Review Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2024.54.008558

What has Genomics Done for us? A Review of the Advancements in the Biomedical Sciences Resulting from the Deciphering of the Human Genome

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ARTICLE INFO

Received: January 02, 2024

Published: January 10, 2024

Citation: Garwin Kim Sing, Dileep Rohra, George Kulik and Ahmad Samir Aljada. What has Genomics Done for us? A Review of the Advancements in the Biomedical Sciences Resulting from the Deciphering of the Human Genome. Biomed J Sci & Tech Res 54(3)-2024. BJSTR. MS.ID.008558.

ABSTRACT

With the completion of the Human Genome Project in 2003, the intense effort in genomics research has provided biomedicine with the tools to manipulate and express the genome. In the past fifty years, there have been three key discoveries which have accelerated this process. The first was the development of monoclonal antibodies, which provided readily available reagents to specifically identify antigens. The second was the development of the Polymerase Chain Reaction (PCR) which made possible the amplification of scarce DNA templates for study and sequencing. And the third has been the development of Next Generation Sequencing (NGS) which has dramatically reduced the time for DNA sequencing and brought costs down. Offshoots from the knowledge gained from the human genome project has rapidly advanced almost all disciplines of biomedical science such as immunology, signal transduction and proteomics, as well as spawned new fields such as gene therapy, regenerative medicine and translation al medicine. But this rapid advance in science and technology should be tempered by concerns from society regarding ethics and safety.

Abbreviations: PCR: Polymerase Chain Reaction; NGS: Next Generation Sequencing; HGP: Human Genome Project; BP: Base Pairs; QPCR: Quantitative Polymerase Chain Reaction; TCGA: Tumours Cancer Genome Atlas Program; RISC: RNA-Induced Silencing Complex; PTMs: Post-Translational Modifications; TH: T Helper; CTLs: Cytotoxic T Cells; N: Natural Killer; EB Cells: Embryonic Stem cells; PRS: Retinal Pigment Epithelium Cells; IPS: Induced Pluripotent Stem Cells; ZMW: Zero-Mode Waveguide

Introduction

In 2003, The Human Genome Project (HGP) was completed [1]. The DNA code was deciphered using Sanger sequencing. The objective was to determine the DNA sequence of the entire euchromatic human genome within 15 yrs., to map all ±3 billion (3 x 10^9) Base Pairs (bp) of DNA of the twenty four human chromosomes. The effort took thirteen years to complete at a cost of three billion dollars, or about $ 1 per base pair. In reality, not all of the 24 chromosomes were sequenced. The aim was to sequence only euchromatic regions of the nuclear genome, which make up 92.1% of the human genome. Euchromatin (also called "open chromatin") is a lightly packed form of chromatin consisting of DNA, RNA and histone proteins. It is enriched with protein-coding genes and is often under active transcription. The remaining 7.9% exists as scattered heterochromatic regions such those found in centromeres and telomeres and are enriched with nucleotide repeat sequences and epigenetic marks. In 2022, the human genome had been sequenced in its entirety [2]. One of the major conceptual outcomes from the HGP was a more realistic estimate of the number of protein-coding genes, currently assumed to be around 19 000. Another finding was that the number of chromosomes does not correlate with the apparent complexity of an animal or a plant. By

knowing the genetic blueprint that dictates who we are, mankind is now on the verge of creating its own version of our biological code. Knowing which sequence of letters on a particular chromosome specifies a protein which may cause chronic inflammatory disease, or lead to the growth of cancer, we can modify the genetic code to disable or enhance the activity of these proteins. Such medical intervention techniques fall under the discussion on translational medicine, which will be covered at the end of this essay.

I will first show how major developments in immunology and molecular biology led to making genomics a possibility. The first was the development of monoclonal antibodies, whereby B lymphocyte clones programmed to make a specific antibody could be fused with leukemic B cells to create a so-called hybridoma. In this manner, the antibody-producing B cell is immortalized thanks to the oncogenes conferred by the leukemic cell. These cells can be cultured on an industrial scale, and their products harvested as monoclonal antibodies. These monoclonal antibodies are the workhorse reagents in biomedical science, because of their specificity due to the antigen-recognition site called the Fab region. Their other usefulness lies with the -COOH-terminal portion of the antibody molecule called the Fc region, which normally serves a number of biological functions in the immune response. But for laboratory purposes, this Fc region can be chemically tagged with fluorochromes, enzymes, radionuclides, toxins, all to identify or kill pathogenic target cells with a high degree of specificity. Target specificity, so important for diagnostics and immunotherapeutics, could not have advanced without monoclonal antibodies. The next breakthrough came in the late 1980's, when K.B. Mullis developed the polymerase chain reaction (PCR) [3]. Specific DNA segments defined by the sequence of two synthetically made oligonucleotides could now be enzymatically amplified up to a million-fold using PCR. One of the most significant uses of this technique is for generation of sequencing templates, either from cloned inserts or directly from genomic DNA. To avoid the problem of reassociation of the linear DNA strands in the sequencing reaction, ssDNA templates can be produced directly in the PCR reaction or generated from dsDNA by enzymatic treatment, and separated by electrophoresis or affinity purification. This was a scientific milestone because it now allowed for small fragments of DNA to be amplified millions or billions of times for study or manipulation. DNA sequencing would not be possible without PCR. This technique has subsequently branched into multiple variations and applications.

Molecular Biology and Genetic Engineering: The Predecessors of Genomics

By 1982, genetic engineering had become so advanced that Stan Cohen and Herb Boyer produced the first recombinant drug, human insulin made from the pig genome [4]. On the technology front, in 1993, Higuchi and co-workers found another use for PCR with Quantitative PCR (qPCR) [5]. By measuring the rate of nucleotide incorporation in the PCR reaction, the kinetic analysis of product formation during amplification reflected the abundance of the template. Since this technique quantifies in real time, it is also referred to as "real-time PCR". Another application is in diagnostics, as demonstrated in the 2020 SARS-CoV-2 pandemic when RT-PCR tests for COVID-19 were being performed worldwide on a daily basis [6]. In 1996, David J. Lockhart and colleagues as well as Joseph DeRisi and colleagues presented DNA microarrays, which allowed the simultaneous examination of thousands of genes [7]. In a so-called biochip, a great deal of information (in this case several thousand proteins, RNA or DNA fragments) is present on a very small space. Thus, even with a small amount of biological sample material, a large number of genomics investigations can be performed simultaneously, and for the most part automated. This idea of a "lab on a chip" has generated a great deal of interest in the study if microfluidics, where chip sensors become more and more miniaturized [8].

In 1970, Francis Crick proposed that genetic information flows in one direction, from DNA to RNA to protein [9]. This was known as the central dogma. But this hypothesis was soon challenged by scientists studying RNA viruses causing cancer in livestock. Research into these pathogens made a remarkable discovery - while the genetic code stored in the viral particles were made of RNA, when the virus infected a cell, the RNA made a copy of itself as DNA which could then insert itself into the host cell chromosome. This backwards flow of information due to these so-called retroviruses required a major rewrite of the Central Dogma. Ten years later, a new retrovirus appeared in the clinics of New York and San Francisco. It did not cause cancer in animals, but destroyed the immune system in humans. It was given the name human Immunodeficiency Virus or HIV, and millions of chronically infected individuals world-wide were soon to die of Acquired Immune Deficiency Syndrome or AIDS [10]. Nevertheless, despite retroviruses being potentially harmful to human health, their ability to convert RNA into DNA make them particularly attractive vehicles for gene therapy and molecular diagnostics. It was the retrovirus enzyme reverse transcriptase that made possible the application of the SARS-CoV-2 RT-PCR tests [11].

Genomics

In the mid-70's, Fred Sanger developed the chain termination method of DNA sequencing [12], while Allan Maxam and Walter Gilbert developed chemical sequencing [13]. In the Sanger method, a labeled complementary DNA strand is synthesized in vitro with the use of so-called dideoxynucleotides that lead to a random chain termination and hence to DNA strands of different lengths. The nucleotides were radiolabeled with ^{32}P which made these experiments potentially hazardous. The resulting fragments are separated by polyacrylamide electrophoresis and evaluated in an autoradiogram. With better dyes, newer methods worked with fluorescence-labeled nucleoside triphosphates in which the fragments are identified in a fluorescence detector and visualized by means of colored peak diagrams, removing the hazards of working with radioactivity. In the method of

Maxam and Gilbert, a DNA fragment is radioactively labeled at one end, denatured, and cleaved at specific bases via a chemical method. The cleavage products of different lengths are then separated by polyacrylamide gel electrophoresis for each base [14]. The base sequence can be identified from the autoradiogram of the four lanes. Sanger's chain termination method with dideoxynucleotides prevailed mainly due to its ability for automation, the quality of the sequences and the longer sequence reads. Indeed, in 1987, Leroy Hood and Michael Hunkapiller while working at Applied Biosystems, built an automated DNA sequencer based on the Sanger protocol. These two techniques dominated DNA sequencing for a decade. Later, the introduction of capillary electrophoresis sequencers automated Sanger sequencing (CE sequencing), heralding a new age of fluidics. Modern CE sequencers achieve read lengths of about 1000 base pairs and read the DNA sequence very precisely. To this day their accuracy is therefore considered a gold standard. However, they can perform only one read operation at a time and therefore work very slowly.

In 2005, new DNA sequencing technologies were introduced called "Next Generation Sequencing" (NGS). Most ultra-high-throughput methods no longer use a separation of the DNA via capillary electrophoresis as in the Sanger method, but a coupling of molecules to surfaces and recordings of rows of high-resolution images. These innovative technologies have the potential to revolutionize biological and biomedical research by significantly accelerating genome analyses and reducing their costs. However, due to technical limitations, a genome cannot be read in a single approach from the beginning to the end in a linear reaction, even by applying the novel sequencing techniques. It must be subdivided into smaller fragments during sequencing. The read lengths of all commercially available NGS devices are considerably shorter than those of the Sanger sequencing method. After sequencing, the pieces have to be combined to a complete genome using bioinformatics. Currently, modern gene sequencers are extremely cheap per base pair. In 2005 it needed 10 years and $ 3,000,000,000, today it is one day and $ 1,000. In 2010, the introduction of its zero-mode waveguide (ZMW) was another innovation for DNA sequencing. ZMW utilises "nanoholes" that contain a single DNA polymerase. Here, the incorporation of a single nucleotide can be observed directly. Each nucleotide is labelled with a different fluorescent dye, and the signal that is emitted during incorporation is immediately recorded and read by very sensitive detectors attached below the ZMW. In 2016, the third generation sequencing technology was unveiled by Pacific Biosciences. With genomics data collected from labs throughout the world, several genome atlases have been compiled from different types of Tumours, such the Cancer Genome Atlas Program (TCGA) which was launched in 2006 [15]. Comparing healthy and mutated DNA sequences can diagnose different diseases including various cancers, or characterize antibody repertoires in a population of antibody-producing B lymphocytes, all of which can be used to guide patient treatment. Having a rapid way to sequence DNA allows for faster and more individualized medical care to be administered, and for more organisms to be identified and cataloged [1].

Epigenetics

In the past fifty years, we have gained a great deal of understanding in the components regulating gene expression. We have discovered many of the transcription factors involved in their activation, particularly in immune responses and the cancer environment. The boosting of RNA replication is controlled by enhancers, and transcription driven by promoters. Transcriptional shutoff is mediated by silencers [16]. But in 2018, (Brown, et al. [17]) reported a new class of DNA with regulatory function, these being called superenhancers. They can act from a distance, upregulating gene expression on another chromosome (trans). Others are active in a tissue-dependent manner. While most act independently or in cooperation with conventional enhancers. We are only now beginning to understand the next level of transcriptional regulation mediated in the nucleosome and chemical changes to the complex of histone proteins exposing specific genes to be transcribed [18]. This has spawned the science of epigenetics – the branch of biology which studies how behaviours and environment can cause changes that affect the way genes are expressed [19]. Epigenetics plays a pivotal role in regulating gene expression in development in response to cellular stress or in disease states, in virtually all cell types [20]. It is the study of heritable changes in gene expression that do not involve changes to the underlying DNA sequence (i.e. change in phenotype but not genotype). One of its primary goals is to dissect the mechanisms behind these changes. One particularly well studied process is the covalent and noncovalent modifications of DNA and histone proteins and the mechanisms by which such modifications influence overall chromatin structure. Chromatin, the complex of DNA and its intimately associated proteins, is thought to be responsible for shaping the features of a cell's epigenetic landscape. By remodeling the chromatin structure or gene expression, epigenetic modifications cooperate with transcription factors and the translational machinery in fine-tuning gene expression. DNA methylation is perhaps the best characterized chemical modification of chromatin. In mammals, nearly all DNA methylation occurs on cytosine residues of CpG dinucleotides. Regions of the genome that have a high density of CpGs are referred to as CpG islands, and DNA methylation of these islands correlates with transcriptional repression [21]. Moreover, the formation of heterochromatin in many organisms is mediated in part by DNA methylation and its binding proteins in combination with RNA and histone modifications characteristic of silent chromatin.

Noncoding RNA

IN 1998, Andrew Fire and Craig Mello made a groundbreaking discovery that double-stranded RNA molecules inhibited gene expression in the nematode worm *Caenorhabditis elegans* [22], and this phenomenon was named RNA interference (RNAi). RNAi is a mediator of gene silencing. There has been rapid progress in identifying RNAi pathway components and elucidating the mechanisms of gene suppression [23] The basic RNAi response starts with long dsRNA being processed into small interfering RNAs (siRNAs) by a ribonuclease (RNase) III enzyme, Dicer. Next, the siRNA is incorporated into the

RNA-induced silencing complex (RISC). For target RNA recognition to occur, the siRNA duplex must be unwound, allowing binding of one siRNA strand to the target mRNA. This is followed by RISC cleavage of the homologous mRNA. Recent work has shown that the RNAi machinery is also involved in antiviral responses, transposon silencing, development and heterochromatin formation [24]. Three years later, a group led by Thomas Tuschl discovered that small RNA duplexes approx. 21 nucleotides (nts) can mediate RNAi in cultured mammalian cells [25]. Thereafter, several groups achieved potent RNAi-mediated gene suppression in vivo, in adult mouse liver and brain [26]. Our current understanding is that RNA found in the body can be divided into coding (mRNA, tRNA, rRNA) and non-coding. Recent research show that only less than 2% of our genome codes for proteins via transcribed mRNA [27]. Most of the rest of the genome codes for so-called non-coding RNA (ncRNA). These ncRNAs do not undergo translation to synthesize proteins but are implicated in gene regulation and human diseases [28].

We now know of several diverse types of ncRNAs of different lengths which may be related to their function. Among the ncRNAs characterized so far are microRNAs(miRNA), long non-coding RNAs, circular RNA's (circRNAs) are also emerging as relevant contributors to human disease. MicroRNAs have generated much attention recently because of their association with cancer. They are regarded as master regulators of the genome due to their ability to bind to, and alter the expression of many protein-coding RNAs [29]. There are now over 2000 miRNAs that have been discovered in humans and it is believed that they collectively regulate one third of the genes in the genome. miRNAs have been linked to many human diseases and are being pursued as clinical diagnostics and as therapeutic targets. Other ncRNAs are involved in chromosomal structure and organization, DNA replication and repair, transcriptional/post-transcriptional regulation, RNA processing and routing, translation and cellular energy/metabolism regulation. There is also evidence to suggest that invading pathogens use miRNAs to disable the immune system of the host [30]. Another important group of ncRNAs is short interfering RNA or siRNA. It also shuts off gene transcription like miRNA, but whereas miRNA is single-stranded, siRNA is double-stranded. Currently trials are being done to assess the feasability of anticancer therapy using siRNA's [31]. Recently, it has become evident that RNA, particularly noncoding RNAs, play a role in controlling multiple epigenetic phenomena [19].

Gene Therapy

Efforts to correct for defective genes causing primary immune deficiency diseases or tumorigenesis by gene therapy began in the 1990's, but progress has been slow due to a host of problems. Firstly, in order to correct for a defective gene containing single-base mutations, a single stranded DNA having the correct sequence, needs to recombine with the defective strand by homologous recombination activities [32]. Much work has been devoted to understanding these repair processes. The other line of investigation is to search for ideal vectors to introduce the therapeutic DNA into the cell. These include viral such as retrovirus, adenovirus, and adeno-associated virus. Non-viral vectors such as liposomes or nanoparticles appear less efficient, but have some advantages in that they are less immunogenic and can accommodate a larger amount of DNA [33]. The process of gene therapy remains complex and many techniques need further developments. There are two types of gene therapy (1) Germ line gene therapy where germ cells (sperm or egg) are modified by the introduction of the functional gene which needs to integrate and replace the defective DNA. This treatment would become hereditary. Because of this, there are serious ethical and legal issues pertaining to this type of treatment. (2) Somatic gene therapy where therapeutic genes are transferred into the somatic cells of a patient. Another major hurdle is to precisely insert the therapeutic gene in the chromosome and avoid off target effects. In the early days of gene therapy, the dangers were highlighted with the tragic case of two patients who were recipients for one of the earliest gene therapy trials, and developed a lymphoproliferative disorder due to inappropriate insertion of the retrovirus vector [34]. This problem has now been avoided with the discovery of clustered regulatory interspaced short palindromic repeat (CRISPR)\ Cas-based RNA-guided endonucleases for precision gene editing [35].

CRISPR)-Cas (CRISPR-associated proteins) modules are adaptive antivirus immunity systems that are present in most archaea and many bacteria and function on the self-nonself discrimination principle [36]. Bacteria have rapid reproduction rates, are metabolically diverse, and can produce complex molecules that cannot be produced through conventional chemical syntheses, such as enzymes and a myriad secondary metabolites [37]. With the development of metabolic engineering, many high-yield strains for industrial production have been established [38]. Bacterial cell factories have broad development prospects in industrial production. In recent years, CRISPR)/Cas systems were widely used for genetic engineering of bacteria, which has greatly promoted their application [39]. According to the structure and function of Cas protein, the CRISPR/Cas systems can be categorized into two classes (class I, class II), which are further subdivided into six types (type I–VI) [36]. Class I includes type I, III, and IV, and class II includes type II, V, and VI [40]. Type I, II, and V systems recognize and cleave DNA, type VI can edit RNA, and type III edits both DNA and RNA. How the effect of type IV system on DNA or RNA is still unknown [41]. In recent years, CRISPR)/Cas systems have been widely used for genetic engineering of bacteria, which has greatly promoted their application [41] (Table 1).

Table 1.

	Class I	Class II
Type	I	II
	III	V
	IV	VI

Proteomics

The genetic code is translated into a heterogenous chain of amino acids. The sequence of the amino acids determines the structure of the protein, due to folds or ridges created by the atomic forces between each amino acid and its neighbours. Just as genomics sequences DNA, proteomics sequences proteins. Understanding how proteins fold and assemble in 3-D shape is crucial in determining ligand-receptor interactions. Knowing such interactions, we can modify the amino acid sequence to alter the structure of the receptor or ligand by genetic engineering. Proteomics is the study of the interactions, function, composition and structures of proteins and their cellular activities [42]. But compared to genomics, progress in proteomics has been much slower due to the technical difficulties associated with limited material and the vulnerability of proteins to degradation over a short space of time and non-ideal environmental conditions. It is estimated that there are almost one million human proteins, many of which contain some modifications such as Post-Translational Modifications (PTMs). However, it is also estimated that the human genome codes for about 26000-31000 proteins [43], and sequencing them all is a daunting task. For years, proteins could only be studied by electrophoresis and sequenced by Edman degradation, where a radiolabeled sequence of protein is enzymatically digested and the order of amino acids read. This method is only able to read peptides up to 50-70 amino acids [44].

The major breakthrough equivalent to monoclonal antibodies or PCR in the field of proteomics was the application of the mass spectrophotometer in amino acid sequencing. Originally developed in the early twentieth century, it had been invented to measure the mass of atoms. One of its first contributions to science was to demonstrate the existence of isotopes. Thereafter it was used in the petroleum industry to measure small hydrocarbons in process streams. Only in the 1960's did chemists begin to understand how complex molecules fragmented inside the instrument and see its potential applications. The success of mass spectrometry is driven both by innovative instrumentation designs and by large-scale biochemical strategies, which use mass spectrometry to detect the isolated proteins. In the past twenty years, increased automation of sample handling, analysis, and the interpretation of results has generated an avalanche of qualitative and quantitative proteomic data. Protein-protein interactions can be analyzed directly by precipitation of a tagged bait followed by mass spectrometric identification of its binding partners. By these and similar strategies, entire protein complexes, signaling pathways, and whole organelles are being characterized. Posttranslational modifications remain difficult to analyze but are starting to yield to generic strategies. Proteomics has made significant strides in understanding disease mechanisms and identifying drug targets [45]. It plays a crucial role in comprehensively understanding cell identity, function, and interactions, which are vital for tissue repair [46]. It uncovers the intricate web of cell-cell interactions in both health and disease states [47]. The dynamic nature of the proteome, which varies with cell type and state, aids in the understanding of various diseases. Despite the technological challenges faced, proteomics accelerates the identification of molecular targets [48]. It also serves as a valuable source for disease markers, particularly in the field of organ transplantation [49], and finds applications in transfusion medicine [50]. Despite advancements, there are still many challenges in extracting low-abundance proteins and integrating omics data [51]. Therefore, the development of effective methodologies is crucial for comprehensive protein analysis [46]. Proteomics techniques can be broadly classified into two categories, untargeted or targeted: Untargeted or shotgun proteomics is aimed at comprehensively identifying and characterizing relative abundances of the totality of a sample. Targeted proteomics is focused on identifying and absolutely quantifying one protein. Targeted proteomics offers clinical insights with a high level of precision, while untargeted proteomics aids in the discovery of biomarkers and provides a global understanding of protein expression [52]. Integrating both approaches provides a holistic view [47], which enhances our understanding of genetic codes and the complexities of proteins [53]. Cutting-edge technologies, such as single-cell transcriptomics are propelling the field of proteomic research. The integration of mass spectrometry, microchip, and reiterative staining techniques enhances the assessment of cellular heterogeneity. Moreover, the incorporation of genomics readouts into multi-modal single-cell methodologies allows for the dissection of regulatory networks [54].

Immunology

The immune system is a giant network controlled by signaling messengers called cytokines. These proteins circulate through the lymph nodes and blood, stimulating B lymphocytes to make antibodies, to instruct T cells to proliferate, to make macrophages more phagocytic, amongst numerous other activities. These instructions are relayed through cytokine-specific receptors. The chemical signals in the form of phosphorylation trigger relaying to the cell nucleus, via intermediaries like G, Jak and Stat proteins which transduce the signal through the cytoplasm to the nucleus. Phosphorylation stimulates signal transduction whereas phosphatases shut down gene expression. Our traditional view of the immune system as a purely defence mechanism to protect from invasive pathogens and sense for damage to healthy tissue has undergone a radical change in thought in the past 20 years. Inflammation is the immunological battlefield for destroying invasive microbes, and immune cells like macrophages and T helper (TH) cells are a major source of inflammatory cytokines. The inflammatory response can also cause collateral damage to healthy tissue, and if left untreated, can cause chronic inflammatory diseases like rheumatoid arthritis, liver cancer or cardiovascular disease, or multi-organ failure in the case of a "cytokine storm" [55].

A newly recognized function of the immune response is to participate in homeostasis of the normal functions of growth and development. Macrophages, with their well-researched function of ingesting microbes and producing inflammatory cytokines, are called M1 mac-

rophages. They are an essential part of the innate immune system. They have an alter-ego called M2 macrophages. The latter suppresses an activated immune system, and the cytokines it secretes block the function of inflammation-causing T helper (TH) subsets and Vice'versa. The role of the M2 macrophages is to remove dead or dying cells or cell debris in the absence of inducing an inflammatory response. Like garbage collectors, they take up biological proteins destined for recycling. Likewise, TH cells also have a suppressor subset called regulatory T cells or TREGs. They train the immune system not to react to innocuous food protein antigens and also suppress inflammatory response after successful resolution of an infection. Any imbalance between the two, namely inflammation vs suppression can lead to disease. As mentioned above, chronic inflammation can cause serious immunopathological diseases. But going to the opposite end of the scale, cancer cells produce an environment of cytokines and cell receptor expression that favours the viability of the tumour cell.

By promoting expression of suppressor cytokines, stimulating the activation of TREG cells, and expressing receptors which block vital signals that cytotoxic T cells (CTLs) and Natural Killer (cells) Need to be fully activated. These signals are called co-stimulatory molecules and are the targets for boosting the immune system to fight cancer cells. By blocking these ligand/receptor interactions, with specific monoclonal antibody, these suppressive signals induced by the cancer cell are inactivated [56] This is the basis for checkpoint therapy. Recent developments in neuroimmunology [57] show that the primary and secondary lymphoid tissue are intricately connected with the the brain, through hormonal, cytokine-mediated interactions or even neural junctions. We should therefore consider the immune system as part of the autonomic nervous system [58]. Understanding how the complex interplay of stimulatory signals works within a network of cells involved requires a reworking of traditional research methodology which employed a reductionist approach. With the age of genomics, bioinformaticians began creating libraries of annotated genes, their DNA sequence, protein structure and function. The different cytokine-mediated signals being transmitted between and inside cells could be mapped as one large system, consisting of nodes and edges. Analysing these networks required the creation of a new science, systems biology.

Systems Biology

Thanks to genomics, immunologists have mapped the signaling pathways involved in diverse cellular processes like responses to inflammatory or suppressor cytokines, and identified the signaling messengers of Jaks and Stats, G proteins in transmitting these signals. This has spawned a new field in the form of bioinformatics and systems biology, which recruited experts in the field of data analysis, statistics and computer programming. Systems biology is a comprehensive analysis of the manner in which all the components of a biological system interact functionally over time [59]. There are several goals in this endeavor. One is to find master controllers that regulate inflammatory responses. Since the biological systems has many built-in redundancies, experiments demonstrating the absence of function as a result of the gene knockout did not occur, because its absence of function was replaced by a signal from another receptor specific for a different cytokine. Nevertheless, a few master regulators have been identified, and are potential tools for immunotherapeutics, either to dampen the inflammatory response as in the case of sufferers of chronic inflammation, as in crippled arthritis sufferers, or to boost the immune system in cancer sufferers.

High Throughput Experiments

This entails high throughput experiments like single cell transcriptomics [60]. Key to driving forward single-cell transcriptomics has been the scaling of technologies to profile large numbers of cells in parallel. During the early developments of this technology, several methods were devised to facilitate sequencing of transcripts (transcriptome) within a single cell. These protocols differed in their amplification technology and transcript coverage, as well as in the exent of robotisation of liquid handling in plates containing the RNA transcripts (transcriptome) in plates containing 384 wells. This was followed by development of nanodroplets, picowell technologies and in situ barcoding which have made it possible to sequence tens of thousands of cells in parallel [61]. In recent years multi-modal single-cell methodologies that measure and integrate genomics readouts from different molecules (RNA, DNA and protein) [62] enabled dissection of the complex regulatory and cell-cell communication networks that drive cell identity to a greater degree than ever before. After years of developing high throughput transcriptome atlases in animal tissue, scaling of single-cell and single nucleus RNA-sequencing (sxRNA-seq) is one of the sequencing technologies that is now applicable to the whole human body. Single-cell transcriptomics are being used to create reference maps of healthy human tissues, organs and systems at single-cell resolution. These approaches are also being applied to understand non-human organisms, including mice and non-human primates. Analysing healthy versus diseased tissues and genetic variation between individuals is also a valuable platform for the understanding of disease mechanisms. Factors identified from in vivo single-cell transcriptomic studies can also be applied to generate improved in vitro models and responses to therapeutic screening can be assayed at the single-cell level. Such experiments yield data which must be processed and analysed, as well as stored and compressed [63]. Amongst the current projects using single-cell transcriptomics, there are efforts to determine the antibody repertoire encoded by B cells in the blood or lymph nodes.

Regenerative Medicine and Synthetic Biology

Regenerative medicine and tissue engineering aims to repair damaged tissue. It is an interdisciplinary field that applies engineering and life science principles to promote regeneration, can potentially restore diseased and injured tissues and whole organs. However, in order to do this, we first need to have a comprehensive atlas depicting cell identity, function and interaction in healthy tissues, as

well as understanding how these change upon tissue disruption, such as that cause by injury, ageing or infection. Tissue injury response is a highly dynamic process driven by complex interactions between immune and stromal cell populations [64]. In 1998, James Thomson succeeded in isolating stem cells from human embryos and cultivating them as Embryonic Stem cells (ES cells) in the laboratory. The ES cells can be transformed into almost all human cell types. Thomson thus created an important experimental base for stem cell research and regenerative medicine [65]. In 2014, Mandai et al successfully transplanted a sheet of Retinal Pigment Epithelium Cells (RPE), which were differentiated from autologous Induced Pluripotent Stem Cells (IPS) into the eye of an elderly woman suffering from age-related macular degeneration [66]. The transplanted sheet remained intact after one year of transplantation, although there was no improvement in visual acuity. After four years at follow-up, the transplanted RPE sheet still survived beneath the retina and showed improved growth with respect to a slight expansion of the pigmented area and no adverse effects. The outer nuclear layer above and adjacent to the graft showed marked thickness and an organized structure, with evidence of vascularization and support of photoreceptors [67].

Genomics has also had a major impact on synthetic biology, whereby artificial proteins are created by expressing different parts of genes to create a chimeric molecule, as exemplified by the chimeric antigen receptor (CAR)-T cell. This product has been shown to be highly effective in treating B cell lymphomas. Using genetic engineering, scientists were able to get T cells extracted from patients with B cell lymphoma to express a hybrid antibody molecule, the CAR. This consists of an artificially created gene coding for an antigen recognition sequence derived from specific antibodies fused to the signaling region of a T cell receptor. This hybrid gene is then introduced into autologous T cells extracted from the patient by retroviral transfer and re-infused into the patient. The T cells expressing the CAR are able to bind to the CD20 antigen expressed by the B cell lymphoma without MHC restriction and activate the CAR-T cell to kill the lymphoma [68]. Another example of a chimeric antibody is in the creation of genetically modified antivenom or anti-tetanus antibodies. Passive vaccination (injection of pre-formed antibodies) is given to patients requiring immediate treatment, as in the case of patients suffering from snakebites, rabies virus infection or exposure to tetanus toxin. The aim is to immobilize the venom, virus or toxin before it spreads throughout the body. Traditionally, these antibodies have been raised in horses which were inoculated with the offending agent. However, some patients react by producing antibodies against these horse antibody proteins, causing a mild fever and rash called serum sickness. To avoid this, humanised antibodies have been created composed of the antigen-binding Fab region of a horse antibody and a Fc region of a human antibody [69]. Recently, another synthetic protein was created when (Mi, et al. [70]) created transgenic silkworms by using CRISPR to replace most of the gene sequences coding for the primary silk protein with a spider silk protein gene called MiSp. The transgenic silkworms produced fibers with high strength and toughness.

Diagnostics and Therapy

Nowhere has the preparedness and speed by which significant discoveries can be made thanks to genomics been demonstrated as during the SARS-CoV-2 pandemic. The virus surfaced in late December 2019 and declared by the WHO as a pandemic in March 2020. Critically ill patients first started to appear at the Wuhan General Hospital. China, on 24 Jan 2020. In the same month, the scientists at Wuhan had succeeded in isolating, culturing, and sequencing three genome sequences which were entered into the public domain [71,72]. The release of this code resulted in the creation and mass distribution of the Pfizer-BioNTech BNT162b2, Astrazenica RNA-based vaccines, amongst others [73]. Meanwhile by 24 March, a serological assay had been developed for one of the viral antigens. On the 24th March, those with severe COVID-19 showed a deficiency in the ability to produce two types of antiviral proteins called interferons. In April, several patients who had died were declared to have experienced cytokine release syndrome, In May, a group announced that those with pre-existing antiviral antibodies and experiencing milder illness had cross-reactive T cells protecting them from SARS-CoV-2. By August, mild disease and infection was associated with a robust B cell-mediated antibody response [74]. Proteomics played a major role in the developing of the vaccines for SARS-CoV-2 as well as understanding the biophysical properties of the virus.

Translational Medicine

With this diverse array of advanced technology and scientific knowledge, many have predicted that the future of medicine lies with translational medicine, the "bench to bedside approach. The basis of "personalized medicine" is that all patients should not be treated in a uniform manner. The toxic side effects of chemotherapy or radiotherapy could be minimized if each patient's characteristics were taken into account. The goal of translational medicine is to provide a precise approach to the prevention, diagnosis and treatment of disease using genomics [75]. The greatest application of genomics and proteomics (panomics) research is in the field of oncology. Cancer can result from an accumulation of mutations in a particular tissue causing uncontrolled cell division. The information gathered from panomic analysis can be used to determine the cause of an individual patient's disease at the molecular level. Once the pathophysiology of the disease process is determined, targets can be identified to utilize specific treatments to address that individual patient's disease process [76,77]. Thanks to panomics, we now have a clearer picture of the heterogeneity of cell types within the tumour environment. These findings lead to a novel approach in pharmacotherapy. Some drugs which have not shown promising results in general might prove to be very successful in a subset of patients with a particular genetic profile. The most notable example use of tastuzumab in a subset of breast cancer patients whose lesion is tested positive for over-expression of the HER2/neu receptor. Another example are tyrosine kinase inhibitors such as imatinib used in the management of chronic myeloid leukemia. In most patients with this malignancy, the BCR-ABL fusion

gene (the product of a reciprocal translocation between chromosome 9 and chromosome 22) is present. Imatinib and other tyrosine kinase inhibitors in this class specifically inhibit the Abelson tyrosine kinase protein and are thus a prime example of targeted therapy based on knowledge of genomics [78].

Conclusion

In this review we have attempted to show how the completion of the Human Genome Project has greatly benefited biomedical research in the past twenty years. Despite these advances, many moral and ethical issues still remain with regards to the role of science and technology in society. This epoch has been labelled as the Anthropocene, an appropriate term for the impact that Homo sapiens has had on the planet. How this powerful knowledge affects life on earth and how to temper it remains to be seen.

Acknowledgement

The APC was financially supported by the Office of Research and Innovation at Alfaisal University, Riyadh, Kingdom of Saud Arabia.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2024.54.008558

Garwin Kim Sing. Biomed J Sci & Tech Res

Submission Link: https://biomedres.us/submit-manuscript.php