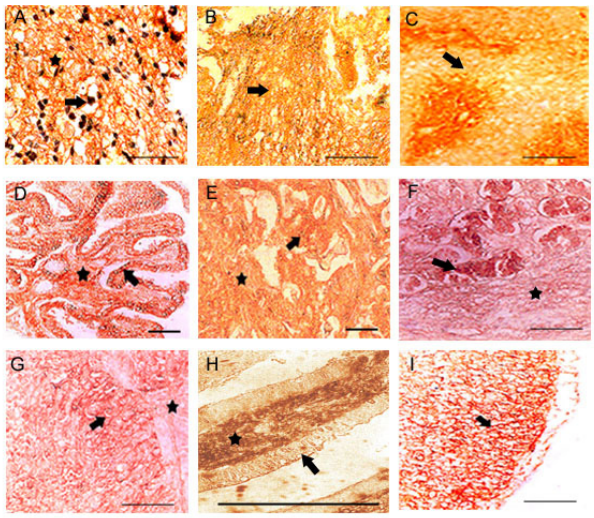

Figure 1
HA expression in well differentiated tumors: Only 8 examples are shown. **Top row, (A, B, C)** represents cerebral astrocytomas, ganglioglioma and salivary gland tumor. In astrocytomas neuroglial fibers and the astrocyte membrane are highly reactive for HA. Neoplastic nerve cells with extensive fibrillar processes are HA positive in ganglioglioma. Benign epithelia surrounding the tumor cells in salivary of showed low HA reaction. **Middle row, (D, E, F)** represents papillary carcinoma of thyroid, infiltrating breast and stomach tumor. Thyroid tumor showed intense HA staining at intraepithelial and in surrounding stromal components. High level HA expression was observed in tumor cells and surrounding stroma in case of infiltrating breast tumor. Tumor epithelia of stomach showed strong HA reaction. **Bottom row, (G, H, I)** represents Transitional epithelia of urinary bladder, descending colon and 5 days old chicken embryo limb. Transitional epithelia of urinary bladder showed intense staining at intra-epithelial and in surrounding tumor components. Invasive colon tumor cells showed strong HA reaction. 5 days chicken embryo limb was used as positive control, where the mesoderm is intensely positive for HA. Arrow shows the tumor cells and the asterisk shows stroma. Scale bars, 50 μm.

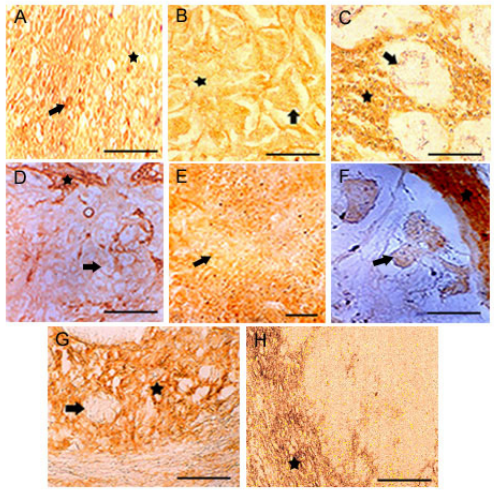

Figure 2
HA expression in poorly differentiated tumors: Only 8 examples are shown. **Top row, (A, B, C)** shows disappearance of HA stain from the tumor epithelia. Fibrillar astrocytomas show intense reaction while astrocytes are negative. Infiltrating breast and stomach tumor epithelia shows the loss of HA while the stromal components shows intense HA stain. **Middle row, (D, E F)** shows the loss HA expression in urinary bladder, pancreas (tumor epithelia) and caecum tumor epithelia while the stromal areas from urinary bladder and caecum were enriched with HA positive materials. **Bottom row, (G, H)** represents prostate and ovary. Prostate is showing numerous invading tumor cells in stromal areas. Only the stromal components are stained with b.PG. Ovary shows total absence of HA expression in tumor cells while the stroma is enriched with HA. Arrow shows the tumor cells and the asterisk shows stroma. Scale bars, 50 μm.

processed like all other human tissues) was used as a positive control. The specificity of HA staining was confirmed by incubating tissue sections with either streptomyces or bovine testicular hyaluronidase (50 U/ml in acetate buffer pH 5.0, overnight at 37°C) prior to the incubation with the HA-affinity probe. In the second set of experiments the specificity of HA staining was additionally confirmed by staining the tissue sections with a pre-incubated complex of biotinylated PG with 100 μg/ml hyaluronan or 300 ug HA-oligomers. In all the cases the probe was applied in PBS containing 1% BSA for 2 hours at room temperature, the slides were washed in PBS and processed as described below.

Chromogen treatment

In all cases, the slides were subsequently incubated with streptavidin-horseradish peroxidase conjugate (Bangalore Genei, Karnataka, India) for 1 hour at room temperature. The color reaction was developed for 15 minutes using diaminobenzidine hydrochloride (DAB) in 0.1 M Tris-Hcl pH 7.4. The substrate solution was freshly prepared by dissolving 6.0 mg DAB in 15 ml of 0.1 M Tris buffer, then 4.5 μl of 30% H_2O_2 was added just before use. The slide was rinsed in distilled water, sequentially dehydrated in graded alcohol and mounted in DPX mounting media. Photographs were taken with a Leica Photovat microscope.