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Research Article

Bio-Informatics - Next Generation Cancer Targeted Therapies

Ayan Chatterjee†* and Uttam Kumar Roy†

†Department of IT, Jadavpur University, Kolkata, India

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Abstract

Since its birth in the 1980s, bio-informatics has been rapidly growing, keeping pace with the expansion of genome sequence data. Latest technological development of large-scale gene expression analysis using DNA micro-arrays and proteomics experiments has further boosted the importance of bio-informatics methods. In Bio-Informatics a lot of tools & algorithms are developed to study detailed shape of proteins & other biological molecules to develop drugs with more disease resistant capacity in reduced cost & time. The integration of wet experiments and the use of bio-informatics analysis have become an important part of the biological and clinical research of this century. The area of cancer research is not an exception. Cancer is one of the most common causes of death, taking nearly 7 million lives each year worldwide. So, methods of cancer treatment have become most challenging. Existing methods are based on chemotherapy drugs and radiation which are not target specific and lead to side effects. New cancer targeted therapies (therapeutic antibodies or small molecules) are more targets specific and less toxic. But, there remain a lot of challenges to the treatment of cancer including drug resistance, and high tumor tissue fluid pressure. In many solid tumors, for example, increased tumor tissue fluid pressure makes the activity of therapeutic agents less efficient. One promising way of meeting such challenges is targeted therapy that may be used to make targeting more specific and carry higher dosages of anti-cancer drug to tumor tissues. This article reviews and discusses recent advances in the treatment of cancer and the challenges that remain, with the help of Bio-Informatics. The statistics shows that the cancer cases will rise to 70-80% in coming two decades. This paper discusses with different possible targeted therapies.

Keywords: *Mutation; target therapy; DNA; mRNA; Vaccine; Hormone; Virus; Spindle; Sequence Analysis.*

1. Introduction

The first description of cancer was found in an Egyptian papyrus and dates back to approximately 1600 BC. It was considered to be an incurable disease until the nineteenth century, when surgical removal was made more efficient by anesthesia, improved techniques and histological control. Before 1950, surgery was most preferred means of treatment. After 1960, radiation therapy was being used to control local disease.

We are still unable to improve the mortality rate or to prolong the survival time for metastatic cancer what we expected after more than 50 years. We have identified the diagnostics and pathways of different tumor entities. This knowledge is analyzed to generate tumor specific targeted therapies (Gibbs, W. Wayt *et al*, 2003). Targeted therapy provides us a wide variety of direct and indirect targeted approaches. Direct approaches target tumor antigens to alter their signaling strategy either by monoclonal antibodies like MoAbs2 or by small molecule drugs or ligands that interacts with target proteins. Indirect approaches rely on tumor antigens on the cell surface acts as target devices for ligands that contain different kind of effectors molecules. In addition to active targeting, tumors can also be targeted by macromolecules but that are passive. Bio-informatics tools have boosted up cancer research. They analyze the global profiles of gene expression patterns of cancer cells and compare it with normal cells or those are subtype of cancer and genes over/under-expressed in the cancer tissue and followed by identification and clustering (identifying cancer signatures) is done.

2. Bio-informatics and cancer research

Bio-informatics has been rapidly growing, maintaining a pace with the expansion of genome sequence data. Modern technological improvement of large-scale gene expression analysis utilizing DNA micro-arrays and proteomics scientific research has further assisted the worth of bio-informatics methods. A lot of tools & algorithms are developed to make out detailed shape of proteins & other biological molecules to design drug with more disease resistant capability in reduced cost & time. The consolidation of wet experiments and bio-

*Corresponding author: **Ayan Chatterjee**

informatics analysis have become an important part of the biological and clinical research of this period. The area of cancer research is not an exception (Thomas Lengauer, Ralf Zimmer *et al*, 2000; Wang XD, Liotta L *et al*, 2011).

A. Cancer Drug Design with Protein Structure Analysis

The molecules whose shape produces interest on structural biologists are proteins as protein molecules do much of the work in the body. The shape or structure of a protein offers hints on the part it plays in the body. It also holds the key to develop new medicines, materials, or diagnostic procedures. Proteins are made of amino acids hooked end-to-end like beads on a necklace. Many scientists believe that if deciphering the structures of proteins from their sequences becomes possible then we could better understand protein functions. Then we could use that knowledge to improve the treatment of diseases (G Roti, K Stegmaier *et al*, 2012; Kihara D, Yang YD, Hawkins T *et al*, 2007)

Research activity at Ohio State University's comprehensive cancer center has determined the 3D structure of the protein produced by the p16 tumor-suppressor gene on the computer. When the p16 protein is missing or inactive because of mutations in the p16 gene, cancer can occur. In fact, more than 70 different types of cancer is caused due to damage to the p16 protein. So, after predicting structure of the protein followed by developing a new drug that mimics p16 will open a new possibility for cancer. But p16 protein structure prediction was a time consuming due to the constant motion in protein molecules. P16 interacts with target molecule, a second protein called cyclin-dependent kinase 4 (cdk4). After having structure of two such proteins we can develop new drug that can block malignant tumors (G Roti, K Stegmaier *et al*, 2012; Kihara D, Yang YD, Hawkins T *et al*, 2007; Thomas Lengauer, Ralf Zimmer *et al*, 2000).

B. Cancer classification based on Gene Expression

Traditional cancer classification methods (morphological & clinical based) are poor and are suffering from limitations in their diagnostic ability. Therefore, accurate prediction of different tumors has become important to offer better treatment & toxicity minimization on patients. Also, the existing tumor classes have been found and they are heterogeneous and consists molecularly distinct diseases that follow different clinical treatments. To gain a better perception into the problem of cancer classification different systematic approaches are taken depending on global gene expression analysis. The expression levels of genes are known to contain the keys to study fundamental problems which are related to prevention, cure of diseases with biological evolution and drug discovery (Azuaje *et al*, 2000)

Microarrays and SAGE are two recent technologies for measuring the thousands of genome-wide expression values in parallel. The first one, which consists of c-DNA microarrays and high-density oligonucleotide arrays, measures the relative levels of mRNA surplus between different samples, while the latter measures the absolute level. DNA microarray has been used by researchers to identify genes involved in metastasis. Researchers investigated the colonial relationship of 22 liver tumor foci from six patients through gene expression profiling by cDNA microarray containing 23,000 genes. This study aimed at identifying metastasis genes for liver cancer. They were able to identify a total of 63 genes (39 known genes and 24 expressed sequence tags) up-regulated and 27 genes (14 known genes & 13 expressed sequence tags) down-regulated in metastasis modules compared to primary tumors. Microarray based expression profiling has been used for studying metastasis in osteosarcoma, colorectal tumor & brain cancer. Microarray analysis also helped in identifying potential biomarker finding of lung, oesophagal, and colorectal cancers. Stanford University is heading a competing method for microarray synthesis. Information can be found at their Stanford Microarray Database. Extensive study reveals challenges with cancer classification problems like available public gene expression data for cDNA microarrays is very small, the attribute domain or the number of genes, of the data is enormous (therefore, developing an effective and efficient classification algorithm for cancer classification is not an easy task), presence of noise inherent in the data set (makes accurate classification of data difficult). Both Accuracy & Biological relevancy are important in cancer classification (Azuaje *et al*, 2007; Berns *et al*, 2000).

C. Current & Future Scope of Targeted Therapies for Cancer

They are new forms of treatment to inhibit specific molecular targets namely altered or deregulated proteins. First of all, it is essential to identify and quantify protein targets in tumor tissues for the reasonable use of such targeted therapies. The main objective of targeted therapy (basically drugs) is to target particular responsible molecules for malignant tumor growth & progression with minimal side effects and more accuracy. Its goal is to cure the cancer, refrain it from developing or spreading caused by cancer. This paper includes present & future scope of different targeted therapies as mentioned below:

Antibody targeted therapy: They are monoclonal antibodies and are used to target antigens found on the cancer cell surface like trans-membrane receptors or extracellular growth factors. They can be conjugated to radio isotopes or toxins to grant specific delivery of these cytotoxic agents to the targeted cancer cells.

Monoclonal antibody has been generated & it is well-tolerated & effective for different cancer target therapy and it has been approved by FDA (USA). Several issues must be considered in antibody target therapy like choice of target antigen, immunogenicity of antibodies, half-life of antibody, penetration into solid tumors and ability of antibodies to recruit

immune effectors functions. The first antibodies studied were murine, rabbit, or rat proteins. Patients often generated antibodies to these foreign antigens, are often referred to as HAMA (human anti-mouse antibody) or HARA (human anti-rat or rabbit antibody). The host antibody reduces the efficiency of therapy either by prematurely clearing the treatment antibody or restricting the possibilities for future immunotherapy. The HAMA or HARA responses can cause adverse events such as serum sickness and anaphylaxis (Dvorak HF, Nagy JA, Dvorak AM *et al*, 1991).

Solid tumors are quite heterogeneous and therefore difficult to target them completely as during targeting them; smaller recombinant Moab structures like single thread antibodies should be able to penetrate into the tumor with higher efficiency than the parental antibody. One promising approach to solid tumors is to target the tumor microenvironment and endothelium of tumor blood vessels in particular, because several tumor endothelial markers are well characterized (Dvorak HF, Nagy JA, Dvorak AM *et al*, 1991; Shockley TR, Lin K, Nagy JA, Tompkins RG, Dvorak HF, Yarmush ML, 1991).

Targeted therapy by Small molecules: They are capable of penetrating the cell membrane and interacting with targets inside a cell. Small molecules are generally designed to communicate with the enzymatic activity of the target protein without causing any detrimental side-effects. Small molecule inhibitors of protein kinase are important for studying target therapy. Protein kinase mostly targeted for drug development are plasma membrane related like tyrosine kinase. The first kinase inhibitors were developed in the early 1980s by Hiroyoshi Hidaka and Naphthalene sulphonamides was developed as antagonists of the calcium-binding protein calmodulin. There are more than 30 such agents in clinical trials now and the most well-known small molecule inhibitors are glivec and gefitinib (Swen Hoelder, Paul A. Clarke, Paul Workman *et al*, 2012).

Ligands targeted therapy: Most cancer cells exhibit many common features with the normal host cells from which they are derived. Therefore, it is a challenge in anticancer chemotherapeutics. To overcome this, unique molecular targets needs to be identified that would distinguish them from normal cells. It can lead to increased toxicities in normal tissues like bone marrow, cells at gastrointestinal tract and hair follicle tissues and to avoid the side effects to normal tissues.

We often provide sub-optimal doses of anti-cancer chemotherapeutics that result in eventual failure of therapy. The selective dosage level of an anticancer drug can be increased by either increasing the amount of the drug, reaches the cancer tissue or decreasing the density of drug that reaches the normal tissues. so, ligands-targeted therapy leads tumor specificity and reduced toxicity and shows promise in the development of noble therapies for cancer. The Ligands targeted therapy can pass higher doses of a drug to the tumor targeted cells and may cross over obstacles presented by cytotoxic chemotherapy (Gibbs, W. Wayt *et al*, 2003; Han-Chung Wu1, De-Kuan Chang, and Chia-Ting Huang *et al*, 2006).

Enzyme Iinhibition therapy: Drugs like Enzyme inhibitors inhibit enzymes that signal for cancer cells to grow. Inhibition stops growing & spreading of cancer cells. The tumor is not getting smaller means its abnormal growth has been interrupted where a regular chemo can give a better chance to work. Slowing or stopping out-of-control growth rate of cells may also help people live longer, without adding other drugs. Enzyme inhibitors are called by different names based on the enzymes they block (Gibbs, W. Wayt *et al*, 2003; Han-Chung Wu1, De-Kuan Chang, and Chia-Ting Huang *et al*, 2006; G Roti ,K Stegmaier *et al*, 2012):

- Tyrosine kinase inhibitors
- mTOR inhibitors
- Proteosome inhibitors
- Growth factor inhibitors
- Signal-transduction inhibitor
- Multi-targeted kinase / multikinase inhibitor (blocks many different enzymes).

Hormone therapy: Prime growth factor of normal cell are hormones. Although cancer cells lose some of the general responses to growth factors, but some cancer cells still require hormones to grow up. Hormone therapy for cancer attempts to starve the cancer cells. This is usually done with drugs that block the mechanism of the hormone, besides some drugs can block synthesis of the hormone. Drugs block the binding site for hormone to slow the growth of these cancers. Research proves that it is effective for prostate & breast cancer (Han-Chung Wu1, De-Kuan Chang, and Chia-Ting Huang *et al*, 2006; G Roti ,K Stegmaier *et al*, 2012).

Angiogenesis inhibitor: Vasculogenesis & Angiogenesis are two tightly regulated processes for vascular system development. Angiogenesis is active under specific physiological conditions in healthy adults where the vasculature can be aberrantly activated to create new blood vessels during pathological conditions such as cancer and chronic inflammation. Making of new blood vessels is a normal physical process with the growth & development of human body. Their formation rate becomes stagnant in adults but never gets stopped as new blood vessels help in healing wounds & repairing damages.

But in a person with cancer, same process creates new blood vessels to provide tumor its own blood supply through which nutrients can flow to cancer cells that can be controlled or stopped by Angiogenesis Inhibitors by formation of new blood vessels for tumor cell growth. Such type of drugs work by blocking vascular endothelial growth factor (VEGF) which is created by some tumors. The vascular endothelial

growth factor proteins can attach to the VEGF receptors of blood vessel cells which causes new blood vessels to form around the tumors. If this process gets blocked it will prevent angiogenesis (Veggeberg, S *et al*, 2002; Alicia S. Chung, John Lee, Napoleone Ferrara *et al*, 2010).

Targeted therapy by virus: Research at Illinois University shows Targeted oncolytic poxviruses or vaccine viruses have a great future for targeting, attacking and eradicating cancer cells due to their enhanced tolerability, efficacy & minimal side-effects which are limited to flu-like symptoms.

The strain of pox virus has been used safely in millions of people as part of a worldwide vaccination program. The biology of pox viruses make them proper nanotech therapeutics to attack cancer with features incorporate 1) rapid and motile spread in tumors , 2) intravenous stability 3) therapeutic trans-gene-arming capacity & 4) antidotes are available to maximize safety. Oncolytic poxviruses can not only vaccinate the cancer patients and induce an anti-tumor immune response that additionally can cause acute tumor lysis through viral replication and cell destruction with disruption of the vasculature to the tumor. After penetrating into tumor cells, virions replicates rapidly and efficiently. Infectious virions are discharged around six hours after tumor cell infiltration, and tumor cell destruction occurs within approx. 24 hours, up to 10,000 particles released upon cell lysis and spread within tumors and to distant metastases [Jennerex Biotherapeutics].

Researches have capitalized on vaccinia viruses to create safe therapeutic viruses which can broadly infect tumors via rapid replication & spreading with the induction of long term anti-cancer immunity.

Apoptosis Iinducing drug therapy: Some targeted therapy forces cancer cells to die by changing proteins within victim cells. They are known as apoptosis-inducing drugs. Normal cancer treatments like chemo or radiation kills normal cells along with cancer infected cells. Targeted drugs are different in this context- they aim at correct victim cancer infected cell and lead it to apoptosis state (Gibbs, W. Wayt. *Et al*, 2003; Han-Chung Wu1, De-Kuan Chang, Chia-Ting Huang *et al*, 2006).

A. Cancer Vaccines

The main objective of vaccination is to build a resistance in body against tumor Specific Antigen (TSA) and Tumor Associated Antigens (TAA). Peptide vaccines was developed based on epitopes. Epitopes based vaccines are designed on the basis of B and T lymphocytes. The T cell epitopes consists of peptide fragment and B cell epitopes usually made of proteins, lipids or nucleic acids or carbohydrates. Peptide vaccines against various types of cancer had been developed due to their easy production, stability and no infectious materials present in them.

Cervical cancer has affected around 500,000 women in developing countries due to genital infection of human Papilloma virus. It is an DNA virus that infect basal epithelial cells. HPV vaccines are prepared from virus like particles using the recombinant DNA technology. HPV vaccine gives protection against the risk types HPV 16 and 18 and the Quadrivalent HPV vaccine also gives protection against risk genotypes like 6,11,16 & 18. Compared to all other treatment modalities like chemotherapy, radiotherapy and adaptive immunotherapy, a vaccine based immune response against the tumor may be the only way to prevent the cancer for lifetime. Prophylactic vaccines are only in research mode in animal models and far away from the application in human subjects but the development of prophylactic vaccine would definitely play a greater role in prevention of cancer.

One of Several mechanisms had been delivered to instruct Antigen presenting cells like dendritic cells to enhance immune response like a) infecting dendritic cells with yeast, viral, bacterial vectors, b) by Pulsing dendritic cell with protein, peptides and capturing dendritic cells with tumor cells or tumor lysates, c) by Transfecting dendritic cells with DNA, RNA.

Therapeutic cancer vaccines uses patients own immune system in pointing and removing cancer cells. Tumor Specific Antigen vaccine is difficult. Tumor Specific Antigen found in tumor cells, varies from individual to individual due to the somatic mutations on the protein sequence, thereby personalized immune response to individuals system is needed. Tumor Associated Antigens discovered in tumors with similar analysis and of different origin are weakly immunogenic because of tolerance to self antigens obtained from immune system in developmental stages, so targeting them for vaccine production is difficult. Nan-particles had been recently used as the delivery system for the Tumor Associated Antigen and adjuvant to dendritic cells so as to elicit an effective immune response against the tumor cells.

Algorithm proposals based on T cell epitope have been set up using the peptide sequence binding data with experimental binding data based on affinity using any of the methods such as Motif based systems, Hidden Markova models, ANN, Quantitative-structure based relationships, structure based approach to T cell epitope prediction and support vector machines. One of the major problems in the development of cancer vaccine is insufficient Tumor specific antigen and lack of the Tumor Associated Antigen, which could be overcome by the invention of Cell based vaccines, RNA, DNA based vaccines, vector based vaccines and peptide based vaccines [Shanju Sankar, Sangeetha K Nayanar, Satheesan Balasubramanian *et al*, 2013].

B. Discovery of Novel Genes

The purpose of this approach is to identify transcription factor of novel genes (tumor suppressor genes & oncogenes) & regulate transcription directly or indirectly to target genes, identified with microarray

related expression profiling. The P53 is a transcription factor of tumor suppressor gene that induces growth arrest/apoptosis by transcriptional activating its target genes.

C. Epigenetic

The aim of Epigenetic research is to make out heritable gene regulation that is not directly caused by changes in the DNA sequence. Epigenetic regulation plays a key mechanism for cellular differentiation and cell fate decisions. Stochastic (random / non-deterministic) and environment-induced epigenetic defects are known to play a major role in cancer and ageing, and they can also contribute to mental disorders and autoimmune diseases. Epigenetic analysis of embryonic stem (ES) cells has started to uncover the basic circuitry of mammalian development and in cancer research, epigenetic opens up novel approaches for early diagnosis and treatment. Promoter prediction is an important topic in bioinformatics since the early 1990s that can be regarded as the first attempt to predict epigenetic states from the DNA sequence.

A considerable amount of bioinformatics research has been committed to the prediction of epigenetic information from characteristics of the genomic sequence. Such predictions serve two purposes- One, accurate epigenome predictions can substitute for experimental data, to some degree that is particularly relevant for newly discovered epigenetic mechanisms and for species other than human and mouse. Two, prediction algorithms build statistical models of epigenetic information from training data and can therefore act as a primary step toward quantitative modeling of an epigenetic mechanism.

Different epigenome prediction (Interfering epigenetic states from the DNA SEQUENCE) methods are proposed on which research is going on (Christoph Bock, Thomas Lengauer *et al*, 2008):

- Promoter Prediction
- CpG island prediction
- DNA methylation prediction
- Prediction of nucleosome positioning

D. Spindle Inhibitor

A class of drugs called spindle inhibitors (stop the synthesis of microtubules) stops cell replication early in mitosis. Experts have suggested that in order to overcome the side effects, efforts should be aimed at finding new and efficacious anticancer drugs based on identification of the cell cycle targets that interfere with the cell cycle regulatory pathway (Gibbs, W. Wayt. Et al, 2003; Han-Chung Wu1, De-Kuan Chang, Chia-Ting Huang *et al,* 2006).

3. Challenges

Although targeted therapy drugs don't directly affect the body the same way as standard chemo drugs do, yet they still cause side effects that depend largely on what the drug targets. Some drugs target substances that are more common on cancer cells and can be found on healthy cells. So these drugs may affect healthy cells, too, causing some side effects and it happens when drugs attack more than one target.

Also, drugs that act as angiogenesis inhibitors that disrupts new blood vessel formation all over the body, not just those near the cancer. This can lead to other side effects, as well like change in skin feel, rash, dry skin, itching, red sore cuticles around the nails, hand-foot syndrome, change in hair growth, and change in hair or skin color, change in and around the eyes, high blood pressure, problem with blood clotting, problem with wound healing, diarrhea, nausea & vomiting, constipation, fatigue, low blood cell count, trouble breathing etc.

4. Death Statistics

Cancer is the most responsible disease for death as researched by WHO and they have prepared below death count statistics. There are more than 200 types of cancer but we have concentrated on most popular cancer types. More than 60% of world's total new cases occur in Africa, Asia and Central and South America per annum. These regions account for 70% of the world's cancer deaths by oesophagal cancers.

Table 1 Statistical data by WHO

S. No	Cancer	Death as per 2012 WHO record
1	Lung	1.59 million
2	liver	745K
3	Stomach	723K
4	Colorectal	694K
5	Breast	521K
6	oesophagal	400K

Conclusions

As per WHO, among women the 5 most general cancer diagnosed are breast, colorectal, lung, cervix, and stomach cancer. Around one third of cancer deaths are due to the leading behavioral and dietary risks like high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, alcohol use as per WHO studies. The Early diagnosis of cancer increases the chance of survival so test for cancer should be conducted on regular basis. Regular genetic tests & cancer risk counseling is required if any family has strong cancer history. Genomic technology has made drug target analysis effective with the help of bio-informatics. Many new molecular targeted cancer drugs have gained approval over the last few years and have improved and extended the lives of a large number of patients.

However, the discovery and development of new targeted drugs have some drawbacks like it is still frustratingly slow and have high failure rates, mainly in

late stage clinical trials leading to a constant challenge. Our understanding of the genetics and molecular basis of cancer initiation and malignant advancement has improved enormously, with the help of bio-informatics. It has opened up astounding possibilities for selective therapeutic targeting to exploit addiction, dependency and vulnerabilities in cancer cells. Scientific and technological breakthroughs have enabled faster efficient drug discovery, together with more refined clinical trials. Progress has been made in large scale genetic and molecular characterization of cancer to accelerate bringing new personalized drugs to cancer patients worldwide.

The link between cell cycle & chromatin control has led to untie the mechanisms to explain abnormalities of tumor cells that can be targeted to design new drugs. Targeting chromatin control may yield valuable information.

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