Summary

The expression of HABPs in the tissues of benign (appendicitis) and in cancers of ovary, cervix, stomach, colon, lymphoma, breast and buccal mucosa were studied by overlay experiment using bHA. Multiple bands were observed. The over expression of these proteins was observed in cancers tissues.

The competition experiment using bHA and cold HA in normal and in different grades of stomach cancer, pull down experiment using bHA and ammonium sulphate in normal, colon and stomach cancers were also studied. In all these tissues, multiple bands of proteins were observed which range from 90 to 20 kDa HABPs. The over expression of these proteins in cancers was found when compared with benign appendicitis.

The expression of HABPs in the blood and serum of normal and in different cancers of different grades were studied by overlay experiment using bHA. Over expression of these proteins were also observed in blood but only two bands were seen in the case of serum.

The competition experiment using bHA and cold HA, pull down experiment using bHA and ammonium sulphate, overlay experiment using bHA oligo and ELISA experiments using bHA were also studied in the serum. In all these experiments, studies were consistent and showed the over-expression and distribution of HABPs in serum. HABPs were increasing in the serum of different cancers and also increasing with the grades when compared to normal serum. Mainly two bands were observed. One is major at 57kDa and other one is minor at 30kDa proteins.

The expression of serum H11 antigen in normal, in different cancers and also in different grades was studied by overlay experiment using mAb H11B2C2, bHA pull down experiment and reacted with mAb H11B2C2 and immunoprecipitation analysis. Here mainly one band is observed in the serum that is 57kDa using mAb H11B2C2 and it is over expressed in cancers.
It was found that 57kDa HABP was increasing in the serum of different cancers when compared to normal serum. It was also observed that HABP was increasing with the progression of the cancers from grade I to III.

Serum proteins from normal and stomach and colon cancer samples were partially purified by G-50 column chromatography. Out of 3 peaks, only the first peak showed H11 antigen. The Ist peak from G-50 column from normal and stomach and colon serum samples were further purified by Q-Sepharose and eluted with different salt concentrations 50, 150, 220 and 300mM NaCl.

When tested with bHA only the first peak fraction from G-50 column and Q-Sepharose fractions eluted with 220 mM shows the over expression of HABPs in cancer serums when tested with mAb H11B2C2.

By pull down (using bHA and mAb H11B2C2), competition (using with and without cold HA and mAb H11B2C2) and immunoprecipitation (bHA and mAb H11B2C2) experiments it was proved that 220 mM Q-Sepharose fraction protein from normal and stomach and colon cancer serum samples is a HABP.

220 mM Q-Sepharose fraction protein was used to check whether the H11 antigen associated with serum albumin or not, Cibacron blue affinity column was used for separation of albumin and associated peptide. It was found that the eluted protein from the Cibacron blue column is a HABP and this HABP (H11 antigen) associated with serum albumin.

220 mM Q-Sepharose fraction protein was used for purification of H11 antigen using mAb H11B2C2 affinity column and maximum H11 antigen was eluted with glycine when compared with TEA buffer indicating it is an acidic protein.
Conclusions

Cancer biomarker is a molecular signature that indicates the physiologic and pathologic changes in a particular tissue or cell type during cancer development. When such molecular signatures can be detected in the cancer serum as biomarkers, can have a significant effect on clinical outcomes. Information on the clinical need to find a serum biomarker for the early detection of cancer is still speculative. The detection of less abundant proteins in serum samples could hold major importance for metastatic tumor diagnosis. Separation of serum proteins using chromatographic or electrophoretic techniques is impeded by the presence of multiple highly abundant proteins such as albumin, immunoglobulin, transferrin, haptoglobulin etc. Analysis of human embryonic, adult tissues and in the human circulatory serum has revealed presence of high amount of hyaluronan. It is well documented that HA is involved in matrix regulation, cell proliferation, migration and malignant tumor progression. This involved with intimate association with its receptors (HABPs). In this investigation, fractionations of human cancer serum by column chromatography and detection of a specific HA binding protein was reported. The protein, as detected by a mAb H11B2C2, is named as H11 antigen. The antigen is over expressed in almost all the human tumor tested. Unexpectedly it was found that the same antigen in circulatory serum component by immunoprecipitation with mAb and by overlay and pull down experiments with bHA probe. It was also observed that over expression of H11 antigen in cancer serum compared to the normal serum and detected H11 antigen was associated with the serum carrier proteins. The present evidence shows that the H11 antigen can be used as a useful marker for the cancer detection.

The data presented here indicate that high-abundance serum carrier proteins such as albumin may act to sequester 57kDa peptide fragments which is a novel hyaluronan binding protein and is overexpressed in the cancer serum. Such sequestered peptides may provide a potentially rich source of cancer-associated biomarkers for clinical evaluation. Further work is under investigation to characterize this 57kDa H11 protein by mass spectrometric analysis and its possible homology with other basement membrane matrix molecules.