Discussion

In the current study an attempt has been made to elucidate the nature of H11 antigen (HABP). In chapter III, it was shown that H11 antigen (HABP) is expressed in all types of human cancer sera of different grades. In addition it is observed that, the expression of H11 antigen (HABP) increases with tumour progression. Biochemical analysis showed the expression of major protein of molecular weight 57kDa as recognized by mAb H11B2C2.

Earlier two specific HABPs (CDC37 and IHABP4) were cloned and characterized by using mAb IVd4. The IVd4 was further sub cloned to obtain with mAb H11B2C2. This antibody is found to be more specific for the detection of H11B2C2 antigen (HABP) and it is different from IVd4 antigen. Because of this it prompted to investigate the nature of this H11 antigen and the extent to which it is a hyaladherins (HABP).

To know the nature of H11 antigen (HABP), the H11 antigen (HABP) was purified in a sequential process. First through gel chromatography (G-50) and then through Ion-exchange chromatography (Q-Sepharose). The mAb H11B2C2 specific antigen (HABP) came out in the first peak fraction of G-50 column. This was re-chromatographed through Q-Sepharose Ion-exchange column. 220mM fraction showed a very high amount of expression when reacted with mAb H11B2C2. By pull down (using and 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. To prove that serum albumin was a carrier protein for the H11 antigen, Cibacron blue gel was used to eliminate most of the serum components and checked for the distribution of H11 antigen. It was found that the eluted protein from the Cibacron blue column is a HABP.

Cancer proteomics has been showing its increasing importance in biomarker discovery. However, the common cancer-tissue-based approach is less optimal in
cancer detection because many proteins are not necessarily detectable in serum or plasma. Essentially, a highly desirable biomarker for cancer screening and monitoring should be measurable in the body fluid samples. Accordingly, it is possible to identify cancer biomarkers directly from the blood proteome. More recently, cancer secretome, (in a broader sense, it is to harbor proteins released by a cell, tissue or organism through various mechanisms including classical secretion, nonclassical secretion, and secretion via exosomes.) all proteins released by cancer cells, has been attracting wide attention. These proteins play an important role in many essential physiological and pathophysiological processes, such as cell growth and differentiation, invasion, and metastasis via an endocrine, paracrine or autocrine way (Xue et al., 2008) more importantly, these cancer secreted proteins or their fragments always enter into body fluids, such as blood or urine and are probably measurable via noninvasive assays. Thus, cancer secretome emerged to be a promising and reliable source of cancer biomarkers. Initial studies on cancer secretome have successfully identified a rich set of potential biomarkers for cancer detection.(Gronborg et al., 2006; Lou et al., 2007; Weng et al., 2008).

Examinations of standard CD44 molecule in tumour tissues and in neoplasm lymph nodes showed its higher expression (Liu et al., 1996; Zalewski et al., 2001; Zhang et al., 2003).The examinations of some isoforms of CD44 in cancer and lymph nodes tissues showed the same results,(Harada et al., 2001; Chun et al., 2000) Especially v5 and v6 CD44 splice variant expression was higher in tumour tissues and depended on cancer progression(Zhang et al., 2003). Elevated levels of sCD44v6 in malignant ascites were also described (Dong et al., 2003). Some publications about expression of v6 CD44 and v8-10CD44 isoform in the serum of patients with colorectal cancer did not show any correlation with pathological features and metastases (Weg et al., 1998, Goi et al., 1997). There is no association between CD44v5 and v6 expression estimated in the serum and any clinicopathological features in patients with colorectal adenocarcinomas the same results in lack of correlation between expression of v5 and v6 variants of CD44 and clinicopathological features could depend on the absence of these molecules in the soluble form in the serum ( Zalewski, 2004).

There is an obvious interest of several cancer studies across the globe to identify a malignant tumour-specific marker independent of its histology, but this has met with
ambiguous results and limited success. There are many cancer markers that are available today but they are limited by their restricted expression in individual tumour types (Bast et al., 2005). Recently Basil et al., reported the identification of a common marker, specifically in colon, melanoma, ovarian and esophageal cancers (Basil et al., 2006). In the present study was carried out to identify and detect a biomarker that is ubiquitously associated with uncontrolled proliferation, metastatic potential and aggressiveness in human malignant tumours independent of histological origin and sex. It was believed that high-abundance of serum carrier proteins such as albumin may act to sequester 57kDa peptide fragments which is a novel HABP and is over expressed in the cancer serum. Such sequestered peptides may provide a potentially rich source of cancer-associated biomarkers for clinical evaluation.