Abstract

Cancer is currently widespread disease and its successful treatment requires the elimination of all cancer cells in the body. One method of cancer treatment is immunotherapy, which seeks to elicit an immune response and activate the body's anti-tumor defense mechanisms. Therapeutic antibodies are used to target tumor cells markers. One of such markers is the HER2 receptor which is overexpressed for example on the surface of breast cancer cells.

Humanized monoclonal antibodies are often used as therapeutic antibodies, but other constructs such as bispecific particles, nanobodies or their analogs are also used. Nanobodies refer to recombinant antibody-derived variable domains that lack light chains in their structure. Such antibodies occur naturally, for example in camelid mammals or in certain cartilaginous fishes, such as sharks.

This work describes the preparation of various glycoforms of the antiHER2 nanobody and verification of the effect of the glycosylation on the ability of nanobody to bind to the cell line that is overexpressing the HER2 receptor on its surface. A nanobody with complex natural glycosylation (produced in the HEK293T cell line) and a nanobody with uniform glycosylation (produced in the HEK293S GnTI$^-$ cell line) were prepared. The work also describes the cloning and production of antiHER2 nanobody with a mutated glycosylation site in the paratope. Glycosylation has been shown to negatively affect binding on HER2 receptor, an important finding for the future use of this nanobody in the preparation of potential immunoactive therapeutics.

Key words

nanobody, HER2, N-glycosylation, mutation, HEK293, recombinant protein expression