Letter to the Editor

Cytosolic prostaglandin E₂ synthase/p23 but not apoptosis-linked gene 2 is downregulated in human atherosclerotic plaques

Wim Martinet a,*, Dorien M. Schrijvers a, Guido R.Y. De Meyer a, Arnold G. Herman a, Mark M. Kockx a,b

a Division of Pharmacology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium
b Department of Pathology, A.Z. Middelheim, Antwerp, Belgium

Received 12 November 2003; accepted 14 November 2003

In the November issue of Cardiovascular Research, we reported a strong downregulation of apoptosis-linked gene 2 (ALG-2) protein in advanced human atherosclerotic plaques as compared with non-atherosclerotic mammary arteries [1]. Because ALG-2 is a positive mediator of apoptosis, we proposed a novel survival mechanism against cell death in human plaques. Downregulation of ALG-2 protein was identified via the Western array screening facility of BD Biosciences. This technique included polyacrylamide gel electrophoresis and Western blotting followed by screening of the blots with unique cocktails of monoclonal antibodies. In our study, 823 monoclonal antibodies were used that were all from BD Transduction Laboratories. To validate the Western array results, traditional Western blotting (one antibody per blot) and real-time quantitative RT-PCR was applied [1]. Surprisingly, we were unable to confirm differential expression of ALG-2 at the RNA level. We proposed that ALG-2 might be regulated by posttranscriptional mechanisms. However, after acceptance of our paper, La Cour et al. [2] demonstrated that three commercially available antibodies against ALG-2 (two antibodies from Santa Cruz Biotechnology as well as the same antibody from BD Transduction Laboratories [clone 22] that we had used) detected neither mouse recombinant ALG-2 nor endogenous ALG-2 in Jurkat cell lysates. Instead, the clone 22 BD antibody recognized a protein in Jurkat cells running approximately 2 kDa higher than the endogenous and recombinant ALG-2 [2]. The sequence of the recombinant ALG-2 was verified by mass spectrometry, which excludes the possibility that mutations and/or other abnormalities had occurred during protein synthesis. Based on these new findings, the protein that we picked up by Western array screening and that was strongly downregulated in human atherosclerotic plaques cannot be ALG-2. Recent immunoprecipitation experiments, however, showed that the clone 22 antibody recognized protein p23 [3]. This finding may be of wide interest in atherosclerosis research as p23 is identical to the cytosolic prostaglandin E₂ (cPGE₂) synthase and is thus capable of converting COX-1-derived PGH₂ to PGE₂ [4]. Moreover, p23 is associated with the 90-kDa heat shock protein (HSP90) and regulated by the latter enzyme [5]. Further research should be conducted in order to understand the effect of p23 downregulation in atherosclerosis.

Of note, several other papers in which the clone 22 BD antibody was used draw wrong conclusions regarding ALG-2 [6–8]. We can imagine that misinterpretation of experimental results due to a specific binding or incorrect specificity of an antibody frequently occurs but that in most studies this problem remains unnoticed. Therefore, we hope that this incident may remind other research groups to be cautious with antibodies, even with monoclonal antibodies that were extensively tested by established companies.

References


* Corresponding author. Tel.: +32-3-820-27-10; fax: +32-3-820-25-67. E-mail address: wim.martinet@ua.ac.be (W. Martinet).

