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Review

Proteomic approaches for drug discovery against tegumentary leishmaniasis

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ABSTRACT

Tegumentary leishmaniases (TL) comprise various clinical forms, in which current therapeutic treatments lack in safety and efficacy. Recently the parasite is developing resistance mechanisms against anti leishmanial drugs startling the scientific community to recruit efforts to search for novel therapeutics. Proteomics hold promises for the treatment of leishmaniasis and investigation of parasite–host interaction since these set of methodological tools have provided a wealth of protein expression data on several *Leishmania* species. Firstly this review puts together the current treatment and challenges to fight tegumentary leishmaniasis. In addition, the 2 dimensional gel electrophoresis and mass spectrometry techniques in protein identification and characterization are described and discussed in the context of proteomics regarding *Leishmania* studies. In this review, we selected literature content on TL causative agents. Important proteomic findings related to differentiation proteome (promastigote and amastigote forms), *Leishmania*-macrophage interaction proteome and secreted and soluble proteins including molecules involved in parasite resistance and potential drug targets are examined and discussed. We also highlight open questions regarding drug research that can be addressed with proteomics approaches.

1. Introduction

The leishmaniasis are a group of diseases caused by protozoan parasites from > 20 different *Leishmania* species that are transmitted to humans by the bite of infected female sandflies. Globally, there are an estimated 1.5–2 million new cases and 70 000 deaths each year, and 350 million people are at risk of infection and disease [1]. The two main forms of the disease are tegumentary and visceral. Tegumentary leishmaniases (TL) comprise various clinical forms that depend on the *Leishmania* species as well as the host response [2].

TL are classified as localized cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis, disseminated leishmaniasis, leishmaniasis recidiva cutis, and mucosal leishmaniasis (ML) [2]. The CL is the most prevalent form and it is caused by all of the dermotropic *Leishmania* species being endemic in many countries [1]. It is specially found in Asia, the Middle East, Southern Europe and South America [1] but it is becoming increasingly reported in urban and peri-urban areas of the Old and New World [2]. TL is caused by *L. tropica* and *L. major* in the Old World and by *L. braziliensis*, *L. guyanensis*, *L. panamensis*, *L. peruviana*, *L. mexicana*, *L. amazonensis* and *L. venezuelensis* in the New World [3]. CL usually produces ulcers on the exposed parts of the body, such as the face, arms and legs [1]. Many lesions can cause serious disability and when ulcers heal, they leave permanent scars, which are the cause of serious social prejudice. The ML, common in Brazil, produces lesions that can lead to partial or total destruction of the mucous membranes of the nose, mouth and throat cavities and surrounding tissues [2]. Secondary infection plays a prominent role in the size and persistence of ulcers. This disabling form of leishmaniasis can lead to the sufferer being rejected by the community [1–3]. *L. braziliensis* is the primary species involved in New World mucosal leishmaniasis, although *L. panamensis*, *L. guyanensis*, and *L. amazonensis*. In the Old World, *L. major* and *L. infantum* also cause ML [2,32]. The *Leishmania* life cycle begins when parasites in their promastigote form are inoculated by a sandfly bite into the skin of a mammalian host. Macrophages phagocyte parasites, which turn into the amastigote form. Many will survive within the macrophages because of a variety of sophisticated defense mechanisms. *Leishmania* then multiply and spread to other macrophages [4,5].

The control of vectors and reservoirs in vector-borne diseases is difficult due to challenges of interventional programs, particularly in developing countries, where the prevalence is high [6]. The control of *Leishmania* also relies on the early diagnosis, vaccines and efficient treatment.

Although there have been early reviews regarding *Leishmania* biology and studies of proteins, recent proteomic approaches against TL deserve particular attention since they have led to a much deeper

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knowledge of its biochemistry [7–13]. The purpose of this review is to encompass important information regarding pre-proteomic and proteomic studies about TL causative agents. The highlight will be on how proteomic studies on *Leishmania* have contributed in the search of potent drug targets aimed to develop more effective and less toxic therapeutics against the disease.

2. Current treatment and challenges

The first-choice treatment for TL in most parts of the world is the pentavalent antimonials which were developed in 1945; amphotericin B and pentamidine are the second-line antileishmanial drugs, although they require long courses of parenteral administration [14]. The choice of treatment also depends on the causative *Leishmania* species [15]. Although spontaneous cure is the rule for CL the rate of recovery varies depending on the species of *Leishmania*, and may require months or years to complete healing. Most of the commonly used drugs are toxic and do not eliminate the parasite from infected individuals [14]. The major side effects of the first line treatment (antimonials) are arthralgy and myalgy but severe side effects related to cardiotoxocity or renal failure can occur mainly in older patients [16].

Current drugs against leishmaniasis lacks in safety and efficacy, which disrupts adhesion to the treatment. Recently, the parasite is developing resistance mechanisms against antileishmanial drugs alarming scientific community to recruit efforts to search for novel therapeutics. For example, free availability of anti-leishmanial drug in India increased the chances of misuse; thereby increasing the emergence of drug resistance [17].

As stated, unfortunately, drug therapy for TL has failed to significantly change since the beginning of the twentieth century, when it started. In addition, knowledge regarding the differences in the drug responses of the *Leishmania* species that are prevalent in different geographic areas and their clinical manifestation is slowly increasing [2]. Sensitivity of antimonials toward different *Leishmania* species varies differently [17]. It is observed that *L. brasilensis* is more sensitive to the treatment in comparison *L. Mexicana* [17]. There is also increasing awareness that drug treatment can be complicated by variation in the sensitivity of *Leishmania* species to drugs, variation in pharmacokinetics, and variation in drug-host immune response interaction [17–20].

Pentavalent antimonials (SbV) are the first choice treatment. The mechanism of action of antimonials is still poorly understood, but they seem to have a dual mode of action. One mode would be the perturbation of the redox-balance of the parasites and the other mode would be imposing extra oxidative and nitrosative stress upon the parasite through interaction with the host cell [21]. An interesting study comparing *L. panemensis* resistant to meglumine antimoniate and the wild type, tried to define the role of parasite sensitivity to SbV in treatment failure and to examine the mode of action of SbV [22]. The study showed the effects of SbV on the stabilization of cleaving DNA protein complexes associated with the topoisomerase, evaluated by same method described for *L. donovani* [23,24]. The median ED50 for the wild-type strain was considerably lower than the line selected for resistance. Treatment with both meglumine antimoniate and sodium stibogluconate stabilized DNA-protein complexes in the wild-type strain but not the resistant line. The ED50s of the SbVs for *Leishmania* strains from patients with relapses was comparable to those for the line selected for in vitro resistance, and DNA-protein complexes were not stabilized by exposure to meglumine antimoniate. The selective effect of the SbVs on the stabilization of DNA-protein complexes in *Leishmania* and the loss of this effect in naturally resistant or experimentally derived SbV-resistant *Leishmania* suggest that topoisomerase may be a target of antimonial drugs [22].

Amphotericin B is a polyene antibiotic that has been used as a second line treatment for leishmaniasis since the 1960s [17]. It has a selective activity against *Leishmania* due to the higher affinity of amphotericin B for ergosterol, the predominant sterol in these microbes, over cholesterol, the predominant sterol in the mammalian host cells [25]. An interest study compared amphotericin-resistant amastigotes and promastigotes of *L. mexicana* with control parasites to evaluate molecular differences, especially in the membrane [19]. Analyses of drug-resistant and control, wild type *L. mexicana* lines revealed dramatic differences in sterol composition, such as ergosta-5,7,24(241)-trienol contributed approximately 85% of the total sterol. In the amastigotes, the major sterols were a mixture of ergosta- and stigmasta-5,7,24(241)-trienols. In contrast, these sterols were undetectable in amphotericin-resistant parasites, which contained instead high levels of methylcholesta-sterols. The amphotericin B is therefore no longer able to enter the cytosol of resistant parasites [19].

Another treatment against leishmaniasis is pentamidine, which belongs to the diamidine class of drugs. The drug enters both promastigote and amastigote forms of the *Leishmania* cell via a carrier-mediated process which recognizes diamidines with high affinity [26]. Efforts to identify a physiological substrate for the transporter failed. Basic amino acids, polyamines and a wide variety of common metabolites also failed to inhibit pentamidine uptake. Resistance in this case was concluded not to associate with alterations at the level of a plasma membrane transporter, but with changes in the mitochondrial membrane potential [26]. The mitochondrion appears to be the target of pentamidine action and plays a crucial role in the mode of action for pentamidine in *Leishmania* parasites [26].

Because treatment is a growing problem, the development of new medicines that can replace or complement the presently available therapeutic alternatives is therefore necessary [27]. The decades of research that go into identifying the key proteins involved in *Leishmania* pathogenesis and intracellular survival are the groundwork for targets of new drug discovery.

3. Proteome findings on parasites causing TL

Previous studies and reviews also provide a framework for proteomics in the study of *Leishmania* [7,10,11,28,29]. In this review, however, we have selected important researches that focus on TL causative agents and discussed the challenges and perspectives for drug development regarding proteomic findings.

In the 1980s, the first proteome maps of *Leishmania* (*L. tropica*; *L. mexicana*; *L. braziliensis*) were published [30–32] before the term proteomics had been created [33]. Handman and colleagues evaluated protein isolates from four *L. tropica* isolates. Samples were biosynthetically labeled with S-methionine or surface radioiodinated, and the detergent lysates were analyzed by 2 dimensional gel electrophoresis (2DE). This study revealed two different protein patterns of four strains of *L. tropica* isolates [30].

Saravia and his team examined the relationship among different *Leishmania* strains and species (*L. mexicana* and *L. braziliensis*) using 2DE to identify subspecies. The study was successful in revealing an unexpected degree of disparity between this two species, at that time, when no further information was available [32].

The identification of large molecules, such as proteins was achieved at 1980's, when mass spectrometry techniques were upgraded to analyze specifically large biomolecules. The identification of proteins was achieved by cleaving an intact protein into its peptides and analyzing these fragments by a new mass spectrometric (MS) technique developed by two independent groups in 1987 [34,35], which was MALDI-TOF (Matrix Assisted Laser Dessorption Ionization time of flight). This ionization technique is used to form intact species the TOF analyser is used to determine exact masses of proteins ions and then correlating such peptide masses against a database of known peptide and proteins expressed in the genome. In this way, it is possible to identify protein sequences to a high degree of accuracy without ever determining more than the masses of the peptide ions in question (Fig. 1a). Later on, in 1989, electrospray ionization (ESI) has emerged as a powerful