Galectin-3 regulates cell surface distribution of neural cell adhesion molecule L1 in human cancer cells

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1. THE KEY PLAYERS

- **L1 CELL ADHESION MOLECULE**
  - 200–220 kDa transmembrane glycoprotein of the Ig superfamily
  - Originally discovered on neural cells
  - Neuronal migration
  - Axon outgrowth and pathfinding
  - Expressed on a wide variety of human tumors
  - Cell migration/invasion/metastasis
  - Tumor growth/chemoresistance/survival

- **GALECTIN-3**
  - 33 kDa evolutionary conserved glycan-binding protein
  - One Carbohydrate Recognition Domain binds to specific glycan-residues of ligands
  - Fused to short stretch of tandem repeats
  - Pentamerization upon ligand binding, formation of Galectin-Glycan lattices on the cell surface
  - To date, 15 Galectins have been identified
  - Divided into 3 subclasses with distinct properties/ligands
  - Mostly with wide tissue distribution

2. THE FACTS

1. **Expression**
   - Galectin-3 is co-expressed with L1 in a variety of human cancer cell lines and human tumors

2. **Secretion**
   - Galectin-3 is secreted into the extracellular space as well as in the ECM

3. **Binding**
   - Several independent techniques for proven binding of galectin-3 to L1 in the cell surface

4. **Clustering**
   - Binding of galectin-3 to L1 and subsequent cell-polarization of galectin-3 leads to a co-localizing of L1 and the formation of galectin-3-L1 lattice

5. **Formation of polarized membrane domains**
   - These galectin-3-L1 lattices form polarized membrane domains which appear on the migratory front of a cell and support endocytosis stimuli, a pre-requisite for L1-mediated signaling

6. **Abolishing these galectin-3-L1 lattices by down-regulation of galectin-3 leads to reduction in migration and invasion and impairs L1-mediated signaling and tumor promoting effects.

3. CONVENTIONAL METHODS

- **Microscopy**
  - Light microscopy
  - Electron microscopy

- **Immunohistochemistry**
  - Detection of antigens in tissues

- **Immunofluorescence**
  - Detection of antigens in fixed cells

- **FACS**
  - Flow cytometry

4. IMAGING FLOW CYTOMETRY

- **The ImageStream® System**
  - ImageStreamX Imaging Flow Cytometer
  - IDEAS® Statistical Image Analysis Software

- **Imaging Flow Cytometry**
  - MULTISPECTRAL IMAGING
    - Simultaneous detection of multiple antigens

- **Quantitative Imaging**
  - Imaging of cell surface antigens provides new insights into cell behavior

- **Cell Surface Analysis**
  - Quantitative analysis of cell surface antigens

5. OUTLOOK and CONCLUSION

Since Galectin-3 has a broad range of ligands, it will be interesting to identify further proteins involved in these polarized membrane domains.

Impairing the formation of these membrane domains proved to have a distinct impact on cell motility and invasion. To accomplish this, signals from these cell surface domains must be translated into intracellular cues. To identify the mechanisms of this connection is of great interest.

It will be interesting to establish the impact of galectin-3 on the endocytosis of L1.

Imaging flow cytometry has proven to be a novel technology allowing new approaches to cell science. We aim to further develop this method and apply it to many other biological questions.