Introduction

Hyaluronan, a component of the extracellular matrix, accumulates in many malignant and inflammatory disease conditions (Jarvelainen et al., 2009; Whatcott et al., 2011). In cancer, the accumulation of HA in the tumor microenvironment has been associated with more aggressive malignancy both in preclinical models and in patients (Jiang et al., 2012; Tammi et al., 2008). The contribution of HA to tumor progression is multifactorial, as a result of its interactions with and cross linking of other matrix components and cellular receptors which perpetuate the protumorigenic tumor microenvironment (Bouzin and Feron, 2007). Accumulation of HA within a tumor, interferes with cell–cell contact, promotes epithelial–mesenchymal transition, recruits tumor-associated macrophages and is associated with tumor drug resistance (Itano et al., 2008).

Hyaluronan interacts with several HA receptors and they have been widely implicated in tumorigenesis. There is large number of HA binding proteins and they have been characterized. They include CD33, RHAMM, LYVE-1, HARE and Toll-like receptors-2 and 3 (Jiang et al., 2007; Jackson et al., 2009). CD33 is widely distributed and particularly important in the immune system and inflammatory processes (Jiang et al., 2007; Johnson and Ruffell, 2009), as well as in diseases such as atherosclerosis and cancer (Toole et al., 2002; Toole, 2003). Unlike CD33, LYVE-1, HARE and RHAMM do not belong to the “link module” family of hyaluronan-binding proteins. RHAMM can be present either in the cytoplasm or on the cell surface, and is an important factor in cell motility, in wound healing and cancer (Maxwell et al., 2008). LYVE-1 is a close relative of CD33, which is mainly restricted to lymphatic vessel and lymph node endothelia, but its function is not well established (Jackson et al., 2009). HARE/stabilin-2 is a scavenging receptor that clears hyaluronan and other glycosaminoglycans from the circulation (Pandey et al., 2008). The Toll-like receptors recognize hyaluronan fragments during inflammatory events (Jiang et al., 2007).

The major receptors implicated in cancer are CD33 and RHAMM and both are p53-regulated genes linked to a role of HA in tumor progression (Godar et al., 2008; Sohr et al., 2008). They can also exhibit both cooperative and interchangeable signaling functions. For example, interactions at the plasma membrane between CD33 and RHAMM have been shown to activate CD33 signaling through ERK1/2 and promote cancer cell motility (Maxwell et al., 2008).
Several findings have shown that HA oligosaccharides of different sizes are also involving several biological processes through their interaction with HA binding proteins. The best characterized receptors for HA oligosaccharides are CD33, RHAMM, TSG-6, HSP90 etc.

The present investigation deals with the histochemical distribution of HA polymer and different size HA oligosaccharide receptors in human benign and malignant tissues of ovary and breast using bHA polymer and different size bHA oligosaccharides bio-affinity probe.
Results

**Fig. 6.1A** depicts H & E staining of serous benign ovarian tumor composed of large and small papillae with minimal epithelial stratification and detaching cellular buds. Destructive stromal invasion was not seen. The lining cells were tall to columnar. The nuclei are uniform mildly to moderately atypical. Stroma contains spindly fibroblasts. The features were of serous cystadenoma. **Fig. 6.1B** shows H & E staining of ovarian cancer sections. Tumor cells arranged in clusters and in solid pattern (**Fig. 6.1B**). Tumor cells were poorly differentiated type with hyperchromatic nuclei and scanty cytoplasm, many mitosis figures were seen. The features were of serous cystadenocarcinoma.

When the serous cystadenoma sections reacted with bHA polymer probe and stained with DAB, they showed heavily stained membranal region of tumor cells (**Fig. 6.2 A**). The stain was heavy in serous cystadenocarcinoma (**Fig. 6.2 B**) than serous cystadenoma. bHA polymer also stained the stroma heavily, which suggest the uniform distribution of HABPs (**Fig. 6.2A** and **Fig. 6.2B**). However, the sections treated with bHA oligosaccharides probe showed heavy reaction in the membranal region of serous cystadenocarcinoma (**Fig. 6.3B**) than serous cystadenoma (**Fig. 6.3A**), and stroma showed moderate reaction in both serous cystadenoma and serous cystadenocarcinoma.

When similar tissue sections were subjected to react with bHA hexasaccharides they showed strong reaction at plasma membranal region and also in the fragmented nuclei. The intensity of the reaction was in heavy serous cystadenocarcinoma (**Fig. 6.4B & Fig. 6.4C**) than serous cystadenoma (**Fig. 6.4 A**). The stroma showed almost very less reaction with bHA hexasaccharides.

**Fig. 6.5A** shows the H&E staining of benign breast tissue sections. They appeared as circumscribed spherical lesion that are well demarcated from the surrounding breast stroma. The epithelial lined interlobular ducts are compressed into slit like spaces by proliferation of surrounding connective tissue. Stromal cell show no pleomorphism and few mitoses. The fibroadenoma of breast shows all these features. When breast cancer tissue sections stained with H&E, they showed tumor cells with moderately differentiated cells with scanty cytoplasm and hyperchromatic nuclei. The tumor cells were seen infiltrating into the stroma in small groups. The adjacent areas of breast
showed lobules of the breast displaying no significant pathology. These were the features of infiltrating ductal adenocarcinoma of breast (Fig. 6.5B).

When the breast sections reacted with bHA polymer probe and stained with DAB, they also showed heavily stained membranal region of tumor cells and the stain was heavy in ductal adenocarcinoma (Fig. 6.6B) than fibroadenoma (Fig. 6.6A). bHA polymer also stained the stroma heavily, which suggest the uniform distribution of HABPs. When the sections treated with bHA oligosaccharides probe they showed heavy reaction in the membranal region with heavy intensity in ductal adenocarcinoma sections (Fig. 6.7B) than fibroadenoma (Fig. 6.7A) and stroma showed light reaction in both fibroadenoma and ductal adenocarcinoma.

However, the breast sections reacted with bHA hexasaccharides showed strong reaction at membranal region and also in the fragmented nuclei. The intensity of the reaction was heavy in ductal adenocarcinoma (Fig. 6.8B & Fig. 6.8C) than fibroadenoma (Fig. 6.8A) and the stroma showed almost very less reaction with bHA hexasaccharides.
Fig: 6.1

H & E staining of serous cystadenoma and serous cystadenocarcinoma of ovary

Fig. 6.1A: Serous cystadenoma of ovary (Benign). H & E stain of tumor areas. The arrows shows the cluster of tumor cells and asterisk shows the stroma.

Fig. 6.1B: Serous cystadenocarcinoma of ovary. H & E stain of tumor areas. The arrows shows the cluster of tumor cells and asterisk shows the stroma.
Fig. 6.2
bHA polymer staining of serous cystadenoma and serous cystadenocarcinoma of ovary

Fig. 6.2A: Serous cystadenoma of ovary (Benign). bHA polymer stain of tumor areas. The arrows show the staining in the membranal region and asterisk shows the stromal stain.

Fig. 6.2B: Serous cystadenocarcinoma of ovary. bHA polymer stain of tumor areas. The arrow shows the staining in the membranal region and asterisk shows the stromal stain.
HISTOCHEMICAL LOCALIZATION OF SIZE SPECIFIC HA OLIGO…

Fig. 6.3
bHA oligosaccharides staining of serous cystadenoma and serous cystadenocarcinoma of ovary

Fig. 6.3A: Serous cystadenoma of ovary (Benign). bHA oligosaccharides stain of tumor areas. Arrow shows the staining in the membranal region and asterisk shows the stromal stain.

Fig. 6.3B: Serous cystadenocarcinoma of ovary. bHA oligosaccharides stain of tumor areas. Arrows shows the staining in the membranal region and asterisk shows the stromal stain.
Fig. 6.4
bHA hexasaccharides staining of serous cystadenoma and serous cystadenocarcinoma of ovary

**Fig. 6.4A:** Serous cystadenoma of ovary (Benign). bHA hexasaccharides stain of tumor areas. Arrows shows the staining in the membranal region and asterisk shows the stromal stain.

**Fig. 6.4B & 6.4 C:** Serous cystadenocarcinoma of ovary. bHA hexasaccharides stain of tumor areas. Arrows shows the staining in the membranal region and asterisk shows the stromal stain.
H & E staining of fibroadenoma and infiltrated breast ductal adenocarcinoma

**Fig. 6.5A:** Fibroadenoma of breast. H & E stain of tumor areas. The arrows shows the cluster of tumor cells and asterisk shows the stroma

**Fig. 6.5B:** Infiltrated ductal adenocarcinoma of breast. H & E stain of tumor areas. The arrows shows the cluster of tumor cells and asterisk shows the stroma
Fig. 6.6
bHA polymer staining of fibroadenoma and infiltrated breast ductal adenocarcinoma

**Fig. 6.6A:** Fibroadenoma of breast. bHA polymer stain of tumor areas. The arrow shows the cluster of tumor cells and asterisk shows the stroma.

**Fig. 6.6B:** Infiltrated ductal adenocarcinoma of breast. bHA polymer stain of tumor areas. The arrow shows the cluster of tumor cells and asterisk shows the stroma.
**Fig. 6.7**

bHA oligosaccharides staining of fibroadenoma and infiltrated breast ductal carcinoma

**Fig. 6.7A:** Fibroadenoma of breast. bHA oligosaccharides polymer stain of tumor areas. The arrow shows the cluster of tumor cells and asterisk shows the stroma.

**Fig. 6.7B:** Infiltrated ductal carcinoma of breast. bHA oligosaccharides polymer stain of tumor areas. The arrow shows the cluster of tumor cells and asterisk shows the stroma.
**Fig. 6.8**

bHA hexasaccharides staining of fibroadenoma of breast and infiltrated breast ductal carcinoma

**Fig. 6.8A:** Fibroadenoma of breast. bHA hexasaccharides stain of tumor areas. The arrow shows the cluster of tumor cells and asterisk shows the stroma.

**Fig. 6.8B & 6.8C:** Infiltrated ductal carcinoma of breast. bHA hexasaccharides stain of tumor areas. The arrow shows the cluster of tumor cells and asterisk shows the stroma.
Discussion

To reveal the possible role of HA oligosaccharides involvement with progressive malignant behavior, the present study was carried out to understand the distribution of HA polymer, HA oligosaccharide and HA hexasaccharide binding proteins in tumor cells and also in intra-tumoral stromal areas of ovary and breast tumor tissues.

HA and HABPs have been implicated in tumorogenesis (Knudson et al., 1993; Sherman et al., 1994) but their involvement varies. The wide spread occurrence of HABPs indicated that the recognition of hyaluronan is important to tissue organization and to control cellular behavior. A number of extracellular matrix and cellular components, which are termed as hyaladherins, have specific affinities for HA. Extracellular hyaladherins include ECM components such as versican, aggrecan, neurocan, brevican, fibrinogen, hyaluronectin link protein and TSG-6. Soluble proteins include α- trypsin inhibitor. Cellular adherins include cdc37, p32, RHAMM, HBP (hepatocyte binding protein), IHABP4 and transmembrane proteins include CD33 and its spliced variants.

A stromal and epithelial expression of HABPs using bHA polymer probe has been studied and showed distribution of HABPs in cell surface and also in stroma. Malignant tumors of ovary and breast showed strong reaction at cell membrane and less reaction in stroma. Whereas, HA oligosaccharide probe also showed distribution of HA oligosaccharides binding proteins mostly in cell surface and less reaction in stroma.

Intracellular HA like its pericellular counter part may also have important role but it is improbable that low molecular weight HA could directly gain access to the intracellular compartments including the nucleus. A previous study using texas red labeled HA demonstrated the accumulation of low molecular weight HA in cell processes, perinuclear areas and the nucleus of transformed cells where it was associated with enhanced cellular motility (Collis et al., 1998). Thus, relatively low molecular weight HA gaining access to intracellular locations (Hua et al., 1993; Evanko and weight, 1999; Inkinen et al., 1999). Similarly in the present investigation, it was observed that HA hexasaccharides binding proteins distributed in the nuclei and at cell membrane. Also, HA hexasaccharides showed less reaction in stroma which
shows the specificity and distribution of binding proteins to the HA hexasaccharides bio-affinity probe.

The current study clearly indicating from the sections of ovary and breast tumors expresses HABPs on tumor cells surface and also in stroma as well in nuclei. They are required for tumor cells enhanced growth and migration. bHA polymer and HA oligosaccharides showed uniform distribution of HABP throughout the tumor sections, however, HABP for HA hexasaccharides also expressed in the nuclei of the tumor cells and their expression was enhanced along with tumor progression.