A custom RabMAb® solution for detection of subtle epitope differences in amyloid beta peptides in Alzheimer's disease research

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"We have repeatedly used Abcam's RabMAb services; they are the only ones who deliver specific, high-affinity antibodies to small peptides, which are critical for our research."

Introduction

Alzheimer's disease (AD) is the most common cause of dementia. Characteristic neuropathological changes in AD include cerebral neuritic plaques, cerebral neuronal loss, and neurofibrillary tangles. A core component of neuritic plaques is amyloid beta peptide (Abeta), which is derived from the amyloid precursor protein (APP) through the sequential cleavages of beta and gamma secretases. Soluble forms of Abeta generated from APP commonly end at C-terminal amino acid residue 40 (Abeta 1-40) and 42 (Abeta 1-42). Abeta 1-42 tends to aggregate rapidly into oligomers and then insoluble amyloid fibrils. The oligomers are more toxic than fibrils and are responsible for the development and progression of AD (1,2).

"The RabMAb products I received are specific to an immunogen with no cross-reactivity to other peptides or proteins and possess higher affinity than mouse monoclonal antibodies."

Although many drug programs are aimed at alleviating AD symptoms, there are few approved therapies. Those that are available are most effective in the earliest stages of the disease, meaning that good prognostic indicators are needed for their success. Several forms of amyloid beta have been investigated for both such purposes (1, 2).

The problem

As diagnostic and therapeutic targets, accurate recognition of Abeta peptides is a critical task. They differ from each other by only a few amino acids (Figure 1), which makes specificity of detection reagents an important factor. The utility of these peptides as fluid biomarkers also depends on the affinity of antibodies used for their immunoassay identification, as they can be of low abundance in such compartments (3).

"The biggest advantage of rabbit monoclonal antibodies is the unlimited, consistent supply: same specificity, same sensitivity, and high affinity."

Dr. Pankaj D. Mehta, an investigator at the New York Institute for Basic Research in Developmental Disabilities, has been studying Abeta peptides in the context of AD for a number of years. Early on, any commercially available antibodies to these peptides were either incapable of differentiating between closely related forms (such as Abeta 1-40 and Abeta 1-42) or were "not sensitive enough to quantitate small amounts of peptide present in body fluids," explained Dr. Mehta.

Figure 1. Sequences of human Abeta peptides mentioned in this article.

Abeta 1-40	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV
Abeta 1-42	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
Abeta pyroGlu 3-42	pEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

For his experiments, Dr. Mehta needed antibodies with high affinity and high specificity that could also be produced in continuous supply. Although rabbits were an attractive host, Abeta sequences in this species are identical to those in humans and hydrophobic as well; together, these factors make immunization a challenging task.

The RabMAb solution

Having seen colleagues attempt and fail to create specific mouse monoclonal antibodies to Abeta isoforms, Dr. Mehta knew he would need another approach and turned to Abcam's RabMAb technology.

Following his extensive efforts to induce sufficient immune responses, together Dr. Mehta and Abcam were successful in generating rabbit monoclonals to Abeta peptides. The key differentiator was the ability of these antibodies to detect the small differences between the various forms of amyloid beta.

RabMAb technology is based on Abcam's proprietary partner cell line. When fused to rabbit B-cells, the result is true rabbit-to-rabbit hybridomas that each produce a single type of antibody (4). The rabbit immune system generates greater antibody diversity than and uses affinity optimization mechanisms different from those of mice and other rodents (5). With a broader range from which to choose, the chance of finding antibodies with the desired properties is higher.

Many mouse antibodies have affinities in the nanomolar range ($K_D = 10^{-9}$ M), but rabbit monoclonals are generally more sensitive, with the K_D values often at the picomolar level ($K_D = 10^{-12}$ M). Through the combination of high affinity and high specificity, rabbit monoclonals can effectively purify the species of interest from complex mixture and enrich it enough for reliable detection. When used for cell staining, these properties translate into stronger signals, lower background, and more readily interpretable data.

"Abcam's RabMAb technology is very effective in producing specific antibodies not only to proteins, but also to small peptides of molecular weight <1 kDa. The rabbit fusion partner provides us with the opportunity to produce RabMAb antibodies specific for mouse antigens, and also for antigens not immunogenic in mice."

Two of Dr. Mehta's custom RabMAb primaries were featured in his recent Journal of Alzheimer's Disease article (6). These reagents distinguish between Abeta 1-40 and Abeta 1-42 in a number of applications.

As Dr. Mehta explained, they "performed well in ELISA, western blotting, immunoprecipitation, and immunohistochemistry techniques…These are high-affinity antibodies and will be very useful in the quantitation of amyloid beta peptides in body fluids and brain tissues of individuals with Alzheimer's disease."

Future directions

Dr. Mehta has also recently generated a RabMAb primary specific to the pyroGlu version of 3-42 (pE3-Abeta). This form is missing the first two amino acids of Abeta 1-42, and the novel N-terminal residue becomes cyclized to create a pyroglutamate. This peptide is a major component of Abeta deposits in post-mortem brains from patients with AD and Down Syndrome (DS). Using his newest RabMAb product, Dr. Mehta has found increased levels of pE3-Abeta in plasma of older persons with DS relative to controls (7).

Given recent studies showing that another antibody to pE3-Abeta removed Abeta plaques from the brains of mice in a transgenic model of AD (8), Dr. Mehta speculates that there is much promise for these reagents as potential treatments. Indeed, Eli Lilly has a therapeutic monoclonal to this target in its clinical pipeline for AD indications (9).

As additional Abeta peptides are discovered and studied, selective and sensitive antibodies will be key tools in linking them to pathological states and developing targeted drugs and diagnostics. Application of RabMAb technology to this research area will open up new avenues of investigation and enhance understanding of Alzheimer's and other neurological diseases.

References

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