Spotlights on new publications

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New drug targets - XVII

Pathogenic trypanosomatids: Several reasons were considered for the unexpected spread of diseases caused by pathogenic trypanosomatids. Among these are the economics of low- and middle-income countries; vector and host transportation facilitating occurrence of new outbreaks; the suboptimal efficacy of the current available therapeutic drugs. Most of those are poisons, *e.g.*, arsenic and antimony, with diverse serious side effects, besides requiring prolonged administration to achieve complete cure, and there is always the threat of development of drug resistance. **Violeta Kourbeli** and her colleagues from Greece discussed the essential roles of 21 potential drug targets and proposed them for further studies to be certified as novel drugs.

All pathogenic trypanosomatids express **trypanothione reductase (TR)** and **pteridine reductase 1 (PTR1)**, while **histone deacetylase enzymes (HDACs)** were only identified in *Trypanosoma* spp. The first two enzymes essentially control parasite' homeostasis as well as survival and growth, respectively. On the other hand, HDACs are enzymes involved in modulation of gene' expression activity during cell division and differentiation. They are grouped into four classes in which class III includes only sirtuins, *i.e.*, silent information regulators (SIRs). They are essential enzymes with a vital cellular function of deacetylation of transcription factors that contribute in cellular responses to DNA-damaging agents under stress conditions.

In ***T. cruzi***, ten additional potential drugs were suggested. **Cruzain** (Cz) is the major cysteine protease and is vital for *T. cruzi* survival and multiplication. Actually, a Cz specific inhibitor (Thiophene–thiazolidine) became available in treatment of Chagas' disease since ~5 years. **Enolase** has an essential role in glycolysis and glycogenesis, and acts as a plasminogen receptor on trypanosomes cell surface. **Ribose 5-phosphate isomerase** enzyme (R5PI) plays a major role in pentose phosphate pathway, *i.e.*, is involved in nucleotide precursors' production. **Isocitrate dehydrogenase 2** (IDH2) is a vital enzyme in NADH or NADPH production. **Dihydrofolate reductase–thymidylate synthase** (DHFR-TS), is a bifunctional enzyme catalyzing reduced folate with subsequent thymidylate synthesis for DNA synthesis. Lastly, five enzymes are involved in sterol biosynthesis pathway: **sterol 14-alpha demethylase** (CYP51), **farnesyl diphosphate synthase** (FPPS), **sterol 24-c-methyltransferase**, **squalene synthase**, and **oxidosqualene cyclase**. The majority of studies focused on *Tc*CYP51, and *Tc*FPPs that act as essential catalyzing enzymes in converting lanosterol → zymosterol, and terpenes → terpenoids, respectively in sterol biosynthesis pathway. The reviewers recommended further studies to identify the role of other enzymes in sterol biosynthesis.

In ***T. bruci***, three additional drug targets were proposed. First is the **RNA-editing ligase 1** (*Tb*REL1), an essential enzyme involved in trypanosome's survival in both vector and host. Second is the **uridine diphosphate glucose 4′-epimerase** (*Tb*UDPGE) involved in galactose metabolism with an essential role in host immunoevasion *via* surface glycoproteins. Third is the **N-myristoyltransferase** (NMT) that plays an essential role in a variety of signal transduction pathways in cell membrane. This enzyme is required for localization, stability and function of cell membrane proteins; *i.e.*, pivotal enzyme for protein-lipid interactin.

In ***Leishmania*** spp., five potential drug targets were proposed. Since they are unable to synthetize purines *de novo*, a purine salvage pathway is utilized to synthesize it from salvaged bases and nucleosides formed during degradation of host' RNA and DNA. In addition to *Leishmania* **nucleotides' transporters** (NTs), protein kinases (PKs) are promising drug targets due to their role in *Leishmania* life cycle stages growth, survival, and differentiation. The most essential PKs are **cyclic-dependent kinase-3** (CDK3), and **mitogen-activated kinase-3** (MAPK3) participating in all division processes, and *Leishmania* virulence, respectively. **Type 2 NADH dehydrogenase**, the enzyme that catalyzes mitochondrial electron chain from NADH, and the enzymes contributing in polyamines biosynthesis, *e.g.*, **arginase** are also suggested as potential drug targets.

Finally, the reviewers claimed the possibility of developing selective inhibitors targeting these enzymes with available identified target' crystal structure and

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characterization of its best selective binding site. Virtual screening accomplished with docking computational studies enabled the investigators to recognize several selective inhibitors with efficient activity against TR, PTR1, SIR, *Tc*R5PI, *Tc*IDH2, *Tc*CYP51, *Tc*FPPs, *Tb*REL1, *Tb*UDPGE, and *Tb*NMT. Etidronate, used in the treatment of Paget's disease, and osteoporosis, was suggested as a potent *T. cruzi* enolase inhibitor. In addition, three computational studies approached developing novel therapeutic drugs, and one of them identified *Tc*DHFR-TS pharmacophore structure and its binding pocket. Compiled from **"An overview on target-based drug design against kinetoplastid protozoan infections: Human African trypanosomiasis, Chagas disease and leishmaniasis. Molecules 2021 Aug; 26(15): 4629."**

Leishmaniasis: The present compilation deals with validation of *Leishmania* BRCA1 C terminus (BRCT) domain as a potential drug target. Notably, BRCT is a family of evolutionarily related proteins, and termed so after identification of the genetic marker of breast cancer susceptibility. It was observed that BRCT domain (~100 amino acid tandem repeat) was predominantly expressed in response to DNA damage, and cell-cycle control. Elucidating the complete genomic characteristics of *L. major* in 2005 enabled investigators to search new potential drug targets for designing and development of novel inhibitors. Two years ago, *Lmj*_04_BRCT was recommended for further pharmacological studies. A group of scientists from Spain and Estonia **(José Peña-Guerrero *et al*.,)** aimed to construct *Lmj*_04_BRCT homology model and identify new inhibitors with anti-leishmanial activity.

To construct stable homology models, the investigators used three phosphorylated ligands incorporated with gossypol. It was observed that *Lmj*_04_BRCT has high affinity for phosphate-dependent interactions. Utilizing the constructed *Lmj*_04_BRCT homology models, docking studies with molecular dynamics' simulations allowed the investigators to select one of those homology models. Although the homology model (Mult1_lr) showed acceptable structure criteria, the investigators selected SwissModel_2 for its high stability as compared to the other models (Mult1_lr and Mult1_lig). *In vitro* virtual screening with SwissModel_2 enabled the investigators to predict and identify suitable compounds for further *in vitro* studies investigating their anti-leishmanial inhibitory activity against promastigotes and amastigotes. A novel compound (CPE2) exhibited significant anti-leishmanial activity against *L. major* and *L. amazonensis* and to a lesser extent against *L. infantum*, *i.e.*, a lower effect compared to Amphotericin B (the reference drug). In addition, CPE2 exhibited potent activity on both intracellular and extracellular stages of several *Leishmania* spp. However, the investigators recommended further studies with additional pharmacological modifications to increase its activity, or investigate its efficacy in combination with the currently available anti-leishmanial drugs. Compiled from **"Discovery and validation of *Lmj*_04_BRCT domain, a novel therapeutic target: Identification of candidate drugs for leishmaniasis. Int J Mol Sci 2021 Oct; 22(19):10493."**

Malignant malaria: Microtubules (MTs) of the *Plasmodium* cytoskeleton play an essential role in the mitotic and meiotic replication required for proliferation. British investigators (**Alexander D. Cook** and his colleagues) hypothesized that molecules involved in *Plasmodium* replicative machinery, *i.e.* MTs, are potential drug targets. Notably, kinesin superfamily includes several families of motor proteins with specialized functions in MTs, *e.g.*, translocation, regulation of polymer dynamics, and organization of mitotic and meiotic spindles. These motor proteins have high binding affinity with MTs converting the energy of ATP binding and hydrolysis into replicative machinery functions. *Plasmodium* MTs have highly conserved α- and β-tubulin, and although there is ~95% sequence conservation of tubulins between *Plasmodium* spp. and *H. sapiens*, kinesin families showed only 40–50% divergence. This encouraged the investigators to develop selective kinesin inhibitors as novel anti-malarial drugs.

On the other hand, members of kinesin-5 (K5) family are involved in cell division and currently utilized in cancer therapy, and as antifungals. Several classes of selective *H. sapiens* K5 inhibitors were characterized and exhibited high binding affinity in an allosteric site (loop5) in HsK5 domain. Allosteric inhibitors bind at a site other than the enzyme active site changing its catalytic activity. These inhibitors are more selective and potent; moreover, there is no gene mutation in presence of drug pressure. Several studies confirmed Loop5 suitability for allosteric inhibition, for example, replacement of loop5 with similar *Hs*K5 sequence allowed inhibition of *Drosophila* K5, previously resistant to classical *Hs*K5 inhibitors.

Utilizing cryo-electron microscopy and MT gliding experiments, the investigators characterized the structural and biochemical properties of *Pf*K5 motor domain, respectively. Next, the investigators demonstrated that insertion in loop5 drug-binding site of *Pf*K5 motor domain revealed good opportunity to design selective allosteric inhibitors. Based on the obtained results, the investigators suggested that *Pf*K5 plays an MT-organizing role within mitotic spindles. They also hypothesized that *Pf*K5 has no role during intra-erythrocytic stages development. Instead, it was observed that knockout of *P. berghei* K5 significantly reduced sporozoites number in mosquito salivary glands. In fact, this emphasizes *Pf*K5 potentiality to be used as selective inhibitors, *i.e.*, therapeutic purpose, and to decrease malaria transmission. Compiled from **"Cryo-EM structure of a microtubule-bound**

parasite kinesin motor and implications for its mechanism and inhibition. J Biol Chem 2021 Nov; 297(5): 101063."

Toxoplasmosis: Portuguese scientist **Marco da Silva** and his colleagues reviewed the promising drug targets against *T. gondii*, as well as the investigated inhibitors over the last decade. They drew a simplified figure representing four categories for targeting tachyzoites; secretory organelles, fatty acids synthesis, DNA expression, and cell respiration (mitochondrial electron transport pathway). The first category included molecules involved in *T. gondii* motility and host cell invasion. Tachyzoites possess unique apical secretory organelles, micronemes (MICs), rhoptries (Rops) and dense granules (DGs). Expressed molecules are essentially involved in motility, invasion and egress cascade, and host-cell manipulation (MICs and Rops), as well as formation of the tubular intra-vacuolar network (DG). Several functions were assigned to this network however, the most essential one is signaling pathways' communications between parasitophorous vacuole (PV) and the whole tachyzoite, and between PV and host cytoplasm and nucleus. In the first category, the reviewers focused in **calcium-dependent protein kinase 1** (*Tg*CDPK1) due to its possession of an active binding site, different from that of mammalian CDPK1.

Since lipid synthesis is essentially required for development of tachyzoites' plasma membrane and its signaling pathways, the second category included molecules involved in lipid synthesis pathways. The reviewers discussed two essential enzymes, **apicoplast-located enoyl-acetyl carrier protein reductase** (ENR), **β-ketoacyl-acyl carrier protein synthase I and II** (KAS I/II). Both are involved in type II fatty acids synthesis (FAS) pathway; ENR catalyzes the last step in FAS, while KAS I/II contribute in fatty acid elongation. Besides, tachyzoites are capable of synthesizing FAS precursors through another pathway using coenzyme A precursor. Tachyzoites utilize pantothenate synthase enzyme to convert host pantothenate to coenzyme A for use in FAS.

The third category includes enzymes essentially involved in post-translational modifications (PTMs) of histone, *i.e.*, regulation of gene expression required for tachyzoites-bradyzoites interconversion process. Two groups of enzymes are known for acetylation and deacetylation of histone residues; **histone acetyltransferases** (HATs) and **deacetylases** (HDACs). Acetylation generates PTM and increases gene expression, and *vice versa*. In this concept, selective HATs and HDACs inhibitors would prevent both bradyzoites and tachyzoites conversion avoiding chronic toxoplasmosis, and reactivated toxoplasmosis in immunocompromised patients, respectively. The reviewers proposed *Tg*HDAC3 as an effective drug target. **Cytochrome bc1 complex** constitutes the fourth category due to its essential role in *T. gondii* respiration through mitochondrial electron transport pathway. It proved to be a promising drug target in all apicomplexans.

The reviewers also tabulated drugs with *in vitro* and *in vivo* inhibitory activity against *T. gondii* tachyzoites. It was concluded that the ideal novel anti-toxoplasmosis drug should possess four essential issues. First is its capability to target acute replicating tachyzoites and latent bradyzoites, *i.e.*, curing chronic and reactivated acute toxoplasmosis. Second, it should be liable to drug molecular modifications to improve its pharmacokinetic characteristics utilizing a suitable delivery system, *i.e.*, liposomal nanoparticles. Third is its ability to reach a therapeutic concentration in host tissues, *e.g.*, brain (crossing blood barrier), placenta and fetal compartments (efficient bioavailability). Fourth, it should prove safe for administration in pregnancy and be well tolerated for newborns. Compiled from **"Promising drug targets and compounds with anti-*Toxoplasma gondii* activity. Microorganisms 2021 Sep; 9(9):1960."**

Schistosomiasis: South African reviewers **(Ndibonani Kebonang Qokoyi *et al.*)** hypothesized that interruption of protein–protein interactions (PPIs) is a crucial step in development of novel drugs. They are functionally linked with parasite survival, growth, and life cycle stages differentiation as well as in disease pathogenicity and progression. Therefore, identifying essential PPIs in *Schistosoma* spp. would facilitate developing inhibitors to decrease transmission, and prevent or cure schistosomiasis. Discussion of the essential roles played by several schistosomal molecules expressed by multiple developmental stages revealed identification of **cercarial elastase** (CE), and **two heat shock proteins** (HSPs) as potential drug targets, and several antigens as promising vaccine candidates.

It was observed that cercaria in contact with human skin release chemical signals from the acetabular gland complex located posteriorly in the cercarial head. Among them are linoleic acid that stimulates and initiates skin invasion minimizing host immune response, and CE that digests dermal elastin, keratin, fibronectin, laminin and collagen IV and VIII, facilitating skin invasion. Since CE is a serine protease (SP), use of a selective SP inhibitor (SPI), *e.g.*, Elafin would decrease schistosomiasis transmission. Elafin is a novel substrate for elastase and is expressed in human skin and lung to protect tissues from destruction by host immune system (neutrophil elastase). Elafin has a proteolytic activity against microbial SPs, and plays an essential role in wound healing. Its application on the skin prior to cercarial contact would prevent infection.

On the other hand, serpins are specific SPIs, and previous studies characterized and sequenced one, three and eight genes encoding serpins in *S. haematobium, S. japonicum*, and *S. mansoni*, respectively. Among them,

the reviewers proposed four serpins as promising drug targets. **Schistosomula endogenous serpin** (SrpQ) was expressed immediately after skin invasion regulating CE expression and protecting schistosomula from its own elastase. **Schistosomula and adult protease inhibitor** (PI56) was reported to degrade host neutrophil elastase (NE) that plays a crucial role in development of innate immune response. To survive a long time in host venous plexus, *Schistosoma* spp. express **KI-1**, a Kunitz-type PI. Its tegmental localization confirmed its essential function; protecting adults from the continuous contact with coagulation factors as well as host immune mediators. Inhibition of the host coagulation factors was suggested as the possible role played by *Schistosoma* KI-1. Expression of KI-1 in eggs was also observed and it was attributed to egg protection either in mesenteric veins (against host immune mediators) or in intestine (against host digestive enzymes). Therefore, use of selective KI-1 inhibitors results in egg digestion, and prevention of fibrotic schistosomiasis sequelae. The fourth one is **B10** that was expressed in all *S. japonicum* developmental stages hence, and its function is still hypothetical. Accordingly, the reviewers recommended further studies on *Sj*B10 elucidating its role as potential drug target. Although genes encoding *Schistosoma* serpins showed low similarity (~50%) to those of mammalian ones, and were functionally characterized since more than 10 years, no further studies were conducted utilizing their use as a potential drug targets.

Schistosoma **HSP60**, accompanied with its co-chaperonin **HSP10**, is expressed in all developmental stages to overcome stress conditions, *e.g.*, change in temperature, in addition to drug exposure. In such conditions, HSP60 is responsible for protein folding *via* stabilizing peptides; a crucial step in developmental stages differentiation. Selective HSP60 inhibitors in combination with praziquantel (PZQ) would potentially decrease schistosomes' resistance to PZQ. The reviewers claimed absence of reports regarding *Sm*HSP60 crystal structure; therefore, no inhibitors targeting *Sm*HSP60 were developed yet.

As potential vaccine candidates, the reviewers discussed six molecules that are expressed only in schistosomula and adults. A highly expressed membrane-bound protein, ***Sm*29** is implicated in several immune response interactions. A fatty acid-binding protein ***Sm*14** is implicated in sterols and fatty acids synthesis required for their complex membrane systems, *i.e.*, lipid anchors for proteins and sexual maturation and egg production. Through dual interaction, **tetraspanins** (TSPs), transmembrane domain proteins, mediate several functions such as regulation of signaling pathways for juvenile flukes' migration. Maintenance of tegument integrity was also assigned to TSPs. **Paramyosin**, a 97 kDa protein (*Sm*97, and *Sj*97) anchored in schistosomes' muscle layers and the tegument, was evaluated in several reports. Results showed 62–86% protection against schistosomiasis *japonicum*, and reduction in worm burden, intestinal egg loads and granuloma size with a range between 44% to 61% in reduction in schistosomiasis *mansoni*. Furthermore, the reviewers proposed two proteases for further evaluation as vaccine candidates: **Calpain**, and **asparaginyl endopeptidase** (*Sm*AE/*Sm*32). It is worth mentioning that schistosomal antigens are currently in different stages of either clinical (*Sm*14, and *Sm*TSP-2) or pre-clinical trials (*Sm*97, *Sj*97, *Sm*29 and *Sm*KI-1). Compiled from **"Proteins as targets in anti-schistosomal drug discovery and vaccine development. Vaccines (Basel) 2021 Jul; 9(7):762."**