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Omics Technologies: A Hope for Translational Research in Bovine Tuberculosis

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Abstract

Bovine tuberculosis diagnosis is one of the main challenges faced by animal and public health systems. The incidence of *M. bovis* infections remains undefined in developed countries. So it is necessary to carry out an extensive study and surveillance to determine the status of bovine tuberculosis as an urgent need for control eradication program. Furthermore, developed countries, microbiological (bacteriological) and immunological (histochemistry) techniques are still used, making more difficult to homogenize epidemiological knowledge of bTB. Recent reports describing the potential of microarray technology not only to explore subunit vaccine agents (biomarkers), but to pinpoint immunomodulation, and signatures in the journey of pathogen interaction with the host in bovine tuberculosis. Omics and next generation high-throughput technologies have risen as promising tools that will enable translational research (development of prognostic and diagnostic methods with high accuracy and sensibility) and in depth molecular analysis even at single cell level to underpin dynamics in the transcripts regulation of the host response in bTB.

Keywords: Bovine tuberculosis; *M. bovis*; RNA seq; Transcritptomic; Genomics; Omics technologies; Printing signatures; Gene expression profiles

Introduction

The diagnosis of bovine tuberculosis in Mexico is one of the main challenges faced by animal and human public health systems. Actually, accurate diagnosis with a high degree of sensitivity, specificity is an urgent need that should be pursued worldwide [1-5]. The conventional methods that have been used for the detection of *Mycobacterium bovis (M. bovis)* as described in the Manual of the World Organization for Animal Health (OIE) on terrestrial animals reside primarily in the delayed hypersensitivity test (tuberculin test), or tuberculin skin test (TST), bacteriological and histopathological exams [5-6]. More recent reports indicate that the molecular detection of *M. bovis* has been possible thanks to the development of multiplex PCR (primers to several targets) [7-23], allowing identification and differentiation between members of the *M. tuberculosis* complex. In conjunction with this molecular methods of detection, a hot spot, is the approach of the study of gene expression profile, using microarrays technologies, that is, hybridization of cDNA with bovine DNA, enabling thus, determination and identification of genes and the pathways that involved and touched by the pathogen interaction with the host response [24-26]. More recently, high-throughput sequencing technologies such as RNA seq (massive, 2do generation) and third generation (single cell sequencing) [27,28] have been approximate to the study of the alveolar macrophages gene expression profile, comparative studies of host preference between *M. tuberculosis and M. bovis* [29-34]. Furthermore, omics technologies as a whole, including, metagenomics, proteomics, metabolomics, transcriptomics arise and could provide a promising tool to define even at single cell level, the spectrum of the sisease (latent versus active) as well as the dynamics of innate and adaptive immune response in either case, that in conjunction might promote translational research in bTB (35-46) (Figure 1).

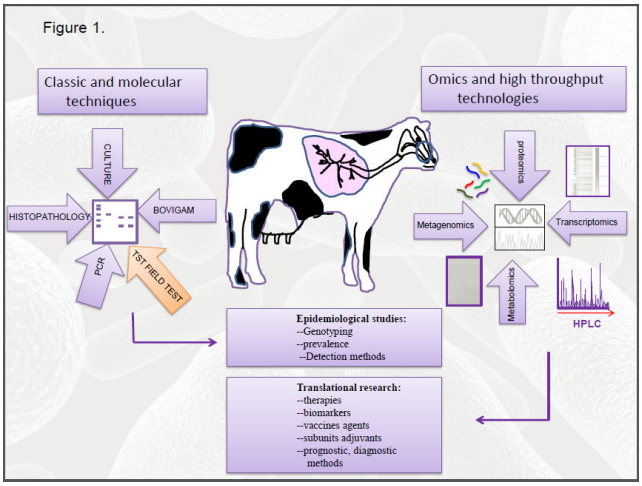

Figure 1: Omics technologies in bovine tuberculosis (bTB). In the scheme is depicted and summarized the main classical techniques that are still in use not only in developed countries but for epidemiological studies. However, due to the increasing development and improvement of new generation technologies, it necessary to approach the study of bTB using them in order to have a whole landscape of the disease spectrum, progression making possible to reach translational research at diferent geographical sites.

What We have in Terms of Detection and Identification of *M. bovis*

In the last decades, a huge of group have been focused in the development of molecular detection of *M. bovis* which in general terms started with isolation from tissue homogenate with lesion, seeding in Middlebrook brook solid medium supplemented with OADC and THF followed by DNA extraction, nested PCR and multiplex PCR amplification of specific regions of the *M. bovis* genome [8-14], a screening test used to prevent infection and introduction of disease in healthy herds. The application of the PCR technology, have been seen as a reliable and accurate diagnostic development [8-14]. Moreover, real-time Multiplex PCR was standardized with reference to Mycobacterium strains and was subsequently tested with 66 clinical isolates [15-17]. The sensitivity and specificity of the designed primers were for each one as follows: 100% for MTC, *M. abscessus*, *M.fortuitum*, *M. aviumcomplex*, *M. kansasii*, and *M. gordonae*. While the sensitivity and specificity of the primers designed for the genus Mycobacterium were between 96 and 100% [15-17]. By other hand, epidemiological analysis using techniques such as spoligotyping, VNTRs, RFLPs, for typification of *M. bovis* substrains and for simultaneous differentiation of other members of the Mycobacterium complex with ther mycobacterial species not included in the complex. Non-tuberculous mycobacterial species (NTM) that may have a clinical significance and interference with the detection and identification of *M. tuberculosis* [18-20] were analysed. Thus, MTBC and NTM were simultaneously evaluated in respiratory specimens using real-time PCR multiplex and RFLPs. and the Geno Blot Advan Sure Mycobacteria trial (LG Life Sciences). The data obtained using this approach, is that species commonly detected in mixed cultures were *M. intracellulare* (29.0%) and *M. abscessus* (29.0%) [18-20] to carry out a rapid and simultaneous detection of the *M. tuberculosis* complex (MTC), as well as of differentiation with *M. bovis*; a multiplex assay based on microspheres was developed using xMAP technology [21]. Briefly, these methods detects 4 target sequences, including the insertion-specific elements IS6110 and IS1081 of the MTC, a specific fragment of 12.7-Kb for *M. tuberculosis*, and an uninterrupted sequence of 229 sub specific for *M. bovis* [17,22]. The specificity of the assay was validated by testing 13 reference strains of mycobacteria; 22 isolates of *M. tuberculosis* and *M. bovis*, and 25 species of non-mycobacterial microorganisms. The assay can be completed within 2 h with a limit of detection per assay above 6 to 10 bacteria per reaction. The sensitivity of the temperate cloned DNA was 0.37 to 0.74 fg of DNA per reaction. The trial also allowed to distinguish between MTC and *M. bovis* with a high percentage, 98.9% (89/90) and 91.9% (34/37) as MTC and/or *M. bovis* in human sputum samples and/or tissue from bovine [17,18,22] (Figure 1).

Why OMICS has Arisen as a Hope for Translational Research in Bovine Tuberculosis?

The increasing necessity not only to detect the pathogen but with the firm aim to control disease and if possible to eradicate due to arise of multi-drug resistant strains of the *M. tuberculosis* complex, which includes *M. bovis* [1]. In addition to other social factors in developed countries. Moreover, while in human TB is very clear and it is very well established [26,27], the spectrum and stages of infection (latent virus active disease, in cattle, is still undefined. Studies highlighted from the literature have evidenced that biological systems based on the integration of data generated by -omics studies are a very useful approach to identify biomarkers with therapeutic potential for human Tb [26] and/or bovine tuberculosis [28,31], to determine gene signatures that can predict and / or correlate with protection following vaccination (either in cows and/or in humans humans) [30,31]. At the beginning, DNA microarrays were used for the rapid and direct detection of *M. tuberculosis* and *M. bovis* in bovine milk has also been reported [20], based on the mtp40 and pncA sequences. The limit of detection for mycobacterial DNA using the DNA microarray was 50 fg (5 tubercle bacilli). *M. tuberculosis* and/or *M. bovis* were detected in 7.1% (24/336) of cow specimens using the DNA microarray compared to 6.0% (20/336) using culture methods [17]. Mixed infections were detected in 3 animals using the DNA microarray method, while mixed infections were detected in 2 animals using either culture or PCR methods. Thus, in vitro detection methods (PCR) together with DNA microarrays increased the detection of cows infected with *M. tuberculosis* and/or *M. bovis* and reduced the number of false positive animals that could be eliminated. [17,19]. Then microarray technologies was used for the development of molecular diagnostic highly sensible and specific [23-31]. In one these reports it has been described the unknown role of type I IFNs in the pathogenicity of *M. tuberculosis* [26] while in the second report, it was revealed the IL-22 role in the protection against *M. bovis* [29]. In order to identify biomarkers profile it is essential to use bioinformatics tools in conjunction with new generation high throughput technologies (massive and RNA seq) to determine a more whole integrated physiologic and immunological (innate and adaptive) response of host to pathogen [28-30]. A wealth of studies have been conducted with bovine peripheral blood mononuclear cells (PBMCs), as well as with alveolar macrophages (are the first cells encountered with *M. bovis*) [32,33]. Indeed, bovine monocytes derived macrophages have been challenged with *M. bovis* and the gene expression signature has been determined [34,35]. Gene signature profile in these cells has been viewed one, as a various tool to study the dynamics of mRNA transcripts in bTB [32,36,37], in particular, innate cytokine pattern induced after infection of alveolar macrophage with *M. bovis* or *M. tuberculosis* [38]. By another hand, RNA seq was also approached in conjunction with microarray technologies, to have a higher accurate and dynamic determination of gene expression transcripts and differential gene expression [39] as Nalpas et al., 2013 [40] showed in an in vitro analysis of the whole transcriptome of *M. bovis* infected macrophages by microarray technologies and later on, using RNA seq, revealed that alveolar macrophage infected with tubercle bacilli have a complex pattern of host immunomodulatory response [40,41], it means to approach dynamics of mRNA. miRNA, sRNA, splicing patterns, expression level, identification of novel transcript, and analyses of global gene expression even at single cell level, any change due to the host-pathogen interaction in bTB by transcriptomics [42] Furthermore, in recent years, microbiome in the different systemic and mucosal compartments have been approached using metagenomics. Thus, in bTB, metagenomics for genetic markers (susceptibility or resistance) using comparative genomic sequence and high-throughput sequencing (NGS) of PBMCs from herds of 1 to 2 months of infection is possible to achieve a more precise accurate and diagnostic of bTB in the early stages of disease progression [43]. Genomic profiles signatures (in terms of innate and adaptive immune response) have recently report to distinguish between active and latent tuberculosis, suggesting that is the genomic data either at nucleotide that information should be flow through to pin point not only to search for gold biomarkers but a set of a more integrated or whole biomarkers tools [44,45]. Malone et al., aimed to compare whether or not are differences in the response to infection by either *M. tuberculosis* or *M. bovis* by comparative omics technologies, they found that depending

of the species, the macrophage gene expression program is different even both pathogens share 99.5% homology, they still have some percentages of different routes depending of the host human or bovine.

Proteomics

Proteomics is also a powerful tool that should be integrated to the study of bTB [45], to deep insight in protein-protein interactions, to characterize proteins that suffer post-transductional modifications, to study stability, abundance of key role of proteins, glycoprotein, when and how are expressed and migrate, protein patterns and if the proteome overall at the level of cells (macrophages, dendritic or lymphocytes cells) or tissues are affected in response to M. bovis infection. All these issues can be studied, by spectrometric mass (SELDITOFF) [27,45]. Moreover, recent research in this aspect indicate that the knowledge of the antigenic targets of T cells in bTB as well as the increasing knowledge of the subset of T cells and their interactions with infected macrophages with M. bovis can help for the development of better methods of control of disease. In biologic systems based in the integration of data generated by omics studies are a potential approach that can be used to identifiy transcriptional gene signatures to predict or to correlate parameters of protection in vaccinated calves versus unvaccinated, and also 188 to predict vaccination protocol effectiveness, until now mostly applied to human tuberculosis [26,27,31,45,46] (Figure 1).

Conclusion

Despite of the development and improvement of the DNA technologies for diagnostic and prognostic test, in the last decade there have been a raise in the technologies of the new generation which certainly are giving an enormous advance either to epidemiological molecular studies as well as in the knowledge of the epigenetics and deep insight in the knowledge of mutations, genetic markers (SNP), biomarkers, definition of spectrum of disease. Omics technologies and third next generation high-throughput technologies have emerged as a potent technologies that cover the totality of the genome wide studies and importantly the functionality and dynamic of the genomes, transcriptomes, and proteomes that will enable to integrate the complete and define as it was possible to determine for humans, the landscape in the spectrum of the infectious disease, the progression and/or the genetic predisposition to mycobacterial diseases for (Figure 1) and make feasible translational research.

Conflict of interest

None.

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