides, attach to stimulatory or inhibitory receptors present on vascular endothelial cell surfaces, signal to either induce or oppose angiogenesis respectively [26,27]. Angiogenesis is stimulated by

Vascular endothelial growth factor-A (VEGF-A) and inhibited by thrombospondin-1 (TSP-1). VEGF signaling is controlled at several stages *via* three receptor tyrosine kinases (VEGFR-1–3). Several factors like hypoxia activation and signaling by proteases is involved in degradation of extracellular matrix (MMP-9) which also contribute to upregulation of VEGF gene expression [28-31]. TSP-1 binds to membrane bound receptors and induce inhibitory signals that may counteract proangiogenic stimuli [32].

vi. ***Activating invasion and metastasis***

Metastasis contributes to the most number of cancer-associated deaths [33]. Cancer cells alter their shape while obtaining attachment to adjacent cells and extracellular matrix (ECM) to activate invasion and metastasis. Downregulation and mutations leading to loss of E-cadherin function, which is a vital cell adhesion molecule observed in human carcinomas, indicating its importance as a key regulator of this characteristic ability [34]. On the contrary, adhesion molecules involved in cell migrations are often overexpressed e.g. N-cadherin, which is otherwise expressed in migrating neurons and mesenchymal cells only during organogenesis, is elevated in numerous invasive carcinomas [35]. The process of activating invasion and metastasis in cancer cells involves a series of processes including cell-biology changes, which initiates with local invasion then proceeds with intravasation into surrounding blood and lymph vessels. Finally extravasation occurs, where it passes through the lymphatic and blood circulatory systems by exiting the vessel lumina into the parenchyma of distant tissues. Thus, these cancer cells forms minor nodules (micrometastases) and eventual colonization involving micrometastatic lesions to form clinically apparent tumors [36,37].

vii. ***Reprogramming of energy metabolism***

The uncontrolled cell proliferation in neoplastic disease involves adjustments of energy metabolism to initiate cell division and growth. Cancer cells can reconfigure their glucose utilization or energy production by shifting their energy metabolism, largely to glycolysis even when sufficient oxygen is available, as the cells must make up for the lowered efficiency of ATP generation afforded by glycolysis. This is achieved by upregulation of glucose transporters (GLUT1) which substantially

enhances glucose import into the cytosol [38-40]. This is related to the activation of oncogenes (e.g., RAS, MYC) and mutated tumor suppressor genes (e.g., TP53). Hypoxic conditions also increase glucose transporters and different enzymes involved in glycolysis [38,39,41]. These lead to increase in transcription factors (HIF1α and HIF2α), which then promote glycolysis [41,42].

viii. ***Evading immune destruction***

Cells and tissues are constantly monitored by the immune system which resist or eradicate formation and progression of neoplasias, late-stage tumors and micrometastases. However, cancer cells manage to avoid detection by the immune system or limit the extent of immunological killing, thus, evading immune destruction, as seen in cases where immunocompromised individuals are at higher risk of developing certain cancers [43]. Increased tumor incidences correspond to deficiencies in the development or function of $CD8^+$ cytotoxic T lymphocytes, $CD4^+$ T_h1 helper T cells or natural killer (NK) cells as observed in experimental mice models [44,45]. Highly immunogenic cancer cells may evade immune destruction by disabling components of the immune system, e.g. cancer cells may secrete TGF-b or other immunosuppressive factors that paralyze infiltration of cytotoxic T lymphocytes and NK cells [46,47]. Additionally, cancer cells may also recruit inflammatory cells like regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) which can suppress the actions of cytotoxic lymphocytes [48,49].

1.4 Anti-cancer drugs and limitations

The quality of life of cancer patients is largely dependent on the efficacy of treatment and the side-effects associated with them. Cancer patients may undergo various modern treatment methods which includes chemotherapy, surgery, and radiotherapy. Despite current progress that helps patients to overcome cancer and extend their life span, numerous complex challenges are still needed to be addressed, such as, drug resistance and a wide range of associated side-effects, which negatively impact the overall treatment efficacy and patient health [50,51]. The side-effects of chemotherapy may be grouped as acute effects, initiating within minutes of chemotherapeutic drug administration (such as nausea and vomiting), and late effects (such as peripheral

neuropathy), which appears weeks, months or years post-treatment [52]. Most medications cause bone marrow suppression to different extents, depending on the drug, dosage, and intrinsic factors. Frequent side effects may also include hair loss and gastrointestinal changes [53]. Another example of a long-term adverse effect is female infertility, which may result from radiation or chemotherapy [54,55]. In view of these considerable health hazards, the decline in quality of life for patients undergoing cancer treatment has become a major health concern.

1.5 Anti-cancer drug development from natural sources

Development of drugs from natural sources, which could be used to overcome the limitations of modern anticancer therapy, have been explored extensively. Natural molecules derived from plant-based sources (phytochemicals) have been studied widely for their anticancer activity. For instance, Curcumin, Hesperidin, Honokiol, Formonetin, Ursolic acid, Capsaicin, Proanthocyanidins, Allicin, Apigenin, Pacitaxel, Tannic acid, Calotropin, Lycopene, Camothothecin, Taxol, and Quercetin are some well-known phytochemicals exhibiting activity against numerous types of cancer [56,57].

Moreover, animal venom has also been reported to exhibit promising anticancer activity in various experimental models [58-60]. Venomous animals significantly contribute to the discovery of novel therapeutic candidates as they produce venoms to immobilize their preys, and also protect themselves from their surroundings, which includes predators [61]. Venoms secreted by animals have various pharmaceutical applications, such as the treatment of cardiovascular, autoimmune and neurodegenerative diseases, as well as cancer [62]. Venom components are characterized by their specificity, stability and potency enabling them to modify molecular targets effectively, thus, making them promising therapeutic candidates. Development of naturally derived small molecules which can be used as sensitizing agents, to target gene products that modulate the process of cell death (apoptosis pathways) and facilitate the effectiveness of therapy. Venom can inhibit the proliferation of cancer cells and promote cell death through apoptosis, cytochrome C release, and the modulation of protein expression that controls the cell cycle [63,64]. Venom from different animals, such as, snakes, scorpions, wasps,

honeybees, frogs, beetles and marine molluscs have been studied for their cytotoxic and anticancer properties [58] (Figure 1.4).

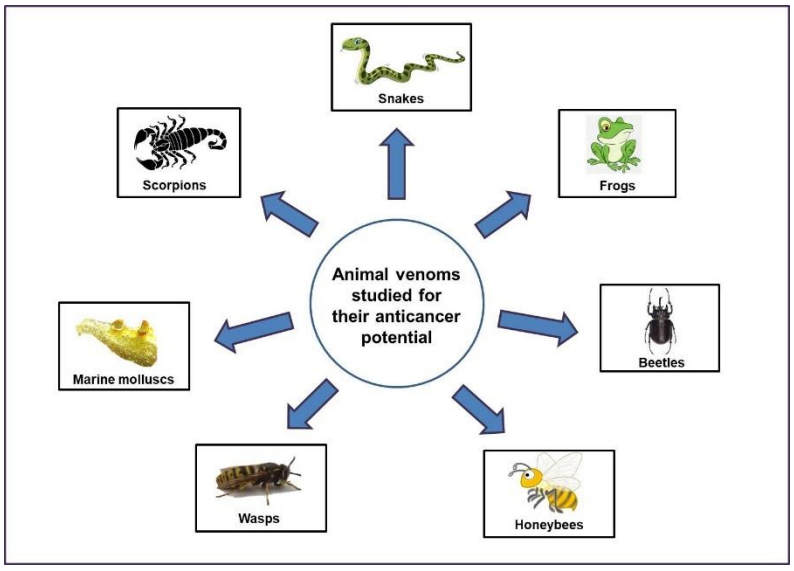

Figure 1.4: Animal venoms studied for their anticancer potential.

1.6 Snake venom: Potential source of drugs

Snake venom is considered as an "advanced biochemical weapon", evolved for the purpose of digestion, predation and protection, and contains various pharmacologically active proteins with target specificity [65,66]. They contain a blend of enzymatic and non-enzymatic components which are highly selective and may be considered as a "mini-drug library" [67]. The pharmacological effects of snake venom include neurotoxicity, cytotoxicity, hemotoxicity, myotoxicity and anti-microbial effects in general. Certain proteins of snake venom target the mammalian homeostasis and vital systems, such as, the cardiovascular system, central and peripheral nervous system, blood coagulation cascade and neuromuscular system [67,68]. Understanding the structure function co-relation of snake venom has led to the development of several drugs, such as, Captopril® which is approved for treating cardiovascular disease and hypertension by the US FDA in 1981. This was derived from bradykinin-potentiating peptides isolated from *Bothrops jararaca* venom [69,70]. In 1998, anti-platelet drugs Aggrastat® and Integrillin®, derived from the snake venom disintegrins of *Echis carinatus* and *Sistrurus miliarus barbouri* respectively were also approved [67,71,72].

Snake venom toxins can induce toxicity in cells and tissues due to their high specificity and affinity for. For instance, cytotoxins isolated from snake venom exert a range of physiological effects primarily by altering the structure of cell membrane [73-76]. Although snake venom proteins contain toxic effects, they also possess pharmacologically useful properties which may be utilized used for treatment of several diseases and ailments, such as, hemophilia, deep vein thrombosis, autoimmune disorders, neurodegenerative diseases and cancer. For instance, various thrombolytic agents developed from snake venom toxins have been reported to treat vascular disorders [77], used against gram-positive and gram-negative bacteria (antimicrobial activity) [78,79], and exhibited antiviral activity against various types of viruses, such as, herpes simplex virus [80], dengue and yellow fever [81]. They also exhibited antiparasitic activity against *Leishmania* [82] and *Plasmodium falciparum* [83] and also exhibited antifungal activities [84]. Snake venom toxins exert their cytotoxicity by altering the cellular metabolism that results in deleterious effects on cancer cells [85]. For example, cytotoxins from cobra venom are widely explored for their anti-cancer effects as they demonstrated considerable cytolytic activity against different cancer cell types including leukemia [74,86]. Based on several studies, researchers have attempted to develop different chemotherapeutic agents depending on the cytotoxic ability of the toxins produced by different venomous snakes. The emerging fields of Nanotechnology and targeted-drug delivery have also brought entirely new perspectives in the preparations of peptide-based drugs, for e.g. venom extracts from the snake *Walterinnesia aegyptia* tagged with silica nanoparticle enhanced the proliferation of immune cell as well as decreased the proliferation of human breast cancer cells [87]. In summary, there is a huge untapped pharmacological potential in snake venom for development of new therapeutic agents for treatment of various human diseases including cancer.

1.7 Need of the proposed study

1. Snake venom proteins provide a promising avenue for drug discovery particularly in the development of novel peptide-based anti-cancer agents that are highly effective and selective towards cancer cells.
2. Thorough investigations into the structure and function of the venom would play a key role in the development of novel anti-cancer drugs.

3. Understanding the mechanism of action of *Naja kaouthia* venom proteins on cancer cells can be used as better therapeutic intervention against cancer.
4. The anti-cancer effect of *Naja kaouthia* venom of the North-East India origin has not been studied yet although the venom of West Bengal (India) origin has been reported.

1.8 Hypothesis

Crude *Naja kaouthia* venom might contain protein/s with potential anti-cancer activity which can be exploited for discovery of anti-cancer agent.

1.9 Aims and Objectives of the study

Aim: To explore the potential of *Naja kaouthia* venom proteins as lead compounds for the development of new anti-cancer agents.

Based on this aim, the following objectives were set for the study:

1. Partial biochemical characterization of crude *Naja kaouthia* venom and determination of its cytotoxic activity against cancer cell lines.
2. Identification of protein/s of *Naja kaouthia* venom with cytotoxic potential.
3. Understanding the mechanism of cytotoxic activity of the identified protein/s.

1.10 Work flow

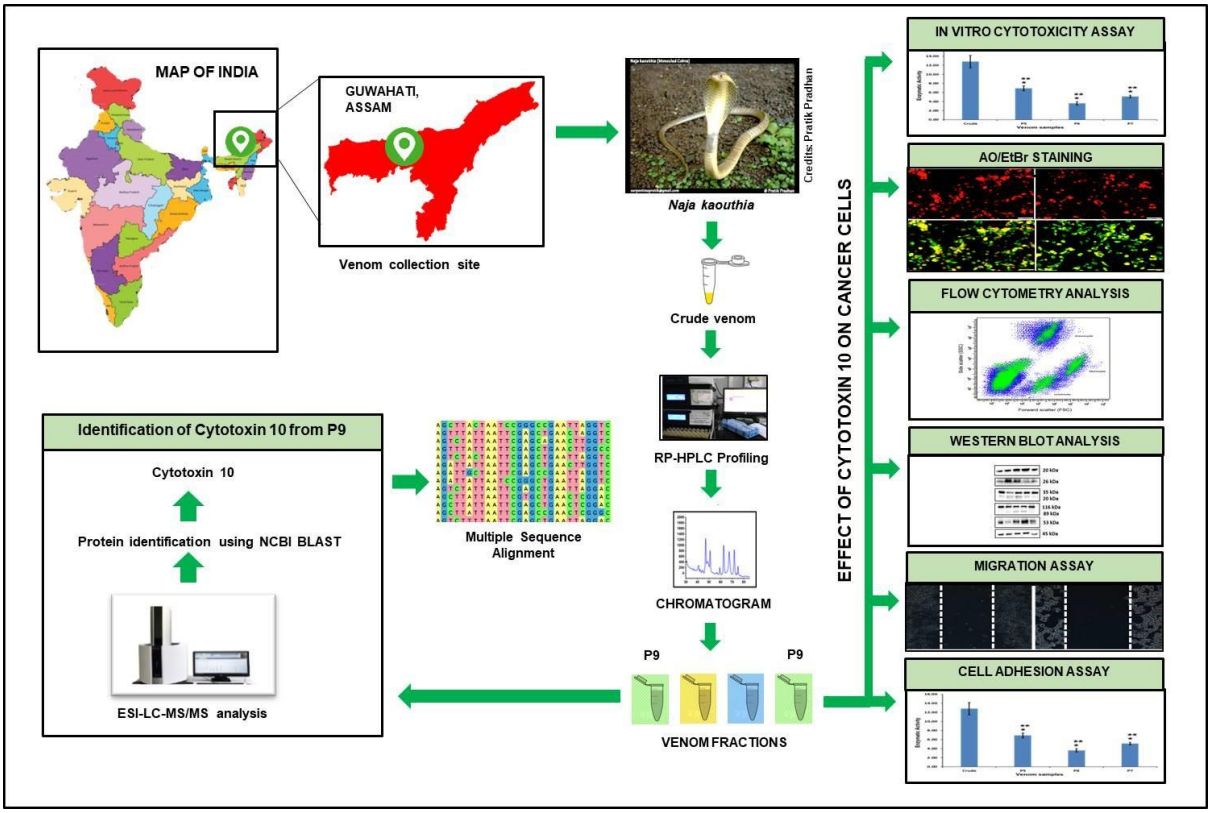

Figure 1.5: Work flow of research objectives.