Disclosing the toxin arsenal of *Acanthoscurria juruenicola* spider venom by in-depth proteomic and transcriptomic analysis

Alexandre Keiji Tashima

Departamento de Bioquímica, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo-SP

Introduction and Objectives

Acanthoscurria juruenicola is a spider from the Theraphosidae family inhabiting the Central and North regions of Brazil and is characterized by a dark brown color and aggressive behavior. One key factor to its evolutionary success is the production of venom, used for subduing prey and as a protection mechanism against predators. In this work we made a comprehensive description of the toxin arsenal present in the venoms from male and female specimens of *A. juruenicola* by proteomic and transcriptomic analysis and evaluated the biological activities against selected substrates in vitro.

Materials and methods

Venoms were extracted by electrical stimulator and stored at -80°C until use. Proteins were digested with trypsin at 37°C overnight and peptides were separated in a capillary C18 column, submitted to electrospray ionization and analyzed by data dependent and data independent LC-MS/MS. Proteins were identified by search against a transcriptomic database made from venom glands. cDNA libraries were obtained in the Ilumina HiSeq 1500 (Ilumina) and assembled in the software CASAVA.

Results and conclusions

Absolute quantification by mass spectrometry revealed that the venom is mainly composed by cysteine-rich venom proteases, neprilysins, carbonic anhydrases, hyaluronidases and theraphotoxins. Total protein quantification showed increased protein concentrations in female crude venoms, however, the main components were detected individually at similar levels on a dry basis. An isoform of carbonic anhidrase was upregulated in female venoms while dystroglycan and a putative toxin were upregulated in males. Zymography assays demonstrated

that *A. juruenicola* venom degrades hyaluronic acid and incubations with peptidic substrates indicated proteolytic activity, supporting the toxin identification results.

Acknowledgements
FAPESP and CAPES