Characterization of YKL-40, SSEA-4 and BLBP Immunostaining in Glioblastomas

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Background

- YKL-40 is a highly conserved glycoprotein produced by cancer cells, neutrophils, macrophages, reactive astrocytes and stem cells and associated with VEGF, angiogenesis, inflammation, proliferation and differentiation.
- Brain specific lipid-binding protein (BLBP) is a marker of radial glial cells, astrocytes and adult neural stem cells.
- Stage-specific embryonic antigen-4 (SSEA-4) is a glycosphingolipid and stem/progenitor cell marker.
- YKL-40, BLBP and SSEA-4 have all, individually, been proposed to function in development of the central nervous system and propagation of malignant brain tumors (glioblastoma (GBM)).
- In recent studies we characterized BLBP, SSEA-4 and YKL-40 in human developing forebrain and outer brain barriers (Figure 1 and 2). YKL-40 stained neuroepithelial cells, radial glial end feet, leptomeningeal cells, pericytes and a population of possible astroglial progenitors. The distribution of SSEA-4 was almost identical with YKL-40 and BLBP defined the radial glial end feet layer in the outer CSF-brain barrier.
- Here we present preliminary immunohistochemistry data from a study, where we in GBM intend to compare immunostainings of YKL-40, SSEA-4, BLBP and markers of “glioblastoma initiating cells”, macrophages/microglia, pericytes and relate this to clinical outcome.

Material and Methods

- Patients: 19 patients with GBM were included in the preliminary study. All patients had received bevacizumab in different combinations at recurrence.
- The paraformaldehyde tissue samples were retrieved from an already existing biobank at Department of Pathology, Rigshospitalet, under approval from the Scientific Ethical Committee. Biopsies had been collected during neurosurgery at diagnosis prior to bevacizumab and radiation treatment.
- Immunohistochemistry: Sections were deparaffinized and rehydrated following 9 weeks of antigenic peroxidase, rinse, inhibition of non-specific binding and overnight incubation with primary antibodies. The REAL® Surface® Selection System was used for detecting these. Sections were incubated with 3,3′-diaminobenzidine chromogen solution and positive staining was recognized as a brown color. The sections were counterstained with Mayer’s hematoxylin.
- Clinical database: Clinical data from all patients were entered into an existing clinical database under approval from the Data Protection Agency. Variables included age, sex, debut, start of bevacizumab, best response, progression free survival (PFS), overall survival (OS), performance status (PS), surgical dose and others.
- Statistical analysis: The statistical software SPSS was used.

Results

- Based on successive sections immunostained for YKL-40, SSEA-4 and BLBP, the immunoreactivity of single markers was heterogeneous both within and between sections from different patients.
- In 8 out of 10 cases YKL-40 and BLBP correlated both in staining intensity and to some extent in localization.
- YKL-40 stained both tumor cells and most probably pericytes and/or endothelial cells in selected areas.
- BLBP stained tumor cells, but not endothelial cells/pericytes.
- Immunoreactivity for YKL-40 and BLBP was most evident in areas absent of Iba1.
- Only 1 patient sample was highly immunopositive for SSEA-4, which was localized mostly in the same areas as YKL-40 and BLBP.
- Increased Nestin staining intensity coincided with YKL-40 and BLBP in several sections.

Conclusion

- YKL-40 and BLBP immunoreactivity often correlate but vary significantly in glioblastoma tumor cells.
- Further optimization of stainings, additional markers, double staining by confocal microscopy and a larger population of patients are necessary to evaluate the clinical significance and exact location of this combination of stem cell markers in glioblastoma.