CHAPTER

1 The Scientific Basis of Oncology

Cancer is a disease that dates back to antiquity. Thus, bone tumors were identified in dinosaurs, animals which have lived 18 million years ago, as well as, in skeletons of Ancient Egyptians (2160 B.C.). Hippocrates (460-375 B.C.) introduced the term cancer, the Greek name for crab, in view of gross morphologic similarity.

Advances of our knowledge on cancer was linked to the progress that have been made on science, technology, as well as, scientific thinking (Table 1-1). This evolution may be divided into three epochs. The first is the period of etiology misconceptions and empirical treatment, extending from ancient times to 1761, the time of the great contribution in the gross pathology of cancer by Morgagni. With the empirical approach, treatment was based upon experience, gained by trials and errors. The second epoch is that of description and classification extending from 1761 to 1900. The third epoch (the 20th century) is a period of great achievements of research with discovery of curative therapy, etiologic factors and molecular pathogenesis of cancer.

A historic review of the subject is important for three reasons. First, nothing gives a better understanding of a subject than a study of the steps taken to reach its present state. Second, appreciation of the current molecular biology techniques and terminology is essential to comprehend current literature on cancer. Finally, knowledge of scientific thinking and scientific method are vital to plan a fruitful cancer research

THE ANCIENT EGYPTIANS

Supernatural Concepts

At the dawn of history, man believed that diseases were due to evil spirits or punishment from gods. According to this supernatural concept, there is only one cause for all diseases and man is helpless to confront it alone, hence he turned to priests and magicians for treatment.

In ancient Egypt, temples were built as houses for gods (usually presented in animal forms or hybrids). Religion was monopolized by priests and pharaohs, the latter were considered half-gods. Ordinary people were not allowed to enter temples except after the middle kingdom (2000 B.C.). Patients used to pray to the falcon-headed Horus, the God of healing, seeking for a cure (Fig 1-1).

Ancient Egyptian medicine was a mixture of religion and magic, dispensed as sacrifices and incantations to expel evil spirits. Even the knife, fire, and drugs were used to drive the spirits away. It is striking that the Ancient Egyptians, in spite of their strong power of observation and extensive

Table 1-1 Time Chart of Advances in Science and Oncology

Period	Background	Concept	Authority
3500 B.C.	Ancient times	Supernatural	Priesthood
400 B.C.	Greek philosophy	Humoral theory	Hippocrates, Aristotle, Galen
200	Medieval Europe	Catholic theology	St. Augustine
1500	Renaissance	Correction of knowledge	Vesalius
1600	Scientific revolution	Scientific method	Galileo, Bacon, Descartes
1700	Autopsy studies	Gross pathology	Morgagni
1800	The microscope	Cellular pathology	Schwann, Schleiden
1900	Discovery of therapy	Cancer curability	Rontgen, Curie, Halsted
1950	Biotechnology revolution	Molecular pathology	Watson, Crick

Fig 1-1 *Supernatural concepts. An Ancient Egyptian seeking cures from Horus, the God of healing. The eye of Horus was a symbol of godly protection*

experience in embalming, acquired little knowledge in anatomy and pathology. This is because, for progress of science, theory must come first before observation. If sickness is thought to result from evil spirits, there is no needed to search the body for natural causes of disease.

THE GREEK CONTRIBUTION

Natural Causes of Disease

The early Greeks (500 B.C.) inherited religious medicine from Egypt, but changed the shapes of gods from animal to human forms. Patients used to worship Apollo and Asclepius, the gods of healing in the Greek mythology. Patients were asked to sleep in the temples and gods were supposed to appear in their dreams and prescribe treatment. The Ancient Greek philosophers, not satisfied with the mythology of their time, turned their attention to nature. Thus, Empedocles (500-340 B.C.) described the four basic elements of nature, namely: air, earth, fire and water. Hippocrates (460-375 B.C.) was the first to consider disease a result of natural rather than supernatural causes, namely an imbalance of the four fluids or humors of the body, namely: blood, mucus, yellow bile and black bile (the Humoral theory). Cancer was considered to be due to excess of black bile which is formed in the spleen.

The great Greek philosopher Aristotle (384-332 B.C.) is considered a pioneer in scientific thinking (Fig 1-2). In contrast to his teacher Plato, he was mainly interested to study natural and biological phenomena rather than ethical matters. Aristotle considered that every aspect of human life, society, or natural world could be a subject of study. New knowledge could be acquired through experience (empiricism) or reasoning (logic). This was a great triumph of human mind over supernatural concepts and dogmatic statements of previous authorities. Aristotle was the first to link the four humors of the body to the four elements of nature (Fig 1-3), thus introducing a natural environmental mechanism of disease. The scientific achievements of Aristotle were remarkable. He wrote 170 books, constructed the first biology museum, classified the branches of knowledge, described the first evolutionary classification of plants and animals (the Ladder of Nature) and considered man an animal for the first time. Darwin called him the world greatest natural scientist.

Fig 1-2 *Aristotle (384-322 B.C.). The founder of the philosophy of logic and world greatest natural scientist*

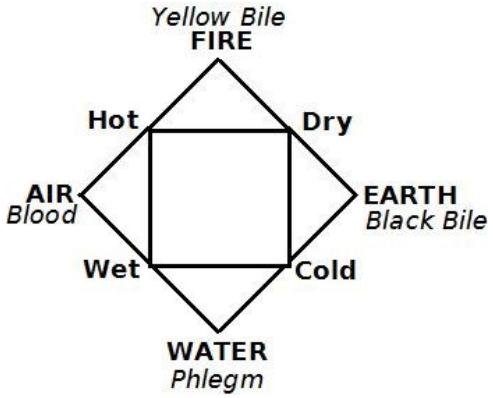

Fig 1-3 *The Humoral theory. The relation of the four body humors to the four environmental natural elements.*

Alexander the great conquered Egypt late in 332 B.C. and founded the Alexandria library and University, which contained half a million manuscripts of Greek culture and was the leading center of learning at that time. In Alexandria, Euclid developed his geometric model of vision (optica), Archimedes contributed in mechanics and physics, and Herophilus dissected the human body and recognized the brain rather that the heart as the seat of intelligence. He emphasized that knowledge is only earned through a balance between theory, experiments and practice.

In the Roman Empire (27 B.C.-476 A.D.), most scholars and doctors were of Greek origin. A leading figure was Galen (130-201 A.D.) who was a devoted and enthusiastic supporter of the humoral theory and he advocated systemic treatment (purgation and bleeding) to eliminate the excessive humor. Galen studied anatomy by animal dissection only and applied the findings to man. According to Galen, the human body contained a five-lobed liver, double bile ducts, a horned uterus and a hole in the septum of the heart. Galen was considered an infallible authority; hence, his erroneous concepts in disease and anatomy dominated medical thought for almost 1500 years after his death.

MEDIEVAL EUROPE

The Hazards of Dogmatism

In Europe during the middle Ages (200-1500 A.D.), philosophy was dominated by the Roman Catholic Church. The pope had more power and wealth than all kings and nobles combined. The theology of that period was introduced by Saint Augustine (354-430 A.D.). In his work (City of God, 410 A.D.) he considered the church above the state, faith above knowledge, disease a punishment from God and no salvation of sins or disease outside the church (Fig 1-4). Religious orders and Devine concepts of the church about nature must be strictly obeyed. Any one who challenged was considered heretic and executed by burning at the stake. In this atmosphere, a progressive deterioration of science occurred in Europe for 1300 years.

In contrast to the gloomy picture in Europe, there was a remarkable development of intellectual activity by Arabs during the Islamic Empire (900-1200 A.D.). The Arabs discovered and translated the works of great Greek philosophers into Arabic and Latin. They also added original scientific achievements in various fields, including: chemistry (AL-Razi, 865-925 A.D.), medicine (Ibn -Sina, 980-1037 A.D.), surgery (AL-Zahrawi, 940-1039 A.D.), philosophy (Ibn-Rushd, 1120-1195) and optics of vision (Al-Haitham, 11th century). All these translated works were introduced to Europe through Spain, thus helping the rebirth of science in the 16th century.

Fig 1-4 *Theology the Queen of all Sciences. A dogma that resulted in deterioration of science in Medieval Europe for 1300 years*

RENAISSANCE EUROPE

The Revival of Learning

During the 16th century, a rebirth of science occurred in Europe (renaissance). That was an age of geographic discoveries, new ideas in arts and scientific freedom. Scientists became more daring and creative in their thinking. They rediscovered and corrected previous classic works of Greeks, Romans and Arabs and also made original contributions. Spread of knowledge was possible through the invention of printing (Gutenberg, 1522).The polish astronomer Copernicus challenged the teachings of the Roman Catholic church and published his heliocentric theory (1543 A.D.), that planets revolve around the sun rather than the earth.

A remarkable scientist of renaissance was Andreas vesalius, a professor of anatomy at Padua University, Italy, then the leading medical school in Europe. He noticed through dissection of human body (Fig 1-5) that the organs did not correspond to the historic descriptions of Galen (no black bile in spleen and no hole in the septum of heart). At the age of 29, he published in 1543 his monumental anatomic studies (The Fabric of Human Body). The immediate effect was the downfall of the Greek humoral theory of disease. Subsequent effects were advances made in surgery and gross pathology.

THE SCIENTIFIC REVOLUTION

A radical change in scientific thinking occurred in the 17th century, replacing the logical reasoning of Aristotle by a new more objective approach (the scientific method) based on experiments, quantitation and mathematical analysis. The three leaders of this scientific revolution were: Galileo Galilei in Italy, Francis Bacon in England and Rene Descartes in France. Their common objective was to liberate science from the influence of philosophy and religion.

Galileo (Fig 1-6) in 1610 was the first to do experiments and quantitate observations. He was a symbol of academic freedom and revolt against dogmatism. Thus, he adhered to his scientific discoveries (that the earth rotates around the sun) despite house-arrest punishment by church authorities. Bacon, in his work (Novum Organum, 1620), stated that knowledge is not something we start with, but something we arrive at after observation and experimentation. Descartes in his work (Discourse on Method, 1637); he invented a skeptical approach to scientific thinking with systematic doubt of all our accepted knowledge since conclusions were based upon subjective unreliable observations. Descartes stressed that we should start by doubt not with belief (the reverse of the attitude of St. Augustine), and we should build our knowledge on solid facts and mathematics.

Fig 1-5 *Andreas Vesalius (1514-1564). He was the first to correct previous misconceptions in human anatomy. His dissection lectures were attended by a large crowd of students, as well as, laymen.*

Fig 1-6 *Galileo Galilei (1564 – 1642). A pioneer of scientific method, the first to quantitate experiments and a symbol of revolt against dogmatism.*

Fig 1-7 *Giovanni Battista Morgagni (1682 – 1771). The founder of gross pathology and clinicopathological correlation.*

The development of science of biostatistics by the end of 19th century introduced a precise objective tool for data analysis. This included: correlation of two quantitative variables (Galton, 1869), X^2 (chi-square) test for qualitative data (Pearson, 1900) and Student t test of significance of difference between means (Student, 1908). Two advanced statistical methods were developed later, namely: survival analysis of treatment outcome (Kaplan and Meier, 1958) and multivariate analysis (Cox, 1972). The latter, tests interaction among multiple variables and identifies the independent variables which are truly significant.

The six main steps of the scientific method as practiced today are: (1) selection of a problem, (2) collection of observations and preliminary data on the problem, (3) construction of a hypothesis. (4) designing an experiment to test the hypothesis, (5) statistical analysis to reach a conclusion (accept or reject the hypothesis) and (6) report of results. When the hypothesis is reproduced by others it becomes a theory, whereas, its global recognition and applicability to different phenomena it becomes a general law.

Gross Pathology and Clinicopathologic Correlation

The rise of gross pathology (Morbid Anatomy) was attributed to Giovanni Battista Morgagni (1682-1771), a professor of anatomy, Padua University, Italy (Fig 1-7). At the age of 79, he published his great work (on the Seats and Causes of Diseases). It contained clinical data and autopsy protocols of more than 700 cases. Morgagni was the first to correlate the symptoms of the patient with the changes in the organs found at autopsy. Thus, diseases, including cancer, were for the first time attributed to local causes. Morgagni was the first to demonstrate the necessity for basing diagnosis, treatment and prognosis on gross anatomic findings.

Abernethy in 1811 introduced the first classification of tumors based on gross appearance. The main groups of tumors were: sarcomas, scirrhous, cancer, osseous, encysted tumors and the gravid uterus (cysts and inflammatory swellings were excluded). This diagnostic system was strongly admired by surgeons, who considered it to be more superior to histopathologic study by the early primitive microscopes.

Epidemiology Reveals Etiology

In 1775, Sir Percival Pott, an English physician, reported on the development of scrotal skin cancer in adults who had been engaged as chimney sweepers during their childhood. He suggested chimney soot (tar) as a causative agent. This report helped to establish laws for the protection of children in hazardous occupations, a pioneer step in cancer prevention. The observation of Pott was confirmed 154 years later by the induction of skin tumors in experimental animals by tar application (Yamagiwa and Itchikawa, 1915).

A link between asbestos and lung cancer was first reported in 1935 (Lynch) and pleural mesothelioma in 1960 (Eagner). To minimize the problem and protect their interests, the asbestos industry in U.S.A supported several research projects to prove the safety of asbestos and blame

cigarette smoking and contaminating impurities for the resulting cancers. After long years of debate, the health risks of asbestos were finally documented (Roggli, 1987), and in 1989 the production and sale of asbestos was banned in U.S.A. Unfortunately, the asbestos industry and its hazards were exported to developing countries, where new asbestos factories were established.

The first reports linking smoking and lung cancer were published in 1950, both from U.S.A (Wynder and Graham) and England (Doll and Hill). However, the cigarette industry is a giant one, with estimated 1.3 billion smokers world wide and 15 billion cigarettes smoked per day. In 1990, a massive lawsuit was brought against the U.S.A. tobacco industry by a coalition of 22 states. Surprisingly, the powerful industry, by the help of efficient lawyers, succeeded in preventing banning of their products and reached a settlement at a rather high cost (immediate payment of 65 billion dollars and annual payment of 10 billion dollars for compensation). They were forced to print on their products that smoking is dangerous to health and my cause cancer.

The Microscope and Cellular Pathology

Technology is the application of science for practical and useful purposes. It is through technology, not science, that progress could be made. In this regard, the microscope was surely the greatest single invention ever made (still in use for more than 1000 years). Invention of the compound microscope was credited to Galileo (1610) and simple microscope to Leeuwenhoek (1721). However, the use of such early primitive instruments was restricted to their inventors, hence, no scientific impact. Not until microscopes were generally available to all scientists following the industrial revolution (1770-1825) could further advances be made. Thus, in Germany, Schwann a zoologist and Schleiden a botanist launched the cell theory in 1838. Accordingly, cells were considered as the units of life of plants and animals. Muller immediately reported that tumors were also composed of cells, and Virchow in 1858 stated that cells can only arise form preexisting cells.

The triumph of cell theory allowed the use of microscopes for practical diagnostic purposes. Thus, in 1843 Adolph Hanover described the specific diagnostic features of malignant cells. Wisk in 1859 classified tumors into 3 groups: benign, semimalignant and malignant. Previous misclassifications of neoplasms were corrected, thus, skin epitheliomas and soft tissue sarcomas were considered malignant tumors (Labret, 1851 and Gross, 1866). The concept of biopsy prior to surgery was introduced (Rage and Viet, 1878) with initial strong opposition from surgeons, but, it was finally accepted and frozen section was used before performing radical mastectomy (Welch and Haltsed, 1891).

Important technical developments were made in histopathology by the end of 19th century, namely: the discovery of Hematoxylin and Eosin stain (1870), the manufacture of microscopes with achromatic lenses (Carl-Zeiss, 1880), the invention of rotary microtomes (Minot, 1885) and the use of formalin for tissue fixation (Blum, 1893). Subsequent major advances included: the introduction of modern classification of tumors (Ewing, 1919), grading of tumors (Broder, 1921), discovery of ultra-structure of cells by electron microscopy (Knoll and Ruska, 1931) and diagnostic use of exfoliative cytology (Papanicolaou, 1943). This represented the maximum which cellular pathology could achieve.

Discovery of Therapy and Increase of Curability

During the 19th century, with lack of any effective therapy, a general impression persisted among the public and doctors that cancer was an incurable disease. However, by the end of 19th century, an optimistic view gradually prevailed with the discovery of different modalities of therapy. Thus, William Halsted in 1891 described radical mastectomy for breast cancer, thus introducing the concept of radical surgery. Moreover, three major discoveries of radiation were accomplished in just three years, namely: x rays (W. Roentgen 1895), radioactivity of uranium (A. Becquerel, 1895) and radium (M. Cuire, 1898). Hormonal therapy of cancer was introduced by ovariectomy for breast cancer (G. Beatson, 1896) as well as, orchidectomy and estrogens for prostatic carcinoma (C. Huggins, 1941). The first chemotherapeutic agent, nitrogen mustard, was used to treat lymphomas (Gilman, 1946) following the war time observation that poisonous mustard gas produced marked inhibition of the hemato-lymphoid system.

The first report on curability of cancer (von Winiwarter, 1878) indicated a 3-year survival of 4.7% in patients with breast cancer treated by

surgery. However, end results improved with discovery of different therapeutic modalities. Thus, the reported 5-year survival was 20% in 1930, 50% in 1980 and 65% in 2004. Such progress in curability was mainly achieved through the wise combined use of surgery, irradiation and chemotherapy (multidisciplinary approach) and management of patients at specialized centers.

Biotechnology Revolution and Molecular Pathology

The last 50 years of the 20th century was the period of biotechnology revolution which enabled us to study cancer at molecular level, with remarkable achievements. The reasons for these accelerated discoveries were: the availability of trained staff, team work of different specialties, availability of sophisticated efficient equipment, as well as, research funds from various sources.

Breakthrough Discoveries

Advances of our knowledge on the molecular pathology of cancer was only possible after several key scientific discoveries, all were awarded Nobel Prizes. The discovery of DNA structure (Watson and Crick, 1953) was an essential first step. It is a double helical structure, composed of a backbone of sugar (deoxyribose) and phosphate, held together by nitrogen-containing bases (Fig 1-8). DNA is the library of genetic information and has two main functions, namely: the passage of genetic information from one cell generation to another during cell division (replication) and the expression of genetic information in the form of protein synthesis (transcription and translation). To accommodate its length in the small nucleus, DNA is in wrapped around histone protein (nucleosomes) which are organized to form 23 pairs of chromosomes (Fig 1-9).

The genetic code (codon) is a sequence of three bases that code for a single amino acid (F. Crick, 1961 and R. Holley, 1968). A gene is a larger sequence of DNA required for the production of a functional product (RNA or protein). A gene includes a structural component (that codes) and regulatory components (repressor, enhancer and promoter) that regulate the structural component. The minority of total DNA length codes for proteins (exons), whereas, the majority are noncoding regions (introns).

For protein synthesis, DNA uses other single-stranded molecules (ribonucleic acids RNAs) of which there are 3 main types, namely: messenger m-RNA, transfer t-RNA and ribosomal r-RNA. The flow of gene expression (Fig 1-10) is from DNA to RNA to proteins (the central dogma of molecular biology). During this process, non-

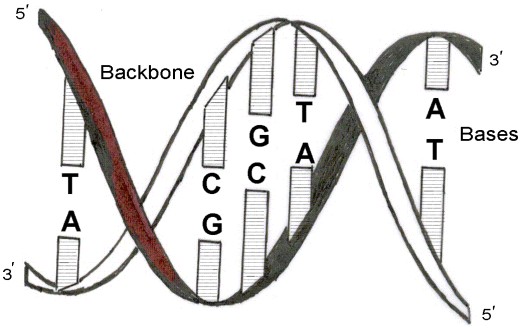

Fig 1-8 *The double helical structure of DNA. A backbone of phosphate and sugar (deoxyribose) held together by a pair of bases. According to the complementarily rule of base pairing, adenine (A) always binds to thymine (T), and guanine (G) binds to cytosine (C).*

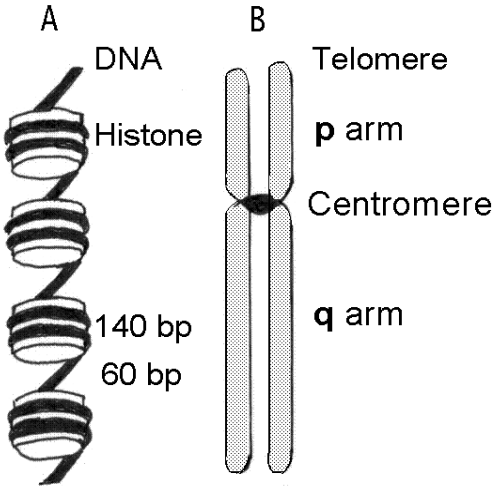

Fig 1-9 *Organization package of DNA. (A) The nucleosomes are the structural units of chromatin formed of 140 base pairs wrapped around a histone protein with spacer connecting strands of 60 base pairs (B) chromosomes are the structural units of DNA only seen at metaphase during cell division. Chromosomal arms are further divided into segments for exact localization of genes*

coding regions (introns) are removed by splicing.

In the cytoplasm, proteins are constructed on ribosomes by the action of t-RNA and r-RNA (R. Green 1997). Two additional RNAs with regulatory function include: small RNA (s-RNA) which controls splicing and micro RNA (mi-RNA) which inhibits m-RNA (silencing) resulting in arrest of protein synthesis. The genotype is the genetic structure responsible for a single specific product (protein), whereas, the genome is the total genetic constitution of the organism. The phenotype refers to the appearance (structure and function) of the cell or organism mainly determined by proteins

Gene regulation is controlled by genetic or epigenetic mechanisms. Thus the transcription signal starts at the promoter region, where RNA polymerase binds to DNA. This enzyme binds only to a promoter primed by a transcription factor (Fig 1-11). Transcription factors (TF) are special proteins expressed by regulator genes, and are capable to combine to DNA, as well as, other proteins resulting in enhancement or repression of transcription. Chromatin remodeling, DNA methylation and mi-RNA interference are other epigenetic mechanisms of controlling gene expression (chapter 6). Thus in the inactive state (deacetylation), the nucleosomes are closely packed together hiding the promoter regions of DNA from transcription factors. However, with histone acetylation, the nucleosomes are dissociated from each other exposing the promoters to transcription factors and hence activating transcription.

Laboratory studies of genetic material were originally faced by four main obstacles, namely: (1) the lack of a method to isolate the nucleic acid segment of interest for analysis, (2) the scanty nature of nucleic acid in samples, (3) difficulty to purify the sequence of interest from all other segments of DNA and RNA in the sample and (4) precise identification of target sequence in the isolated sample.

The discovery of bacterial enzymes (restriction endonucleases) that can split DNA at specific points between codons (Fig 1-12) helped to isolate specific DNA sequences for study (Cohen, 1974 and W. Arber 1978). A

At present, about 100 endonucleases are in commercial use. A restriction endonuclease will

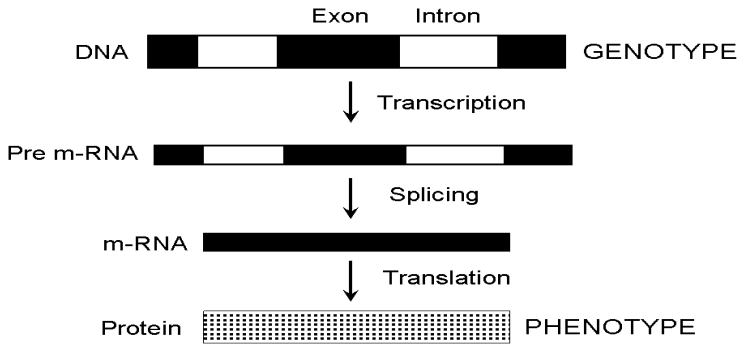

Fig 1-10 *The expression of DNA to form proteins. The flow of information is from DNA to RNA to protein. Transcription and splicing occur in the nucleus, whereas, the translation process occurs on ribosomes in cytoplasm.*

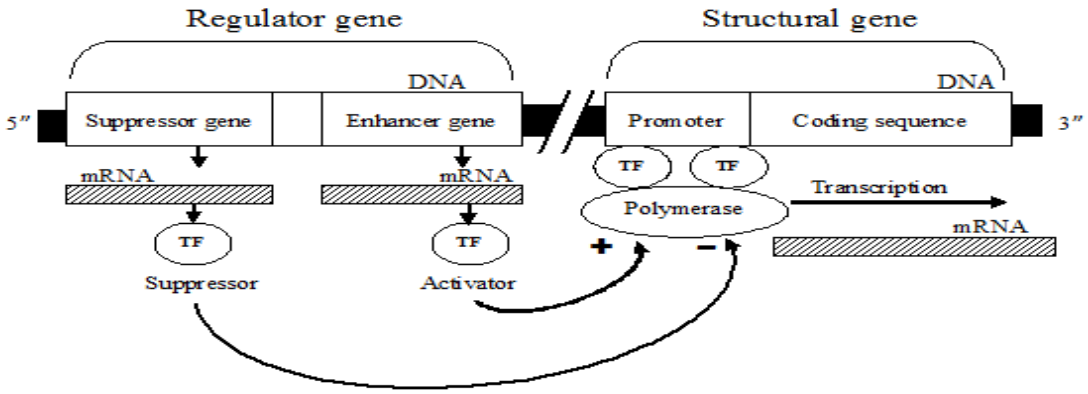

Fig 1-11 *Control of gene expression by regulator genes mediated by transcription factors (TF) needed by RNA polymerase (Pol) to start transcription.*

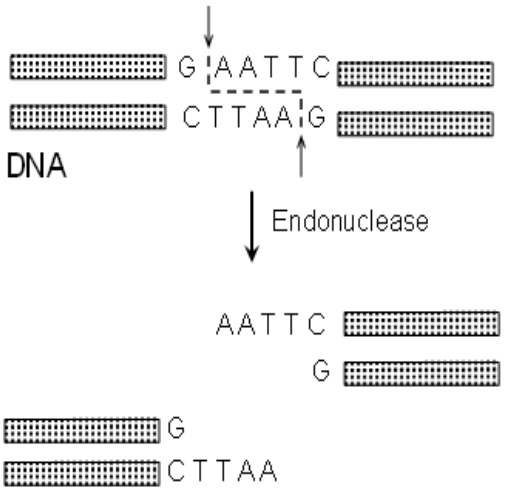

Fig 1-12 *Cleavage of DNA by restriction endonuclease enzyme. The cleavage yields fragments with single-stranded tails (sticky ends), which may be joined to other DNA segments by the enzyme ligase to produce recombinant DNA. In this way, synthetic DNA of any required sequence could be obtained (genetic engineering).*

not cleave DNA if there is a mutation at its restriction point; hence, this phenomenon is used to detect mutations and their exact sites. It is also possible to cut and join (by ligase enzyme) DNA segments, thus manufacture a new molecule of a required base sequence (genetic engineering).

Two methods are available for quantitative increase (amplification) of the DNA sequence of interest, namely: the polymerase chain reaction (PCR) and DNA cloning. In PCR reaction (Kasy Mullis, 1993), amplification is accomplished through cyclic heating and cooling of DNA sequence after adding primers and precursor reagents (Fig 1-13). In DNA cloning, the target DNA sequence is isolated and integrated into a carrier (plasmid) then introduced into an organism (bacteria or yeast) where it undergoes independent multiplication (Fig 1-14).

Separation of DNA sequences according to their size is possible by gel electrophoresis (Southern blot). The target sequence is roughly identified by comparing its migration band with that of a standard control ladder. Precise identification of sequence however is possible by either DNA sequencing technique or hybridization with a labeled probe (Fig 1-15).

Advances in biotechnology have led to two important discoveries related to oncology, namely, cancer genes and monoclonal antibodies. Cancer genes (viral src) were first discovered in experimental animals in the seventies (Bishop and Varmus, 1976), but, the first human oncogene (ras) was discovered in 1982 (R. Weinberg and G. Cooper) and the first human tumor suppressor gene (Rb1) discovered in 1986 (A. Knudson). The production of monoclonal antibodies (G. Kohler, 1976) was possible by using the hybridoma technology (Fig 1-16). Hybridomas represent a fusion of two B-lymphocytes, one containing the genetic information (splenic lymphocytes) and the other cells (immortal myeloma cells) manufacture the antibodies. Monoclonal antibodies are highly specific against the epitope of the antigen, hence, their importance as diagnostic and therapeutic tools in cancer

Discoveries in physics also had a considerable impact on biotechnology. The laser beam discovery (Charles Towns, 1964) allowed rapid quantization of data in flow cytometry technology (W. Gohde, 1968). It was also used in laser capture microdissection (M. Immest- Buck, 1996) which allowed the selection and isolation of individual cells from tissue for study. Laser was also used to produce photothermal effect on tumor by using gold nanoparticles (M. EL-Sayed, 2006). Nuclear magnetic resonance (NMR) revealed the three dimensional structure of proteins (Kurt Wuthrich, 2002). The discovery of silicon chip microprocessor (Robert Noyce, 1959) started an electronic revolution. A microprocessor is a miniature device capable of performing all the logical functions of a computer.

Genomics

The human genome project was completed in 2003 with a total cost of 3 billion dollars, hence establishing the complete DNA nucleotide sequence and gene map of human chromosomes. The haploid human genome contains about 3 billion nucleotides or 25,000 genes. About one half of the genome contains a unique non-repeating sequence of nucleotides, and another half whose sequence is repeated (tandem repeats or microsatellites). Only 1.5% of the human genome is a coding sequence and 98.5% is noncoding and plays a regulatory function. Genes are not distributed at random, but tends to cluster in specific regions of DNA or chromosomes.

Genetic polymorphism refers to alteration of base sequence of a gene in one individual or between two individuals. Single nucleotide polymorphism (SNP) occurs at a frequency of one

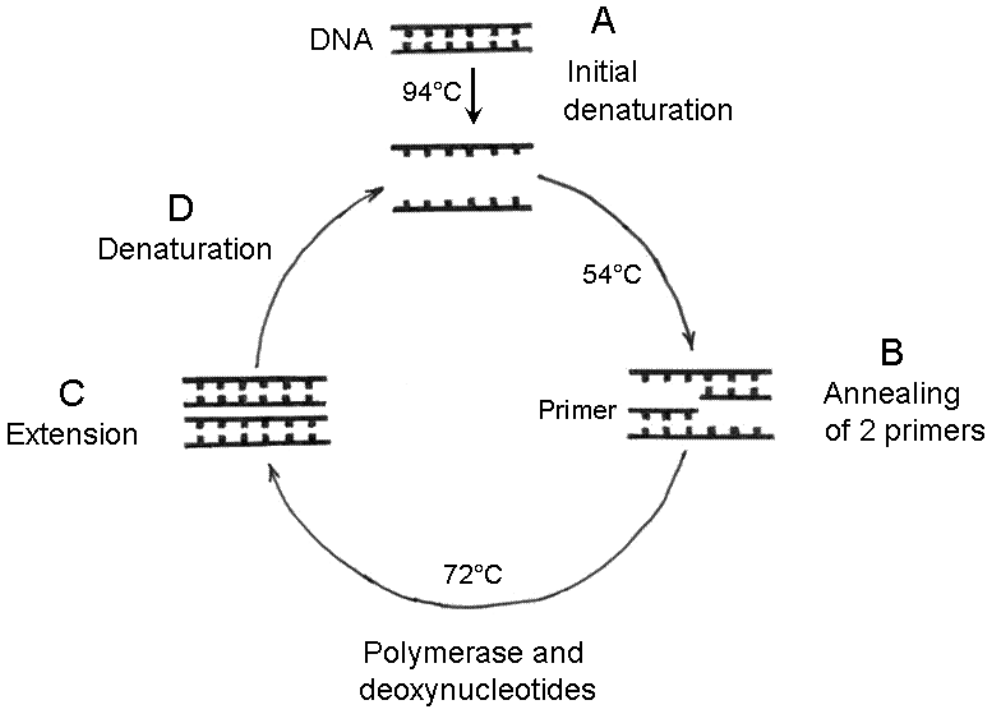

Fig 1-13 *Amplification of DNA segment by the polymerase chain reaction (PCR). (A) Heat denaturation separates DNA into single strands. (B) Annealing of two synthetic oligonucleotide primers are required to specifically target the DNA region of interest. (C) Extension yields two new complementary DNA extending from the two primers. (D) Repeated cycles result in exponential amplification of the initial DNA sequence, producing a million copies in 20 cycles.*

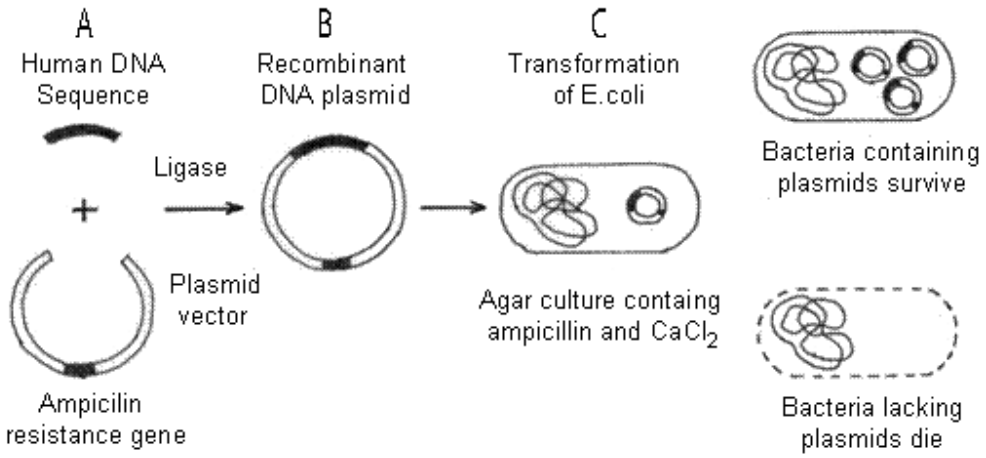

Fig 1-14 *DNA cloning in bacteria. (A) Human DNA sequence and circular DNA bacterial plasmids are cut by restriction endonucleases. (B) Insertion of DNA sequence into plasmid result in a recombinant DNA plasmid. (C) Transformation: plasmids are introduced into bacterial cells (E. coli) then grown on agar culture containing ampicillin. Selection of (E. coli) containing the recombinant plasmid is possible since they survive and multiply in the culture due to their protection by the ampicillin resistance gene, whereas, bacteria lacking plasmids will die.*

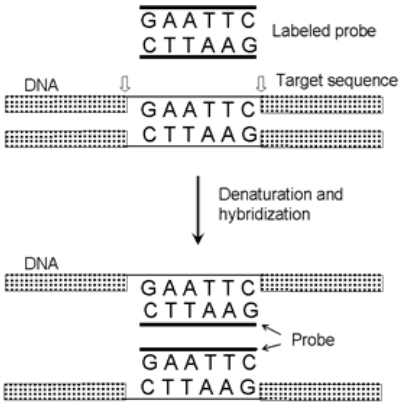

Fig 1-15 *Identification of DNA target sequence by hybridization with a labeled probe. The latter is a synthetic DNA molecule with base sequence complementary to target sequence and labeled by either isotope or fluorescent dye.*

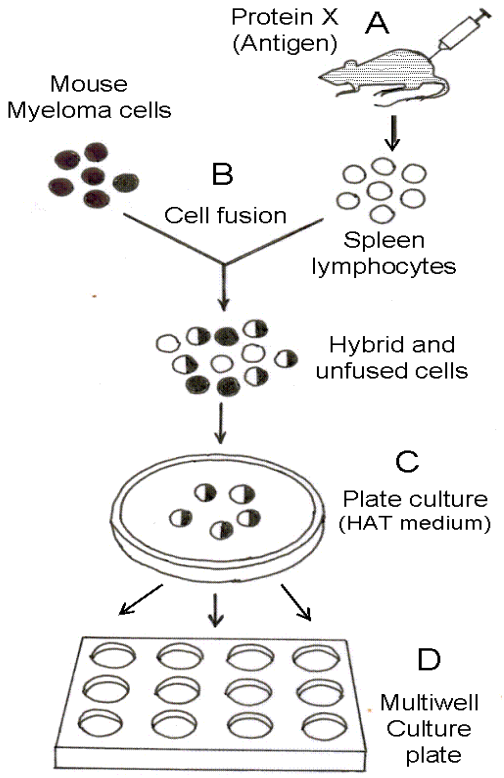

Fig 1-16 *Production of monoclonal antibodies by the hybridoma technology. (A) A mouse is immunized with protein X. (B) Spleen lymphocytes are fused with the immortal mouse myeloma cells with the help of propylene glycol. (C) Plate culture on HAT medium, only fused (hybrid) cells will grow, and (D) Culture of each cell clone in multiwell culture and each well is tested for specific antibodies against protein X.*

SNP/kb throughout the genome when the sequence of any two individuals is compared. The biomedical importance of SNP includes: its relation to risk to develop disease, response to drugs and ethnic variation.

The two methods of genetic analysis are: chromosomal analysis (Karyotyping) and molecular genetic methods. Chromosomal analysis (Table 1-2) studies gross chromosomal aberrations at metaphase for any numerical change (gain or loss) or structural changes (deletion or translocation). Such morphologic changes usually point to an underlying genetic abnormality.

Conversely, molecular genetic methods (Table 1-3) target DNA base structure itself to detect any genetic aberration (gain, loss or translocation) or any epigenetic abnormalities (e.g. methylation status of cytosine). Mutation scanning is a two-step process: first, the target DNA strand is separated by gel electrophoresis (Southern blot), then subjected to further analysis (sequencing) to identify any specific base sequence alteration. Since human disease (including cancer) is usually the result of multiple gene affection, gene expression profile by microarray has gained high popularity in cancer classification, despite its high cost.

Proteomics

DNA represents an information archive, whereas, proteins do all the work of the cell, by playing a key role in both structure and function. This includes: gene regulation, cell cycle regulation, cell differentiation, transmission of signals to cell from its microenvironment (signal transduction), metabolic regulation by enzymes and hormones, immune mechanisms and programmed cell death (apoptosis). Accordingly, proteins must play a basic role in malignant disease.

The estimated one million different proteins in the human body do not match with the 25,000 genes in human genome. This discrepancy is explained by two facts. First, some genes can code for several proteins through a process of gene rearrangement (e.g. immunoglobulin gene of plasma cells which produce thousands of different antibodies, as well as, surface receptors of T-lymphocytes which contribute to the production of numerous cytokines). Second, many proteins undergo post-translation modification with change of their function (e.g. addition of reactive groups

Table 1-2 Chromosomal Analysis

Method	Scope
1. Conventional karyotpying	Visualize chromosomal aberrations by Giemsa stain using light microscope
2. Multicolor FISH, spectral Karyotyping (SKY) multiple metaphase FISH	Visualize chromosomal aberrations using fluorescent Microscope
3. Comparative genomic hybridization (CGH)	Analyse copy number changes (gain or loss) in DNA in each of chromosomes

such as phosphorylation, split of molecule into smaller polypeptide chains, interaction with other proteins with loss of function referred to as dominant negative effect and finally folding of the three dimensional structure).

The life-span of proteins varies according to its function from few minutes to several months and are ultimately degraded by one of two pathways, namely: the ubiquitin-proteasome pathway (UPP) and the autophagy-lysosome pathway (ALP). The UPP is the major pathway involved in intracellular protein degradation and is energy (ATP) dependent (Ciechanover, 2004). Also, cell antigens are degraded by this system and presented on MHC-I for CD8 T-lymphocytes. In this pathway, damaged or misfolded proteins are transferred by carrier proteins (chaperone and ubiquitin) to the proteasome for degradation (Fig 1-17). The resulting degradation products (peptides and ubiquitin) are recycled. Conversely, the autophagy-lysosome pathway (B. Levine, 2004) is energy independent and is the main degradation system operable in apoptosis, starvation (autophagy means self-eating) and phagocytosis of extracellular antigens thus, antigens are endocytosed by macrophages, degraded to peptides in lysosomes and presented on MHC-II for CD4 T-lymphocytes. In this system, damaged protein is engulfed in cytoplasmic double membrane vesicle which fuses with lysosome (autolysosome) where the contents are degraded. If the contents are too abundant to handle, they are expelled from the cells to be immediately phagocytosed by surrounding macrophages.

The various methods available for protein analysis are presented in (Table 4). Major obstacles in protein analysis include the dynamic variation in protein structure and lack of a PCR-like method to increase the quantity of target protein in the sample. For these reasons, recent analytic methods are based on analysis of peptide degradation products of proteins (peptidome profile in the serum).

FUTURE PROSPECTS

Gene and protein expression profiles offer a promising and probably more accurate approach for tumor classification which may possibly replace the current traditional morphologic histopathologic techniques of tissue analysis. Genomic and proteomic methods generate information (tumor imprint) that correlates with the biologic behavior of a given tumor, hence provide a useful guide for therapy selection to the individual patient (individualized therapy), predict prognosis and help to discover new therapeutic agents. However, gene expression profile has not been found to correlate with protein expression (mainly due to post-translational modifications of proteins). For this reason, proteomic techniques are gradually replacing genomic analysis. Technical advances may solve the problem of studying whole protein molecules and its dynamic variation with time (e.g. the four dimensional (4D) electron microscope, A. Zewail, 2009). An alternative approach is to study the peptide degradation products of tumor proteins that are liberated into the blood. This peptidome profile in serum may correlate with tumor type and its biologic behavior.

Nanotechnology has recently proved to be a valuable diagnostic and therapeutic tool in cancer (e.g. diagnostic imaging and targeted therapy). A nanoparticle is an inorganic particle of minute size, 1-100 nm (a nanometer is one billion of a meter or one thousands of a micron). Such particles may acquire new properties different from the original material. For example, gold nanoparticles will emit heat when subjected to a laser beam. Gold nanorods conjugated to monoclonal antibodies will selectively localize to tumor cells, hence, allowing photothermal therapy when laser irradiation is applied (M. El-Sayed, 2006).

Table 1-3 Molecular Genetic Methods

Method	Scope
1. Southern blot	Separates double stranded DNA fragments by electrophoresis
2. Northern blot	Separates RNA segments by electrophoresis
3. Polymerase chain reaction (PCR)	A given segment of DNA is amplified exponentially in vitro by synthesis of complementary strands
4. In situ PCR	Amplification of a target nucleotide sequence in tissue sections
5. Multiplex PCR	Allows screening of multiple DNA sequences from one a sample
6. Real time PCR	To quantitate a given DNA sequence by comparing it to a PCR control sample
7. Reverse transcriptase (RT-PCR)	Makes DNA copy (c DNA) from mRNA molecule to allow its amplification
8. In situ hybridization (ISH)	Visualize a specific nucleotide sequence (interphase or metaphase) by hybridizing it with a labeled probe
9. Split Signal FISH	Identifies translocations by using two differently fluorescent labeled probes
10. Chromogenic in situ Hybridization (CISH)	Identifies a specific gene sequence by light microscopy hybridization (CISH) in interphase nucleus using DNA-labeled probe
11. Restriction fragment Length pleomorphism	Separates DNA fragments of different sizes by Length polymorphism (RFLP) electrophoresis to identify the one containing the mutation
12. Allele-Specific Oligonucleotides	Allows the detection of specific single base - pair mutations oligonucleotides (ASOs)
13. Sequencing	Determine the nucleotide sequence of the coding region of gene (exon)
14. Microsatellite Instability	Identify mismatch repair gene mutation by comparing Instability the number of nucleotide repeats in microsatellite markers in normal and tumor tissue from same patient
15. Methylation analysis	Detects hypo or hyper methylation of cytosine of DNA which is associated with gene dysfunction (epigenetic changes)
16. Gene expression microarray	Detects the expression of thousands of genes from one tumor sample microarray
17. Flow cytometry	Quantitate individual cell DNA in a large population of tumor cells

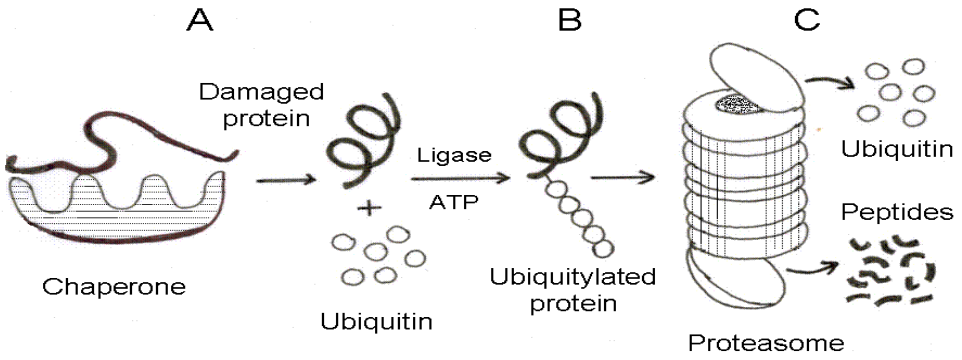

Fig 1-17 *Ubiquitin-proteasome pathway (UPP) of protein degradation. (A) Misfolded or damaged protein is carried by chaperone to ubiquitin. (B) Ubiquitation of protein is accomplished through the action of enzymes ligases and adenosine triphosphate (ATP). (C) In the proteasome, ubiquitylated protein is enzymatically degraded to ubiquitin and peptides which are recycled.*

Table 1-4 Proteomic Analysis Techniques

Method	Scope
1. Sanger analysis	Determine the sequence of polypeptides and amino acids content
2. Column chromatography	Separates proteins according to their mass, charge or binding affinity
3. Western blot and 2 dimensional (2D) electrophoresis	Separates proteins by electrophoresis according to their charge and mass
4. Mass spectrometry	Estimates peptide mass
5. X-ray crystallography	Determine the three dimensional relations of electrons (electron density map)
6. Nuclear magnetic resonance (NMR) spectroscopy	Determine the three dimensional relations of atomic nuclei
7. Bioinformatics	The use of computer programs to determine protein structure by matching it with standard protein models
8. Ultrafast four dimensional (4D) electron microscopy	Visualize dynamic changes in protein molecules with time
9. Immunohistochemistry	Determine protein type using labeled monoclonal antibodies
10. Fluorescent flow immunocytometry	Detect and quantitate different proteins in cell populations
11. Image immunocytometry	Quantitate cellular proteins in tissue sections or cytology samples

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