CRIPTO-1
A POSSIBLE NEW BIOMARKER IN GLIOBLASTOMA MULTIFORME

PIA OLESEN, MD, PHD STUDENT
Glioblastoma

- WHO Grade IV Glioma
  - Heterogenic
  - Undifferentiated phenotype
- 50% of all Gliomas
  - Around 600 patients each year in Denmark
- Incurable – despite multimodal treatment with both aggressive surgery (Stummer), chemotherapy and radiotherapy (Stupp)
- Mean life expectancy of 12-14 months
Glioblastoma

- No knowledge about:
  - Cause
  - Risk behavior
  - Prevention

- Focus on:
  - Early diagnosis
  - Treatment
Glioblastoma growth

• Spreads throughout the surrounding healthy brain tissue:
  • Membrane extensions helps find and travel along white matter tracts and endothelial lining
  • Secreting metalloproteases breaks up the extracellular components

• Normal brain structure provides the perfect microenvironment facilitating migration of glioblastoma cells
Cancer stem cells

- Cancer stem cells (CSC) – cancer initiating cells:
  - Acute myeloid leukaemia:
    - AML cells have limited proliferative capacity
    - The leukaemia clone is maintained by a rare population of stem cells
  - Breastcancer:
CSC in Glioblastoma

• Have not yet been identified – numerous candidates
  • CD133 (prominin-1, a cell membrane protein)
  • Cell membrane growth factor receptors
• What is being done to find these?
  • Relate cell surface characteristics to functional characteristics such as migration and tumorigenicity.
Or not

- Only a minority of cells have the ability to form tumors – but CD133- can also do that
- Several markers are needed
- Analyzing premalignant stages to identify the cancer cell of origin
Gameplan

Cure Glioblastoma
Gameplan

• A way to identify tumor initiating cells
• A way to sort these cells from the rest of the tumor cells
• Improve treatment
Why Cripto-1

- Because it is there
- Cripto-1 linked to increased proliferation and invasiveness in Glioma
- Upregulated in other cancers
  - Breast
  - Colon
- Poorer prognosis and aggressive clinical pathological parameters:
  - Elevated depth of invasion
  - Positive lymph node involvement
  - Metastasis
- Serological marker of malignancy and disease progression
- Targeted therapies showes promising results
Hypothesis

• The presence of Cripto-1 in Glioblastoma is part of the underlying mechanism for the invasive growth pattern and regrowth
• This makes Cripto-1 a possible diagnostic and predictive biomarker and a possible candidate for targeted therapy
Studyplan

• Localize and characterize Cripto-1 in Glioblastoma tissue and in blood:
  • Primary cell lines
  • Cripto-1 levels

• Relate these levels to:
  • Tumor volume
  • Clinical status

• Histopathological staining
What is Cripto-1

- Teratocarcinoma-derived growth factor-1
- Glycoprotein
- Very low concentrations in normal adult blood and tissue
- A soluble ligand and a cell membrane anchored protein
What is Cripto-1

- Important during embryogenesis:
  - Formation of primitive streak
  - Patterning anterior/posterior axis
  - Establishing left/right symmetry

- Promotes EMT
- Poorly defined regulatory mechanisms
Setup

• Inclusion criteria:
  • All patients with suspected Glioblastoma on MRI
  • Verified by histopathological examination
  • Blood and tissue
• 16 patients (now more than 40, including relapse)
• Median age 57.4
• Gender ratio 1:1
• Protein concentrations in blood and tissue (ELISA)
• Gene product in tissue – and primary cell lines (RT-PCR and qPCR)
• Xenografts – tumorigenicity
Results

Primary cell lines

• Mechanical dissociation and enzymatic digestion. The remaining nucleated cells are purified by centrifugation and filtration before seeding.
• Grown in DMEM-F12 with FCS and AB
• Spherecultures without FCS
RT-PCR

A.

Cripto-1 RT-PCR product in GBM tumor samples depicted on an agarose gel.

A blank control and the embryonic cell line CLS2 was included as negative and positive controls respectively.

Expression of the housekeeping gene GAPDH was used as reference.
B.
Western Blot: Cripto-1 protein in GBM tissue was detected. U87 served as positive control, and a secondary antibody as negative control. 15-20kDa (not shown)

C.
Cripto-1 protein in GBM tumor samples (n=12), in blood (n=17) and in control plasma samples (n=8)

Significantly higher (p<0.05) Cripto-1 Concentration in blood from GBM patients than controls
Xenografts

• Nude mice
• 500,000 cells injected in the flank (primary cell line and U87 model cell line) or 50,000 cells injected in the striatum (U87)
• Set to 21 days
• Only spheres form tumors
• Immunohistochemistry
  • Cripto-1 in xenograft brain tumor and patient samples
• Immunofluorescence
  • Cripto-1 and laminin
Results

• Cripto-1 is present in both GBM tissue (gene and protein) and blood (protein)
• Not all GBM patients have high Cripto-1 levels in tissue and blood
  • Could represent tumor progression???
  • A distinct subtype of GBM???
• Located along axons and perivascular niche
  • Indicates role in migration and invasion
• Adrenalcorticol hormone (solucortef):
  • No effect on Cripto-1 expression
• Upregulated by hypoxia (2.5 fold) and in spheres (5.5 fold). When combined 19 fold
  • In the primary GBM culture – not in model cell line
  • Indicates role in migration and invasion - can be blocked by knockdown of HIF
    • Mendez, O., et al., Knock down of HIF-1alpha in glioma cells reduces migration in vitro and invasion in vivo and impairs their ability to form tumor spheres. Mol Cancer, 2010
Keep in mind

- Location in the tumor
- Biopsy – resection
- The small number of patients
Future goals and hopes

- Cripto-1 in relations to:
  - Clinical outcome
  - Tumor volume
  - Ethical application has just been approved

- Primary cell lines
  - Sphereformation

- Targeted treatment
  - Liposomes – exosomes
Thank you for listening

- Laboratory for Cancerbiology, Aalborg University, Denmark
  - Meg Duroux, Associate Professor
  - Linda Pilgaard, Assistent Professor
  - Michael Henriksen, PhD fellow
- Department of Neursurgery, Aalborg University Hospital
  - Preben Sørensen, MD, Consultant in Neurosurgery, assistant professor
- Department of Pathology, Aalborg University Hospital
  - Mogens Vyberg, MD, Professor