Molecular and cellular learning on extracellular matrix and toxins of venomous animals.

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Cell-extracellular matrix interactions have been implicated in a wide variety of cellular properties such as cell adhesion, migration, growth and differentiation, being important for tissue architecture and remodeling. These cell-extracellular matrix interactions are possible because the existence of cell surface receptors such as integrins and proteoglycans. Integrins are dimeric glycoproteins that link extracellular matrix domains to cytoeskeleton. Proteoglycans are complex molecules formed by a core protein to which one or more glycosaminoglycan chains are linked. They have been involved in several biological events. Working with human tumor cell lines, by using metabolic radiolabeling with sodium sulfate, immunoprecipitation reactions and affinity chromatography we characterized alpha5/beta1 integrin as a part-time proteoglycan containing both heparan and chondroitin sulfate residues. Moreover, using and immunoprecipitation reaction with a specific monoclonal antibody, we report that alpha5/beta1 integrin from different cell lines is post-translationally modified by incorporation of sulfate groups. Cell-haptotactic motility experiments on fibronectin in the presence of xyloside or chlorate (inhibitors of sulfation) showed an important decrease when compared to control. These data together lyases treatments indicated the proteoglycan nature and the involvement of glycosaminoglycan chains of the alpha5/beta1 integrin on fibronectin cell motility. Additionally, studying the human melanoma cell line Mel-85, we were able to detect Mel-85-LBM, a specific laminin-binding molecule structurally resembling a chondroitin sulfate. Mel-85-LBM is a cell surface molecule that could function as a laminin co-receptor and cooperate with the laminin-integrin interaction necessary for cell adhesion and locomotion. Venom toxins are excellent tools for investigating molecular mechanisms in the cell biology field, as well as for pharmaceutical applications of newly discovered medicines. In this area, venom toxins could be used as starting materials to design new drugs or directly for therapeutic use. Defibrinogenating toxins have been used for a number of clinical conditions such as deep vein thrombosis, myocardial infarction, pulmonary embolus, acute ischemic stroke, angina and central retinal vein occlusion. By studying *Loxosceles intermedia* (Brown spider) venom, we reported the identification of two gelatin binding metalloproteases with fibronectinolytic and fibrinogenolytic (loxolysin A) and gelatinolytic (loxolysin B) activities, probably involved in deleterious effects of the venom. Similarly, we were able to detect metalloproteases in *L. laeta* and *L. gaucho* venom. In addition, we detected a disruptive activity of *L. intermedia* venom toward EHS-basement membrane, with a hydrolytic effect on entactin and heparan sulfate proteoglycan. The venom also alters the integrity of blood vessel walls *in vivo* and endothelial cells in culture, causing a hydrolysis of extracellular matrix and cell surface heparan sulfate proteoglycans and shedding these molecules to the culture medium. Experiments using venom gland extract confirmed hydrolytic activities, the presence of metalloproteases and refuted criticism against contamination with digestive enzymes during venom harvesting. Proteolytic activities of the venom could be related to its deleterious effects such as loss of vessel and glomerular integrity and spreading of the venom toxins to underlying tissues. Histopathological findings in the skin of rabbits after acute experimental exposure to the venom revealed a massive acute inflammatory reaction with coagulative necrosis of affected areas. Additionally, crude venom induces a directly nephrotoxicity in mice. Results pointed to brown spider dermonecrotic toxin cytotoxicity upon renal structures and provide experimental evidence that this toxin is directly involved in nephrotoxicity evoked during accidents. Finally, we were able to identify hyaluronidases in *Lonomia obliqua* caterpillar bristle extract (lonoglyases) probably involved in deleterious activities disseminated by the venom. Supported by grants from CNPq, Capes, Fundação Araucária-Paraná e Secretaria de Estado de Ciência, Tecnologia e Ensino Superior do Paraná.