We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Impact of Sequencing Human Genome on Drug Design to Treat Oral Cancer

Abdul Hameed Khan

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.80231>

Abstract

Of all the known cancers, oral cancer is the most preventable and it is the second most deadly cancer after the breast cancer. Out of 609,640 deaths of overall cancers, 13,500 died of oral cancer. In spite of this enormous increase in loss of life, there are no useful drugs to treat oral cancer. Sequencing human genome identifies with precision and accuracy the specific mutations responsible for causing oral cancer. In this chapter, a novel approach to design drugs to attack mutated genes in squamous cell carcinoma responsible for causing oral cancer is proposed. Alkylating aziridines attack single-stranded DNA shutting off genes. Using dinitrobenzamide dye as a carrier for aziridine, we successfully made a novel class of drugs (CB 1954) which shuts off gene of a solid tumor, Walker Carcinoma 256, in rats. We translated the animal work in humans by using quinone as a carrier for aziridines making AZQ (US Patent 4,146,622) for attacking glioblastoma for treating brain cancer in humans. We propose to search for a carrier for aziridines to attack squamous cell carcinomas to treat oral cancer. Ethical issues are discussed. Since tobacco smoking causes oral cancer, it is the most preventable disease.

Keywords: nicotine, nicotine N-oxide, aziridines, quinone, squamous cells carcinoma, glioblastoma, melphalan, pharyngeal carcinoma, nitrogen mustard, adeno-carcinoma, nitro-benzamide, nucleotide, codon, oncogene, carbamate, mitochondrial cells, AZQ

1. Historical background

Use of tobacco products will kill you. It does not matter either you smoke tobacco or you use a smokeless tobacco or nicotine patch or smoke e-cigarettes. Last year, 13,500 smokers died of oral cancer. Out of 450,000 new cancer cases diagnosed this year, 37,000 new cases will be diagnosed for 13 different types of oral cancer which include cancer of the lips, salivary glands,

buccal cavity, pharynxes, oropharyngeal, laryngeal, nasopharyngeal, hypopharyngeal, etc., and respiratory tract and finally lung cancers. It was Professor Ross of London University who developed oral cancer drug called melphalan (phenylalanine moiety is the carrier for the nitrogen mustard) to treat pharyngeal carcinoma [1]. Melphalan cross-links both strands of DNA shutting off genes. Statistics is worst for smokers. Over 155,700 Americans died of lung cancer last year and over 222,500 will be diagnosed this year. Compare the US mortality, the worldwide figure is ten times as high. While squamous cell carcinoma is responsible for causing oral cancer, adenocarcinoma is one of the deadliest forms of lung cancers. Most patients die within 5 years of their diagnosis. Tobacco smoking kills faster.

For years, we have been trying to answer three important questions: What is cancer? What causes cancer? And how could we diagnosed treat and prevent cancer. For this chapter, I have divided my presentation in three parts. First, I will provide some historical background; second, I will describe how efforts are being made to develop novel drugs to treat cancer; and finally, I will share some ethical problems we are facing today.

Today, we must tell all smokers in the world that tobacco smoking whether it is the regular cigarette or e-cigarette or nicotine patch or the use of smokeless tobacco, and all tobacco modifications contains nicotine, and it is the nicotine which causes oral and lung cancers. If you are diagnosed with lung cancer, you are most likely to die within 5 years. Tobacco companies have known this fact for more than half a century. In the late 1930s, German scientists discovered the fact that tobacco tar causes lung cancer [2]. When Adolf Hitler was elected as the Chancellor of Germany, he authorized 100,000 Deutsch Marks to a science research institute in Munich to isolate and identify all components of tobacco tar which causes lung cancer. He ordered all his uniform officers including pregnant women not to smoke tobacco. These facts are still available in the National archive. Tobacco companies are aware of these facts. For the past 60 years, tobacco companies have lied to us. No one was spending more money on tobacco research than tobacco companies themselves and yet when they confirmed German's finding in early 1960s that smoking causes lung cancer, they hid the fact from general public. For almost 50 years, the greedy tobacco companies exposed millions of smokers around the world with thousands of deadly chemicals with disastrous consequences. Up to this time, more than 6000 toxic chemicals have been isolated and identified from tobacco tar and 60 of those chemicals are deadly and known to cause cancer. The main constituent of tobacco tar nicotine is one of the most addictive chemicals isolated and is probably more addictive than opium. In India, the second most populous nation on Earth, last year, of the 1 million people who died of cancer, almost a quarter million died of lung cancer. Last year alone, about 3 million people died of lung cancer around the world. By the year 2020, smoking will most likely kill about 10 million people worldwide. Most smokers will prematurely die in their middle ages, cutting 20–25 years from their life expectancy. If we examine the death rate during the last 48 years, from 1950 to 1998, you will be shocked to learn that about 50 million men and 10 million women died of cancers by tobacco smoking and related illnesses.

The tobacco companies have flooded so much money in the Congressional and Senatorial election campaign, in the US alone, the US senate had voted not to ban tobacco sale to school-aged teenagers. Greedy tobacco executives won the day and needy millions around the world lost the day. Now, tobacco companies will be exporting over 150 billion cigarettes per year to

the Asian continent particularly targeting India and China where almost half of the world's population live. It is a sad day for all of us. The young people, who are getting addicted to smoking today, will start dying 20 years from now. Of all cancers, tobacco smoking remains the single major preventable cause of cancer deaths in the world.

2. What is cancer?

Cancer is a very ancient disease. The early Greek coined the term cancer. They thought that this unusual disease spread like a crab so they were the first to call it a cancer or crab. The answer to the first question is that cancer is the abnormal growth of the normal cells due to accumulation of mutations over lifetime.

Our body is made of about 100 trillion cells. These cells are constantly being replaced by similar young fresh cells. For example, your white blood cells (WBCs) live for about 120 days. They are replaced by other white blood cells. Liver cells make more liver cells and replace old liver cells with new liver cells; lung cells make new lung cells. When this normal cell regulation breaks down, liver cells do not produce liver cells; they produce altered cells called mutant cells. Mutation is caused by exposure to radiations, chemical pollutions, viral infection, or genetic inheritance. The changed cells grow much faster than the normal cells and they form a lump, we call it a tumor. As tumor grows, it spreads over to the nearby blood circulating vessels. When tumor grows over these vessels, it draws more nourishment than the normal cells. Some of the tumor cells break off and split and plunge in the blood stream. These live tumor cells flow in the blood stream and travel wherever the blood goes. If the blood goes to the brain, the free floating tumor cells deposit in the brain and start growing as the brain tumors called metastasized cells. Although the cancer may have started in the lungs from smoking, it may spread to the brain or to other organs of our body. Only cancer cells spread this way; we call these migratory deposits metastasized cells, and only metastasized cells invade neighboring organs causing spread of cancer. We have no cure against metastasized cancers.

How many kinds of cancers do we have? There is a cancer for every tissue type in our body. There are 220 types of tissues in our body. Every tissue type in our body can suffer from cancer because every tissue in our body is replaced cells by cells with younger cells; they can either be replaced by normal cells in healthy people or they can also be replaced by abnormal or mutated cells when we get cancer. Would you believe that even your bones are being replaced? You could also get bone cancer.

3. What causes cancer?

Short answer is exposure to radiations, chemical pollutants like smoking, viral infection, or genetic inheritance. This is the most important question and I want to spend more time explaining the causes of cancer. This is where we spent most of the \$30 billion during the lasts 30 years trying to understand how normal cells become abnormal. If we understand how they become abnormal, we should be able to treat them.

In 1971, President Richard Nixon declared war on cancer and released hundreds of millions of dollars for cancer research. He challenged Americans, the way President John Kennedy had challenged Americans a decade earlier to land men on the moon and bring them back safely. Although both presidents had great ideas, but there was a major difference.

At the time President Kennedy made that famous speech in the US congress, most of the engineering problems had already been worked out. For example, we already knew the engine thrust and its lift off power needed to leave Earth's gravity. It was calculated to be 7 miles/minute or burning fuel in the absence of air to excel the spacecraft. These engineering problems had already been solved and the knowledge was already available. Only money and trained men power were needed to build the spacecrafts. Within 10 years of that speech, President Kennedy's dream was turned to reality. On July 20, 1969, Americans landed men on the moon and brought them back safely.

But when President Nixon declared war on cancer, money was made available, but the knowledge was not there. We did not exactly know the inner working of a single living cell and how a normal cell functions, and we did not know why the normal cell becomes abnormal or cancerous. Some basic knowledge was available. In 1953, the big discovery was made in Cambridge University, England. Crick and Watson had determined the double helical structure of the genetic material DNA and postulated how the living cells divide and cells grow, and they were awarded Nobel Prize for their discovery [3]. Armed with this knowledge, we were ready to understand how a normal cell functions. Crick and Watson also opened the doors for the Nobel Prize Club. Every year, since then, a new Nobel Laureate was added to the genetic club. Soon after, Marshall Nirenberg broke the genetic code and unlocked the secret of life by showing that only three nucleotides code for an amino acid, the building block of protein. The remaining codes were deciphered by Salvador Ochoa, followed by an Indian Scientist, Govind Khorana who shared the Nobel Prize with Walter Gilbert.

After President Nixon's speech, it had taken about 20 years to understand how a normal cell functions and how it becomes abnormal by exposure to radiations, chemical pollutants, viral infection, or genetic inheritance. Let me summarize below the work of a dozen Nobel Laureates: To understand cancer, you have to understand how a normal cell functions and how it becomes abnormal. We made step by step progress over the past 30 years. First, you might ask why we study a single cell and why a single cell is so important. The fact is our life begins with a single cell. You and I are the loving union of our parents. Both parents contribute half the genetic material to each cell. Our father contributed one sperm and our mother contributed one egg. When the egg and sperm join together, we were conceived. We grow as a single cell in our mother's womb. This single cell has a set of complete instructions to construct us within in 9 months. By the time we are grown up to adulthood, that single cell makes over 100 trillion copies of itself.

During replication, if we introduce slight error in the nucleotide sequence called mutation by exposing to radiations or chemical pollutants including nicotine, viral infection, or genetic inheritance, the error is copied in every other cell. At this stage, pregnant mothers should be extremely careful what they eat and what they drink and to avoid exposing the fetus from the secondhand smokers and should stay away from smokers as far as they could. Every cell in our body has a complete library of our genome and carries complete instructions to make our brain, our nose,

our ears, our arms, and our legs. Although each cell carries complete instructions to make all the organs, not all cells make all organs, but each cell begins to receive specific instructions to take a different role as it begins to make more copies. We call this process the cell differentiation.

3.1. Mutations

Damage to DNA nucleotide called mutations produces disastrous change in the information molecules. As I said above, mutations are caused by exposure to radiations, chemical pollution, viral infection, or genetic inheritance. In addition to hundreds of chemicals isolated from tobacco tar, the most potent carcinogen is nicotine N-oxide. As mutation begins in a single biological molecule, it is called a point mutation. To study changes in genetic profile of a single cell, we examine the entire genome of the same single cell. As cells grow rapidly, other mistakes in DNA replications are most likely to occur such as deletion, insertion, or inversions of nucleotide sequence. Such additional mutations are responsible for causing major diseases. Before the completion of Human Genome Project, NCI screened thousands of chemicals, plant extracts, and animal extracts for their antitumor activity. By trial and error, one in several thousand turned out to be useful. There was a need to make a rational approach to design drugs.

In 1990, United State Congress authorized 3 billion dollars to NIH to decipher the entire human genome within 15 years that is the total genetic information that makes us human called the Human Genome Project. Thousands of scientists from 6 industrialized nations and 20 biomedical centers joined our effort, and within 13 years, the entire human genome was deciphered and published in the scientific journal *Nature* [4–8] and linked to website. If you have an access to a computer keyboard, you have access to all that information.

On April 3, 2003, we read the Human Genome, the entire book of life. We found that less than 2% of the genome codes for proteins and the rest is the noncoding region which contains switches to turn the genes on or off. We can cut and paste genetic letters in the noncoding region which could serve as markers and which has no effect, but a slight change in the coding region makes a normal cell abnormal or cancerous.

A single cell is so small that we cannot even see with our naked eyes. We have to use a powerful microscope to enlarge its internal structure. Under an electron microscope, we can enlarge that one cell up to nearly a million times of its original size. Under the electron microscope, a single cell looks as big as our house. There is a good metaphor with our house. For example, our house has a kitchen, the cell has a nucleus. Imagine for a moment that our kitchen has 23 volumes of cookbooks which contain 24,000 recipes to make different dishes for our breakfast, lunch, and dinner. The nucleus has 23 pairs of chromosomes which contain 24,000 genes which carry instructions to make proteins. Proteins interact to make cells; cells interact to make tissues; and tissues interact to make an organ and several organs interact to make a man, a mouse, or a monkey. In every cell of our body, we carry 16,000 good genes, 6000 mutated genes responsible for 6000 diseases, and 2000 pseudogenes that have lost their functions, during evolutionary time.

Our entire book of life is written in four letters, and they are A (adenine), T (thymine), G (guanine), and C (cytosine). These four chemicals are called nucleotide and they are found in the nucleus of all living cells including humans, plants, and animals. Instruction in a single

gene is written in thousands of AT/GC base pairs that are linked together in a straight line and we call them DNA (deoxyribonucleic acid—Nobel prize was awarded to Crick and Watson for describing the double helical nature of the DNA structure). When thousands to millions of AT/GC base pairs contain information to make a single protein, we call that portion of AT/GC base pairs a gene (Nobel Prize was awarded to Khorana and Gilbert for making a functional gene). The genes begin to function with a start codon (AUG) and stop working at the following three stop codon: UGA, UGG, and UAG. After the stop codon, no more amino acids are added and DNA synthesis stops. If we count all the AT/GC base pairs in a single cell of our body, we will find that there are 3.2 billion pairs present in every cell. The entire AT/GC sequence of 3.2 billion base pair is called the human genome or the book of our life which carries total genetic information to make us.

We deciphered all 46 chromosomes. What surprises us most is that our genome contains 6,400,000,000 nucleotide bases, half from our father and half from our mother. Less than 2% of our genome contains genes which code for proteins. The other 98% of our genome contains switches, promoters, terminators, etc. The 46 chromosomes present in each cell of our body are the greatest library of the human book of life on planet Earth. The chromosomes carry genes which are written in nucleotides. Before sequencing (determining the number and the order of the four nucleotides on a chromosome), it is essential to know how many genes are present on each chromosome in our genome. The Human Genome Project has identified the following genes on each chromosome. We found that the chromosome (1) is the largest chromosome carrying 263 million A, T, G, and C nucleotide bases and has only 2610 genes. The chromosome (2) contains 255 million nucleotide bases and has only 1748 genes. The chromosome (3) contains 214 million nucleotide bases and carries 1381 genes. The chromosome (4) contains 203 million nucleotide bases and carries 1024 genes. The chromosome (5) contains 194 million nucleotide bases and carries 1190 genes. The chromosome (6) contains 183 million nucleotide bases and carries 1394 genes. The chromosome (7) contains 171 million nucleotide bases and carries 1378 genes. The chromosome (8) contains 155 million nucleotide bases and carries 927 genes. The chromosome (9) contains 145 million nucleotide bases and carries 1076 genes. The chromosome (10) contains 144 million nucleotide bases and carries 983 genes. The chromosome (11) contains 144 million nucleotide bases and carries 1692 genes. The chromosome (12) contains 143 million nucleotide bases and carries 1268 genes. The chromosome (13) contains 114 million nucleotide bases and carries 496 genes. The chromosome (14) contains 109 million nucleotide bases and carries 1173 genes. The chromosome (15) contains 106 million nucleotide bases and carries 906 genes. The chromosome (16) contains 98 million nucleotide bases and carries 1032 genes. The chromosome (17) contains 92 million nucleotide bases and carries 1394 genes. The chromosome (18) contains 85 million nucleotide bases and carries 400 genes. The chromosome (19) contains 67 million nucleotide bases and carries 1592 genes. The chromosome (20) contains 72 million nucleotide bases and carries 710 genes. The chromosome (21) contains 50 million nucleotide bases and carries 337 genes. Finally, the sex chromosome of all female called the (X) contains 164 million nucleotide bases and carries 1141 genes. The male sperm chromosome (Y) contains 59 million nucleotide bases and carries 255 genes.

If you add up all genes in the 23 pairs of chromosomes, they come up to 26,808 genes and yet we keep on mentioning 24,000 genes. The remaining genes are called the pseudogenes. For

example, millions of years ago, humans and dog shared some of the same ancestral genes; we both carry the same olfactory genes. Since humans do not use these genes to smell for searching food, these genes are broken and they lose their functions in humans, but we still carry them. We call them pseudogenes. Recently, some Japanese scientists have activated the pseudogenes; this work may create ethical problem in future as more and more pseudogenes are activated.

The above DNA nucleotide bases constitute the genetic map of the normal human being; what makes them abnormal and makes us sick is the mutation in the coding regions of the genome. Now, we can examine the tumor genome of the oral cancer patients to identify specific mutations responsible for causing the disease. As I said above, less than 2% of the genome codes for amino acids. Slightest damage to the coding regions of the four nucleotides A, T, G, and C either by radiations, chemical pollution (from tobacco tar), genetic inheritance, or viral infection or by insertion, deletion, or inversion of the nucleotide bases code for wrong or abnormal amino acids resulting in diseases.

Although you and I are both human and yet no two individuals look the same because the AT/GC base pairs in each of us are arranged slightly differently, a difference of one nucleotide in a thousand base pair. The amazing fact is that out of 3,000,000,000 AT/GC base pairs, only 3 pairs of AT/GC code for a single amino acid, the building blocks of protein, called a codon (Nobel Prize was awarded to Nierenberg and Ochoa). Codons are the most important collections of AT/GC base pairs because they have correct instructions to make the right amino acids (there are only 20 amino acids; they randomly combine to make a protein). Thousands of amino acids make a protein and thousands of proteins make a cell; billions of cells make a tissue and hundreds of tissues make an organ and several organs make an individual such as you and me. This is how normal cell functions and we begin to grow.

As I said above, the old cells begin to die and they are constantly being replaced by healthy cells. Why do the normal cells become abnormal or become cancerous? Any factor that disrupts the 2% of the coding region of our genome will alter its function by slightly altering its code; an altered codon will code for a wrong amino acid and a wrong amino acid will give a wrong protein and it will make normal cell abnormal. When the functions of codons are disrupted intentionally or unintentionally, we alter the codon's function. For example, intentionally we alter a codon by smoking and unintentionally by exposure to environmental pollution such as chemicals or radiations. Altered codons have wrong information to make wrong amino acids. Wrong amino acids make wrong proteins and wrong proteins make wrong cells and wrong cells grow much faster than the normal cells and become abnormal or cancerous and they form a lump, we call these lumps tumors.

Four factors will disrupt a codon's function. Two are minor such as viruses and inherited oncogenes, and two are major such as radiations and chemical pollutants in our environment. Let me explain how a codon is altered:

3.1.1. *Virus*

HPV causes more than 32,000 cases of cancer including oral cancer every year in the US. It is also very preventable by giving HPV vaccine for children at ages 11–12 which can protect them.

Viruses are not considered germ in the classical sense because they lack the ability to reproduce its progeny independently. To reproduce their own kind, viruses attack DNA of living cells, whether they are humans, animals, or plant cells. Viruses are also fragments of DNA that have the ability to merge in the host cell DNA and become integrated and invisible. They infect (merge) host's DNA and alter its functional machinery in such a way as to make protein and progeny for themselves. Not all viruses are bad. If all viruses kill their host cells, they will die too. Only a handful of virulent viruses destroy host cells. For example, AIDS viruses in human disrupt the codons and cause a unique cancer in AIDS patients called Kaposi carcinoma. Hardly anyone survives that cancer.

3.1.2. *Oncogene*

Oncogenes are also fragments of mutated DNA, but they are always bad. They are complete genes that have the instructions to make a specific bad protein. Such genes are called oncogenes (cancer-causing genes). Our institute's, NIH's, past Director, Dr. Harold Varmus, was the first man to identify an oncogene in humans. For his work, Dr. Varmus shared a Nobel Prize with Dr. Michael Bishop.

From here begins one of the most exciting stories that explain the causes of cancer. In the early 1990s, some scientists in England were studying cancer-causing viruses. When scientists injected cancer-causing viruses to animals, they found that sometimes viruses grow and other times they do not. On close examination, they detected a background protein in all cells. Whenever the background protein is absent, cancer-causing viruses grow and the animal develops cancer. Whenever the background protein is present, cancer-causing viruses will not grow. They called it the background Protein 53 or (P53). They identify the gene that makes P53 protein and they named P53 gene. Since cancer is suppressed in the presence of P53, scientists named P53 as cancer suppressor proteins. When a normal cell is damaged, the surrounding cells grow to make P53 protein to repair the damage. When healing is complete, the P53 protein stops the cells from further growth. If there is a mutation in the P53 gene, P53 loses its function and the cell growth will not stop and grow continuously and become cancerous.

As I said above, that gene is a collection of codons and each codon is made of three pairs of AT/GC. Hundreds of thousands to millions of AT/GC base pairs combine to form gene-53. Now, we know that the codon in P53 is sensitive to mutation by chemicals, radiations, viruses, and oncogenes. If you cause a slight defect in the codon by altering one letter of AT/GC base pair, the entire P53 gene becomes defected. Defected P53 cannot produce background protein that suppresses cancer. In the absence of P53 protein, patients develop cancer. The lesson we learn is that if a single letter of this four-letter AT/GC base pairs is altered by virus or by chemicals or by gene or by radiation, first a single normal cell becomes abnormal or cancerous. Over many replications, the mutated cell becomes cancerous. Repeated exposure to chemicals such as smoking tobacco several times a day, you could alter a single letter of AT/GC base pairs. If a single cell becomes defected, it will multiply and accumulate and an entire organ becomes cancerous. When the defected organ cannot function, we become ill.

Unfortunately, cancer is not localized at one place for long (we could have cut out the defected organ and throw it away); it spreads or metastasizes as I described above. Once it is

metastasized, the other organs lose their function; with too many defected organs, a patient cannot survive for long. When we examine the internal structure of tumors of a dead patient, we find it mostly consists of abnormal cells that have been altered.

Let me tell you what you can do to protect yourselves and what we can do to help you? If you want to protect yourselves from oral or lung cancers, stop taking tobacco in any form and in any kind. The best way we can help you is to pursue as vigorously as we could do to find other sensitive genes and try to replace them with healthy genes by a method called gene therapy. Gene therapy could work if a single gene is mutated. Unfortunately, several genes are mutated in oral lung cancers. Gene therapy fails, but drug therapy works. From here my work begins. Professor Ross and I design drugs to shut off multiple defected genes.

As I said above, although the book of life is written in 3.2 billion AT/GC base pairs, about 2% of the AT/GC base pairs contain 24,000 genes (specific instructions to make proteins), the rest of DNA is called the junk DNA. As I said above, it is not garbage that we throw away; it contains important gene switches, promoters, enhancers, etc. We keep it because someday we might be able to find out what additional information it may provide about us. We carry all 24,000 genes in every cell of our body, but less than 6000 genes are probably bad genes (mutated) that are linked to a variety of genetic defects leading to 6000 different diseases. Each of us does not carry all 6000 bad genes, but we do carry a single copy of at least 4–5 bad genes in our genome. They remain dormant because only one parent carries a single copy of a mutated gene. We need both bad copies of bad genes, one from each parent to get sick. We have inherited these bad genes from our parents. Our parents inherited these genes from their parents. One ancestor can pass on the same bad genes to her children and children's children. Therefore, it is a wise idea not to marry within the same family tree. The more intermarriages among the same family members, the more bad genes tend to concentrate among fewer and fewer children of that family.

3.1.3. *Nicotine N-oxide is a carcinogen*

I could take tobacco extract samples to the lab and analyze its ingredients. First, I soak the tobacco in ammonia and extract with chloroform. All nitrogen bases are extracted in chloroform. I could wash the extract with water to remove all water-soluble impurities and dry the extract and distill off the chloroform. I place the residue in long glass column filled with silica gel. I pour a solvent mixture which carries the residue down the column. It is called the separation of different components by chromatography. I shine the UV light on the glass column as the solvent flows down. Hundreds of different bands appear in different colors, corresponding to hundreds of components present in the tobacco tar. The largest band is nicotine. To confirm, I take a little sample from the band and inject in the MS (mass spectrometer: the largest peak corresponds to the molecular weight of the nicotine). I could make the radiolabeled nicotine by adding C-14 diazomethane in sodium hydroxide. The reaction adds a radiolabeled methyl group to nicotine molecule. I inject the radiolabeled methyl nicotine to half a dozen mice. I collect their urine samples separately. Analysis of the urine sample shows that some mice produce no change and others produce a new chemical called nicotine N-oxide. All those mice in which the gene that produces monoamine oxidase was activated developed cancer. If you inject nicotine N-oxide to another set of a dozen mice, they all come down with various cancers. All aromatic N-oxides are carcinogens.

Figure 1. NIH Scientific Achievement Award. Aziridines as single strand DNA binding agent.

Now, you know why Sir Winston Churchill, who smoked cigar all his life, never developed cancer, but film actor Yul Brenner smoked cigarettes and died of lung cancer. If you were to analyze their urine samples, you will find that Mr. Brenner urine contains nicotine N-oxide. The gene monoamine oxidase is activated in Brenner to make nicotine N-oxide not in Sir Winston. While Sir Winston lived, Brenner died.

While I was busy designing drugs, such as AZQ, to shut off genes which cause brain cancers, my colleagues in the other labs at NCI (National Cancer Institute) have isolated hundreds of chemicals from the tobacco tar which contains dozens of carcinogenic chemicals. If you would apply the tobacco tar on the skin of mice, within a few weeks, tumor develops on the skin surface. The major culprit is nicotine which is considered as one of the most addictive chemicals. Some studies showed that it is even more addictive than many known narcotics such as marijuana, opiates, and heroin. Oral cancer (OC) is caused by chemicals released by chewing tobacco. Most football players chew tobacco; they call it smokeless tobacco. Smoking burns tobacco generating even more aromatic amines which are known carcinogens. Nitrosoamines bind to DNA producing mutations.

After the completion of the Human Genome Project, we have identified specific mutations responsible for a specific disease. Now, we design drugs to attack that specific mutation to shut off that gene. The completion of the Human Genome Project has the greatest impact on

developing drugs on a rational basis and for treating various cancers including the oral cancer. The technologies developed during the completion of the Human Genome Project (the tool kits which contain hundreds of restrictions enzymes to cut DNA ligase to join DNA pieces; using these tool kits, we can cut, paste, and copy genes) could be used to treat and prevent oral cancer. The recently completed Thousand Genome Project will pinpoint with precision and accuracy the specific damage to DNA nucleotides responsible for causing various oral cancers. Once the mutated genes on a specific chromosome are identified, we can design drugs to shut off those genes like we designed AZQ (US Patent 4,146,622) to attack brain tumor (for structure, see **Figure 1**).

Once the mutation sites and chromosome numbers are identified, we can diagnose, prevent, and treat the oral cancer either by gene therapy if a single gene mutation is responsible for causing any of the above cancers or by drug therapy if multiple mutations are involved. As I stated above, French Anderson and his colleagues have successfully developed gene therapy for treating SCID (severe combined immunodeficiency) syndrome; we could use the same method to cut and paste and replace the bad gene with the good gene in a virus which is used to infect the WBC obtained from the same patient. After harvesting the infected WBC, the transgenic WBC was injected back in the same patient to treat SCID. It worked and patients fully recovered. Several thousand SCID children are living a normal life. Gene therapy works with a single gene mutation, but not if the multiple mutations are responsible for causing diseases.

4. Historical background for drug design

On the other hand, if cancer is caused by multiple mutations, we could use drug therapy by preventing malignant cell replication developed by Ross by cross-linking both strands of DNA. Using dyes specific to OC cells as carriers for nitrogen mustard, as done by Ross in making Melphalan, we could also develop new class of drugs to attack cancer cells in the other parts of the oral cavity. The bad news is that there have been 13 different forms of oral cancer identified. The good news is that for designing a drug, we have to find a dye which colors one of these tumors. There are hundreds of dyes available for testing. Once we succeed in finding a dye, we could design drugs by using our method by attaching aziridines to attack that specific oral cancer by shutting off mutated genes by binding to a single strand of DNA. What would happen if we succeed and when next-generation sequencers produce inexpensive and fast sequencing genomes is that we could identify all mutated genes on all 13 oral cancers with precision and accuracy and design drugs to shut off those genes.

In the laboratory of the Sir Walter Ross at the Royal Cancer Hospital of London University, England, I was trained to design drugs to attack mutated DNA shutting off mutated genes. Professor Ross had spent all his life working on “Biological Alkylating Agents” and published a series of paper including a book [9–13]. Using the same rationale, I worked with Professor Ross for almost 10 years at London University developing anticancer drugs. Instead of cross-linking DNA with nitrogen mustards, I used aziridines to bind to a single strand of DNA shutting off the genes.

During 10-year period in the Professor Ross' Lab, I made over 120 such drugs to attack a solid tumor called the Walker carcinoma 256 in rats. The most effective drug was called CB 1954 (2,4-dinitrophenyl aziridiny benzamide) (see structure in **Figure 1**). It was 70 times more toxic to the tumor cell [14]. It is the most effective drug ever made against the solid experimental tumor for which I was honored with the Royal Cancer Hospital's Institute Cancer Research postdoctoral award. Why mutated DNA must be attacked is because mutated DNAs code for wrong amino acids which produce wrong protein and cause abnormal growth leading to cancers. The reason why we work on mouse model is that if you would compare the genome of man, mouse, and monkey, they are all mammals and their genomes are very similar. Once you succeed in attacking mouse tumor, it opens gate to attack human tumors. Now, you know how challenging it is to shutting off a mutated gene and how easy it is to introduce mutations in the same gene by smoking.

The good news about smokers is that they could see their own genome on their computers and could also see the progress of mutations of their own genomes before and after smoking. Now, I do not have to tell my best friends not to smoke. All I have to do is to give the two CDs of their genomes and let them see for themselves. Let them see on their own computers and compare the two genomes. First, CD taken soon after their birth and second taken after they become smokers. Before I talk about the sequence-specific tumors of oral and lung cancers, let me share with you how our genome, the book of our life, looks like before you smoke.

The basis of OC is that people who are chewing tobacco or inhaling burning tobacco by smoking (as in India) or chewing betel quid, betel nut, etc. (as in Taiwan) causing major mutations in their genomes producing a host of chemicals which damage the normal function of the cell causing them to become abnormal or cancerous. To understand the molecular basis of cancers, we have to sequence the normal as well as cancer cell genomes for comparison.

To refresh your memory, I repeat. Carcinoma is the most common type of cancer. It begins in the epithelial tissue of the skin, or in the tissue that lines the internal organs, such as mouth, oral cavity, reparatory tract, and lungs. There are 220 different types of tissues in the normal human being. We have sequenced (read letter by letter the entire script of nucleotides, their numbers, and their orders in which they are arranged). After genome sequencing, we can compare with the sequence of oropharynx carcinoma with normal cell from nonsmokers. I am happy to inform you that we just completed the Thousand Genome Project. Now, we can compare thousands of the same mutated sequence a time. We can locate the specific mutations with precision and accuracy. To locate specific mutations, in all oral cavity cancers, similar comparison can be studied in several types of salivary gland cancers including adenoid cystic carcinoma, mucoepidermoid carcinoma, and polymorphous low-grade adenocarcinoma. Tonsils and base of the tongue tissues also develop lymphomas.

Once a mutation is identified in a specific oral cancer, my job begins trying to find a dye which colors these tumors. Once a dye is found, I could attach the toxic groups to shut off their genes. There are hundreds of dyes and there are hundreds of their combinations. With AT and GC, four nucleotides of DNA, I get 64 combinations; imagine how many combinations I get from hundreds of dyes. Fortunately, there are finite combinations. I could find it. Suppose I want to design drugs to treat cancers of the oral cavity. The cancer begins in the epithelial tissue of the skin, or in the tissue that lines the internal organs, such as mouth, oral cavity, reparatory tract, and finally reaching lungs. We have been designing drugs to attack toughest solid tumor like

Walker carcinoma 256 in rats or glioblastoma in humans. It would be much easier to attack carcinoma. And also suppose that I find a single dye which specifically colors a specific carcinoma cell. Now, my work begins to attach aziridines or carbamate or both as we succeeded in making AZQ for attacking brain tumor. Nowadays, I have to submit the research proposal to the Safety Committee (IRB: Institutional Review Board) for their approval. Since I will be using highly toxic nerve gases and nitrogen mustard, the proposal will be rejected.

5. Drug design for treating cancer

Fritz Heber, a German Army officer, worked on the development of chemicals as a weapon of war. He was responsible for making deadly nerve gases and nitrogen mustards. Before the WWI, he was honored with a Nobel Prize for capturing nitrogen directly from the atmosphere by burning the element magnesium in the air forming its nitride. Upon hydrolysis, nitride is converted to its nitrate which is used as a fertilizer. Using this method, we could make unlimited amount fertilizer. Nitrate is also used for making explosive. Soon after the WWI, Heber was charged with a crime against humanity for releasing hundreds of cylinders of chlorine gas on the Western front killing thousands of soldiers in the trenches. When allied forces reached his residence, his son shot himself and his wife committed suicide. Heber went in hiding in Switzerland. After the war, German government got his release as a part of the peace negotiations. Heber returned home to hero's welcome. Although he promised never to work on the nerve gases again, secretly he continued to develop more lethal analogs of highly toxic nitrogen mustards. It was Heber who first made the notorious bis-dichloroethyl methyl amine. Because it smells like mustard seeds, it is called as nitrogen mustard. During the next 20 years, before the beginning of the WWII, hundreds of more toxic analogs of nitrogen mustard were developed. The bad news is that they are highly toxic and the good news is that they shut off genes.

6. Rationale for developing anticancer drugs

Nitrogen mustard was mercilessly used during the WWII by both German and Italian armies against allied forces. Most soldiers exposed to nitrogen mustard were frozen to death. Their blood analysis showed a sharp decline in white blood cell (WBC). Since patients with the cancer of the blood called leukemia showed a sharp increase of WBC, Professor Ross and his group wondered if minimum amount of nitrogen mustard could be used to control leukemia in cancer patients. It was a success. For the following 30 years, Ross developed hundreds of derivatives of nitrogen mustard to treat a variety of cancers. His most successful drugs are chlorambucil, melphalan, and merophan [2–6, 14].

Radiolabeled study showed that nitrogen mustard shut off genes by binding to DNA by cross-linking. At London University, I work for Professor Ross for almost 10 years first as his graduate student, then his post-doctoral fellow, and then as his special assistant. I worked with the deadliest nerve agents such as nitrogen mustards, carbamates, and aziridines developed during Hitler's time for evil purposes. We are converting evil into good. These agents easily

pass through various layers of our skin from ectoderm to mesoderm to endoderm. They easily enter the cell nucleus destroying the beta and gamma cell which develop immunity. Then they enter the nuclear membrane where they find the stem cells. Stem cells differ from say skin cells. In stem cells, all 24,000 genes are functioning and they have not yet differentiated, but skin cells are differentiated and they have shut off all other genes except the skin cell genes.

To the above toxic agents, I attach dyes to attack one of the carcinoma cells. The toxic group is activated by cell's enzyme to produce a positive carbon ion called carbonium ion. It is extremely reactive; it binds to all four nucleotides (AT and GC) which form the DNA. But it preferentially binds to N-7 of guanine killing the stem cells. Professor Ross and I have demonstrated the attack on N-7 of guanine using the radiolabeled study.

If I am accidentally exposed to any of the above toxic agents, I do not die of mustard poisoning; I would be frozen to death. What happens is the following: each cell carries hundreds of mitochondrial cells (mitochondria are foreign cells called prokaryotes without nuclear membrane captured by human cells during the evolutionary period, millions of years ago; they live in symbiotic relationship; to perform daily function, mitochondria provide energy to human cells and human cells provide free food and free housing to the mitochondria) which carry energy-rich phosphate bonds. They produce energy by breaking phosphate bonds in which a chemical called ATP (adenosine triphosphate) is broken down to ADP (adenosine diphosphate) which is further broken down to AMP (adenosine monophosphate). As normal cell grows, an enzyme attaches inorganic phosphate to the AMP regenerating ATP. If mitochondrial cells die, there is no energy available.

As I said above, NIH is the largest biomedical center in the world. It has unlimited facilities (chemicals, equipment, and personnel). Twenty-one thousand best and brightest scientists selected from Ivy League schools work in 26 institutes in more than 3000 labs. I had sent NIH over 120 drugs for NCI screening program [14–16]. During the 3-year period at NIH labs, I

Figure 2. 2004 NIH Scientific Achievement Award presented to Dr. Hameed Khan by Dr. Elias Zerhouni, the Director of NIH during the NIH/APAO award ceremony held on December 3, 2004. Dr. Khan is the discoverer of AZQ (US Patent 4,146,622), a novel experimental drug specifically designed to shut off a gene that causes brain cancer for which he receives a 17-year royalty for his invention (License Number L-0I9-0I/0). To this date, more than 300 research papers have been published on AZQ. The award ceremony was broadcast live worldwide by the Voice of America (VOA). Dr. Khan is the first Indian to receive one of the America's highest awards in Medicine.

Figure 3. His excellency, Dr. A.P.J. Abdul Kalam, the President of India greeting Dr. A. Hameed Khan, discoverer of anticancer AZQ, after receiving 2004, Vaidya Ratna, the Gold Medal, one of the India's highest awards in medicine at the Rashtrapathi Bhavan (Presidential Palace), in Delhi, India, during a reception held on April 2, 2004.

made AZQ (US Patent 4,146,622) and 45 patentable analogs [17–19]. Years later, I was honored for my work on developing AZQ. Almost 20 years later, for translating my work from mouse to man and making AZQ (US Patent 4,146,622) for treating brain cancer, I was honored with the “2004 NIH Scientific Achievement Award,” one of the America's highest awards in medicine (**Figure 2**). I was also honored by the Government of India with Vaidya Ratna (Gold Medal) (**Figure 3**).

In spite of all the risks, fear, and challenges, the zeal and the enthusiasm that I had to design drugs to attack brain cancer like AZQ is not there to treat oral or lung cancers. Do you know why because I know in my heart, the patient once cured will go back to smoking again. He cannot help it; it is an addiction (**Figure 4**) [20, 21].

Figure 4. Gold Medal for Dr. Khan. Dr. A. Hameed Khan, a scientist at the National Institutes of Health (NIH), USA, and an American Scientist of Indian origin, was awarded on April 2, 2004. Vaidya Ratna, the Gold Medal, one of the India's highest awards in medicine for his discovery of AZQ (US Patent 4,146,622) which is now undergoing clinical trials for treating brain cancer.

7. Forbidden area

Fritz Haber was a hero to some for getting a Nobel Prize for capturing nitrogen directly from atmosphere by burning magnesium metal in the air and hydrolyzing magnesium nitride to produce nitrate the fertilizer. To others, he was the greatest criminal because he released hundreds of cylinders of chlorine gas at the Western front killing thousands of soldiers in the trenches. It was Haber who made the nitrogen mustard and its deadly analogs which were used during WWII. Soldiers exposed to nitrogen mustard burns died of a sharp drop in the white blood cell (WBC) count. Since all cancers showed a sharp increase in WBC count, Ross decided to use modified derivatives of nitrogen mustard to control the cancer growth. Ross was successful. He attached amino acid phenylalanine as a carrier for the nitrogen mustard moiety to make melphalan for treating pharyngeal carcinoma. Over a 10-year period, I made dozens of analogs of nitrogen mustards for Ross. The deadliest among them was the phenyl-enediamine mustard. We use these compounds to check the sensitivity of the tumors in the tumor bank. If tumors in the tumor bank become resistant, we have to replace them with fresh more sensitive tumors for testing other compounds.

Proteins in our body are made of 20 amino acids. When Ross made melphalan by using 1 amino acid out of 20, most of my colleagues thought that they could use the following 19 amino acid to treat all 13 forms of oral cancers including lung cancer: alanine, arginine, aspartic acid, asparagine, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine (Ross used this amino acid to make melphalan for treating pharyngeal carcinoma), proline, serine, threonine, tryptophan, tyrosine, and valine.

Aliphatic nitrogen mustards are deadlier than aromatic nitrogen mustards. They decided not to proceed because they do not want to risk their life to save the life of nicotine addicts. If you want to save smokers' life by risking your own, you are welcome to it. I will show you how to make the nitrogen mustard by describing Haber's crudest method. Haber reacted methylamine with ethylene oxide to make 2-bis dihydroxy ethylene methyl amine. It was chlorinated by heating with phosphorus pentachloride in the phosphoric acid. If you noticed a faint smell of mustard seed, Congratulations, you got nitrogen mustard; you are dead. No matter how much precaution you take, after the experiment, if you take an alcohol swab of walls, doors, and knobs and run mass spectra of alcohol extract, you find a line corresponding to nitrogen mustard. If you are exposed to nitrogen mustard and cross the threshold level, your WBC drops sharply and the energy-providing mitochondria die and you are most likely to freeze to death.

Will anyone approve this study, probably no one. Your IRB will reject your proposal; your safety committee will reject it and who will provide the funds for such study. No one.

Tobacco companies are rich and can afford to invest money in curing cancers. Since most of their work is conducted behind closed doors, why not make nitrogen mustard analogs of all other 19 amino acids. Like melphalan, they might find some of these analogs of amino acids of nitrogen mustard to cure oral to lung cancers. They could make money not only by giving cancers by selling tobacco products but also by treating some cancers.

8. Ethical issues

Some of my best friends smoke and most of them are not scientists. Do I tell them not to smoke and keep their friendship? Not a chance. So I tell them experimental facts as gently as possible. My readers are my best friends. All I want is to make them think. If they think, my job as a friend is done.

Scientists in our group are working on different kinds of cancers. As I stated above, there are more than 220 different types of tissues and they could all become cancerous if they are exposed to radiations or chemical environmental pollution. We are all working to cure those cancers. Unfortunately, there is no great enthusiasm for working on either oral cancer or lung cancer. Such diseases are considered self-inflicting wounds. The users of tobacco products are addicted and frequently developed these types of cancers. Many scientists believe that all of us have a free will. We have a right to live and we have a right to die. If you do not smoke or chew tobacco, you will not expose yourself to a host of carcinogens. Some of us believe that you are addicted to nicotine if we cure your oral cancer, you will go back and chew tobacco again. How can we protect you from yourself? If we protect you from yourself, we create a monster. On the other hand, if you are one of those unfortunate persons who inherit a mutated gene, or exposed to secondhand smoking or exposed to radiations or heavy metal particles, you deserve all our help and many of us have been designing drugs for treating oral and lung cancers for these innocent victims.

Author details

Abdul Hameed Khan

Address all correspondence to: hameedkhan111@comcast.net

National Center for Medical Rehabilitation Research (NCMRR), National Institutes of Health (NIH) Bethesda, MD, USA

References

- [1] Ross WCJ, Mitchell M. Melphalan. *Lancet*. **370**(9594):1209-1218
- [2] The anti-tobacco campaign of the Nazis: A little known aspect of public health in Germany, 1933-45. *British Medical Journal*. **313**(7070):1450-1453, 1933-1945
- [3] Watson JD, Crick FHC. A structure for deoxyribose nucleic acid. *Nature*. 1953;**171**:737-738
- [4] *Nature*. 2001;**409**:934-941
- [5] *Nature*. 2001;**409**:660-921

- [6] Nature. 2004;**431**:931-945
- [7] Nature. 2005;**438**:803-810
- [8] Nature. 2017;**550**:345-353
- [9] Ross WCJ. The chemistry of cytotoxic alkylating agents. In: Greenstein JP, Haddow A, editors. *Advances in Cancer Research*. New York: Academic Press, Inc.; 1953. pp. 397-449
- [10] Ross WCJ. *Biological Alkylating Agents*. London: Butterworth; 1962
- [11] Ross WCJ. *Journal of Chemical Society*. 1949;183
- [12] Ross WCJ. *Journal of the Chemical Society*. 1950;2257
- [13] Ross WCJ, Mitchley BCV. Annual Report of the British Empire Cancer Campaign. 1964;**42**:70
- [14] Cobb LM, Connors TA, Elson LA, Khan AH, Mitchley BCV, Ross WCJ, et al. 2,4-Dinitro-5-ethyleneiminobenzamide (CB 1954): A potent and selective inhibitor of the growth of the Walker Carcinoma 256. *Biochemical Pharmacology*. 1969;**18**:1519-1527
- [15] Chlorambucil - CancerConnect News. CancerConnect News. Retrieved: 2015-12-21
- [16] Hameed Khan A, Ross WCJ. Tumour-growth inhibitory nitrophenylaziridines and related compounds: Structure-activity relationships. Part I. *Chemico-Biological Interactions*. 1969/1970;**1**:27-47
- [17] Hameed Khan A, Ross WCJ. Tumour-growth inhibitory nitrophenylaziridines and related compounds: Structure-activity relationships. PART II. *Chemico-Biological Interactions*. 1971/1972;**4**:11-22
- [18] Hameed Khan A, Driscoll JS. Active antitumor components in a decomposed amino sugar. Part 1. Effect of sugar structure on activity. *Journal of Pharmaceutical Sciences*. 1975; **64**(2):295-299
- [19] Hameed Khan A, Driscoll J. Potential central nervous system antitumor agents: Aziridinylbenzoquinones. Part I. *Journal of Medicinal Chemistry*. 1976;**19**(2):313-317
- [20] Chou FT, Hameed Khan A, Driscoll J. Potential central nervous system antitumor agents: Aziridinylbenzoquinones. Part II. *Journal of Medicinal Chemistry*. 1976;**19**:1302
- [21] Driscoll JS, Hameed Khan A, Chou F-T. Aziridinyl quinone: Anti-transplanted tumor agents. Unites States Patent # 4,146,622; March 27, 1979