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Desenvolvimento de nanoimunossensores de microscopia de força atômica para estudo da esclerose múltipla

Tese apresentada ao Instituto de Medicina Tropical da Universidade de São Paulo para obtenção do título de Doutora em Ciências.

Área de concentração: Doenças Tropicais e Saúde Internacional.

Orientador: Prof. Dr. Fabio de Lima Leite
Co-orientadora: Profa. Dra. Doralina G. Brum Souza

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Somewhere, something incredible is waiting to be known."

Carl Sagan

Resumo

Garcia PS. Desenvolvimento de nanoimunossensores de microscopia de força atômica para estudo da esclerose múltipla (tese). São Paulo: Instituto de Medicina Tropical da Universidade de São Paulo; 2018.

A glicoproteína oligodendrocítica da mielina (MOG) e proteína básica da mielina (MBP) têm sido implicadas como os antígenos-alvo mais importantes nos processos desmielinizantes do sistema nervoso central (SNC), e mais importantes autoantígenos que surgiram dos estudos com o modelo animal para a esclerose múltipla (EM), a encefalomielite autoimune experimental (EAE). Os primeiros autoanticorpos detectados no soro e liquido cefalorraquidiano (LCR) de pacientes com EM foram anticorpos contra antígenos da mielina. O diagnóstico diferencial da EM inclui a presença de bandas oligoclonais (BOCs) no LCR e ausência no soro, demonstrando dessa forma síntese intratecal de imunoclobulinas G (IgG). As técnicas de detecção de anticorpos mais utilizadas atualmente são ELISA, ensaio baseado em células e *western blot* (WB). Neste contexto, o estudo da anti-MOG e anti-MBP e seu papel na EM podem ser estudados através da técnica de espectroscopia de força atômica (AFS). Esta é uma técnica altamente sensível que permite a detecção molecular, com a interação de uma ponta funcionalizada de microscópio de força atômica (AFM) com uma amostra, a qual fornece desta forma a força de adesão (Fad) específica para o sistema. Nesta pesquisa, foi inserido na ponta de AFM funcionalizada os peptídeos encefalitogênicos MOG_{92-106} e MBP_{85-99}, para detectar e estudar os anticorpos específicos IgG anti- MOG_{92-106} e MBP_{85-99}, no soro e LCR de pacientes na amostra, utilizando a técnica AFS. Sendo assim, este estudo foi realizado de forma inédita utilizando a AFS, auxiliando diretamente na investigação da EM e doenças desmielinizantes relacionadas.

Descritores: Microscopia de Força Atômica. Espectroscopia Atômica. Esclerose Múltipla. Bainha de Mielina. Sistema Nervoso Central. Sensor.

Abstract

Garcia PS. Development of atomic force microscopy nanoimmunosensor applied to the survey of multiple sclerosis (thesis). São Paulo: Instituto de Medicina Tropical da Universidade de São Paulo, Brazil; 2018.

Myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) have been implied as the most important target antigens in demyelinating processes of central nervous system (CNS) and the most important antigens candidates whom arised from the animal model for multiple sclerosis (MS), the experimental autoimmune encephalomyelitis (EAE). The first autoantibodies detected in serum and cerebrospinal fluid (CSF) of MS patients were antibodies against myelin antigens. Differential diagnostic to MS includes the presence of oligoclonal bands (OCBs) in CSF and absence in serum, which demonstrate intrathecal IgG synthesis. Most applied techniques to detection of antibodies nowadays are ELISA, cell-based assay and western blot (WB). In this context, the study of anti-MOG role in e disease may be supported through its detection by atomic force spectroscopy (AFS) technique. AFS is a highly sensitive technique that allows molecular detection as a functionalized atomic force microscope (AFM) tip interacts with the sample, providing the system specific adhesion force (Fad). In this research, it was attached in the functionalized AFM tip the notable encephalitogenic peptides MOG_{92-106} and MBP_{85-99} to detect and study the specific antibodies anti-MOG_{92-106} and anti-MBP_{85-99} on the sample with AFS technique. Thus, this study was applied for the first time in research with AFS, assisting directly to MS and other demyelinating diseases investigation.

Descriptors: Atomic Force Microscopy. Force Spectroscopy. Multiple Sclerosis. Myelin Sheath. Central Nervous System. Sensor.

CONCLUSÕES

Este foi o primeiro trabalho experimental realizado pelo nosso grupo, o GNN, para o estudo de doenças desmielinizantes utilizando sensores de ponta de AFM com enfoque na EM. As sequências da MOG e MBP foram escolhidas por serem amplamente estudadas na indução de EAE e os primeiros autoanticorpos detectados, tanto no soro quanto no LCR de pacientes com EM.

Nesta pesquisa, foi desenvolvido com sucesso um protocolo adequado de funcionalização de superfícies para a detecção da anti-MOG, validado pelos métodos AFS e caracterização aplicados, e corroborado pelo método *in silico* para o mesmo sistema; assim, os seguintes pontos puderam ser concluídos:

a) Foi desenvolvido um protocolo eficiente para funcionalização química de pontas de AFM e substratos, para o estudo de autoanticorpos envolvidos na EM;

b) A anti-MOG_{92-106} e anti-MBP_{85-99} foram detectadas com êxito utilizando o sensor desenvolvido, tanto no controle específico quanto nas IgGs purificadas dos pacientes;

c) uma vez que os anticorpos específicos foram detectados somente nos pacientes que estavam em surto e no LCR, pôde-se concluir que o nanossensor desenvolvido é capaz de detectar síntese intratecal de IgG, e dessa forma, pacientes em processos desmielinizantes, em especial na EM no início da doença;

d) utilizando as técnicas de caracterização, juntamente com os resultados satisfatórios de AFS, foi possível verificar que as superfícies estavam devidamente funcionalizadas;

e) foi possível comparar os resultados teóricos obtidos por SMD para os valores de força de adesão com os resultados experimentais, dando suporte à detecção dos autoanticorpos com o nanoimunossensor desenvolvido.

Foi verificado que, com o sensor desenvolvido, é possível detectar surto (estágios iniciais da EMRR), e pode ser utilizado como um sensor de anti-MOG e anti-MBP para verificação de síntese intratecal e desmielinização, juntamente com o quadro clinico estabelecido pelo neurologista com a aplicação dos critérios de McDonald. Estudos futuros serão realizados com um n amostral maior. Sendo assim, o estudo destes anticorpos auto-reativos em pacientes portadores de doenças desmielinizantes com esta técnica de vanguarda é uma ferramenta importante para o estudo antígeno-anticorpo neste contexo, e ainda poderá em breve auxiliar a elucidar ainda outras questões da presença dos mesmos nos pacientes.

Dessa forma, os achados podem fornecer pistas importantes para a participação dos autoanticorpos estudados nas doenças desmielinizantes, bem como na EM, uma vez que o valor da anti-MOG e anti-MBP ainda são conflitantes na literatura; mas seu envolvimento nos processos neurodegenerativos é inegável.

Assim, o estudo da presença de anticorpos contra sequências da MOG e MBP, em particular das sequências MOG_{92-106} e MBP_{85-99} na EM, é substancial tanto para o entendimento da doença, quanto para o desenvolvimento de novos sensores, a busca de biomarcadores para sua detecção ou contribuição para o tratamento da EM. Neste contexto, a microscopia de força atômica pôde ser aplicada como método de detecção para estudar e entender os mecanismos moleculares envolvidos com a EM.

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