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Review Article

Basics of Hybridoma Technology for The Generation of Monoclonal Antibodies

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Abstract

Hybridoma technique is perhaps of the most well-known strategy used to deliver monoclonal antibodies. In this method, B lymphocytes, a type of white blood cell which produces antibodies are obtained from mice subsequent to vaccinating the mice with explicit antigen and are fused with myeloma cell lines to produce hybrid cells (hybridomas).

Keywords: myeloma cells; antibodies; hybridomas; antigen; monoclonal

Introduction

Hybridoma technology is a technology used for making monoclonal antibodies [1]. The word hybridoma comes from hybrid which means a combination of two different organisms or cells [2]. It is called hybridoma technology because in these monoclonal antibodies are produced from hybrid cells or hybridoma, which is formed by the combination of two different cells such as one cell is an immortal myeloma cell and the other one is a type of white blood cell, which is activated and can produce antibodies [3,4]. The hybridoma technology help biotechnologist in the making of a large amount of homogeneous antigen-specific antibodies known as monoclonal antibodies [5]. Monoclonal antibodies are used for many purposes such as disease treatment, diagnosis etc [6].

Principle of Hybridoma technology

In Hybridoma technology an immune response is provoked inside the mouse by injecting the antigen of interest in the mouse [7]. As a result of this, the B lymphocyte (a type of white blood cell) inside the mouse spleen produced antibodies against the foreign antigen [8]. And later on, these antibodies are harvested from the injecting mouse [9]. Due to the short life span of B lymphocyte cells, they cannot be cultivated so that's why they are fused with a cancerous cell known as a myeloma cell [10]. As a result of this fusion hybrid cells or hybridoma cell lines are produced [11]. Hybridoma has the ability to divide repeatedly (because of myeloma cells) and can produce antibodies (because of B lymphocyte cells) [12]. Then these hybrid cell lines are incubated in a HAT medium and the selection of these is carried out by ELISA technique for the production of monoclonal antibodies [13]. The type of monoclonal antibodies produced as a result of hybridoma Technology depends on the type of antigen used [14].

Antibodies and monoclonal antibodies

Antibodies are molecules produced by the immune cell known as B lymphocytes against the specific antigen that invades the body [15]. The nature of these molecules is a glycoprotein and their structure contains four polypeptide chains. Of these four polypeptide chains, two are long or heavy chains and the two are short chains [16,17]. These chains have constant and variable regions [18]. Monoclonal antibodies are derived from a single clone of plasma cells having mono specific nature [19].

Discussion

Process

In Hybridoma Technology the following steps are as follows.

- **Injecting A Mouse with Desired Antigen** The first step of Hybridoma technology is the injecting of a mouse with the desired antigen as a result of this the mouse's immune system is provoked and antigens are trapped in the spleen [20]. The mouse splenic B cells start producing antibodies against the foreign antigens [21].
- **Collection of Splenic B Cells** The splenic B cells (splenocytes) which are activated and can produce antibodies are collected from the mouse after 72 hours of immunization [22].
- **Preparation of Myeloma Cells** Before fusion with plasma cells, metastatic tumour cells are incubated in 8-azaguanine to get non-functional HGPRT genes in Myeloma cells so after fusion, they cannot grow alone on the HAT medium due to the inhibition of the Salvage pathway due to lack of HGPRT for nucleotide synthesis [23,24].
- **Fusion (Hybridoma Production)**

Splenocytes (splenic B cells) have ability to produce antibodies but they can not divide repeatedly and they died after some time so they can not be cultivated as they are not immortal [25]. For their cultivation, they are fused with immortal cancer cells known as myeloma cells because myeloma cells have the ability to divide repeatedly and can be cultivated [26] and immortal cells have a similar structure to that of spleen cells that's why we choose immortal cells for the fusion with splenocytes as both of them are compatible with each other [27]. The fusion of splenocytes (B cells) and myeloma cell occur in the presence of polyethylene glycol [28]. During the process of fusion 5 types of cells are produced including fused plasma cell, unfused plasma cell, fused myeloma cell, unfused myeloma cell and hybridoma [29]

- **Cultivation of Fused Cells** These five types of fused cells are then cultivated on the HAT medium [30]. Hypoxanthine, aminopterin, and thymidine are the three main components of the HAT medium [31]. As we know that nucleotide within DNA and RNA contain purine and pyrimidine and their synthesis occurs by the De novo pathway or salvage pathway thus the component of HAT medium aminopterin has ability to stop the De Novo pathway so the synthesis of purine is now dependent on the Salvage pathway [32,33]. But the Salvage pathway only occurs in the presence of enzyme HGPRT and thymidine kinase [34]. From the five types of cells which are produced as a result of the fusion the unfused and fused myeloma cell lack HGPRT so these two cells cannot grow on the HAT medium [35]. Fused and unfused plasma cells, due to their short life spans will also die within a few weeks [36]. So hybridoma is the only cell that can grow on the HAT medium because in hybridoma the myeloma part has the ability to divide repeatedly and the plasma cell part has HGPRT that's why hybridoma cells will grow and remain alive for a long time on the HAT medium [37,38].
- **Screening** In the medium containing hybridoma cells, each hybridoma cells have specificity for one epitope so the medium containing hybridoma cells is diluted into multi-well plates to such an extent that each contains only one cell [39]. Using the ELISA technique or radioimmunoassay, the hybridoma cells are selected using specific antigen fragments and they are proliferated selectively [40]. Screening is performed after the isolation and separation of different hybridomas. The result of this screening the desired antibodies targeting specific epitopes for an antigen are produced [41].
- **Cloning of Hybridoma** This can be done in vivo or in vitro [42]. The in vivo method includes a usage of mouse for the production of monoclonal antibodies. In this Hybridoma cell are injected into the mouse and monoclonal antibodies are produced inside the mouse are then purified [43]. This in vivo method may be inconvenient due to contamination even after purification [44]. In in vitro method the hybridoma cells are cultured in laboratory on a large scale. These are cultured on a media and then monoclonal antibodies are isolated from this media [45].

Pros of Hybridoma technology

Hybridoma Technology has many advantages some of them are mentioned below.

- Hybridoma technology gives a large quantity of pure and specific antibodies thus an unlimited and large number of monoclonal antibodies can be produced that bind to a single epitope of an antigen. [46].
- Hybridoma technology is an appropriate method in the production of monoclonal antibodies which are used in chemotherapeutics to treat cancer [47].
- Monoclonal antibodies produced by Hybridoma technology can be used for [i]disease treatments and diagnosis purposes as well [48]
- Hybridoma technology is also useful in the differentiation between two strains of a single pathogen [49].

Cons of Hybridoma technology

Hybridoma technology also have some drawbacks such as it is a time-consuming process and an expensive method to execute and poses high risk of contamination [50].One of the big disadvantages of this technique is that it can transfer disease from mice to humans [51].Besides the antibodies produced in this technology are of mouse origin that's why even after purification there is no guarantee that the produced monoclonal antibodies are virus free [52].

Conclusion

Thus, monoclonal antibodies could be produced using hybridoma technology for the treatment and diagnostics of various diseases

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