HYBRIDOMA TECHNOLOGY USED FOR THE SCANNING OF VARIOUS DISEASE & CURE WITH THE HELP OF MABS.

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Abstract: Hybridoma technology are mostly used for the innate functions of both immune cells and testing of the cancer, with the help of production of Hybridoma cells, which are regularly generate the monoclonal antibodies specific to antigen of interest. Generating of the Hybridoma cells with the help of Hybridoma technology, and the helping of this technology are formed of the B-lymphocytes must be somatically fused with myeloma cells. Some scientist has been developed a new Hybridoma technology and this is used for the involves preselection of B-lymphocytes with target antigens based on the immunoglobulin receptors and the helping of the electrical pulses are fused of the B-cells-myeloma complexes, some monoclonal antibodies formed as anti-CEA, Ca1 and HMEG-2, which are used for the search of the malignant disease. The advancement methodology is formed with the help of this technology; may be applicable to simultaneous production of monoclonal antibodies, multitargeting and stereospecific targeting, termed B-cell targeting, selective production of stereospecific monoclonal antibodies for clinical purposes.

Keywords: Hybridoma cells, CEA and B-lymphocytes.

1. INTRODUCTION

Hybridoma technology was firstly discovered by G. Kohler and C. Milstein in 1975, which is used for the hybrid cell helping of the fusions of the B-lymphocytes with tumour or myeloma cells. Hybridoma technology is formation of the hybrid cells and this is cultured in laboratory or sub cultured using mouse peritoneal cavity. Kvalheim *et. al.*, (1996) were search of clinical significance, which are used for the detected to the ocult tumour cells in bone marrow and blood in breast cancer. The hybrid cells can produce the antibodies being present of B-lymphocytes genetic material. The B-lymphocytes is responded to the single type of antigen or antigenic determinate and they produce the single type of antibody that shows specificity for a specific antigen. So, the formation of an antigen reaction with B-lymphocytes receptors triggers the firstly division of lymphocyte. The specific antigen produced by generate antibodies of B-cells of a clone. Nelson *et al.*, (2000) was performed a sensitive antibody, which is known as monoclonal antibodies. B-Lymphocytes cells are produced a single type of antibodies specific to a single type of antigen or antigenic determined. This process known as clonal selection. If the B-lymphocytes are fully producing the determinate antibody (known as plasma cell) but this division cannot culture in laboratory. Riesenberg *et al.,* (1993) double stained of the Immunocytochemical of cytokeratin and prostate specific antigen in individual prostatic tumour cells. The myeloma cells are used for the Hybridoma technology but should not use for the synthesis of their own antibodies. The Hybridoma cells are synthesising machinery selected base on inhibiting the nucleotide (subsequently the DNA). De novo synthesis or salvage pathway are very important process of mammalian nucleotide selection.

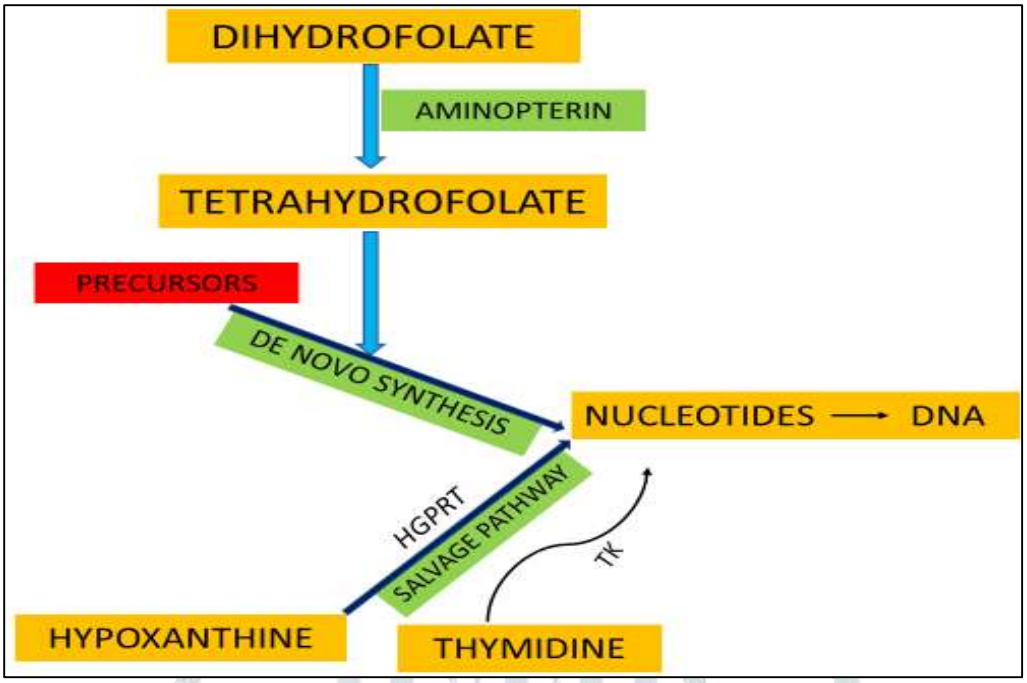

Fig: 1. Pathways for the synthesis of nucleotides

Tetrahydrofolate formed from dihydrofolate is required in the De novo synthesis of nucleotides. Aminopterin (which is an inhibitor) is used to block the formation of tetrahydrofolate (and therefore nucleotide). The purines and pyrimidines are exchanged into the corresponding nucleotides in the salvage pathway. Salvage pathway of purines is the HGPRT are involved the key enzyme which converts Hypoxanthine and guanine to inosine monophosphate and guanosine monophosphate respectively. Thymidine kinase which is known as pyrimidines. This is involved in the salvage pathway and converts Thymidine to Thymidine monophosphate (TMP). The salvage pathway is blocked than when the other enzymes are produced in this pathway (leave of HGPRT and TK). If the chance of the mutation, so cells are growing in medium containing Hypoxanthine Aminopterin and Thymidine. (HAT medium), But th8s medium belong to the fail to survive as the De novo synthesis of purines nucleotides is inhibited. The Hybridoma cells to possess the ability of myeloma cells it enhances in vitro with a functional HGPRT gene, which is obtained from the lymphocytes fused myeloma cells. These types of technique used for the only Hybridoma cells can proliferate in HAT medium and this process is used for their selection.

2. PRODUCTION OF MABs

The establishment of Hybridomas and production of MAbs involves some following steps are used: -

2.1. With the help of immunisation

The immunisation a mouse with a suitable antigen are formed with the help of Hybridoma technology. The subcutaneous route (Adjuvants are non-specific potentiators of specific immune response) are injected from antigen an adjuvant (Like Freund's complete or in incomplete adjuvants). The injections can repeat short times of many sites. The antigen response from B-lymphocytes are increases to the stimulation. Three days ago, the animal slayed assay and final dose of antigen given via intravenous route. The process of synthesize of antibodies and gives rise to large number of immune-stimulated are using this approach. The immunisation is used the concentration of desired antibodies is assayed the animal serum at frequent interval. The method of mechanically or enzymatically to release the cell and can removed of the spleen. Density gradients centrifugation separation technique can be used to the separate of the spleen lymphocytes.

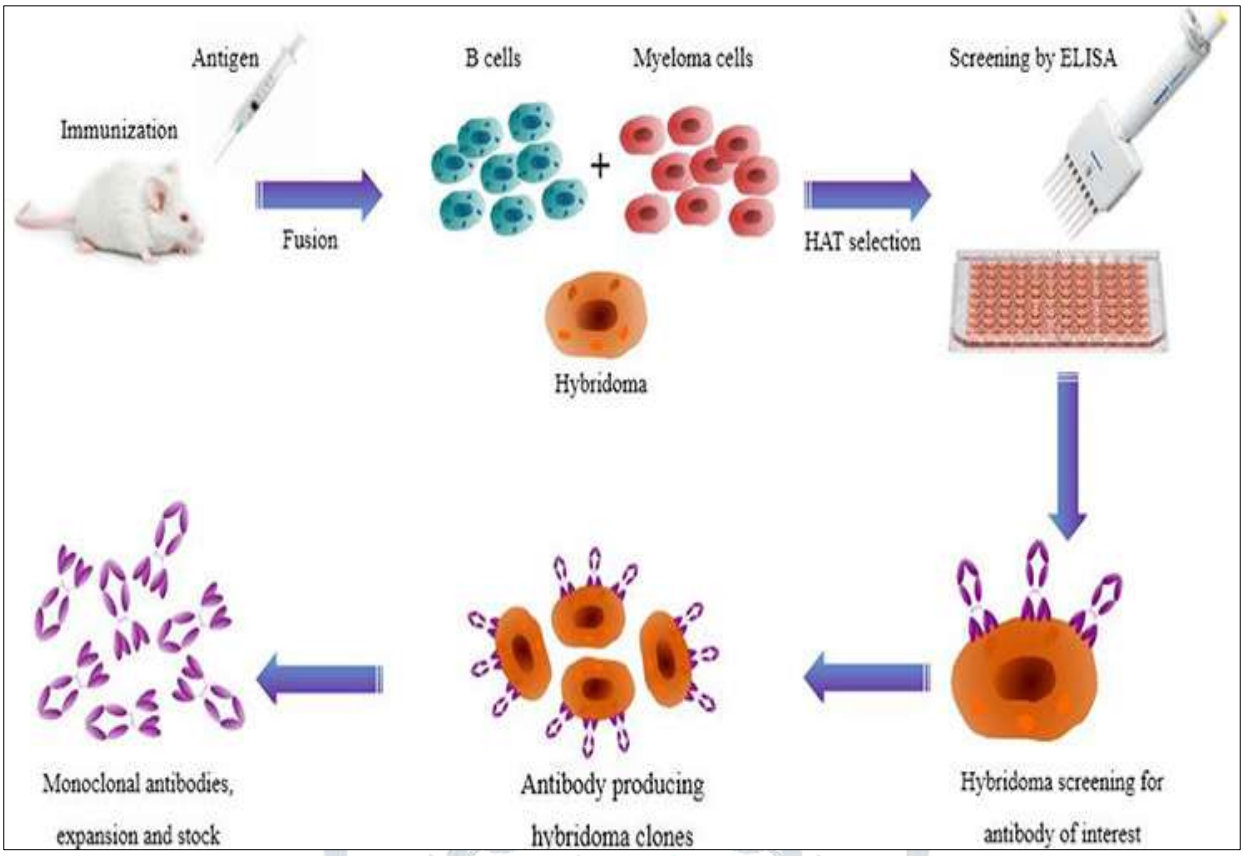

Fig: 2. Protocol for the derivation of monoclonal antibodies from hybrid myeloma

2.2. With the help of cell fusion

The lymphocytes cells ar3 maximum washed and mixed from the HGPRT defective myeloma cells. The fusion of cells used for the polyethylene glycol (PEG) but this is due to the toxicity for few minutes. The cells after the washed it remove the PEG and rest in a fresh medium.

2.3. With the help of selection of Hybridomas

The HAT medium is used to the culturing of the cells and the Hybridoma cells are growing and remaining cells preparing slowly within 7-10 days in this medium. The hybrid cells is very essential which is produced from the selection of a single antibody and when this is possible if when the Hybridoma are isolating and growth fatly. The function of desired antibodies when these cells are growing in a regular culture medium.

2.4. With the help of screening and products

The antibody of the desired specificity is secretion from the Hybridomas with the help of screened method and the tested for (using ELISA and RIA) the desired antibody specificity used to the Hybridomas culture. Korbakis *et al.*, (2015) monitored screening facility the development of ELISA for the measurements of native TEX101 in biological fluid. The technique of ELISA and RIA are using to the bounding of antigen and unbounded of the mixture can be washed. This technique is used to the antibody binds to the specific antigen (coated to plastic plate) and unbounded antibody or some other components in the medium to washed off. The monoclonal antibodies secreted from the antigen is based on the hybrid cells.

2.5. With the help of cloning and propagation

The two techniques used for the single hybrid cells producing the desired antibody isolated and cloned. **i) Limiting Dilution Method (LDM): -** The suspension of the Hybridoma cells is serially diluted and aliquots of each

dilution are transferred into the microculture wells could be the used in this method. In this dilution method are made up of each aliquot in a well contains a single hybrid cell and ensuring that the antibody is produced of monoclonal.

ii) Soft Agar Method (SAM): - The soft agar is using in this method and cultured of Hybridoma cells. Some cells are works for the making of the monoclonal colonies from semisolid medium. The maximum monoclonal antibodies formed with the help of in this method.

2.6. With the help of characterisation and storage:

Biochemical and Biophysical characterisation are shows for the desired specificity from the obtain monoclonal antibodies. The MAbs are also used to the elucidated for the immunoglobulin class or sub class. This is specific epitope and this is number of binding site's, both are characters to check with the help of stand freezing thawing by freezing desired cell lines in liquid nitrogen as several stages of cloning and culture.

3. LARGE SCALE PRODUCTION OF MONOCLONAL ANTIBODIES

The culture bottles are low approx (5-10ug/ml), Which in the production of MAbs and this culture are working for the increase the hybrid cells yield and grown the peritoneal cavity of mice. In vitro cultivation techniques could be used to the superior ascitic fluid contains 5-20 mg of MAbs/ml. The heavy risk of the collection of MAbs from ascitic fluid for the contamination by pathogenic organisms of animal.

3.1. Encapsulated Hybridoma Cells for Commercial Production of MAbs

The suspension culture is used for the increase of the Hybridoma cells density. The containing of poly-lysin are using of the coating solution and encapsulated in alginate gels from Hybridomas. The gels are used to the nutrient's antibodies enter in the Hybridomas. Helping of this technique can be increase the yield of MAbs production approx-(10-100 ug/ml).

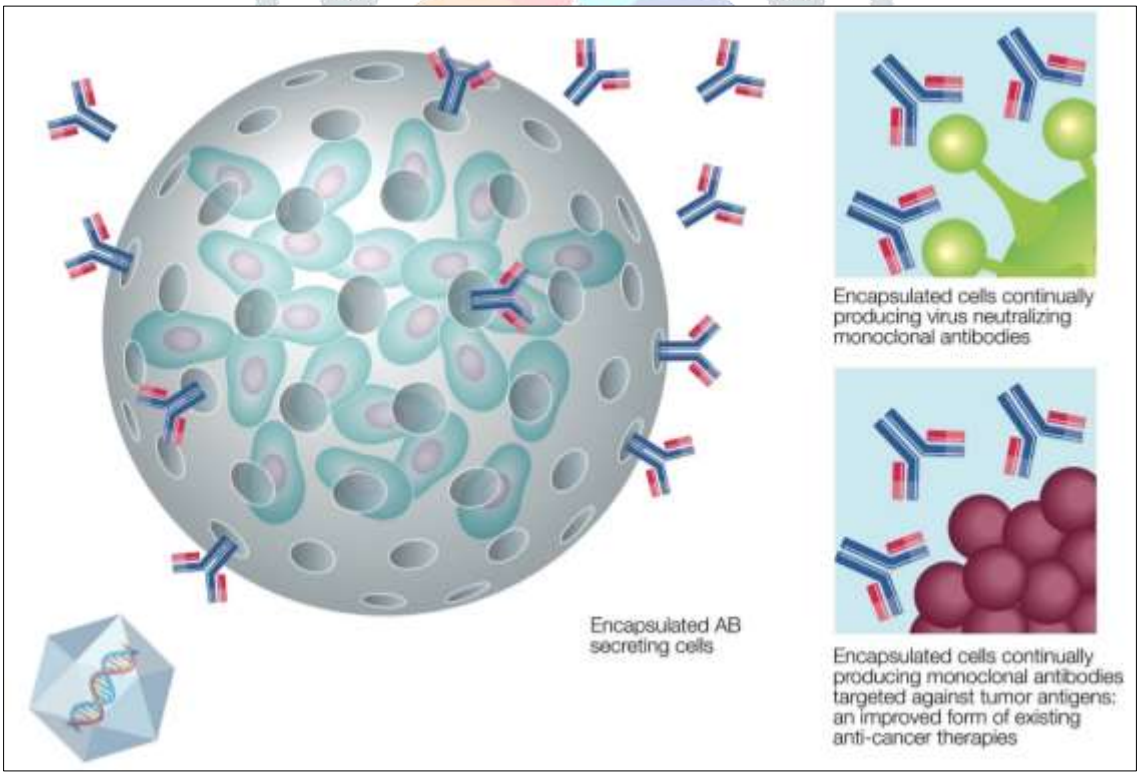

Fig: 3. Production of encapsulated hybridoma cells

3.2. Purification of MAbs

The desired antibodies are present in the extracted from a media and some contaminants in the cell culture which was growth factors, hormones and transferrin. The contains of host antibodies, proteas, nucleus and nucleic acid and viruses'

cultures in vitro. Some other secretions are secret from the Hybridomas (like cytokines). The endotoxin may be present in the bacterial contamination which is secret by bacteria. The purification of MAbs are used many separation techniques that as remaining cell debris, lipids and clotted materials through centrifugation and size of filter 0.45um are using for filtration. Some many technique as chromatography are used to the charged impurities and subsequently low PH. - Mostly charged impurities are used to the ion exchange chromatography (IECG) And formation of the low PH condition with the help of the cation and anion exchange chromatography. Many proteins and Amin's can be separated on the base of isoelectric point (PI). The removing transferrin could be confirmed with the help of size exclusion chromatography. If we want the maximum purity of the MAbs so we can used to the affinity chromatography. The mostly important role of the agarose gel in this purification technique. And if we want the final purity of the analysis so we can separate with the help of the gel electrophoresis and capillary electrophoresis separation technique.

4. APPLICATION OF MABs

4.1. Diagnostic Application

Diagnostic tool is known as in imaging of disease and diagnostic reagents known as the Biochemical analysis used for the MAbs.

4.1.1. The Biochemical analysis used for the MAbs:- some different diagnostic tests employing in MAbs as diagnostic reagents are used in the RIA and ELISA techniques. Process of the circulating concentration of hormones (human chorionic gonadotropin thyroxine, growth hormone, insulin, progesterone, trridothyronin, renin and gastrin) and many micro cells and tissues products (blood clotting factor, interleukin, interferon, blood group antigen and tumor markers) can be determined with the help of this technique.

i) Pregnancy: - MAbs can be used for the detection of pregnancy by measuring by the urinary level of human chorionic gonadotropin.

ii) Cancers: - The estimation of plasma carcinoembryonic antigen (CEA) in colorectal cancer, prostate specific antigen (PSA). In prostate cancer and tumor markers for prognosis of cancers can be checked with the help of MAbs. Some scientist has 53 samples of pleural or peritoneal fluid will be analysed which are Ghosh, Mason and Springs. And they are searching 41 patients effected with the malignant disease from given in the total 53 samples. Any neoplastic cells could not be revealed conventional cytological. Some MAbs, anti -CEA, Ca1, and HMEG-2 can be used to search for the malignant cells. The Immunocytochemical lebeling will be performing on the unstained Smear's on the stored of temperature -20℃ up to 18 months. Twelve of the forty-one cases were stored which in the Immunocytochemical stained, which are revealed malignant diseased cells. Approximately 20% accuracy increase of the represented of result. The Immunocytochemical lebeling can be used routinely in the examination of cytologically negative samples and respect to the patient of important implications in this study concluded that in patient with suspected malignant disease.

iii) Hormonal Disorder: - The analysis of thyroxine, triiodothyronine and thyroid stimulating hormone for detecting thyroid disorders. These all analysis and synthesis can be done with the help of MAbs.

iv) Infectious Diseases: - The MAbs are very important role in the detection of the infectious disease by determining the circulatory levels of the antigen specific to the infectious agent e.g herps, simplex virus for the diagnos of SIDs and antigen of neisseria gonorrhoeae.

4.1.2. MAbs In Diagnostic Imaging: - The radio labeled are used in the diagnostic imaging of disease, which is help of immunoscintigraphic technique. The radio labeled MAbs are labelled from example Iodin-131 and Technetium-99 and

injected in the patient with the help of intravenous route, which are restricted at the target sites (like a tumour) and showing by radioactivity imaging.

i) Myocardial Infraction: - Antimyosin MAbs are used in this technique which are labelled with indium chloride -111 radioisotope. And this radioisotope is used in the detection of myosin and the Myocardial Infraction site. Antimyosin MAbs are used for the location and the degree of damage to the hear. Detection of the Heart attacks can be check with help of this technique.

ii) Deep Vein Thrombosis: - The fibrin specific MAbs used for the detection of the clots in thighs, pelvis, calves, and knees.

iii) Atherosclerosis and Cancers are detected with the help of Radio labelled MAbs. The thickening and elasticity of arterial walls in the Atherosclerosis and tumours of membrane origin can be detected with the help of Radio labeled MAbs, and the tumour markers example 1. a- fetoprotein, 2. Carcinoembryonic antigen (CEA), 3. Human chorionic gonadotropin (HCGT), are using of the MAbs detecting of the cancer example 1. cancer of colon, stomach, pancreas, 2. Cancers of liver and germ cell of tests, 3. Choriocarcinoma. Some effectively as - breast cancer and asses the efficiency of purging regiment prior to autologous stem cell infusion, this process is very important for the detection of even small quantities of breast cancer cells. The immunohistochemical method are ideal because that they are simple sensitive and quite specific. Franklin *et al.*, 1996 evaluated sensitive Immunocytochemical history by using a combination of four monoclonal antibodies (260Fa, 520Ca, 317G5, and BrE-3) this tumour cells surface glycoprotein to identify breast tumor cells in bone marrow and peripheral blood. They are concluding from the results that Immunocytochemical on which staining of the bone marrow and peripheral blood is a sensitive and simple way to detect and quantity breast cancer cells.

4.2. Therapeutic Application

The therapeutic application are as follows: -

i) The Use of MAbs As Direct Therapeutic Agent's: - The direct enhancement can be used of for the immune function and host with the help of MAbs. The process of the enhancement of the immune function could be help in the toxicity to the target tissues or the host. The direct therapeutic agents are using for the disease-causing organism that as enhance the phagocytosis. These agents are used in the cancer treatment with the help of MAbs as leukemia, lymphoma, myeloma and colorectal cancer are treating in many patients. This agent can be used for the treatments of the AIDS. AIDS mostly courses immunosuppression by CD4 cells of T-lymphocytes.The surface membrane glycoprotein (gp120) are helps binding to the HIV from specific receptors on the CD4 cells. This agent is used to the making of clinical trial of rheumatoid arthritis patients with the help of MAbs.

ii) MAbs are used as a targeting agent in therapy: - The MAbs can be used for the many toxins, drugs and radioisotopes. The MAbs and toxins with the help of conjugating are produced to the immunotoxin as toxins in cancer treatment, pseudomonas exotoxin and diphtheria toxin, which is used in the therapy. These antibodies are used as in the dissolution of blood clots. The high concentration of the tPA (tissue Plasminogen Activator) are used on the target sites, and Plasminogen gets converted to the plasmin which are dissolve in the blood clots (fibrin).

4.3. Protein Purification

The chromatographic matrix (sepharose) are activating with the help of cyanogen bromide which are combining from the MAbs. The immunoaffinity can be apply for purification of protein which are confirmed help of the immobilise MAbs. Main advantages as high degree of purification, specificity of the binding of MAbs to the desired protein and effective elution from the chromatographic column. More than 5099-fold purification of interferon-a2 are formed by the recombinant interferons, immunoaffinity chromatography. Disadvantages of the 100 % purification of protein is not possible with the

help of immunoaffinity method because that the small fraction of MAbs leaks into the elution. Impact target protein and a fragment of the binding of antigen site cannot be difference with the help of MAbs. So, some disadvantages leave in the purified protein

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors express their sincere gratitude to C.C.S. University Meerut-250004, U.P., India for their remarkable support.

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