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Recent developments in systems biology and genetic engineering toward design of vaccines for TB

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Abstract

Tuberculosis (TB) is one of the most prevalent diseases worldwide. The currently available Bacillus Calmette-Gúerin vaccine is not sufficient in protecting against pulmonary TB. Although many vaccines have been evaluated in clinical trials, but none of them yet has proven to be more successful. Thus, new strategies are urgently needed to design more effective TB vaccines. The emergence of new technologies will undoubtedly accelerate the process of vaccine development. This review summarizes the potential and validated applications of emerging technologies, including: systems biology (genomics, proteomics, and transcriptomics), genetic engineering, and other computational tools to discover and develop novel vaccines against TB. It also discussed that the significant implementation of these approaches will play crucial roles in the development of novel vaccines to cure and control TB.

Keywords

Mycobacterium tuberculosis ; systems biology; bioinformatics; proteomics; genomics; genetic engineering

Introduction

Mycobacterium tuberculosis (Mtb) is a globally important pathogen that causes tuberculosis (TB) in susceptible humans. The World Health Organization (WHO) estimates that 9–10 million patients are diagnosed with TB and 1.3–1.5 million die of TB each year.

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India, China, the Russian Federation, and sub-Saharan Africa have high TB prevalence rates, where Mtb infection is acquired in childhood, and disease peaks in middle-aged adults [1]. Mtb is transmitted by aerosol droplets produced by coughing, and disease is characterized by: fever, coughing, night sweats, and weight loss in approximately 10% of those infected [2]. Severely immune-compromised people are at increased risk of TB disease progression because they cannot generate protective immunity to primary Mtb infection [3]. As multidrug-resistant (MDR), extensively drug-resistant (XDR), and totally drug-resistant (TDR) cases increase, development of new vaccines (prophylactic and therapeutic) is urgently needed [4].

Vaccination has undoubtedly been of tremendous benefit in promoting a healthy population of the world. This has dramatically saved lives, lowered medical costs and increased the quality of life for men and women [5]. Till now, the 100-year-old vaccine, *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) is the only commercial vaccine against TB. It is a live-attenuated strain of *Mycobacterium bovis* [6]. However, in normal, immune-competent adults, BCG's protective efficacy ranges from nearly 0% to 80% against pulmonary TB. Unfortunately, BCG is neither safe nor effective for immune-compromised individuals [7]. Also, the WHO Global Advisory Committee recommends against BCG vaccination of HIV-positive individuals because exposure to live mycobacteria can cause BCGosis [8]. Thus, there is a need to focus on discovering new TB vaccines that are safe and effective for immune-compromised people.

New technologies, including systems biology and genetic engineering, facilitate vaccine development [9]. Systems biology approaches include: genomics, proteomics, and transcriptomics, which will significantly accelerate our understanding of the various biological features of Mtb [10]. These approaches, together with genetic engineering, will empower the development of more potent vaccines. Vaccine development has changed from empirical testing to rational strategies using recombinant DNA, conjugation, and smart epitope design to improve efficiency and translational potential [11]. Moreover, vaccine designs by systems biology will significantly save time and labour. Vaccinomics combined with bioinformatics for epitope predictions have led to the emergence of new vaccine design platforms [12]. Immunological experiments produced a large amount of data that needs a bioinformatic approach for analysis with precision and speed [13]. Using immunoinformatics, vaccine candidates can be predicted by analyzing the systems biology of the pathogen [14].

Moreover, computational approaches significantly reduce the cost of searching for specific antigens [15,16]. Successful implementation of systems biology approaches and computational tools (i.e. omics studies) has revolutionized vaccine design against TB. This review provides insight into the systems biology coupled with the genetic engineering of Mtb with a focus on vaccine development. Furthermore, we include important information on the bioinformatics tools and techniques involved in studying systems biology and genetic engineering to facilitate the development of vaccines for TB.

Systems biology approaches for vaccine development

The complex pathogenesis of Mtb has involved a broad range of interactions among different immunogenic factors and the host immune system. The dynamic mechanism of Mtb pathogenesis makes it challenging to predict whether a particular target will affect the host system [17]. It will be easier to study the complex mechanism if it is disintegrated into several small components. Considerable information has been produced while analyzing these mechanisms from individual components, creating a challenge to integrate these datasets to predict intervention outcomes. Systems biology aims to understand, explain, and predict various biological phenomena produced from the dynamic interaction of the components [18]. Basically, systems biology involves computational approaches to analyzing complex biological data, including: genomic, transcriptomic, proteomic, and metabolomic data (Figure 1). This allows a comprehensive study of: vaccine–host, host–pathogen, and vaccine–pathogen interactions. Systems biology can identify interactions, predict molecular and cellular outcomes, and guide the selection of vaccine candidates [19]. Furthermore, this approach may also identify correlates of vaccine-induced protection from bystander immune responses and identify immunologic correlates of vaccine failure [20]. Systems biology can be further empowered by incorporating aggregate databases and computational tools (Table 1) to predict new vaccine targets [36].

Immunomics comprises all the molecules related to the immune system and involves all the technologies of bioinformatics, genomics, and proteomics that help study [37] the immune system's response against pathogens. Moreover, it also involves systems biology methods to analyze the immune system related molecules [38]. Compared with other body systems, the immune system is highly diverse, and traditional approaches are insufficient to study such a highly complex system. Therefore, immunomics become a powerful tool in vaccine design and target identification [39]. With the incorporation of genomics, proteomics, molecular biology and immunomics provides insight into immune function and identifies immune targets with antigenic diversity [40]. Many genes are highly polymorphic in the human immune system, contributing to diverse immune responses against the diseases [41]. The outcome of vaccination is also affected by these variants. Around 3.1 million SNPs were identified in the International HapMap Project in 270 people [42]. An essential component of immunogenetics is vaccinomics, which analyzes variations in host genetic markers affecting the immune response against specific antigens [43]. Besides that, it also predicts and minimizes the failure of vaccines and their adverse effects. Likewise, pharmacogenetics also detects genetic differences in individuals based on metabolism reactions toward a particular vaccine [44]. Isoniazid is the first-line drug of TB linked with high variability in human metabolism consisting SNPs encoding arylamine N-acetyltransferase [45,46]. All the aspects of systems biology like genomics, transcriptomics, and proteomics are discussed sequentially in this review.

Genomics

Genomics is the study of genes and methods used to determine interrelated functions in the whole organism [47]. The most important step in vaccine design is to identify the antigens that can trigger the host's immune response [48]. Genomics is the backbone of the omics study and its implementation enhances vaccine design [49] as shown in Figure

2. The availability of genomic sequences help in analyzing genetic patterns and identifying virulent factors [50]. For each major pathogen, at least one genome sequence is available in the genome repository [51]. The complete genetic information of antigens or novel vaccine targets can be identified by exploring genome sequences of Mtb. Databases and software are also available on mycobacteria, such as Mycobrowser (https://mycobrowser.epfl.ch/) [52]. These databases are repositories for genome, proteome, and transcriptome information of Mtb, providing rich sources of data for vaccine design against TB.

In concern with vaccine development, the study of host genetic factors is equally important and plays a significant role in understanding TB infection [53]. The availability of the human genome project (http://www.1000genomes.org/) is a valuable source to obtain complete information on human genome sequences [54]. Therefore, the presence of both host and pathogen sequences can accelerate the detection of novel vaccine candidates. Moreover, the vaccine projects based on genomics are considerably helpful in increasing the understanding of mycobacterial epidemiology, physiology, and pathogenesis [55]. Genomic variation within the species is very challenging in the selection of potential vaccine targets. It is somehow resolved by the pangenomic reverse vaccinology, which is a powerful budding tool for the detection of new vaccine targets for multiple genome sequences present for single species [56]. Particularly, pangenomics is useful in the identification of a complete set of genes in a single species. A comparative genomics study between pathogenic and nonpathogenic strains helps in virulent protein identification and thus aids the process of vaccine development [57]. Advent of new sequencing technologies will definitely open a gateway to screen pathogenic genomes while selecting the vaccine candidates. Moreover, the availability of diverse bacterial sequences will assist in antigen identification by observing a cluster of single nucleotide polymorphisms (SNPs) and various mutations affecting protein sequences under immune selection [58].

The most important vaccine development process is the implementation of systems biology to investigate the host–pathogen interactions to discover novel vaccine candidates and develop *in silico* based testing models for their analysis [59]. Mtb and host interaction is a complex topic, and is important to study in terms of vaccine design. To design a vaccine, it is important to know the evolving strategy of Mtb to escape from the host immune response [60]. It is also important to understand the behavior of Mtb in the host cell. Moreover, the interaction mechanisms also hint for the design of novel vaccines and drugs [61]. Host–pathogen interaction studied by protein–protein interactions and knockdown screening technologies is helpful in identifying virulent factors, pathogenesis pathways, and candidate genes involved in infections [62]. In addition, gene signatures are an important approach of systems biology for identifying and characterizing the immune responses in humans who are vaccinated with the yellow fever vaccine YF-17D [63]. Initially, Gaucher *et al.* [64] applied functional genomics and a polychromatic flow cytometry approach to identify unique signatures in the human response of YF17D. Querec et al. [65] used the computational study to demonstrate the induction of gene responses in YF-17D.

Transcriptomics

Transcriptomics deals with the study of the complete set of RNA transcripts during defined conditions such as infection with a pathogen [66]. The common methods used to analyze transcripts are microarray and RNA sequencing. Santoro et al. [67] used transcriptomics to identify the Mtb antigen H56, as a potential vaccine candidate, and immunogenicity results showed that H56-induces antigen-specific CD4+ T cells, B cells, and antibody responses. To our knowledge, the protective efficacy of H56 against the virulent Mtb challenge has not yet been published. Roy et al. [68] applied Cap Analysis Gene Expression (CAGE), a transcriptome technology to identify the promoter-based transcriptional landscape of interferon-gamma (IFN-γ) or interleukin (IL)-4/IL-13 stimulated macrophages infected with Mtb *in vitro*. Although fewer databases are freely available to identify immunological pathways of infected hosts or infected host cells, the databases InnateDB, MSigDB, and Ingenuity possess manually annotated genes and provides valuable information about pathways related to the immune functions [69]. Computational methods for the identification of dormant antigens and T cell epitopes were used to predict a potent vaccine target by Sundaramurthi et al. [70].

Implementation of genomics, proteomics, and transcriptomics, collectively termed as omics, is growing in different areas to fight against various infectious diseases. The primary step is to collect genetic information, and single-cell sequencing is the best option. CITE-seq and REAP-seq are the best methods to study infectious disease models involving the interconnection of genomics and proteomics [71]. Analysis of scRNA-seq of the peripheral blood mononuclear cell (PBMC) was performed in patients suffering from active and late TB and some healthy individuals [72]. Data showed regular depletion in the natural killer (NK) cells in samples of LTBI and TB. Thus, NK cells were subsequently used as a biomarker to differentiate active TB from late TB. The interaction of different immune cells at the single cell level helps to understand the response of vaccines with the advancement of single-cell technology. Additionally, it will also help to recognize the effect of heterogeneous vaccine responses in the immune cells [73].

Many challenges come with the implementation of scRNA-seq, such as the question regarding experimental techniques [74]. The scRNA-seq starts with the preparation of a single-cell suspension, which must have the exact property of the original tissue. However, different scRNA-seq can be studied by applying the transcriptome approach [75]. Besides that, the half-life of mRNA, post-translational modification and cellular trafficking affects the protein expression and cellular function [76]. Furthermore, REAP-seq and Ab-seq are used to study the RNA-based experiments [77].

The application of transcriptomics to analyze differential gene expression under various growth conditions is helpful in antigen selection [78]. An essential step in the identification of vaccine antigens is to understand the upregulation of genes in the infection process as they can act as a potential vaccine candidate. In addition, the systems biology approach is also applied to evaluate the efficacy of BCG [79]. With the help of the microarray transcriptome, Cortes et al. analyzed BCG vaccination in mice and also defined bio-signatures. The success of BCG and sub-unit vaccination in cattle is predicted in the murine *M. bovis* infection model. Whole transcriptome of the lung and spleen of BALB/c mice was

studied by using microarray technology to obtain the biosignature. Afterwards, they carried out a study of *M. bovis* infection and BCG vaccination. They found precise pulmonary gene expression signatures dealing with the development of connective tissue and Th17-related cytokine profiles. However, the infection study with virulent *M. bovis* helps in the prediction of the success of this vaccine by inducing protective immunity [80]. Zárate-Blades et al. applied systems biology to characterize the transcriptome of mice infected with Mtb, and reasoned the heat shock protein 65 as a potential DNA vaccine [81]. Thus, it can be concluded that the transcriptomics study facilitates the identification of immunological signatures that are associated with the immune response during Mtb infection [82]. The potential application of the transcriptomics approach on Tb vaccines is still in the growing stages and needs some more effort to build a model for the analysis of vaccine efficiency.

Proteomics

Proteomics is also a budding approach along with bioinformatics tools that enhance the progress of vaccine development. Compared with genomics and transcriptomics, proteomics study is not stable and dynamic in nature [83]. Undoubtedly, proteomics is a powerful tool to study the various aspects of cellular proteins, including their identification, localization, modifications, and functions. Various researchers established the proteome databases of different pathogens such as *Bacillus anthracis*, Mtb, *Helicobacter pylori*, and *Salmonella enteric* [84]. Secretory proteins of Mtb are essential for its pathogenesis and also were identified as potent antigens [85]. However, different novel protective antigens of Mtb were identified through traditional methods. However, genomics and proteomics analysis enhanced the research at the vaccine level to confirm the usefulness of selected antigens and thus expand the list of new candidates in order to construct subunit vaccines [86].

Furthermore, Mtb secretary proteins were also proved as T-cell antigens. However, more effort is needed to understand the secretome of Mtb. Culture supernatant proteins of Mtb and *M. bovis* BCG which have been comparatively analyzed by Mattow et al. and 22 novel secreted proteins were identified in the Mtb sub-proteome. Moreover, they also detected the existence of five proteins encoded by ORFs earlier reported to be absent in *M. bovis* BCG compared with Mtb [87]. Also, Målen et al. [88] applied the proteomics approach and identified 257 secreted proteins in Mtb and most of them are lipoproteins. Apart from these secreted proteins, more secreted proteins of Mtb have to be explored for their functional roles. Therefore, the immunological features were screened out in order to uncover novel protective antigens. The multi-component subunit vaccine consists of five secreted proteins (CFP-25, CFP-20.5, Ag85B, Ag85A, and CPF-32) and is highly immunogenic and stimulates both humoral and cell-mediated immune responses and provides better protection when compared to the BCG vaccine [89]. Mtb proteins present in the outer membrane could be studied as a potential vaccine candidate [90]. Therefore, the approach of proteomic experiments is required to identify the novel antigens in Mtb. However, the proteomics approach is quite challenging to identify specific antigens, which are sufficient to stimulate T-cells. Furthermore, these challenges can be preferably overcome by the implementation of *in silico* tools. McMurry et al. [91] used bioinformatics tools to identify Mtb secreted proteins and analyzed them as potential multi-epitope vaccine candidates.

Epitope identification is important in vaccine design, and bioinformatics tools can identify potentially interacting host and pathogen molecules [92]. Multiple epitope prediction tools (Table 2) may help inform TB vaccines, including “immunoinformatics” algorithms to predict B/T cell epitopes [92]. The advent of novel techniques such as protein conjugation and recombinant DNA technology help to design vaccines against hepatitis B, pneumonia, meningitis, and human papillomavirus [48]. Meningococcus B is the first for which reverse vaccinology was applied [104]. The genome sequencing identified 600 antigens, among which 90 were surface proteins. These antigens induced a protective immune response in mice. This technology is also implemented in other sequenced pathogens such as *Staphylococcus aureus*, group B *streptococcus*, Chlamydia, *Streptococcus pneumonia*, and group A streptococcus. Reverse vaccinology and antigenome techniques identify *Staphylococcus aureus* antigen as a vaccine in phase III clinical trials [105]. In the case of the respiratory syncytial virus (RSV), the immunogen designed by computational methods mimics the binding site RSV neutralizing monoclonal antibody and induced a positive immune response in monkeys [106].

Reverse vaccinology is a powerful tool to predict MHC class I and class II binding domains [107] as explained in Figure 3. Proteomics has also been used to predict T-cell epitopes and MHC-II antigen-binding from the very large *M. bovis* proteome, which has provided many antigen candidates for vaccination or diagnostics for *M. bovis* [108]. The epitope prediction studies that have been performed so far in Mtb are tabulated in Table 3. Based on these studies, novel vaccines against TB are in clinical trials and few vaccine candidates are also designed to treat the reactivation of latent TB. Exploring the immune response against Mtb and the pathogen response against this response could open the gateway and provide new strategies to develop novel vaccine candidates [116].

Proteomics is the large-scale study of proteins that can be used to study the host and pathogen singly or host–pathogen interactions. Proteomics has identified new antigens and amino acid sequences used in vaccine development [117]. Proteomics on host cells and tissues provides in-depth information on innate and adaptive immune responses and a sophisticated means to assess host outcomes to vaccination and the pathogen challenge. Tucci et al. [118] profiled the proteome of the Mtb culture filtrate. They found 33 new O-glycosylated proteins, including polyketide and non-ribosomal proteins, while others predicted T-cell epitopes for these O-glycosylated proteins and polyketides. Jungblut et al. [119] performed a comparative proteomic analysis in *Mycobacterium* bovis BCG and Mtb and predicted eight proteins specific to BCG while 13 were specific with Mtb. These proteins could be used for vaccine design and drug development. Recently, research is moving in the direction of structural proteomics to understand a 3D model of the identified antigens with the help of X-ray crystallography and NMR [120]. Genuinely, it will guarantee the progress of identifying novel vaccines based on fusion proteins. The combination of two fusion molecules comprises Ag85B and ESAT-6 whereas the other consisting of Ag85B and TB10.4 was demonstrated to induce a positive immune response in various animal models [121]. A TB Structural Genomics Consortium was established in 2000 with the aim to eliminate TB through the progress of new intervention against Mtb [122].

Genetic engineering approaches for vaccine design

Genetic engineering is a novel approach for vaccine design. Advancement in understanding the pathogenesis of Mtb facilitates the design of new vaccines [86]. As an example, the functional role of the esx-3 locus was studied in mycobacterial pathogenesis by Sweeney et al. [123]. They found that esx-3 is functionally involved in the evasion of innate immunity. *Mycobacterium smegmatis* with esx-3 gene inactivated could serve as a new vaccine vector while enhancing the innate immune response activation. Furthermore, the expression of esx-3 in Mtb resulted in a more effective TB vaccine in comparison with BCG when administered through the intravenous route. In another study, Ahn et al. [124] overexpressed phoP or phoPphoR in *M. bovis* BCG and showed that recombinant BCG was safe in CB-17/IcrIco SCID (severe combined immunodeficiency) mice and provided better protection against Mtb. More importantly, this approach has improved vaccination against a number of diseases and many vaccines in clinical trials against TB have been genetically engineered. Some of the proposed vaccines against TB are described in Table 4.

Modern technologies such as transcription activator-like effectors nucleases (TALENs), zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats CRISPR Cas technologies are valuable tools (Figure 4) and have been used in genetic engineering [132]. TALENs cleave DNA nonspecifically [133], while ZFNs mediate insertion, deletion, inversion, point mutation, duplication, and translocation events [134]. CRISPR Cas is a recent technology, and advantageous during gene modification to construct recombinant vaccines. Generally, genes can be inserted by two different means; error-prone non-homologous end joining (NHEJ) and the high-fidelity homology-directed repair (HDR) pathway [135]. Mostly, HDR is used in studies for high fidelity in vaccine design and NHEJ is advantageous due to its high efficacy. CRISPR and protein associated with CRISPR are capable of providing immunity [136]. The CRISPR-Cas technology has been used in Mtb for vaccine design. CRISPR-Cas10 was used in Mtb genome manipulation by Rahman et al. [137]. They functionally explored the genomics of Mtb to identify genes related to growth that is helpful in the discovery of mycobacterial vaccines.

Furthermore, these studies suggested that CRISPR-mediated vaccine development is a rapid and effective way for vaccine design. Genetically, engineered subunit vaccines are advantageous as they do not require adjuvants and are highly stable when lyophilized. The modified vaccinia Ankara virus (recMVA) poxvirus is a well-accepted virus vector and has been used to deliver Ag85A, a vaccine tested in human clinical trials against TB, and is safe and immunogenic, although it was not more efficacious than BCG vaccination [138].

Metabolic engineering

The systems biology approach has been successfully used to analyze host–pathogen interactions and identify metabolic pathways of Mtb [116]. Understanding the interactions of host–pathogen is helpful in the identification of potential vaccine targets. Metabolic engineering is also an essential approach to target metabolic pathways [139]. It is a powerful tool to manipulate microorganisms' cellular and metabolic character to produce the desired product. At present, this technology has been used in different aspects of biology. It can also be explored for vaccine development by generating live-attenuated strains or producing

pathogen metabolites that act as novel antigens [140]. This approach has also been used to genetically modify Mtb metabolism to generate a source of novel antigens and potential vaccine candidates [141]. The tricarboxylic acid cycle (TCA) of Mtb is an essential pathway and involves isocitrate lyase (Rv0467) and the malate synthase (Rv1837c) [142]. Succinate, a product of the TCA, produced under hypoxic conditions, and provides Mtb a means to maintain ATP synthesis [143]. It was observed that inhibitors of isocitrate lyase are lethal for Mtb, thus suggesting a potential target against TB [144]. P-type ATPases encoded in the genome of Mtb in large numbers reveal the evolutionary significance of cation homeostasis. The CtpV deletion, which is one of P-type ATPases in Mtb, results in virulence attenuation [145], providing a promising alternative for BCG. PhoP is a transcription factor in Mtb, and regulates approximately 80 genes involved in virulence. FadD26 encodes an enzyme participating in the phthiocerol dimycocerosates biosynthesis. In light of these findings, metabolic engineering was also used to generate MTBVAC (another live vaccine candidate), a double-deletion Mtb mutant in which PhoP and FadD26 were removed [146] following the WHO guidelines that Mtb-based live vaccines have a minimum of two mutations. Currently, this vaccine is in phase I clinical trials. These studies suggest that metabolic engineering is an essential tool and opens a new doorway in vaccine development.

Limitations and challenges of systems biology

Genomic and proteomics are valuable tools to characterize the functional molecules of different signaling pathways in biomedical research [147]. However, these technologies are costly and need well-specialized facilities along with a skilled staff for data analysis. Systems biology and genetic engineering need to be further developed for searching for therapeutics at a more reasonable cost [148]. Progress is still being made to apply the systems biology approach in finding novel vaccine candidates for TB, while the application of systems biology is successfully applied in various fields. The advantages and disadvantages of systems biology and genetic engineering are tabulated in Table 5. Systems biology generates large amounts of data while studying genes, proteins, and RNAs. Therefore, sufficient and efficient computational tools are needed to understand the gene’s virulent nature and host–pathogen interactions. One of the significant challenges is the calibration of computational tools, models, and algorithms. Thus, the approach of systems biology in vaccine design is so far limited as it is an emerging approach that required wet-lab data validation and specialized instrumentation to identify a novel vaccine. For these reasons, there is still limited use of systems biology in vaccine development.

Conclusions and future perspective

TB vaccines are under a pipeline with considerable new preclinical and clinical trials in the last few decades, but none of them have been successful. It needs special attention along with novel strategies to identify novel vaccine candidates. Proper use and the application of genetic engineering, metabolic engineering, and systems biology approaches are essential to address the critical need for new, protective vaccines against TB. These methods will continue to provide a theoretical basis for the rational selection of novel vaccine antigens/candidates for experimental and clinical testing and mechanistic insight into pathogen survival, metabolism, and pathogenesis as a host. In particular, metabolic engineering

focused on manipulating pathogens' metabolic pathways for either live strain attenuation or generating novel antigens is an exciting framework to guide the selection and development of future vaccines. Furthermore, the coupling of systems biology, genetic engineering, and metabolic engineering will be helpful during vaccine development. Eventually, these combinations will be important in the future for the successful eradication of TB.

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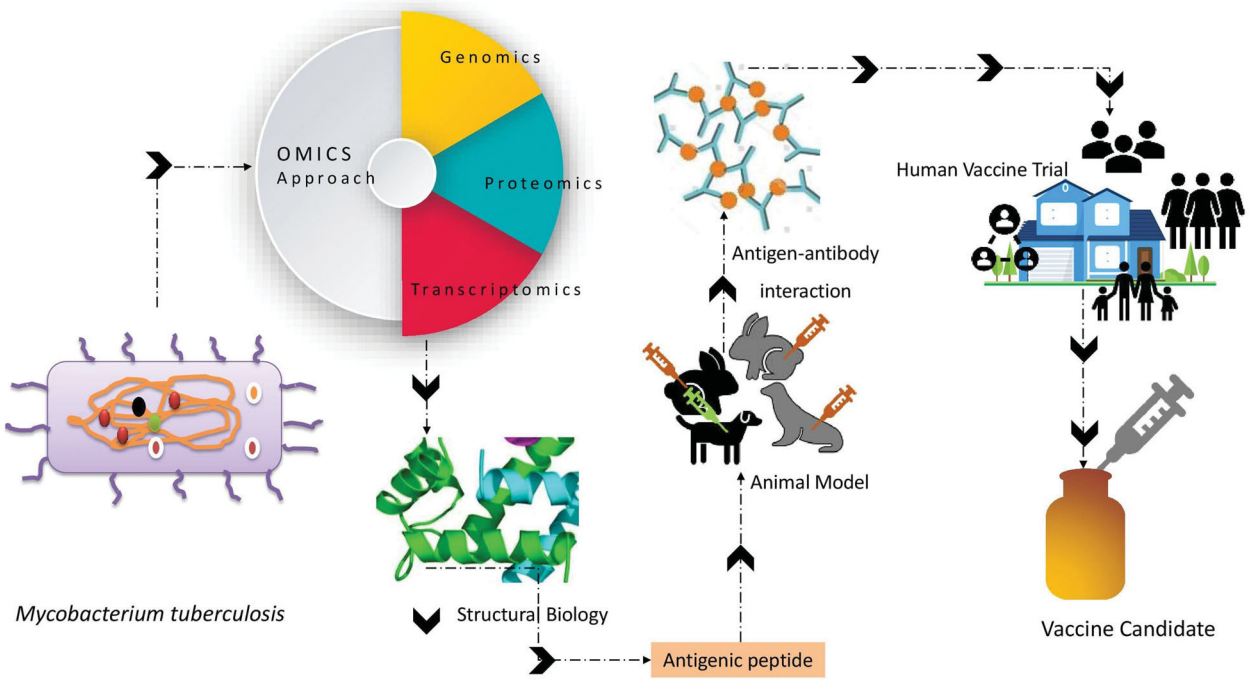

Figure 1.

Overall workflow using multi-omics approach in the process of vaccine designing. The multi-omics approach is used for vaccine designing. Initially, Mtb is subjected to omics study including genomics, proteomics, and transcriptomics. Furthermore, structural biology is applied to find out the antigenic peptides. The selected antigen is carried out for animal testing and after getting specific antigen–antibody reactions, the human trial started. The positive immune reactions against the selected peptide in the human body make it a potential vaccine candidate.

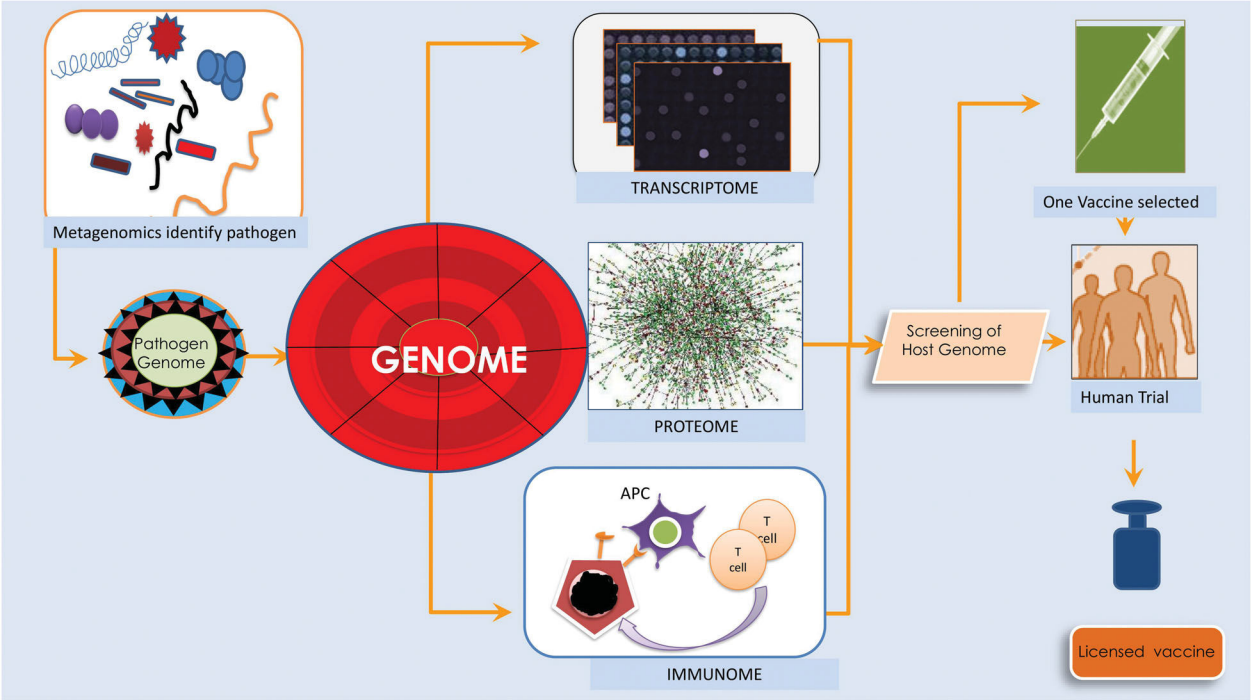

Figure 2.

Genomics approach for the development of the novel vaccine. The metagenomics approach is helpful in the identification of the pathogen. Afterward, genome sequencing was performed to analyze the various aspects of proteomics and transcriptomics. Eventually, the genome sequencing of host and pathogen is essential to study host immune response which is a basic step in vaccine designing. Overall, it all signifies that the genomics approach is an origin to identify novel vaccine candidates.

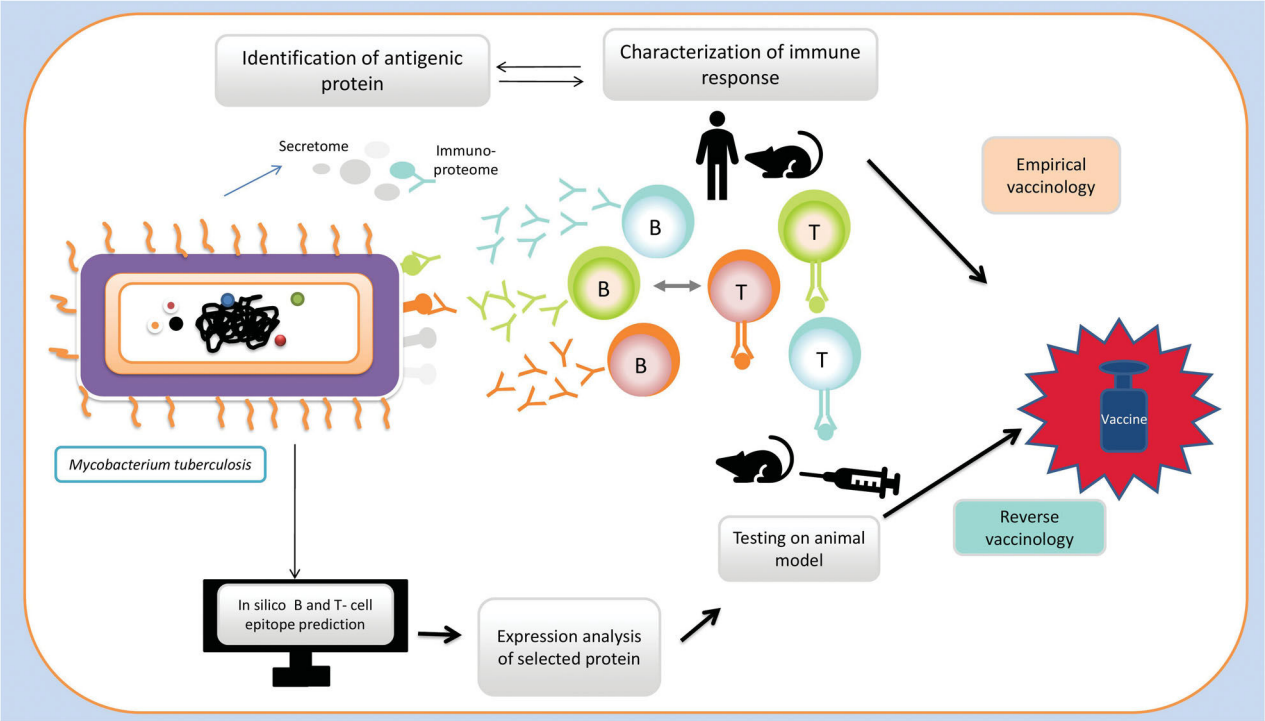

Figure 3.

Representation of reverse vaccinology for epitope prediction in Mtb. Reverse vaccinology is a recent approach in epitope finding and vaccine designing. Computational studies have been performed on Mtb to analyze the particular antigen target. B-cell and T-cell epitope was predicted using various bioinformatics tools. Then, it is experimentally tested to study the immune response of the host. After getting the specific immune reaction, it was allowed for further analysis to prove as a potential vaccine candidate.

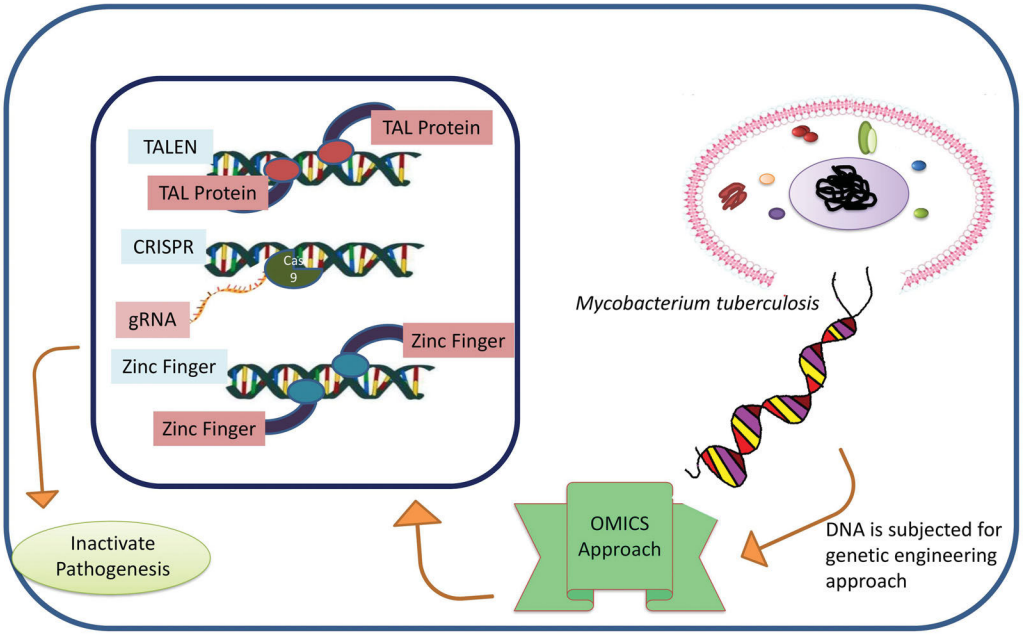

Figure 4.

Systems biology approach using genetic engineering to deactivate the pathogenesis of Mtb. Systems biology helps in the studying of Mtb and using genetic engineering. Novel gene-editing tools such as TALENs, ZFN, and CRISPR Cas can potentially be applied to the genomic content of Mtb. However, the application of the omics approach by using these gene-editing tools is helpful in designing avirulent strain.

Table 1.

Computational database and their significance in vaccine designing.

S. no.	Database	Link	Significant in vaccine development	Reference
1	VIOLIN	http://www.violinet.org/faqs.php	Database of vaccines in research and clinical trials	[21]
2	VFDB	http://www.mgc.ac.cn/VFs/	Bacterial virulence database	[22]
3	BROP	http://www.brop.org/	Detection of oral pathogens	[23]
4	NERVE	http://www.bio.unipd.it/molbinfo	Vaccine candidates identification through proteomics analysis of bacterial pathogen	[24]
5	VaxiJen	http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html	Identify antigenic property of microorganism	[25]
6	SPAAN	https://sourceforge.net/projects/adhesin/files/SPAAN/	Identify adhesins and adhesin-like proteins by neural networks	[26]
7	Pfam	https://pfam.xfam.org/	Protein family database	[27]
8	PSORTb	https://www.psort.org/psortb/	Predict localization of proteins	[28]
9	Allertop	https://www.ddg-pharmfac.net/AllerTOP/	Allergen prediction tool	[29]
10	Protparam	https://web.expasy.org/protparam/	Predict the physiological property of protein	[30]
11	Mycobrowser	https://mycobrowser.epfl.ch/	Mycobacterial repository database	[31]
12	Gene designer	http://www.DNA20.com	Designing of synthetic DNA segments	[32]
13	DyNAVacs	http://miracle.igib.res.in/dynavac/	Optimization of codon sequences	[33]
14	ExactAntigen	http://www.exactantigen.com/	Provide information about monoclonal antibody products	[34]
15	Motifscan	https://myhits.isb-sib.ch/cgi-bin/motif_scan	Prediction of motifs	[35]

Table 2.

List of *in silico* epitope prediction tools.

B-cell epitope prediction tool	Weblink	Description	Reference
DiscoTope	http://www.cbs.dtu.dk/services/DiscoTope/	Use the 3D structure of protein to predict discontinuous B-cell epitope predicts epitopes in complexes of multiple chains.	[93]
IEDB	https://www.iedb.org/	Predict and analyzed B/T-cell epitope. Freely available and as easy to search for the users.	[94]
Bepipred	https://bio.tools/bepipred	Used antigen sequence to predict conformational B-cell epitope. Based on random forest algorithm. Informative and user friendly.	[95]
ABCPred	http://crdd.osdd.net/raghava/abcpred/	Identify linear B-cell epitope using sequence of antigen. Used artificial neural network for prediction.	[96]
CEP	http://bioinfo.ernet.in/cep.htm	Predict conformational epitope, antigenic determinants and sequential epitopes. Used 3D structure of the protein to predict epitopes. Predict epitopes with the accuracy of 75%.	[97]
Tepredict	http://tepredict.sourceforge.net/	Predict T-cell epitope using partial least squares regression method predict proteasomal processing of protein antigens.	[98]
ProPred1	http://crdd.osdd.net/raghava/propred1/	On-line web tool to predict peptide binds with MHC class-I alleles using antigenic sequence, predict standard proteasome and immunoproteasome cleavage site.	[99]
SVMHC	http://www-bs.informatik.uni-tuebingen.de/Services/SVMHC/	Predict peptides bind with MHC class I and II. Examine the effects of single nucleotide polymorphisms on MHC-peptide binding.	[100]
SYFPEITH	http://www.syfpeithi.de/bin/MHCServer.dll/EpitopePrediction.htm	Predict MHC ligand along with peptide motif and T-cell epitopes bind to class I and class II MHC molecules.	[101]
MHCPRED	http://www.ddg-pharmfac.net/mhcpred/MHCPred/	Predict B and T-cell epitope using antigen sequence.	[102]
SVRMHC	http://svrmhc.biolead.org/	Predict T-cell epitope. SVR-based quantitative modeling method.	[103]

Table 3.

List of epitopes identified using Mtb proteins as vaccine target.

S. no.	Epitope prediction tool	Gene name	Description	References
1	IEDB	Mtb39a, Mtb32a, Ag85B, ESAT-6, TB10.4, Rv2660, Rv2608, Rv3619, Rv3620, and Rv1813	Rv2608 was identified as the best non-latency related protein antigen. In comparison with Rv2660, Rv1813 is identified as a better latency-related protein antigen. ID93 vaccine comprises Rv2608 and Rv1813 are the best multistage MTB vaccine candidate.	[109]
2	ProPred and PropPred1	Rv1088, Rv3923c, Rv3235, Rv2871 Rv2731, and Rv0707	Computationally DNA binding proteins of Mtb were identified. Prediction of B and T-cell epitopes from DNA binding proteins. Modeling and docking with HLA to validate them as a vaccine target.	[110]
3	PropPred1	Rv3852, Rv2706c, and Rv3466	Identification of a total of 54 antigenic proteins was performed in Mtb. Nineteen promiscuous, novel and widely conserved were identified from four proteins. Among 4 proteins, the identification of 19 CTL epitopes was done. Proteins Rv3466 and Rv3852 showed population coverage >90%, better than that for ESAT-6, CFP-10.	[111]
4	NetMHC 3.4, ProPred, and IEDB	Rv3083	*mymA* operon proteins was subjected for T cell epitopes bind with MHC class I and II. Rv3083 is recognized as the best vaccine candidate in *mymA* operon proteins.	[112]
5	BIMAS, SYFPEITHI, and NetCTL	Rv1258c and Rv1410c	Epitopes bind with HLA-A*0201 molecule are stable. Identified epitopes from efflux pump Rv1410c capable for the induction of CTL response both *in vitro* as well as *in vivo*. The identified novel epitopes are potential peptide vaccine candidates against drug-resistant Mtb.	[113]
6	FPEITHI, BIMAS, and NetCTL	CFP10 and ESAT-6	Mtb-derived peptides bind to HLA-A24 was studied. Considerably, peptide P5 activates IFN-producing cytotoxic T lymphocytes from HLA-A24^{+} TB donors. In a mini refolding assay, peptide P5 is feebly binds with HLA-A24. The selected epitope is significant in immunotherapy and vaccine designing.	[114]
7	FPEITHI, BIMAS, and NetCTL 1.2	CFP21	Novel T-cell epitope was identified from secreted protein, CFP21 of Mtb. p134, novel epitope expressed potential activity in cytotoxic and ELISPOT assays. Moreover, p134 broadly induced significant responses in PBMCs of individuals.	[115]

Table 4.

List of proposed vaccines in the pipeline against tuberculosis.

S. no.	Vaccine	Description	References
1	Live attenuated vaccine	Inactivation of Rv1503c gene, responsible for surface glycolipid synthesis, and global regulator PhoPR. Double mutant is as safe as BCG in immunodeficient SCID mice.	[125]
2	Live attenuated vaccine	MTBVAC discovery based on *M. tuberculosis* in human clinical trials.	[126]
3	Rational design	Rv2299c is an excellent candidate. Fused HtpG-ESAT6$_{Mtb}$ incorporates the antigenic character of ESAT6 and Hsps.	[127]
4	Multiepitope subunit vaccine	Engineered vaccine possesses B cell, T_C and T_H cell epitopes along with bacterial 50s ribosomal L7/L12 sequence as adjuvant.	[128]
5	Live attenuated vaccine	LC3-traffick was inhibited by auxotrophic Mtb vaccine, mc^{2}6206. CpsA and ESX-1functionally hampered LC3-trafficking.	[129]
6	Genome-based vaccine	Analysis of pathways for down-selecting gene candidates, including splenocytes and peripheral blood lymphocytes. CXCL9, IFN-γ, and CCL5, were important to protect with high specificity.	[130]
7	DNA vaccines	Codon optimized of Ag85B-ESAT-6-Rv2660c was selected as DNA vaccine BER^{opt}. BER^{opt} DNA vaccine is efficient to be used in the future against TB.	[131]

Table 5.

Advantages and disadvantages of systems biology.

S. no.	Systems biology technology	Advantage	Disadvantage	References
1	Genomics	Detect abnormalities in the entire genome. Identification of SNPs gives important information to diagnose, prevent, and treat diseases. Gene polymorphism studies analyze the susceptibility of individual to different drugs.	Genomic analysis predict limited information because of post-transcriptional and post translational events.	[149]
2	Proteomics	Identification of novel antigens Identification, quantification and modification of proteins. High throughput. Data of different samples can be compared on same gel. Different samples can be analyzed at once by MS.	High sensitive. Require huge amount of time. Required manpower. Relatively expensive.	[150]
3	Transcriptomics	Identification of gene expression and its localization. More than 1000 genes can be used for the analysis by microarray and commercial chips. Simultaneously, 100 samples can be detected	Applicable for a small number of genes. Very complicated process. Need an expert for library preparation and data analysis.	[151]
4	Genetic engineering	Produce live recombinant vaccines. Generation of genetically engineered microorganism. Applicable for controlling genetic disorders. Production of novel medical treatments.	Ethical, legal, and social challenges. Animals model are required for vaccine validation. Need a lot of volunteers for final trials of vaccines.	[152]
5	Metabolic engineering	Manipulation of metabolic pathways. Enhanced production of pharmaceuticals and medicines by altering metabolic pathways.	Identification of optimal organisms and targets determination to manipulate genes, pathways, or even in transcriptional and translational control elements.	[153]