Anchorage Independent Growth of Tumor Cells is Mediated by Proteins that are Concentrated in Serum Exosomes.

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Anchorage dependent growth
Anchorage independent growth
Anchorage dependency to anchorage independent

Malignant transformation

1) EMT
2) Loss of contact inhibition.
3) Activation of new signaling pathways
Anchorage independent growth

• Growth in soft agar mimics in vivo growth
• Factors that promote growth in soft agar most likely promote in vivo growth
• These protein factors can be identified by a proteomics approach.
Influence of Exosomes on Growth of Tumor Cells on Plastic

<table>
<thead>
<tr>
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<th>EFM</th>
<th>EEM</th>
<th>CM</th>
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<tbody>
<tr>
<td>MDA-MB-435</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
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<td>MDA-MB-231</td>
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Exosomes mediate growth of normal breast epithelial cells.

MCF-10A + SFM  MCF10 + Serum exosomes  MCF-10A + serum w/o exosomes
Exosome Purification protocol

Serum (1 or 2 ml)

Centrifuge at 2,000xg for 10 min

Supernatant

Centrifuge at 21,000xg for 20 min.

Sup.

Dissolve exosomes in 1 or 2 ml of SFM and filter
Sterilize (0.2 microns)

Centrifuge at 200,000xg for 1 hr

Ppt (micro vesicles)
Exosomes

- Nanoparticles (50-100 nm)
- Secreted by most living cells
- A way of getting rid of obsolete proteins
- Could act on intercellular communication
- Other functions?
Exosome enriched medium mediate growth of breast carcinoma cells in soft agar

A

EFM

EEM

B

Optical Density (570 nm)
Urine and serum derived exosomes mediate anchorage independent growth of breast tumor cells.
Uptake of serum exosomes by MDA-MB-231 cells
Uptake of serum exosomes by BT-549 breast carcinoma cells.
Fetuin-A (ahsg) is expressed both in the supernatant and exosomal fractions of human serum.
## Proteins found exclusively in Bovine Serum Exosomes.

### A) Enzymes

1. Peptidyl-prolyl cis transferase
2. Lysosomal alpha mannosidase precursor
3. Glyceraldehyde-3-phosphate dehydrogenase.
4. Glutathione-S-transferase
5. Carboxypeptidase.
6. ADAM metallopeptidase

### B) Extracellular matrix proteins:

1. Von Willebrand factor 1 alpha.
2. Lumican
4. Extracellular matrix protein
5. Collagenous repeat isoform.
6. Alpha type VI collagen
8. Complement C9

### C) Cytoskeleton protein:

1. Non-muscle mysosin heavy chain.
2. Contactin-1-precursor
3. Tubulin alpha 2 chain.
4. Fibulin-1
5. Beta tubulin.
6. Filamin-A
7. Talin

### D) Intracellular proteins.

1. Ferritin light chain.
2. Thyroglobulin precursor
3. IGHM protein.
4. FGG protein
5. HSP-90.
6. Stem cell growth factor precursor
7. Neogenin precursor.
8. Clq and tumor necrosis factor
9. Plexin B2
Fig. 3. Electron microscopy of urinary vesicles

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<th><strong>Most abundant Proteins in both Exosomes and Soluble Fractions of Serum.</strong></th>
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<tr>
<td>1) Alpha feto protein precursor</td>
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<tr>
<td>2) Alpha 2-macroglobuling</td>
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<tr>
<td>3) Complement C3</td>
</tr>
<tr>
<td>4) Alpha 2HS glycoprotein (fetuin-A)</td>
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<tr>
<td>5) Inter alpha (Globulin) inhibitor H4</td>
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<tr>
<td>6) Gelsolin</td>
</tr>
<tr>
<td>7) Fibronectin</td>
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<td>8) Fetuin-B</td>
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<td>9) Apolipoprotein E</td>
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Inhibition of exosomal mediated growth of tumor cells by anti-ahsg antibodies

Control + IgG

10 ug/ml exosomes + IgG

20 ug/ml exosomes + IgG

Control + anti-ahsg + anti-ahsg (10 ul/well)

10 ug/ml exosomes + anti-ahsg (10 ul/well)

20 ug/ml exosomes + anti-ahsg (10 ul/well)
Inhibition of exosome mediated growth by anti-ahsg
Acknowledgements

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