ABSTRACT

Carcinoma of the breast has been the focus of much scientific interest because of its high and increasing incidence rate as well as due to its unusual characteristic of being a neoplasm which is often hormone dependent. Estrogen receptor plays a critical role in the etiology of breast cancer. It is widely accepted that the determination of estrogen receptor serves as a valuable clinical predictor of hormone dependency of breast cancers. The conventional ligand binding procedures are not entirely satisfactory since they disclose only free binding sites and not sites that are already occupied by endogenous estrogens. It is clear that immunological evaluation of ER content and its distribution in breast lesions are simple, rapid, sensitive and are less likely to be affected by chemical and conformational changes in receptor than steroid binding assays. A positive correlation between the ER content of the breast tumors and response to endocrine therapy has been observed. Primary as well as metastatic breast carcinomas which are ER⁺ have been found to show better response to endocrine therapy with long disease-free interval and survival.

The present study has been undertaken to generate Monoclonal antibodies to estrogen receptor protein by the hybridoma technology and to evaluate their clinical application in the study and management of carcinomas of the breast. Analysis of 500 cases of South Indian female
breast cancers for their ER profile by the radioligand assay has indicated that more than 60% of these tumors are ER+.

In order to render the identification of ER+ tumors, more specific immunoassay procedures have been developed using estrogen receptor monoclonal antibodies. The ER protein has been isolated from MCF-7 cells (human breast cancer cells) and ER+ breast tumor tissues. The fresh cells from culture as well as surgical specimens obtained fresh by surgery were snap frozen in liquid N2, pulverized using TED buffer and ER isolated by ultracentrifugation, all processes being carried out at 0-4°C. The purification has been achieved by gel chromatography using ultrogel AcA 44 and high performance liquid chromatography (HPLC) and the characterization of the ER protein has been carried out by sucrose density gradient centrifugation (SDGC) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis (Chapter 2).

Using ER protein (both cytosolic and nuclear) as immunogen, BALB/c mice have been immunized. The immune spleen cells have been fused with Sp 2/0.AG-14 mouse myeloma cells using 50% PEG as fusing agent. Of the 41 positive clones identified by ELISA using 96 well falcon plates coated with ER protein, three hybrids designated ERD7, ERE5 and ERE10 have been found to exhibit specific staining of ER protein with MCF-7 cells and breast tumor tissue sections by Perioxidase-Anti-Peroxidase (PAP) Immunohistochemical assay. All the three clones exhibited intense staining patterns of cytosolic ER. ERD7 is also found to exhibit weak positive cytoplasmic staining with paraffin embedded breast tumor tissue.
sections. These 3 ERMAbs have been stabilized by limiting dilution and used for the generation of hybridomas in BALB/c mice (Chapter 3).

These 3 ER MAbs have been found to be very specific to ER as revealed by analysis of large number of ER$^+$ tumors of breast, ovary, endometrium, FNAB specimens of the breast cancers and controls by PAP and Avidin-Biotin (ABC) method of immunocytochemical study. These results have been confirmed by other sensitive and specific immunological techniques like Immunoprecipitation, Western Immunoblot analysis and Dot blot analysis. An increase in the sedimentation coefficient of ER MAb-Receptor-Hormone complex, compared to that of Receptor-Hormone complex by sucrose density gradient centrifugation has further revealed the formation of a ternary complex when the Receptor-Hormone complex has been incubated with the primary antibody. In immunohistochemical assay, the ER MAb incubated with excess cytosolic ER has been shown to give a negative result, indicating competition between cytosolic and tissue ER (Chapter 4).

Immunocytochemical study of 250 cases of human breast tumors has been carried out by PAP and ABC method and the cytosolic ER of some of these tumors has been determined by developing serological assays using all the three ER MAbs generated. Such a study has revealed a very good correlation between the immunocytochemical and enzyme immunoassay and the conventional biochemical ligand assay. These 3 ER MAbs seem to have great potential for the study and management of human breast cancers and other ER$^+$ tumors (Chapter 5).
An experimental study has been carried out using 7, 12 DMBA induced mammary tumors in female Wistar rats using 'Air pouch technique'. The hormonal regulation and the polyamine biosynthetic pathway of mammary tumors have been evaluated by the antiestrogen, Tamoxifen and antipolyamines using D, L, α-Difluoromethylornithine (DFMO) and (2R, 5R)-6-heptyne-2,5 diamine (Methyl acetylenic putrescine). This experimental study has been shown to reveal the hormonal regulation of estrogens and their interaction with other factors help in the better understanding of hormonal status of breast cancer (Chapter 6).