Selection of an anti-modified citrulline antibody by phage display

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Introduction
The post-translationnal conversion of peptidylarginine to peptidylcitrulline, a process also known as citrullination, is catalyzed by the enzyme family of peptidylarginine deiminases1 (PADs; Fig. 1). To get more insight into the role of PADs and citrullination in both healthy and diseased individuals, experimental strategies to characterize citrullinated proteins in complex biological samples are crucial2.

Fig. 1 Citrullination. Arginine residues are converted to citrulline residues by PAD enzymes.

A method described for the detection of citrullinated proteins is based on the selective chemical modification of the citrulline side-chain with 2,3-butanedione monoxime and antipyrine in an acidic environment (Fig. 2). Chemically-modified citrullines were subsequently detected by rabbit polyclonal anti-modified citrulline antibodies and this procedure appeared to be very sensitive and specific3. However, for unknown reasons, these rabbit antisera are no longer commercially available. Therefore, novel antibodies for the detection of citrullinated proteins are required.

Fig. 2 Chemical modification. Citrulline residues are chemically modified by 2,3-butanedione monoxime and antipyrine under highly acidic conditions.

Methods
Target peptide for phage display

citrulline-containing peptide

Fig. 3 Target modification. A citrulline-containing peptide was chemically modified by 2,3-butanedione monoxime and antipyrine and the modified peptide was used as a target in the biopanning procedure.

Fig. 4 The biopanning procedure.

Results

Human anti-modified citrulline antibody

Fig. 5 Cloning strategy. Reactive scFv antibodies were cloned into ModiQuest’s proprietary human IgG1 and mouse IgG2a vector set to obtain either human or mouse full-length monoclonal anti-modified citrulline (AMC) antibodies.

Fig. 6 Specificity of the human monoclonal AMC antibody, as determined by ELISA. The human monoclonal AMC antibody was tested on various modified citrulline-containing peptides and their non-modified forms.

Fig. 7 Specificity of the human AMC antibody, as determined by Western blot. In vitro citrullinated recombinant proteins (A) and in vitro citrullinated cell lysate (B), were separated by SDS-PAGE and transferred to Western blots. Blots were chemically modified and citrullinated proteins were visualized with the human monoclonal AMC antibody.

Mouse anti-modified citrulline antibody; preliminary data

Fig. 8 Specificity of the monoclonal mouse AMC antibody, as determined by ELISA. The mouse monoclonal AMC antibody was tested on various modified citrulline-containing peptides and on their non-modified forms.

Conclusions
Our novel monoclonal human anti-modified citrulline antibody shows a high sensitivity for modified citrulline residues in ELISA and on Western blot. Preliminary results show that the monoclonal mouse anti-modified citrulline antibody has a high sensitivity and specificity in ELISA. These antibodies are therefore excellent replacements for the discontinued rabbit polyclonal anti-modified citrulline antibodies.

References