Heparin blocks uptake of glioma-derived extracellular vesicles

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Glioblastoma multiforme (GBM)

- Most frequent primary brain tumor
- Incidence is 2-3 new cases per 100,000 people per year.
- Median survival with standard-of-care 15 months, without treatment is 4½ months

- Tumor micro-environment
  - Secretion of cytokines
  - Protein presentation on cell surface
  - **Release of extracellular vesicles (EVs)**
Extracellular vesicles (EVs)

EXOSOME BASICS
Exosomes are small membrane vesicles secreted by most cell types. Internal vesicles form by the inward budding of cellular compartments known as multivesicular endosomes (MVE). When MVE fuse with the plasma membrane, these internal vesicles are released as exosomes, which can travel to distant tissues to influence various aspects of cell behavior and physiology.

FROM FORMATION TO TARGET
In the first step of exosome formation, MVE bud inward to form small internal vesicles containing proteins, mRNAs, and miRNAs from the cytoplasm 1. These internal vesicles are released as exosomes when MVE fuse with the cell membrane 2. Alternatively, MVE can fuse with lysosomes, which degrade MVE contents 3. Upon reaching their destinations, usually determined by the binding of specific ligands on their surfaces, exosomes can enter target cells in one of two ways: by being taken up by the target cell's endocytic pathway 4 or by fusing to the target cell's membrane and releasing its contents directly into the cytoplasm 5. Cells also secrete other membrane-derived vesicles, such as ectosomes, shed vesicles, or microvesicles, which bud directly from the cell's plasma membrane 6. These vesicles are also known to carry active proteins and RNAs, as well as some compounds never before described in exosomes, but little is known about their effects on distant tissues.
EVs: A New Hallmark of Cancer

1 Tumorigrowth
2 Angiogenesis
3 Invasion/migration
4 Tumor resistance
5 Cancer stem cells
6 Microenvironment: Cell to cell communication
Connecting The Dots

GBM

EVs

Heparin
Hypothesis: Heparin can be used to block transfer of glioma-derived EVs
Heparin

- Highly sulfated glycosaminoglycan
- Negative charge density
- Biological role not clear
  - 3000-30000 kDa
- Medical use
  - Anticoagulant
EV transfer and block in recipient cells

Transwell system

- i
- ii
- iii

Recipient cell
Labeled donor cell
Labeled donor cell EVs
Soluble heparin
Heparin blocks transfer of labeled EVs into glioma cells
Heparin blocks transfer of labeled EVs into glioma cells

PKH67-labeled in recipient cells (%)
Heparin blocks uptake of labeled EVs into primary HUVECs

Heparin (µg/ml)

<table>
<thead>
<tr>
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<th>20</th>
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<tbody>
<tr>
<td>293T EVs</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td>HUVEC EVs</td>
<td><img src="image3.png" alt="Image" /></td>
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Integrated Density

- 293T EVS
- HUVECS EVS

- heparin
+ heparin

* indicates statistical significance.
Heparin causes EV aggregation

Heparin concentration (µg/ml)

- GBM11/5-EVs
- U87-MG-EVs

Percentage of area covered by clusters

Extracellular vesicles population in clusters
Heparin causes EV aggregation

GBM11/5 EVs

U87-MG EVs
Heparin binds EVs

FITC-Heparin

U87-MG EVs

U87-MG EV-Heparin complex
Heparin-mediated inhibition of EV-mediated EGFRvIII transfer

Normalized EGFRvIII Ct

- Donor cell
- Donor cell EVs

Relative recovery of EGFRvIII

- EVs
- Cells + EVs
- Cells - EVs (n.d.)

Relative EGFRvIII levels

- Heparin -
- Heparin + (*)
Conclusion

- Heparin binds EVs
- Heparin blocks transfer of EV-derived cargo
- Heparin Inhibits of EV-mediated EGFRvIII transfer
- May have therapeutic implication in glioma or other type of tumors
- Heparin may be used as a tool in basic research to study EV function
Clinical implication

- 15% cancer patients → Coagulation problems
- Studies cancer patients treated with heparin increased survival (3 months)
- Cancer patients elevated levels of EVs → EV-heparin complex
  - Systematic effect → Platelets → Platelet-derived EV-heparin complex → aggregation
  - Local effect → Tumor → Tumor-derived EVs-heparin complex → tumorigenesis
Scientific implication

- Extracellular vesicles:
  - In vitro:
    - Cell lines require heparin as growth factor → HUVEC
  - In vivo:
    - Animals?
    - Patient material → High levels of heparin →
      - OR
      - Biomarker
  - Study function of the EVs
- If heparin is more than a anticoagulant
Is heparin more than a anticoagulant?
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