A

Seminar report

On

Hybridoma Technology

Submitted in partial fulfillment of the requirement for the award of degree
of Bachelor of Technology in Computer Science

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Preface

I have made this report file on the topic **Hybridoma Technology** ; I have tried my best to elucidate all the relevant detail to the topic to be included in the report. While in the beginning I have tried to give a general view about this topic.

My efforts and wholehearted co-corporation of each and everyone has ended on a successful note. I express my sincere gratitude to ……..who assisting me throughout the preparation of this topic. I thank him for providing me the reinforcement, confidence and most importantly the track for the topic whenever I needed it.

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Introduction

Hybridoma Technology is a method for producing large numbers of identical antibodies (also called monoclonal antibodies). This process starts by injecting a mouse (or other mammal) with an antigen that provokes an immune response. A type of white blood cell, the B cell that produces antibodies that bind to the antigen are then harvested from the mouse. These isolated B cells are in turn fused with immortal B cell cancer cells, a myeloma, to produce a hybrid cell line called a **hybridoma**, which has both the antibody-producing ability of the B-cell and the exaggerated longevity and reproductivity of the myeloma.

The hybridomas can be grown in culture, each culture starting with one viable hybridoma cell, producing cultures each of which consists of genetically identical hybridomas which produce one antibody per culture (monoclonal) rather than mixtures of different antibodies (polyclonal). The myeloma cell line that is used in this process is selected for its ability to grow in tissue culture and for an absence of antibody synthesis. In contrast to polyclonal antibodies, which are mixtures of many different antibody molecules, the monoclonal antibodies produced by each hybridoma line are all chemically identical.

The production of monoclonal antibodies was invented by César Milstein and Georges J. F. Köhler in 1975. They shared the Nobel Prize of 1984 for Medicine and Physiology with Niels Kaj Jerne, who made other contributions to immunology. The term **hybridoma** was coined by Leonard Herzenberg during his sabbatical in César Milstein's laboratory in 1976–1977.

Methadology

- A hybridoma, is produced by the injection of a specific antigen into a mouse, procuring the antigen-specific plasma cells (antibody-producing cell) from the mouse's spleen and the subsequent fusion of this cell with a cancerous immune cell called a myeloma cell.
- Once splenocytes are isolated from the mammal's spleen, the
- B cells are fused with immortalised myeloma cells.
- The hybrid cell, which is thus produced, can be cloned to produce many identical daughter clones.
- These daughter clones then secrete the immune cell product. Since these antibodies come from only one type of cell (the hybridoma cell) they are called monoclonal antibodies.
- The advantage of this process is that it can combine the qualities of the two different types of cells; the ability to grow continually, and to produce large amounts of pure antibody.
- HAT medium (Hypoxanthine Aminopetrin Thymidine) is used for preparation of monoclonal antibodies.
- Laboratory animals (eg. mice) are first exposed to an antigen to which we are interested in isolating an antibody against.
- Once splenocytes are isolated from the mammal, the B cells are fused with immortalized myeloma cells - which lack the HGPRT(hypoxanthine-guanine phosphoribosyl transferase) gene - using polyethylene glycol or the Sendai virus.

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- Fused cells are incubated in the HAT
- (HypoxanthineAminopetrin Thymidine) medium.
- Aminopterin in the myeloma cells die, as they cannot produce nucleotides by the de novo or salvage medium blocks the pathway that allows for nucleotide synthesis. Hence, unfused D cell die.
- Unfused B cells die as they have a short life span.
- Only the cell-myeloma hybrids survive, since the HGPRT gene coming from the B cells is functional. These cells produce antibodies (a property of B cells) and are immortal (a property of myeloma cells).
- The incubated medium is then diluted into multiwell plates to such an extent that each well contains only 1 cell.
- Then the supernatant in each well can be checked for desired antibody.
- Since the antibodies in a well are produced by the same B cell, they will be directed towards the same epitope, and are known as monoclonal antibodies
- Multi-well plates are used initially to grow the hybridomas and after selection, are changed to larger tissue culture flasks.
- This maintains the well being of the hybridomas and provides enough cells for cryopreservation and supernatant for subsequent investigations.

Applications

The use of monoclonal antibodies is numerous and includes the prevention, diagnosis, and treatment of disease. For example, monoclonal antibodies can distinguish subsets of B cells and T cells, which is helpful in identifying different types of leukaemias. In addition, specific monoclonal antibodies have been used to define cell surface markers on white blood cells and other cell types. This led to the cluster of differentiation series of markers. These are often referred to as CD markers and define several hundred different cell surface components of cells, each specified by binding of a particular monoclonal antibody. Such antibodies are extremely useful for fluorescence-activated cell sorting, the specific isolation of particular types of cells.

In Diagnostic Histopathology

With the help of monoclonal antibodies, tissues and organs can be classified based on their expression of certain defined markers, which reflect tissue or cellular genesis. Prostate specific antigen, placental alkaline phosphatase, human chorionic gonadotrophin, α-fetoprotein and others are organ-associated antigens and the production of monoclonal antibodies against these antigens helps in determining the nature of a primary tumor.

Monoclonal antibodies are especially useful in distinguishing morphologically similar lesions, like pleural and peritoneal mesothelioma, adenocarcinoma, and in the determination of the organ or tissue origin of undifferentiated metastases. Selected monoclonal antibodies help in the detection of occult metastases (cancer of unknown primary origin) by immuno-cytological analysis of bone marrow, other tissue aspirates, as well as lymph nodes and other tissues and can have increased sensitivity over normal histopathological staining.

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One study performed a sensitive immuno-histochemical assay on bone marrow aspirates of 20 patients with localized prostate cancer. Three monoclonal antibodies (T16, C26, and AE-1), capable of recognizing membrane and cytoskeletal antigens expressed by epithelial cells to detect tumour cells, were used in the assay. Bone marrow aspirates of 22% of patients with localized prostate cancer (stage B, 0/5; Stage C, 2/4), and 36% patients with metastatic prostate cancer (Stage D1, 0/7 patients; Stage D2, 4/4 patients) had antigen-positive cells in their bone marrow. It was concluded that immuno-histochemical staining of bone marrow aspirates are very useful to detect occult bone marrow metastases in patients with apparently localized prostate cancer.

Although immuno-cytochemistry using tumor-associated monoclonal antibodies has led to an improved ability to detect occult breast cancer cells in bone marrow aspirates and peripheral blood, further development of this method is necessary before it can be used routinely.One major drawback of immuno-cytochemistry is that only tumor-associated and not tumor-specific monoclonal antibodies are used, and as a result, some cross-reaction with normal cells can occur.

In order to effectively stage breast cancer and assess the efficacy of purging regimens prior to autologous stem cell infusion, it is important to detect even small quantities of breast cancer cells. Immuno-histochemical methods are ideal for this purpose because they are simple, sensitive, and quite specific. Franklin et al. performed a sensitive immuno-cytochemical assay by using a combination of four monoclonal antibodies (260F9, 520C9, 317G5 and BrE-3) against tumor cell surface glycoproteins to identify breast tumour cells in bone marrow and peripheral blood. They concluded from the results that immuno-cytochemical staining of bone marrow and peripheral blood is a sensitive and simple way to detect and quantify breast cancer cells.

One of the main reasons for metastatic relapse in patients with solid tumours is the early dissemination of malignant cells. The use of monoclonal antibodies (mAbs) specific for cytokeratins can identify disseminated individual epithelial tumor cells in the bone marrow.

One study reports on having developed an immuno-cytochemical procedure for simultaneous labeling of cytokeratin component no. 18 (CK18) and prostate specific antigen (PSA). This would help in the further characterization of disseminated individual epithelial tumor cells in patients with prostate cancer. The twelve control aspirates from patients with benign prostatic hypertrophy showed negative staining, which further supports the specificity of CK18 in detecting epithelial tumour cells in bone marrow.

In most cases of malignant disease complicated by effusion, neoplastic cells can be easily recognized. However, in some cases, malignant cells are not so easily seen or their presence is too doubtful to call it a positive report. The use of immuno-cytochemical techniques increases diagnostic accuracy in these cases.

Ghosh, Mason and Spriggs analysed 53 samples of pleural or peritoneal fluid from 41 patients with malignant disease. Conventional cytological examination had not revealed any neoplastic cells. Three monoclonal antibodies (anti-CEA, Ca 1 and HMFG-2) were used to search for malignant cells. Immunocytochemical labelling was performed on unstained smears, which had

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been stored at -20°C up to 18 months. Twelve of the forty-one cases in which immuno-cytochemical staining was performed, revealed malignant cells. The result represented an increase in diagnostic accuracy of approximately 20%. The study concluded that in patients with suspected malignant disease, immuno-cytochemical labeling should be used routinely in the examination of cytologically negative samples and has important implications with respect to patient management.

Another application of immuno-cytochemical staining is for the detection of two antigens in the same smear. Double staining with light chain antibodies and with T and B cell markers can indicate the neoplastic origin of a lymphoma.

One study has reported the isolation of a hybridoma cell line (clone 1E10), which produces a monoclonal antibody (IgM, k isotype). This monoclonal antibody shows specific immuno-cytochemical staining of nucleoli.

Tissues and tumours can be classified based on their expression of certain markers, with the help of monoclonal antibodies. They help in distinguishing morphologically similar lesions and in determining the organ or tissue origin of undifferentiated metastases. Immuno-cytological analysis of bone marrow, tissue aspirates, lymph nodes etc. with selected monoclonal antibodies help in the detection of occult metastases. Monoclonal antibodies increase the sensitivity in detecting even small quantities of invasive or metastatic cells. Monoclonal antibodies (mAbs) specific for cytokeratins can detect disseminated individual epithelial tumour cells in the bone marrow.

References

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