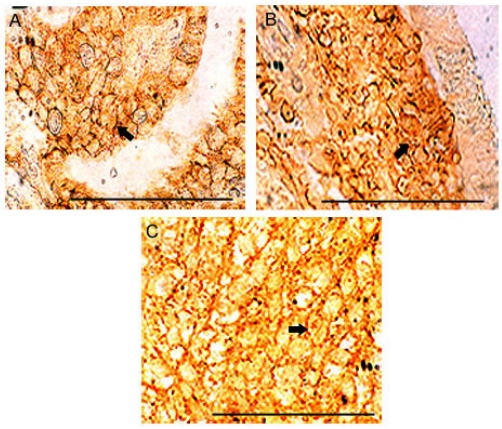

Figure 5
HA on tumor epithelial cell surfaces: Three examples are shown. **(A, B, C)**. **A.** Thyroid, **B.** Descending Colon, **C.** Urinary bladder. Tumors derived from well differentiated stage. Only 3 examples are shown. High accumulation of HA reactive materials observed on the cell surfaces of all the tumors. Arrows shows the cell surface. Scale bars, 50 μm.

This dilemma along with our previous observation of linear over expression of HA-binding protein on the tumor cells during tumor progression prompted us to investigate more clearly the spatial and temporal expression of HA on both tumor cell surfaces and stroma during the progression of tumor to understand its possible role during tumor progression. We have concentrated specifically on well and poorly differentiated tumors from carcinomas, sarcomas and various squamous cell carcinomas. Our results from the present investigation clearly showed a total absence of hyaluronan on tumor epithelia in the poorly differentiated tumor as shown in figure 2, except in astrocytoma (fig. 2A) and a definite trend in the continued expression of hyaluronan in the intratumoral stroma (fig. 4). The increased accumulation of HA on tumor cells surfaces (fig. 5) and associated stroma during early and mid-cellular differentiation of tumors (fig. 1) of different origins irrespective of tumor types was observed. Indeed, in well-differentiated tumors the enriched accumulation of hyaluronan was observed and is well in accordance with previous investigations. However some of the poorly differentiated tumors such as liver, gallbladder, prostate, endocervix, ovary, testes and osteosarcoma showed negligible presence stromal HA. The results of the present investigation are condensed in to Table 1, which shows differential HA expression between well-differentiated and poorly differentiated tumor with respect to tumor cells and tumor associated stroma.

The observations from our previous and present investigations as well as the observations from different laboratories around the world enticed us to speculate the existence of intricate multiple pathways towards the regulation of HA synthesis and degradation. Such pathways might involve temporal over-expression of *Has* [42]; Hyaluronidase enzyme [37,40]; and HA-binding proteins [4,9] during tumor development and progression. Evidence is mounting that suggests the extracellular matrices degrading enzymes are involved in controlling multiple steps in tumor invasion, metastasis and adhesion [33,34]. The observation that the differential clinical significance of HA and HYAL1 (Hyaluronidase 1), the correlation of Hyaluronidase enzyme in prostate tumor, its application for detecting intermediate and in high-grade bladder cancer [37] indicated the possible down regulation of HA in poorly differentiated tumors. Additionally, it was also shown that the accumulation of HA fragments is accompanied with tumor metastasis and neovascularization [18,40]. The above observations partially answered our questions about the loss of HA on the tumor cells during the progression of tumor from well differentiated through poorly differentiated stages.

There are a number of ways the high levels of HA might promote tumorigenesis. First, the increased detachment and motility of malignant cells during early and mid-stages of the tumor are due to the increased accumulation of HA, which in turn caused the deformation of restrictive architecture of the ECM components and provides a hydrated environment to facilitate migration of tumor cells to distant sites [4,28-30]. Second, increase in the interaction of HA with intracellular and surface receptors (CDC37, IHABP, RHAMM & CD44) activates the HA mediated signaling for the active invasive nature of the malignant tumor cells during early stage of tumor progression. Third, increased tumor invasion and migration may be due to increased synthesis of stromal HA through the interactions of stromal fibroblasts with tumor cells. It is possible that in addition to the above factors, there are key events such as temporal expression of metalloproteinase [31,32], the interaction between HA-HABP in early malignancy and subsequent loss of this interaction during late malignancy and the increase in stromal synthesis of HA may regulate the tumor cell behavior during the progression of tumors. The growing evidence of the presence of intracellular hyaluronan and its interaction with intracellular hyaladherins such as CDC37, IHABP4 [5,8] and further the subsequent loss of HA interaction with its receptor during the late malignancy led us to compare the distribution of H11B2C2 (one of the HA receptor detected from IVd4 hybridoma) receptor and hyaluronan expression in multiple cancer tissues in later invasive stages.