Hybridoma Technology

History of Hybridoma Technology

What is hybridoma technology? Hybridoma technology is a well-established method to produce monoclonal antibodies (mAbs) specific to antigens of interest. Hybridoma cell lines are formed via fusion between a short-lived antibody-producing B cell and an immortal myeloma cell. Each hybridoma constitutively expresses a large amount of one specific mAb, and favored hybridoma cell lines can be cryopreserved for long-lasting mAb production. As a result, researchers usually prefer generating hybridomas over other mAb production methods in order to maintain a convenient, never-ending supply of important mAbs.

Fig 1. Hybridoma technology Inventor: Georges Kohler and Cesar Milstein

Hybridoma technology was discovered in 1975 by two scientists, Georges Kohler and Cesar Milstein. They wanted to create immortal hybrid cells by fusing normal B cells from immunized mice with their myeloma cells. For incidental reasons, they had all the requirements fulfilled and it worked in the first attempt. By cloning individual hybrid cells, they established the first hybridoma cell lines which can produce single type of antibody specific to the specific antigen.

Their discovery is considered one of the greatest breakthroughs in the field of biotechnology. For the past decades, hybridomas have fueled the discovery and production of antibodies for a multitude of applications.

By utilizing hybridoma technology, Sino Biological provides cost-effective mouse monoclonal antibody service, and we can deliver you purified antibodies in 60 days.

Steps Involved in Hybridoma Technology

Hybridoma technology is composed of several technical procedures, including antigen preparation, animal immunization, cell fusion, hybridoma screening and subcloning, as well as characterization and production of specific antibodies.

mAb generation by the hybridoma approach requires knowledge of multiple disciplines and practice of versatile technical skills, ranging from animal handling, immunology to cellular and molecular biology. Generation and identification of high-quality hybridoma clones is a comprehensive and labor-intensive process, and requires months of work during the time frame from immunization to specific hybridoma identification.

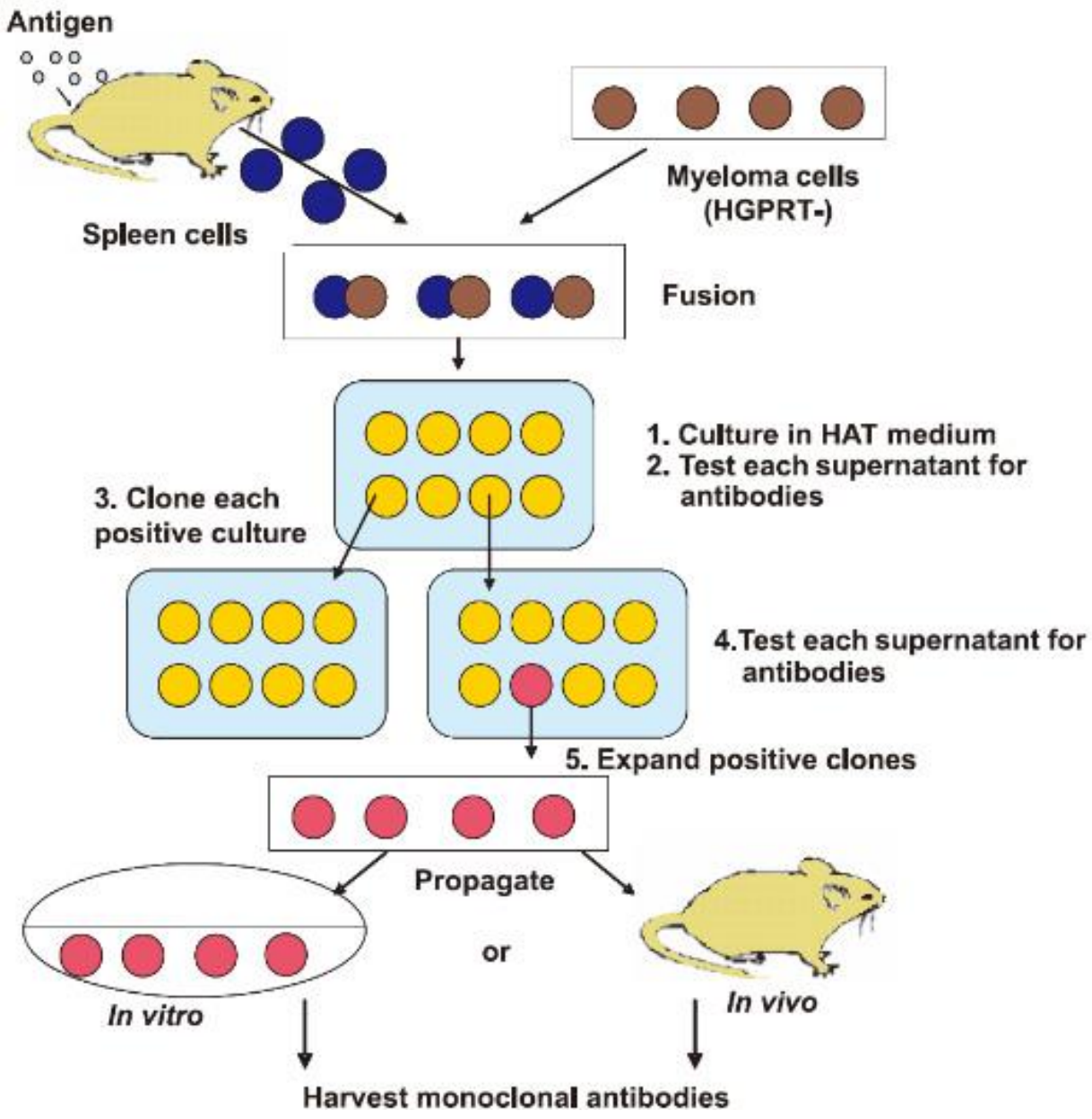

Fig 2. mAb generation by the hybridoma technology

1) Cell fusion

Polyethylene glycol (PEG) and electrofusion are commonly used to induce cell fusion in hybridoma production. PEG fuses the plasma membranes of adjacent myeloma and/or antibody-secreting cells, forming a single cell with two or more nuclei. This heterokaryon retains these nuclei until the nuclear membranes dissolve before mitosis. Electrofusion joins the membranes of neighboring cells by the application of a pulsed electrical field. Electrofusion is more efficient than PEG and the results are reproducible.

2) Hybridoma screening

Even in the most efficient hybridoma fusions, only about 1% of the starting cells are fused, and only about 1 in 10^5 form viable hybrids. This leaves a large number of unfused cells still in culture. The cells from the immunized animal (antibody secreting cell) do not continue to grow in tissue culture and so do not confuse further work. However, the myeloma cells are well adapted to tissue culture and must be killed, which can be achieved by drug selection.

Commonly, the myeloma cells have a defective HGPRT enzyme (hypoxanthine-guanine phosphoribosyl transferase), blocking their ability to use the salvage pathway. These cells containing a non-functional HGPRT protein will die in HAT medium. Only the hybridoma cells have got the ability to divide and proliferate on the HAT medium because genome from the B-lymphocyte makes them HGPRT positive and genome from the myeloma cells they can divide indefinitely.

3) mAb production

Hybridoma antibodies can be produced in vitro and in vivo.

For production of monoclonal antibodies in vitro, hybridomas are expanded by transfer to 24 well tissue culture plates followed by 25 cm^2 flask and a 75 cm^2 flask containing suitable medium. The cell density is maintained between 10^5 and 10^6 cells/ml. Typical culture supernatants yield up to l00μg/ml of antibody, the exact amount depending upon the cell density and rate of growth. Culture in vitro provides a more pure preparation of antibody. Sino Biological can offer serum-free hybridoma production serivce by the use of serum-free medium.

For producing monoclonal antibodies in vivo, mice are primed by intraperitoneal injection with 10^5 - 10^7 hybridoma cells. The rate of growth of the resulting ascites tumour is in general very variable and can be from less than two or more than five weeks. The ascites fluid can be collected from an anaesthetized mouse. It is possible to obtain 10 ml of ascites fluid or more from a mouse by regular tapping. Ascites fluid will be contaminated with mouse imunoglobulins to a small extent and if a very pure antibody is required this may prove inconvenient.

Applications of Hybridoma Technology

mAb therapeutics

Compared with other biologics, mAbs are able to maintain an extremely high affinity towards their target. Due to this high affinity and specificity, researchers began investigating the therapeutic potential of mAbs as metabolic activators, inhibitors and immuno-modulators. While the first few US FDA-approved mAb therapeutics, such as muromonab-CD3, were generated solely in mice, it became evident that in order to avoid immune rejection, future mAb-based therapeutics needed to undergo humanization. Since the approval of muromonab-CD3 in 1986, the FDA has approved approximately 80 more mAb therapeutics for diseases ranging from autoimmune disorders, to inflammatory diseases, HIV and cancer. Interestingly, despite the

discovery of combinatorial display libraries in 1984 as an alternative mAb discovery platform, the majority of these mAb therapeutics were originally discovered using hybridoma technology in either fully murine or humanized mice. The reason for this preference is likely attributed to the natural ability of the murine immune system to generate highly specific mAbs that elicit strong constant domain functionality with limited immunoreactivity after humanization.

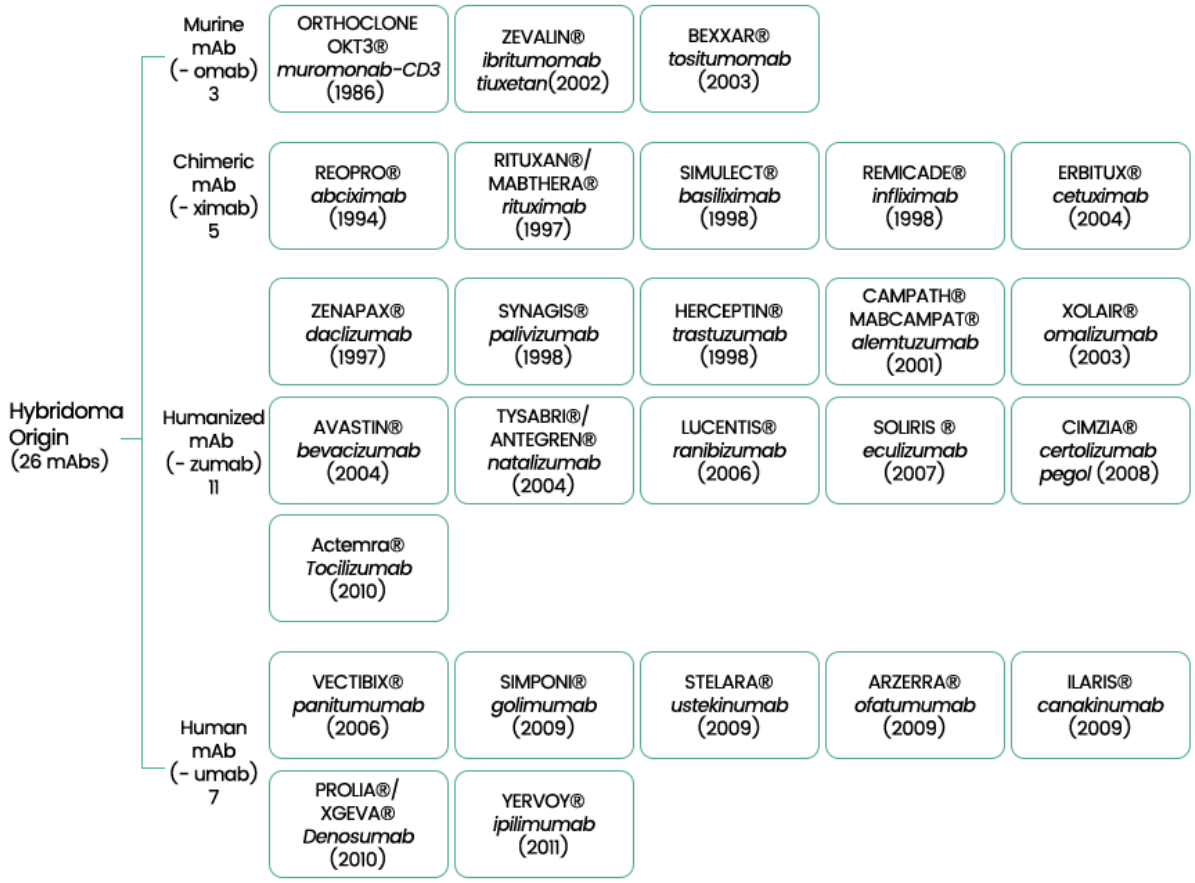

Fig 3. A list of FDA-approved therapeutic mAbs by hybridoma technology

In vivo diagnostics

In vivo diagnostics are a noninvasive way for clinicians to diagnose disease progression through analysis of biomarkers within the body rather than through biologic samples inside a laboratory. Most antibody-based in vivo diagnostics are used for highly specific imaging. Some common imaging methods include positron emission tomography (PET), magnetic resonance imaging (MRI), fluorescent molecular tomography (FMT) and ultrasound. The main difference between immune imaging and standard imaging is that, rather than imaging a large, nonspecific section of the body, a tagged antibody targets a precise location for diagnostic imaging instead. This idea of conjugating a full-length antibody or an antibody fragment to a nanoparticle, be it a radioisotope, fluorophore or positron emitter, would have not been possible without hybridoma-based antibody discovery. It was only through hybridoma technology that fully natural mAb variable domains that did not adversely impact a patient's own immune system during an examination were discovered.

References

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