CHAPTER 1

Introduction

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1.1 Introduction

Cancer is considered as one of the significant mortality which impermanence within the human civilization since long ago [1-5]. Cancer is not a single disease, but a term used to describe hundreds of diseases. It is characterized by either unrestrained cell growth and/or sustained survival of cells [6]. This may originate from almost any type of tissue and cancer cells may spread to distant tissues [7] and mainly induced by interaction of genetic susceptibility and environmental factors [8, 9]. In 2012, the estimates of the World Health Organization (WHO) for global cancer incidence and associated mortality were 14.1 million and 8.2 million, respectively. Although cancer can affect all ages, there is a steep increase in incidence with age. The hallmarks of cancer include eight biological capabilities acquired during the multistep development of human tumors sustaining proliferative signalling, evading growth suppressors, resistance to cell death, replicative immortality, induction of angiogenesis, activation of the propensity to invade and metastasize, reprogramming of energy metabolism and evasion of immune destruction [10]. A major problem in the clinical management of cancer is that malignant cells are not confined to their tissue of origin, but can spread to other parts of body via the lymphatic system and bloodstream, creating secondary deposits known as ‘metastases’ [11-13]. Identification of malignant cells and kill them is a very difficult task. Hitherto, several anticancer have been reported, but, there are numbers of causes to work on predictive performance and resist the desirable result stability such as most of them have developed resistance due to (1) Drug Inactivation. (2) Alteration of Drug Targets (3) Drug Efflux (4) Cell Death Inhibition (5) Epithelial-Mesenchymal Transition and Metastasis (7) DNA Damage Repair and (8) Cancer Cell Heterogeneity [14-15]. Thus, the emergence of multidrug resistance has emphasized the need to understand and development of effective prophylactic means [14-15]. From the reported studies, it is clear that an improved

chemotherapeutics, offering efficient and consistent protection is important [16]. Cancer is often considered as the re-emerging disease as a significant increase is recorded in the number of cancer cases around the globe [17]. Genetic and environmental factors are responsible for the genomic lesions that cause cancer [18, 19, 20]. There are many types of cancers as many types of organs. Cancer risk in patients with cirrhosis could be modified by factors such as changes in hormonal levels, impaired metabolism of carcinogens, or alteration of immunological status.

1.2 Cancer treatment

Present day cancer therapies include four major types: immunotherapy, surgery, radiotherapy and chemotherapy. *Immunotherapy*, a systematic therapy uses the immune system to fight the infection and disease [21]. The immune system detects and destroys abnormal cells and most likely prevents the growth of many malignant cells by stimulating the patients' own immune system or by administering immune system components. Surgery can cure successfully but in primary stage it is very difficult to identify the each one of the tumor cell due to irregular shape and inaccessibility (e.g. brain tumors), the old age and poor health of patient are also the important issues [22-24]. Furthermore, many tumors have metastasized at the time of diagnosis, leading to widespread disease which is no longer treatable by surgery. In these situations surgery is not a prime option, other therapies must be considered. *Radiotherapy* has been used to the treatment of cancer since long time. In this therapy, to destroy the affected cells ionized radiation deposits, with high energy target tissue and breaks the DNA of cells in a way that disrupts their growth and division [25-28]. However, radiation damages healthy cells with cancer cells, the latter are able to repair themselves and function properly. *Chemotherapy* is a drug treatment that inhibits the different types of chemicals to destroy cancer cells. Drugs

are administered via the vascular system and able to target both the primary as well as cancer cells that have spread to distant tissues [16]. Chemotherapy can be given orally, subcutaneously, intravenously, directly into a body cavity or topically applied to the skin. These different types of therapies are often combined during clinical management of cancer. Reducing cancer morbidity and mortality still requires prevention and earlier detection.

1.2.1 Principle of chemotherapy

Chemotherapy can be used as the primary or sole treatment for cancer. Also it can be used after other treatments such as surgery and radiotherapy to kill cancer cells that might remain in the body. In order to the therapy, chemotherapy alone and combined with other therapy is given at alternate intervals after evaluation of the response of patient. The efficiency of chemotherapy depends on the type of cancer and the stage. The aim of chemotherapy is to remove the diseases completely. Chemotherapy is usually classified as follows:

1) Induction chemotherapy: This is the initial treatment of untreated patient to cure the cancer. Induction chemotherapy relies on the principle of spatial cooperation. The delivery of induction chemotherapy can apply before definitive surgery or radiation therapy.
2) Consolidation/intensification chemotherapy: Administered after remission to prolong the diseases free survival. Consolidation uses the same drugs while intensification uses same therapy with different drug.
3) Combination chemotherapy: The therapy includes the number of different drugs simultaneously. Although single agent chemotherapy may be used in some situations, combination of drug therapy has advantage of minimizing the resistance of developing to any single agent [29].

4) Adjuvant chemotherapy: This therapy is designed to give after local treatment such as surgery and radiotherapy. It can be applied with evidential support of cancer with the risk of recurrence. It is also useful to kill the cancerous cells that have spread to other parts of body.
5) Neoadjuvant chemotherapy: Administer to reduce the burden of local therapy such as surgery. Moreover, it can cure the high risk metastatic diseases.
6) Maintenance chemotherapy: This is repeatedly, given in low dose to during remission with curative purpose.
7) Salvage chemotherapy: This is required after all failed therapy to decrease the load of tumor and control the disease and /or provide palliation.

1.2.2 Types of chemotherapy agents

Since the discovery of the cytotoxic agents, numbers of different kinds of chemotherapy drugs administered differently by screening the large number of compounds by experimental methods. They are classified based on different in-vitro effects on dendritic cells and mechanism of action.

1.2.3 Alkylating agents

Alkyl agents group is most active and have been used since long. These types of drugs are cell-cycle nonspecific and have ability to alkylate many molecules including protein, DNA and RNA. The ability to bind covalently to DNA is prime cause to their anti-cancer effects. If the cell tries to replicate crosslinked DNA during cell division, or tries to repair it, the DNA strands can break. This leads to a cell death [30, 31].

1.2.4 Antimetabolites

The group of drugs has a similar structure to the building blocks of DNA and RNA and cell cycle dependent. These drugs exert their effect by either blocking the enzyme required for DNA synthesis or becoming incorporated into DNA or RNA. When inhibiting the enzymes in this mechanism, DNA damage can occur as DNA cannot duplicate itself and programmed cell death is induced [32-34].

1.2.5 Anti-microtubule agents

These agents are plant derived drugs that can block cell division by preventing microtube function which is made of two proteins α tubulin and β-tubulin. Vinca alkaloids and taxanes are the prime groups of anti-microtubule agents. Both agents cause the microtubule dysfuction. The vinca alkaloids prevent the formation of the microtubule agents, whereas the taxanes prevent the microtubule disassembly [35, 36].

1.2.6 Topoisomerase inhibitors

DNA dependent inhibitors group consists of two enzymes topoisomerase I inhibitors and topoisomerase II inhibitors. All events such as DNA replication, transcription, recombination, repair and nucleosome remodelling, chromosome condensation and segregation are DNA dependent [37]. Inhibitors produce the stress by this effect and DNA breaks in single or double strands. This class of drugs is phase-specific and prevents cells from entering mitosis [38].

1.2.7 Cytotoxic antibiotics

Group of all drugs has various mechanism of action. Most cytotoxic antibiotics are derived from bacteria and fungi. This group of drugs includes anthracyclines, actinomycin,

bleomycin and mitomycin c. They affect the function and synthesis of nucleic acids in various ways [33, 39].

1.3 Resistance to chemotherapeutic agents

1.3.1 Drug Inactivation

Drug activation involves complex mechanism in which drug interacts with targeted protein. The interaction depends on the environmental circumstances which can modify, partially degrade, or composite the drug with other substances or protein. Although, cancer cells contain a high degree of molecular heterogeneity, which can develop the drug resistance during treatment through decreased drug activation [40]. Platinum drugs can be inactivated by the thiol glutathione [41]. Nucleoside analogues (NAs) can be deactivated by deoxycytidine kinase which catalyses the rate-limiting step of conversion of most NAs to their corresponding monophosphates. Decreased or absent Deoxycytidine kinase (dCK) activity will therefore confer resistance to NAs [42-46]. The gene encoding thymidine phosphorylase can be inactivated by methylation and therapy causing capecitabine resistance [47]. Another example of drug activation and inactivation includes the cytochrome P450 (CYP) system, glutathione-S-transferase (GST) superfamily, and uridine diphospho-glucoronosyltransferase (UGT) superfamily.

1.3.2 Alterations in drug targets

The alteration of drug targets may be a secondary mutation in the target protein. Drug expressions can be affected by the alteration of the direct target, such as mutation and response level. In cancers, these types of target alterations can ultimately lead to drug resistance. For example, Warburg effect, a phenomenon of cancer cells with elevated aerobic glycolysis [48-50]. Tamoxifen (TAM) is commonly used for patients with ER-

positive breast cancer, relying on its ability to compete with estrogen for the ligand binding site of ER.

1.3.3 Adaptive response

Active drugs acquire their target, the efficiency of drug and potency of treatment depends on how the cancer cell responds. Many anticancer drugs act via DNA damage, directly or indirectly. There are two ways that DNA receives the responds by cells 1) cell death and 2) cell repair. The response of cancer cells to DNA damage is a major factor determining the effectiveness of DNA-damaging drugs. Although deregulation of DNA damage response (DDR) may remit the resistance induced by DNA repair, it may also increase the risk of developing new mutations due to genomic instability, the accumulation of which may initiate a new round of carcinogenesis. Therefore, DNA damage response is a complex mechanism in cancer treatment and recurrence, and it requires thorough consideration when used as an anticancer therapeutic target. Examples are deficiencies or mutations in mismatch repair (MMR) genes or/and p53, which may confer resistance to DNA damaging drugs [51-53].

1.3.4 Drug influx and drug efflux

Both decreased drug influx and increased drug efflux can produce drug resistance. Drug efflux is a key mechanism of resistance in Gram-negative bacteria. Moreover, efflux pumps allow the microorganisms to regulate their internal environment by removing toxic substances, including antimicrobial agents, metabolites and quorum sensing signal molecules [54]. Intrinsic resistance is usually the result of the reduced permeability of the bacterial envelope and the activity of multidrug efflux pumps [55]. This suggests that the main physiological role of the components of intrinsic resistance involves the prevention of influx of toxic components by restricting the permeability of the cell or the active

export of toxic compounds or their metabolites out of the cell. Many gloomy drugs, including anti-infectives, can simply be seen as xenobiotics, and systematic studies have been performed to see the extent to which the loss of effluxers (and occasionally of influxers) regulate their toxicity [56, 57]. In particular, the AcrAB-TolC complex, which spans inner and outer membranes, is constitutively expressed, and is considered to play a major role in multidrug resistance [58, 59, 60, 61, 62]. Consequently, Major mediates of microbial resistance to antibiotics and as targets for ameliorating are major activities which are done by efflux transporter.

1.3.5 Deregulation of Apoptosis

Too much death can result in one of many degenerative diseases. In the case of cancer, for example, this can result in a block or inhibition of apoptosis leaving a buildup of 'un-dead' cells. Cancer is one such disease where apoptosis is often deregulated. The Bcl-2 family of proteins plays a key role in the normal regulation of apoptosis and aberrant expression of members of this family has been associated with several tumours. The apoptosis program and members of the Bcl-2 family control this release. Some members of the family, such as Bax, drive cytochrome c release whereas other members, such as Bcl-2 itself, prevent this release. Experiments involve the knockout mice display a hyperplasia which leads to increased tumor development [63]. Over-expression of Bcl-2 has been observed in many tumors such as lung, renal, stomach, and brain cancer. Surprisingly, lower than normal levels of Bcl-2 have also been observed in breast cancers [64].

1.3.6 Autophagy

Autophagy plays a housekeeping role in removing misfolded or aggregated proteins, clearing damaged organelles, such as mitochondria, endoplasmic reticulum and peroxisomes, as well as eliminating intracellular pathogens. Moreover, its deregulation has

been linked to the non-apoptoic cell deaths. There are three defined types of autophagy 1) macro-autophagy 2) micro-autophagy and 3) chaperone-mediated autophagy; all three culminate in the delivery of cargo to the lysosome for degradation and recycling. However, as a cell survival mechanism, that autophagy may promote drug resistance and tumour cell adaptation to stress [65]. To understand the malignant transformations among the cells by the changes of glycosylation pattern is an important task [66]. The glycosylation is a key fact with highly regulated mechanism of secondary protein processing within the cells. It plays a vital role in determining the protein structure arrangement, its stability and activity particular during the protein-protein, protein-ligand and other receptors interaction to create large macromolecules or complexes. Glycosylation is a form of co-translational and post-translational modification and is also present in the cytoplasm and nucleus as the O-GlcNAc modification. There are five classes of glycans:

- **N-linked glycosylation:** N-linked glycans are attached to nitrogen of asparagine or arginine side-chains. This pattern is very prevalent form of glycosylation and is important for the folding of many eukaryotic glycoproteins and for cell–cell and cell–extracellular matrix interaction. The N-linked glycans of a protein can modulate a protein's function.
- **O-linked glycosylation:** O-linked glycans are attached to the hydroxyl oxygen of serine, threonine, tyrosine, hydroxylysine, or hydroxyproline side-chains, or to oxygens on lipids such as ceramide. For example, eukaryotes in the Golgi apparatus.
- **Phosphoserine glycosylation:** phosphoglycans are linked through the phosphate of a phosphoserine.

- **C-mannosylation:** This is the rare form of glycosylation where a sugar is added to a carbon on a tryptophan side-chain.
- **Glypiation:** Glypiation is an addition of a GPI (Glycosylphosphatidylinositol) anchor that links proteins to lipids through glycan linkages.

1.4 Objectives

The present work aims to clarify a deep understanding and identifying the malignant cells during glycosylation. In addition, the work also aims to investigate the different characteristics and dramatic effects of targets with respect to the pH and temperature and analyse their binding energy with various ligands as well as modified ligands. Study has been carried out of selected targets under the framework of first-principles density functional theory (DFT) [67]. For the investigation of their ground state energy, structural, electronic and vibrational properties have been computed and analysed. Simultaneously to achieve the goal without any loop hole we have incorporated the body environment using molecular dynamics simulation (MDs) [68]. Following specific objectives were fulfilled, the analysis in the response of drug against the target at given time.

- To find the structure-based inhibitors against the selected target and analysis their dynamical behaviour of the target with respect to time.
- Check the capacity of strengthening in drug-target interactions; compare the Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (ROG) and Hydrogen bond analysis of solid bodies.
- Calculate the most interacting region of druggable targets.
- Verify physiochemical properties of selected drug molecules.

Here, we have demonstrated the N-linked glycosylation pattern in the form of sialylation configuration. Sialylation, or the covalent addition of sialic acid (SA) to the end of glycoproteins terminal, is a biologically important transformation that is involved in embryonic development, neurodevelopment, reprogramming, oncogenesis and immune responses. It was first isolated by Gunner blix in 1936 [69]. Since the discovery of sialic acids in vertebrates by Blix and Klenk in 1941, extensive effort has been focused on identifying the structure and the functional roles that these ubiquitous carbohydrates play in mammalian biology [70]. SA is found widely distributed in animal tissues and to a lesser extent in other organisms, ranging from fungi to yeasts and bacteria, mostly in glycoproteins and gangliosides. Moreover, it is a structurally unique family of 8 and 9-carbon monosaccharides and characteristically contains an anomeric carboxylate, a deoxygenated methylene C-3 ring carbon, an oligohydroxylated side chain at C-6 and is differentially functionalized at C-5. In human, the brain has the highest sialic acid concentration, where these acids play an important role in neural transmission and ganglioside structure in synaptogenesis [71]. Among all the structure, N-acetylneuraminic acid (Neu5Ac) is the most common SA which carries negative charge at physiological pH, SA takes place in many tumor- associated carbohydrate antigens, such as, sialylatedTn antigen and sialyl Lewis X. Its anti-adhesive properties present a mechanism for cancer cells to separate from a primary tumour and initiate metastasis [72]. We have attempted to outline how simulation data with varying temperature and pH may be used as a tool to elaborate the question of ligand interaction with SA as the target and to evaluate if our observations reasonably reproduce the experimental findings. Development of new drug molecule is expensive and time consuming. Improving safety efficacy ratio of “old” drugs has been attempted using different methods such as individualizing drug therapy, dose titration, and therapeutic drug monitoring. Delivering drugs at controlled rate, slow

delivery and targeted delivery are other very attractive methods and have been pursued vigorously. The major aim of developing nanocarrier drug delivery systems is to enhance the therapeutic effect or reduce toxicity of therapeutically active materials.

1.5 Structure of thesis

The present thesis is divided in six chapters. The Chapter 1 presents the expression of cancer cells accordance with organs and their representation in human body. Cell surface proteins are elaborated with covalently attached complex array of N-linked glycans. Glycans offer correct folding of the protein, provide resistance to proteases and facilitate their interaction with ligands. The dynamical behaviours and glycosylation changes often show the malignant transformation. This is characterized by an increased branching of N-linked glycans thus creating additional sites for terminal sialic acid (SA) residues (73, 74, 75). Further, it is shown that over-expression of SA on malignant colonic cells and tissues, in vitro, correlates with the metastatic stage [76]. Moreover, the challenge now days is to directly pass macromolecules to cell membrane without any active process. It is found that the paclitaxel (PTX) is dynamic against a wide range of cancers that are considered to be an intractable to conventional chemotherapy. This has led to the regulatory approval of paclitaxel in the palliative therapy of patient with breast cancer [77], ovarian cancer [78], lung cancer [79], pancreatic cancer [80] and many more. Unfortunately, Cremophor EL itself is toxic, which makes finding a suitable alternative a high priority. Therefore development of novel techniques for introducing bioactive molecules inside the living cells is an active area of research. Finally study shows the newly predicted drug and drug carrier to control the malignant changes with respect of body environment, different physiological variables such as pH and temperature.

Theoretical description of computational methodology used throughout the work is presented in **Chapter 2**. In this chapter, theoretical concepts which are the basis of density functional theory (DFT) based first-principles calculations and Molecular dynamics (MD) simulation based on Newton's second law of motion are presented and discussed. In particular, all quantities which help to calculate the electronic, structural and dynamical properties of complexes on the basis of the DFT and MD simulations are discussed. We present all the body environment and chemotherapy conditions as well. To know the dynamical behaviour we have employed the GROMOS 53a6 [81] and OPLS_2005 force fields, an enhanced version for all atom force field developed by Schrödinger [82]. The constant temperature and pressure Nose-Hoover thermostat and Berendsen barostat have been utilized respectively. Moreover, energy-minimized system using the leap-frog algorithm in the NVT canonical and NPT isothermic ensemble has been used.

In **Chapter 3,** we report the results of our systematically investigated the recognition of pH and temperature dependent glycosylation pattern. Results verify the dual approaches of cationization and attachment of identification peptide in the targeting of colon cancer cells, exhibiting metastatic-stage dependent expression of SA. Our calculations on electronic and surface properties using density functional theory (DFT) based first-principles approaches demonstrate that the carriers decorated single antennary saccharides and mimic exhibit high affinity towards over-expressed SA and galectin residues on cancer cell surface. We have also included the pH, physiological temperature (37˚C) and chemotherapy temperature (42 ˚C) to obtain the accurate absorption energy. Our calculations demonstrate a stronger D- galactose- SA interaction at tumor-relevant low pH and hyperthermic condition. Furthermore, basis set superposition error (BSSE) of intermolecular potential function was corrected by Boys-Bernardi counterpoise method [83]. We found that the D-galactose is 1.67 Å close to SA with 0.22297 eV energy gap

and -26.52 kcal/mol interaction energy at 6.0 pH and 42 ˚C temperature. Overall, our results reveal that; (i) increasing temperature and decreasing pH, in general, have a favourable effect on binding affinity, and (ii) induction of hyperthermia at tumour-relevant pH offers pronounced enhancement in the binding affinity.

Chapter 4 presents the comparative study of single and bi-antennary saccharides and mimics with extra cellular SA using DFT and MD simulations to evaluate the quantum mechanical and classical based properties respectively. Bi-antennary PBA domain is the primary region of interest for probing the impact of SA activators, and binding affinities of this ligand to cellular SA domain. The binding of three complexes have been compared amongst themselves. The bi- antennary phenyl boronic acid (2PBA) displays the ability to form reversible covalent interaction with SA. Further we have employed the different time slots to know the way of act and predict the properties of complex under the physiological environment such as 1 bar pressure and 37 ˚C temperature. We adopted the OPLS_2005 force fields [82]. The time course of the SA-ligand interaction for 5, 15 and 25 ns is presented. Our results indicate that in the presence of strong interaction energy, bi-antennary PBA molecules would spontaneously move towards the SA. 15 ns structure quickly interacts to SA with -181.2479 kcal/mol binding energy with 1.579(Å) distance. Finally our results indicate that after 15ns in the presence of strong interaction energy, bi-antennary PBA molecules would spontaneously move towards the SA.

In **Chapter 5,** We have found the potential drug delivery systems to free the drugs due to longer circulation time, higher drug uptake and selectively, lower dosage and better therapeutic efficiency [84]. To understand the protonation and diameter effects on drug loading and releasing we have taken paclitaxel (PTX) loaded with three armchair chirality (n,n) as (12, 12), (13, 13) and (15, 15) size single wall carbon nanotube (SWCNT). We have chosen the loading instead of side wall adsorption to know the temperature effect and

PTX loaded various diameters sized SWCNT. We have incorporated the physiological temperature and chemotherapy temperature to check the structural and mechanical properties with solvent accessibility. Higher pair distribution function of the complex per atom and interaction energy at 315.15K indicate the strong interaction between (15, 15) armchair SWCNT and PTX. Furthermore, the 20.53 Å diameter is optimal for the encapsulation and at 315.15 K temperature drug delivery time is 440 ps. Finally in chapter 6, the present summery of the work carried out in the present study and future scope of the present study.

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