UNIVERSITI PUTRA MALAYSIA

PRODUCTION, ESTABLISHMENT AND CHARACTERISATION OF MONOCLONAL ANTIBODY AGAINST BREAST CANCER CELL LINE (MCF-7)

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By
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Thesis Submitted in Fulfilment of the Requirements
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Specially for.....

My respected parents,

Irene,

brothers B.Keang, B.Ping, B.Ching

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LIST OF ABBREVIATIONS

CGM	Complete Growth Medium
DMSO	Dimethyl Sulphoxide
ELISA	Enzyme-linked Immunosorbent Assay
FBS	Fetal Bovine Serum
HAT	Hypoxanthine, Aminopterin and Thymidine
HGPRT	Hypoxanthine-guanine-phosphoribosyl-transferase
HT	Hypoxanthine and Thymidine
ITES	Insulin, Transferin, Ethanolamine and Selenium
kDa	kiloDalton
MAb	Monoclonal Antibody
mg	milligram
ml	millimetre
MOPC	Mineral Oil Plasmacytoma
OD	Optical Density
PBS	Phosphate Buffer Saline
PEG	Polyethylene Glycol
RIA	Radioisotop Assay
RPMI	Rosewell Park Memorial Institute
TK	Thymidine-kinase
ul	microlitre
um	micrometer
%	percent
°C	degree Centigrade

Abstract of the thesis presented to the Senate of
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for the degree of Master of Science.

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October, 1995

Chairman : Abdul Manaf bin Ali, Ph.D.

Faculty : Food Science and Biotechnology

Ever since hybridoma technology was introduced by Kohler and Milstein in the 70's, numerous efforts have been undertaken to produce monoclonal antibodies (MAbs) against mammary cancer cells. However, even to this day, all the MAbs produced still possess cross-reactivities toward other types of cancerous cells and also normal mammary cells.

In this study, the breast cancer cell line MCF-7 was used as an immunogen to raise MAb against mammary cancer cells. Fusion between lymphocytes sensitised with MCF-7 cell line and myeloma cells, SP2, was performed using 50% of polyethylene glycol (PEG). The hybridoma secreting MAb against MCF-7 cell line was selected using cell-ELISA technique. Limiting dilution of five times was performed to yield a stable hybridoma clone secreting the MAb.

The selected clone, C2E7, which secreted MAb of the IgM class and lamda light chains was chosen for further studies.

MAb secreted by C2E7 was found to react with an antigenic determinant located in the cytoplasm of the MCF-7 cell line. Immunocytochemical studies showed that apart from the MCF-7 cell line, the antigenic determinant was also present in mammary cancer cell line of T47-D. Weak cross-reactivities were also observed against cell lines Panc-1 and Ova-3. Immunohistochemical studies using the immunoperoxidase technique showed that staining occurred in the cytoplasmic region of all mammary lobular carcinoma and 90% of mammary ductal carcinoma examined. Staining was also found in 50% of mammary fibroadenoma cases studied. On the contrary, no staining of tissues was found in uterine leiomyoma, stomach showing intestinal metaplasia, cervical carcinoma, tonsillitis, neurofibroma, ductal papilloma of the breast and normal mammary tissues. Biochemical studies showed that the antigenic determinant on the MCF-7 cell line with reactivity towards MAb C2E7 was composed of endopeptide chain having arginine and lysine as the side chains, and possessed a specific conformational order which was disrupted when the determinant was electrophoresed on SDS-PAGE. Consequently, characterisation of the determinant using Western Blotting technique could not be performed.

The hybridoma clone C2E7 was able to grow and proliferate in serum-free medium of EDRF supplemented with ITES. Purification technique using a combination of ammonium sulphate precipitation and gel filtration on Sepharose 6B enabled the separation of IgM from MAb secretion of C2E7 hybridomas cultured in serum-free medium.

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keperluan untuk Ijazah Master Sains

**PENGHASILAN, PEMBENTUKAN DAN PENCIRIAN
ANTIBODI MONOKLON
TERHADAP TITISAN SEL BARAH BUAH DADA MANUSIA (MCF-7)**

Oleh

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Semenjak teknologi hibridoma diperkenalkan Kohler dan Milstein pada tahun 70'an, pelbagai usaha telah dilakukan untuk menghasilkan antibodi monoklon (MAb) terhadap sel barah buah dada. Namun demikian, sehingga kini, hampir semua MAb yang telah dihasilkan itu masih mempunyai tindak-balas silang terhadap sel barah jenis lain dan juga sel normal buah dada.

Dalam kajian ini, titisan sel barah buah dada, MCF-7 telah digunakan sebagai immunogen dalam penghasilan Mab terhadap sel barah buah dada. Perlakuran antara sel limfosit yang telah diaruhkan dengan MCF-7 dan sel mieloma, SP2 dilakukan dengan 50% PEG. Teknik sel-ELISA telah digunakan untuk memilih hibridoma yang merembes antibodi terhadap titisan sel MCF-7. Pencairan terhad sebanyak lima kali dilakukan, agar klon

hibridoma yang stabil dalam rembesan MAb diperolehi. Seterusnya, hibridoma klon C2E7 yang merembes MAb kelas IgM dan rantai ringan lambda telah dipilih untuk kajian selanjutnya.

MAb dari C2E7 didapati bertindak dengan suatu penentu antigenik di sekitar sitoplasma titisan sel MCF-7. Kajian immunositokimia juga menunjukkan, selain di titisan sel MCF-7, penentu antigenik ini juga hadir di titisan sel barah buah dada T47-D. Tindak balas silang yang lemah juga didapati berlaku terhadap titisan sel Panc-1 dan Ova-3. Dalam kajian immunohistokimia dengan menggunakan teknik immunoperoksidase, didapati pewarnaan berlaku di kawasan sitoplasma pada semua tisu barah buah dada jenis karsinoma lobular dan 90% daripada tisu barah buah dada jenis karsinoma lobular yang telah diuji. Pewarnaan juga didapati pada 50% daripada kes fibroadenoma buah dada yang diuji. Sebaliknya, tiada sebarang pewarnaan berlaku pada tisu seperti uterine leiomyoma, karsinoma servik, tonsillitis, neurofibroma, perut menunjukkan intestinal metaplasia, duktal papilloma buah dada dan juga tisu normal buah dada. Ujian biokimia menunjukkan, penunjuk antigenik di titisan sel MCF-7 yang ditindak oleh MAb C2E7, terdiri dari rantai endopeptid dengan arginin dan lysin sebagai rantai sisi serta berada dalam keadaan konformasi spesifik. Konformasi spesifik ini akan berubah, andai kata ianya dielektroforeskan dalam SDS-PAGE. Oleh yang demikian, kaedah Western Blotting tidak dapat digunakan dalam pencirian penentu antigenik tersebut.

Hibridoma klon C2E7 berupaya hidup dan melakukan pembahagian sel dalam medium bebas serum, ERDF yang ditambah dengan ITES. Teknik penulenen yang melibatkan kombinasi pemendakan ammonium sulfat dan penurasan gel Sepharose 6B, membolehkan IgM, hasil rembesan hibridoma C2E7 yang dikultur dalam medium bebas serum, diasingkan.

CHAPTER 1

INTRODUCTION

Today, cancer is a major public health problem (Lim, 1991). Globally, out of the fifty million deaths that occur annually, five million is attributed to cancer. The World Health Organisation estimated that, by the year 2000, this figure will increase to eight million whereby 5 to 25% of this number is due to breast cancer (Management, 1994). The incidence of breast cancer is high in most of the industrialised and developed countries (Harris *et al.*, 1992). In the United States, breast cancer is the leading cause of death among women who are about forty to fifty-five years of age. The incidence rate of this disease has increased steadily, since formal tracking of such cases through cancer registries began in the 1930's. These incidences and mortality rates indicate that annually out of the 12% of American women diagnosed of having breast cancer, 3.5% of them will die of the disease (Harris *et al.*, 1992). In addition, according to Baum et al. (1991), about one in every 12 women in the United Kingdom will eventually develop this disease. Breast cancer is also one of the most common type of malignancy among women in Malaysia. About 1200 new cases of breast cancer are reported annually. Statistical data from the National Cancer Registry showed that cancer of the breast accounted for 10 and 18% of the total reported cancer and total female cancer cases respectively (Management, 1994).

Basically, death as a result of breast cancer is due to the distant spreading or metastasis of malignant tumour cells from the breast to the other vital organs of the body like the liver, lungs, bone and brain (Rosai, 1989). The progress of cancer can clinicopathologically be divided into four stages, namely, Stage I, II, III and IV (Chandrasoma and Taylor, 1991). Statistics showed that a patient suffering from Stage I of the disease and having a mass of less than 5 cm localised in the breast has 85% of the 5 year survival rate. However, patients with Stage IV of the disease where distant metastases have occurred, have only 10% of the 5 year survival rate (Chandrasoma and Taylor, 1991). Therefore, early detection of breast cancer is very important because the smaller the lesion, the greater is the likelihood of cure.

The detection and diagnosis of breast cancer is dependent upon the ability to discriminate between normal and neoplastic tissues. At present the histologic examination of a biopsy of a tumour mass is the most definitive diagnostic method of breast cancer (Chandrasoma and Taylor, 1991). For almost a century, routine histopathological diagnosis has been based upon the examination of haematoxylin and eosin stained paraffin embedded tissue sections. Although a majority of the tissues received in routine histopathology laboratories can be reliably diagnosed in this way, there are a number of cases whereby a firm diagnosis cannot be made on morphological grounds alone (Gatter *et al.*, 1982). To overcome such diagnostic problems diagnosis based on

immunohistological staining techniques were introduced. This approach although of genuine diagnostic value in some cases, has been limited in its scope by the relatively small number of tissue antigens which could be detected with conventional antisera. However, with the advent of hybridoma technology (Kohler and Milstein, 1975), the range of antigenic constituents which can be detected by immunohistological techniques in human tissue has been dramatically expanded (Gatter *et al.*, 1982).

Several attempts to establish monoclonal antibodies (MAbs) specifically towards antigenic constituents in the breast cancer have been reported (Schlom *et al.*, 1980; Taylow-Papadimitriou *et al.*, 1981; Foster *et al.*, 1982; Cordell *et al.*, 1985; Pancino *et al.*, 1989; Peterson *et al.*, 1990; Pancino *et al.*, 1991; Nuti *et al.*, 1992; Modjtahedi *et al.*, 1993). These MAbs differed in their binding capacities and their relative abilities to be sensitive and specific in recognising malignant cell lines as well as tissues of mammary origin. However, most of the reported MAb that react primarily with human breast carcinoma also showed reactivities towards other tumours and have considerable cross reactivities with normal human tissues. Consequently, to date, no MAb has yet been conclusively proven to be tumour specific for human breast carcinoma (Yuan *et al.*, 1982; Plessers *et al.*, 1990; Blottiere *et al.*, 1991).

Thus, the objectives of this study are :

a) to produce murine MAb against breast cancer cell line MCF-7;

b) to characterise the selected hybridoma clones; and

c) to cultivate a selected hybridoma clone in serum-free media and purify the MAb produced.