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Technical note

Don't trust an(t)ybody - Pitfalls during investigation of candidate proteins for methylmercury transport at the placental interface

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ABSTRACT

While investigating placental mercury transport, we validated specificity of commercial antibodies against four candidate transporters (Large neutral amino acids transporter (LAT)1, LAT2, 4F2 cell-surface antigen heavy chain (4F2hc), and multidrug resistance-associated protein (MRP)2) by immunoblotting and small interfering RNA (siRNA)-mediated protein knockdown. An anti-4F2hc- and one anti-LAT1-antibody were specific. Another anti-LAT1-antibody reacted with LAT2. Two anti-LAT2-antibodies detected mainly albumin in placental lysates. A specific anti-MRP2-antibody hardly detected MRP2 in human placentas, contradicting published data. We recommend testing any unknown antibody by western blotting for 1/specificity for the protein of interest using *e.g.* siRNA knockdown and 2/cross-reactivity with albumin.

1. Introduction

The toxic organic mercury compound methylmercury crosses the placenta barrier most likely through active transport [1]. Our knowledge on the involved transporters is poor [2]. Mercury strongly adheres to sulfhydryl groups. Methylmercury-cysteine, a molecular mimicry of methionine [3], is transported into cells by L-type amino acid transporter (LAT) light chains LAT1/SLC7A5 and LAT2/SLC7A8 [4] dimerized to the 4F2 surface antigen heavy chain (4F2hc/CD98/SLC3A2). Exit from cells is assumed to occur as a complex with glutathione (GSH) recognized by GSH-carriers such as multidrug resistance-associated protein 2 (MRP2/ABCC2) [5].

In preparation to functional studies on placental mercury toxicokinetics, we aimed to verify specificity of commercial primary antibodies against LAT1, LAT2, 4F2hc, and MRP2 combining the methods of immunoblotting [6] with target-specific small interfering RNA (siRNA)-mediated knockdown [7]. Current literature suggests that antibody specificity is valid only in 50% of cases [8]. Many researchers worldwide, who invested incredible amounts of money and time into validation, realized this [9–11]. Despite our awareness that antibodies do not necessarily recognize (only) their specified target, we were quite surprised by the results obtained during validation and want to share the most striking observations with the research community.

2. Materials and methods

We selected one antibody against 4F2hc, two antibodies against LAT1 and LAT2, respectively, and one further against MRP2 according to two criteria, i.e., recommendation for immunoblotting

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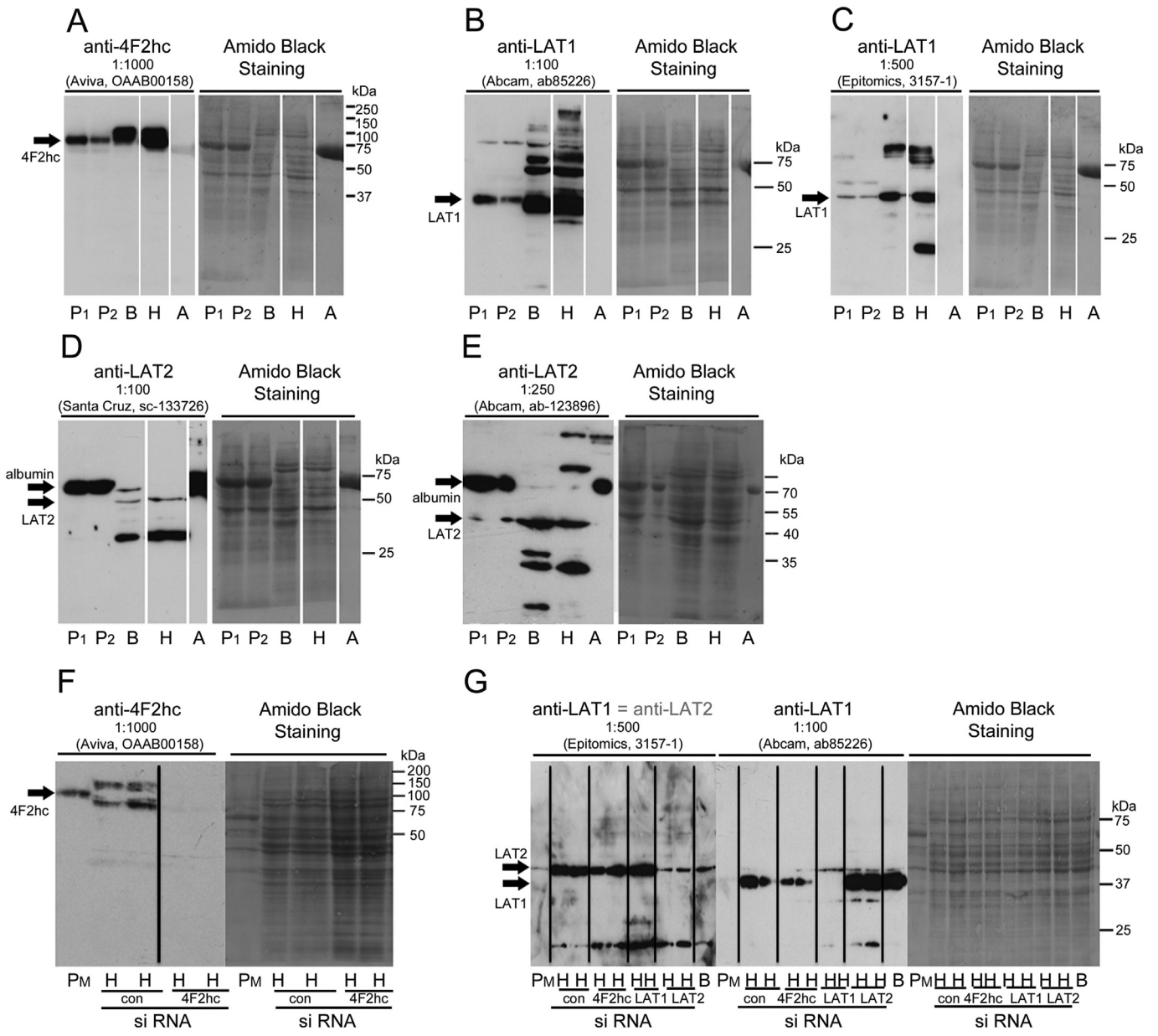

Fig. 1. Evaluation of specificity of antibodies against system L amino acid transporter subunits (LAT1, LAT2, 4F2hc). A–E: Representative samples of human term placentas (40 μg total lysates) (P1, P2), BeWo (B) and HeLa (H) cells and purified human albumin (A, 1 μg) were processed by reducing SDS-PAGE and western blotting. F–G: siRNA-mediated knockdown of 4F2hc, LAT1 and LAT2 in HeLa cells (H). Controls (con) were treated with non-targeting siRNA. Lysates (40 μg) of HeLa cells as well as placental membrane fractions (P_M) and untreated BeWo cells (B) were subjected to reducing SDS-PAGE and immunoblotting. F: Western blot was performed with anti-4F2hc antibody G: Membrane was first blotted with Epitomics anti-LAT1 antibody, Surprisingly, the major reactive band was reduced in the LAT2 siRNA-transfected but not in the LAT1 siRNA-transfected cells. To exclude any mix up of the siRNA samples, the blot was stripped and re-incubated with the Abcam anti-LAT1 antibody. One major band was observed that, however, had a lower molecular weight (about 40 kDa) as that observed with the Epitomics antibody (40–50 kDa). Moreover, with the Abcam anti-LAT1 antibody the expected reduction of antibody-binding in LAT1 siRNA-transfected cells is observed. All membranes were finally stained with Amido black. Molecular weights are indicated by numbers at the right side of blots, proteins of interest are marked by arrows.

and provision of immunoblot(s) indicating specificity for a protein of the appropriate size.

Healthy term placentas were obtained after elective cesarean sections. Prior to, all patients gave written informed consent and the study was approved by the ethics committee of the Medical University Vienna (EC-number 833). Preparation of highly enriched apical membrane fractions of the placental syncytiotrophoblast was done as described [12]. For comparison of protein expression pattern and for knockdown experiments we employed BeWo, HeLa and Caco-2 cells. The cells were transfected according to protocol [7]. Detailed information on the used reagents, antibodies and siRNAs, on cell culture, preparation of placenta tissue and lysates from human term placentas and cell lines as well as on immunoblotting are provided in Supplemental Data. In brief, proteins were separated by SDS-PAGE and then transferred onto PVDF transfer membranes. Individual proteins were detected by incubation with primary antibodies diluted in Blotto overnight. Thereafter, blots were washed and incubated with matching secondary HRP-conjugated antibodies. Amido black or Ponceau-S staining was performed to visualize and compare the protein amounts loaded [13]. Bands were detected by chemoluminescence.