

Combination of atomic force microscopy and mass spectrometry for the detection of target protein in the serum samples of children with autism spectrum disorders

A L Kaysheva^{1,*}, T O Pleshakova¹, A T Kopylov¹, I D Shumov¹, I Y Iourov²,
S G Vorsanova², Y B Yurov², V S Ziborov³, A I Archakov¹ and Y D Ivanov¹

¹Institute of Biomedical Chemistry, Moscow, Russia.

²Mental Health Research Center, Moscow, Russia.

³Joint Institute for High Temperatures RAS, Moscow, Russia.

*kaysheva1@gmail.com

Abstract. Possibility of detection of target proteins associated with development of autistic disorders in children with use of combined atomic force microscopy and mass spectrometry (AFM/MS) method is demonstrated. The proposed method is based on the combination of affine enrichment of proteins from biological samples and visualization of these proteins by AFM and MS analysis with quantitative detection of target proteins.

1. Introduction

According to WHO, about 67 million people worldwide suffer from autism, and this level increases by 14% every year. Due to the complications in the diagnosis, in Russia there are no official data on the number of autistic children. In the early 2000s, the genes associated with the development of autism spectrum disorders (ASD) were annotated [1]. The genes associated with the development of ASD are, however, inhomogeneous and can participate in the development of other psychiatric and neurological disorders [2]. A full understanding of the causes of autism can be achieved by the revelation of functional protein markers along with behavioral reactions and the development of highly sensitive and efficient methods of their quantitative detection in biomaterial [3, 4].

Earlier, the authors carried out comparative panoramic mass spectrometric (MS) analysis of serum samples from three families, in which children with ASD were brought up [5]. As a result of the performed comparative analysis of the protein composition of the serum samples, a small group including 13 conventional marker proteins was sorted out; in this group, one *LIM domain-containing protein 1* was identified in four of five samples. To date, changes in the levels of GFAP, apoptosis factor Bcl-2, glutamate metabolism factor GAD-2, metallothioneins and thymidylate synthases, etc. [4, 6] in blood samples of children suffering from ASD are announced in scientific literature. These proteins are attributed to potential markers of development of autism in children.

The aim of the present study is the development of sensitive multiplexed method of target protein registration in serum samples of children suffering from ASD. The following objects were used as target proteins: apoptosis regulator (Bcl-2, UniProt AC P10415), metallothionein-3 (MT3, UniProt AC P25713), and thymidylate synthase (TYMS, UniProt AC P04818). The developed method is based on affine concentration of target proteins from the serum onto the surface of chips for atomic force



microscope (AFM) with subsequent visualization of the affine complexes formed on this surface in a combination with MS quantitative analysis of the content of target proteins in biological samples [7].

2. Materials and methods

Similar to panoramic analysis [5], serum samples of children suffering from ASD were tested. Four control serum samples of healthy volunteers (C1, C4, C6, and C8) and five samples of children with ASD (S2, S3, S5, S7, and S9), provided by Separated Structural Unit “Clinical Research Institute of Pediatrics” at Pirogov Russian National Research Medical University Named After Y.E. Veltishev, were tested.

AFM chip represented an affine reagent, on which surface four sensor areas were formed: three working areas with immobilized monoclonal antibodies against three target proteins, and one control area without immobilized affine reagents. The procedures of AFM chips sensibilization are described in detail elsewhere [7]. AFM scanning of each area was carried out before and after the chip incubation in the analyzed sample [7].

Preparation of AFM chip for MS analysis included hydrolytic cleavage of proteins on the chip surface according to [7]. Mass spectrometric selected reaction monitoring (SRM) of the target proteins was carried out using Agilent 6490 mass spectrometer (USA), equipped with Agilent 1260/1290 HPLC system (USA) for unique peptides of target proteins according to the technique described in [7].

3. Results and discussion

AFM chips fabrication and analysis procedure (including the step of specific enrichment of AFM chip surface with target protein molecules) are described in our papers [5, 7, 8]. The identification of protein objects captured onto the AFM chip surface was carried out by SRM mass spectrometry [7].

The AFM scanning of working areas indicated that the affine complex «antibody/target protein» is hindered due to insufficient contrast of the images on the background of antibody molecules. That is, the changes in the heights of objects visualized in the working areas after the chip incubation in the target protein solution are insufficient for unambiguous evaluation of complex formation. Earlier, with HIV-1 gp120 glycoprotein we demonstrated that the best contrast of AFM images is observed for small probe molecules – aptamers (short single-stranded DNA sequences specific against the target protein). The contrast of «aptamer/target protein» images is twice higher than that of «antibody/target protein» images [9]. For this reason, in our present research AFM chip served as efficient affine reagent [5, 8]. Subsequent SRM analysis of the AFM chips has revealed the presence of Bcl2 protein on the surface of three chips and TYMS protein on the surface of one chip at picomolar concentrations (Table 1). Identification of MT3 protein on the AFM chips' surface was not possible.

It is interesting to point out that AFM/SRM method allowed detection of Bcl-2 protein in serum samples of brothers (S2 and S3), and also in S5 sample.

Table 1. Results of AFM/SRM analysis of 5 serum samples of children suffering from ASD, and of 4 control samples.

Sample No.	Protein	Results of AFM/SRM analysis	
		Amino acid sequence of the revealed peptide	Concentration of the revealed peptide ($\times 10^{-12}$ M)
S2	Bcl-2	FATVVEELFR	2.10
	TYMS	—	—
S3	Bcl-2	FATVVEELFR	4.20
	TYMS	DFLDSLGFSTR	20.8
S5	Bcl-2	FATVVEELFR	1.20
	TYMS	—	—

4. Conclusion

Thus, we have demonstrated the efficiency of SRM with preliminary affine enrichment and concentration of target protein molecules from serum onto small area of AFM chip surface for target protein detection.

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