PREFACE

Detection of a protein biomarker in cancer serum samples could hold major importance for progressive tumors and for disease detection. The need is particularly urgent in research related to progressive disease. The early diagnosis is a major importance of successful patient management. The search for biomarkers in serum begins with a separation step to remove the abundant high molecular mass proteins. However the circulating carrier proteins, such as albumin, acquire the carriers longevity in the serum, can become the reservoir for the accumulation and amplification of bound biomarkers. These biomarkers are secreted from cells of metastatic origin into the circulatory system.

In accordance with our previous observations of the linear over-expression of H$_{11}$ antigen recognized by mAb H$_{11}$B$_2$C$_2$ during tumour progression the investigation showed a decline in HA expression by the tumour cells while maintaining its presence in the tumour associated stroma. The discovery of H$_{11}$ antigen is one more addition to the previously observed HA-binding proteins such as IHABP4, CDC37, The significant role of these proteins in vertebrates is well explained (Grammatikakis et al., 1995; Huang et al., 2000). Based on the previous evidence about HA and its receptors in human cancer tissues, we are hypothesizing that sequestered peptides may provide a potentially rich source of cancer-associated markers for clinical evaluation. Therefore, cancer serum samples are selected to carry out the research program. In the present study it was decided to heighten the importance of the involvement of HA and HABP (H$_{11}$ antigen) in human cancer serum. The assumption that HABP (hyaluronan binding protein) acts as a protein biomarker and may give an important diagnostic information in cancer detection led to investigate the present study to fractionation of serum proteins, mainly HABP, from various cancers and normal patients using strong anion exchange chromatography and single dimension electrophoresis and elimination or reduction or even association of albumin bound protein biomarker from serum samples using Cibacron blue and affinity antibody related methods.

**Present study is divided into 4 chapters.** Chapter I deal with the introduction and gives a review of literature on cancer metastasis. This chapter also provides
detailed information about hyaluronan and its receptors hyaladherins in cancer and circulatory system. The role of HA and hyaladherins in tumor progression is discussed. Information is also given on serum markers in various cancers.

Studies carried out to look at the expression of hyaluronic acid binding proteins (HABPs) using biotinylated probes bHA in different cancer tissues and also in the serum. These studies also included the differential expression of HABPs in tissues and serum samples. These results are compared with the normal values in both tissues and also in the serum are discussed in the second chapter.

Chapter III deals with detection of specific HABP in normal and different types of cancer serum and in different grades using mAb H₁₁B₂C₂. The present chapter deals with the identification H₁₁ antigen and its expression during cancer progression with the assumption to find common biomarker.

In the fourth chapter studies are carried out to purify and characterize this novel HABP (H₁₁Antigen). HABP is purified using G-50 and Q-Sepharose column chromatography. Cibacron blue affinity column is also used to find out its association with albumin. To find out this protein is HABP, studies are carried out using cold HA, probe bHA and mAb H₁₁B₂C₂. Comparison was also drawn with the differential expression of this novel antigen in normal and cancer serum samples.