

Paper presentation JC Current Topics in Applied Immunology WS

Systemically Injected Exosomes Targeted to EGFR Deliver Antitumor MicroRNA to Breast Cancer Cells

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09.12.2019 by Moritz Bechtold

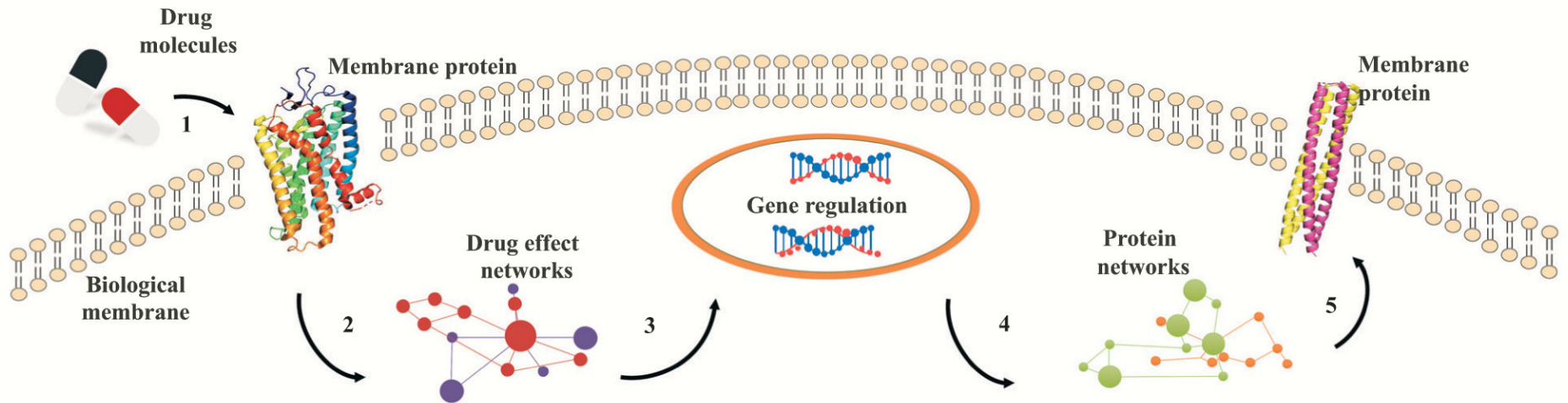
Exosomes = drug delivery carrier in a model of cancer



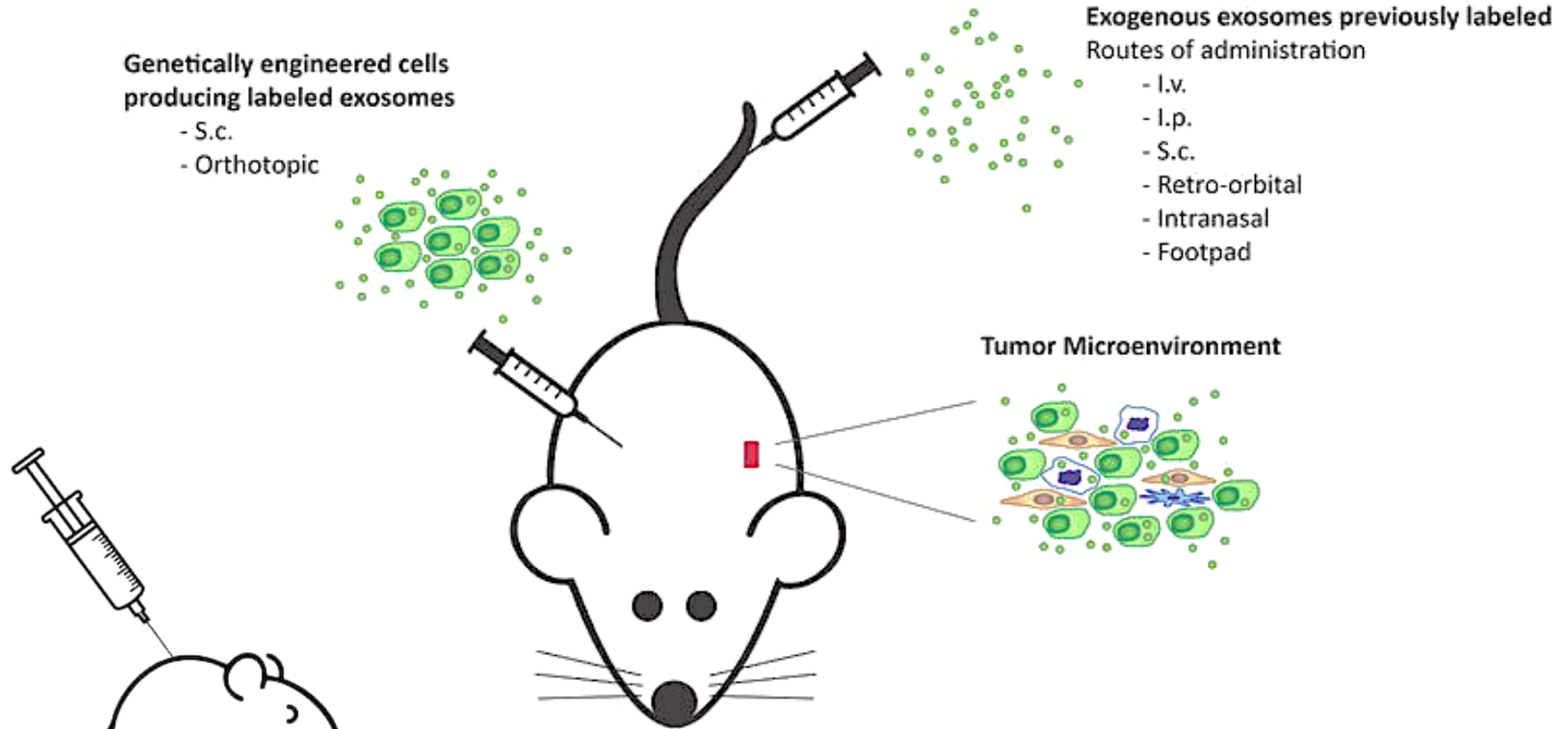
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Molecular Therapy (MOL THER)

Year	Bioxbio Journal Impact*	IF	Total Articles	Total Cites
2018/2019	-	8.402	219	16991
2017	-	7.008	216	16013
2016	-	6.688	187	15093
2015	-	6.938	170	14136
2014	-	6.227	189	13077
2013	-	6.425	213	12709
2012	-	7.041	219	12759
2011	-	6.873	230	11679
2010	-	7.149	235	11493



Gong J, Chen Y: Understanding Membrane Protein Drug Targets in Computational Perspective, *Curr Drug Targets*. 2019;20(5):551-564.



RAG2 ^{-/-} (recombinase activating gene)
 → keine reifen B-/T-Zellen, lymphopen

Adem B, Melo SA: Animal Models in Exosomes Research: What the Future Holds,
 July 12th 2017, DOI: 10.5772/intechopen.69449

EGFR (epidermal growth factor receptor)

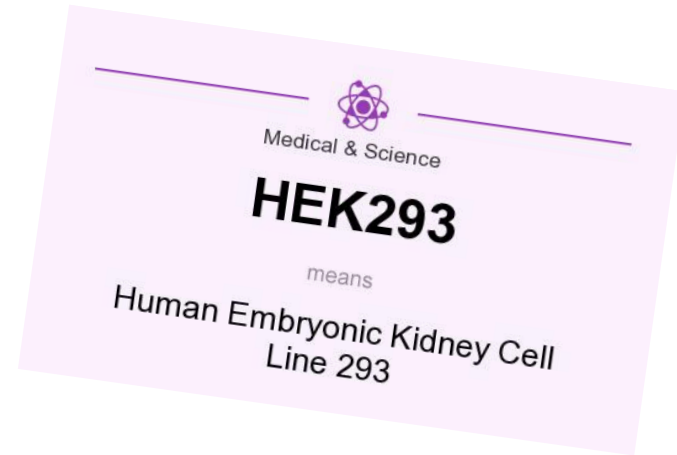
- expression in tumors of epithelial origin
- ligand (EGF) strongly mitogenic / neoangiogenic
- **GE11 specifically binds EGFR** (less mitogenic than EGF)
 - expression on exosome plasma membranes by pDisplay vector

Material & methods

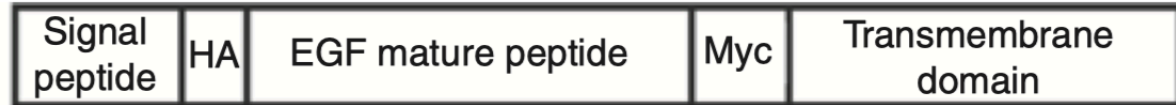
(in-vitro)

- HEK293 cell line

→ transfected with pDisplay encoding GE11 / EGF



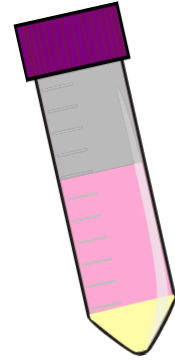
pDisplay-EGF
(insert size 478 bp)



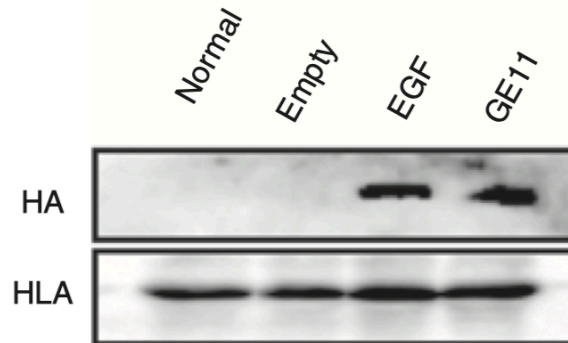
pDisplay-GE11
(insert size 445 bp)



Material & methods



- cells cloned
- GE11/EGF-positive exosomes derived from supernatant
- GE11/EGF expression levels
→ western blot (anti-hemagglutinin antibodies)



Material & methods

- FACS

→ confirmed presence of GE11 / EGF

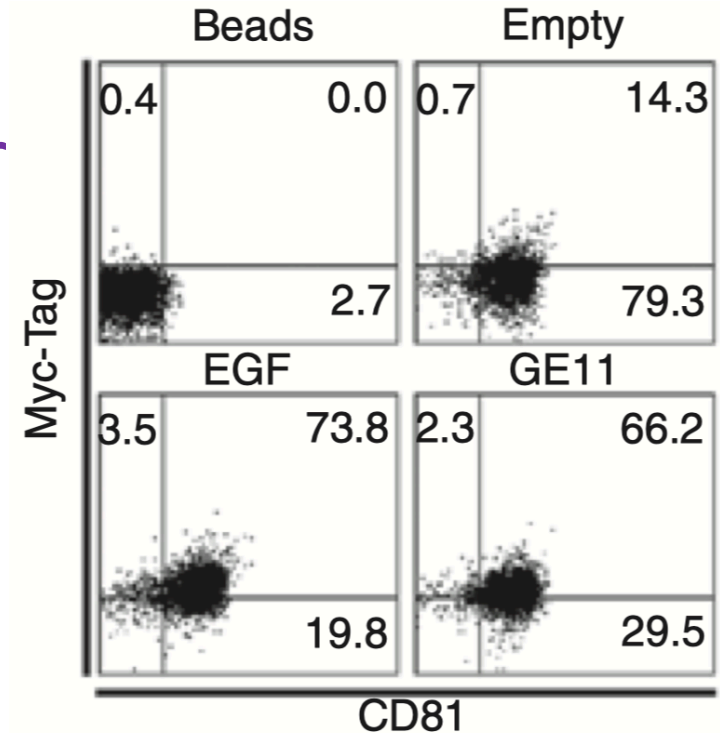
(on the outer membranes of exosomes)

→ CD81 = exosomal marker

(positive control)

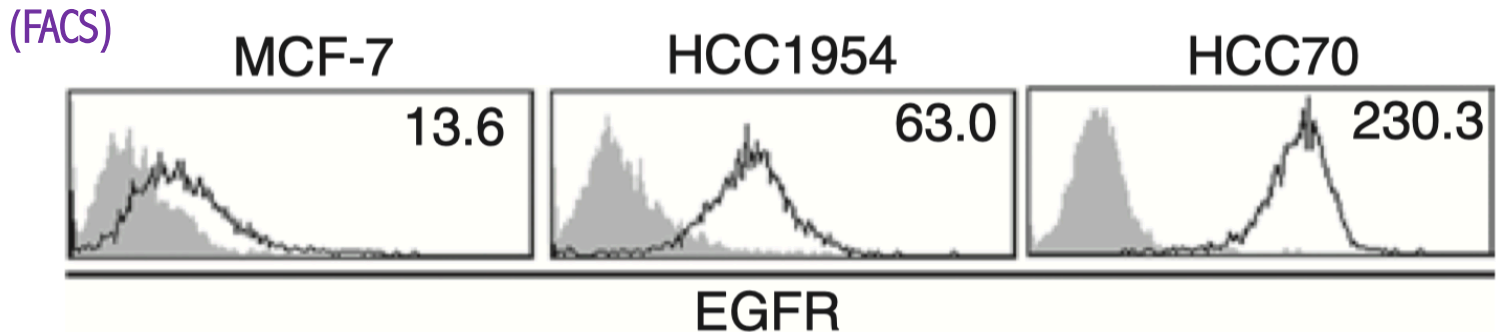
- latex beads = Träger

→ anti-Myc-tag antibodies to
detect exosome-complexes



Material & methods

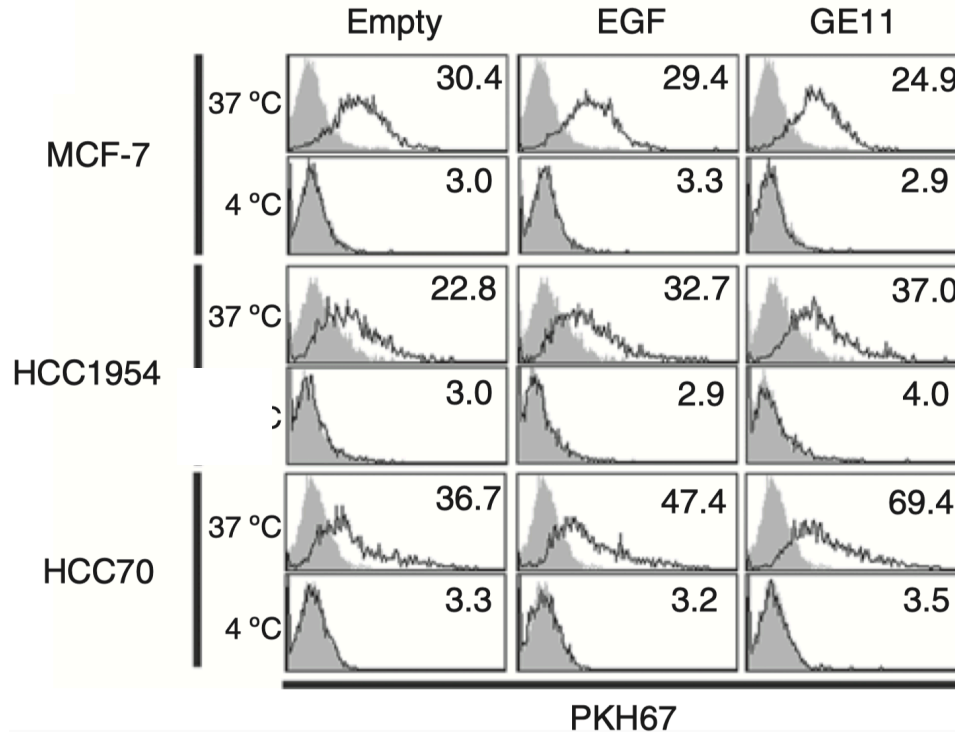
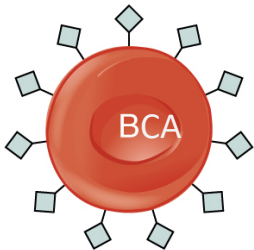
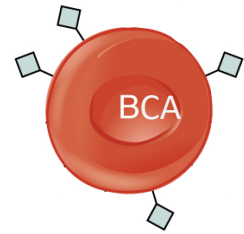
- EGFR-dependent binding of the exosome-supernatant?
- EGFR-expression of 3 human breast CA cell lines



Material & methods

- labeled exosomes incubated with the CA cell lines

(FACS at 37°C vs. 4°C / non-biologically active)



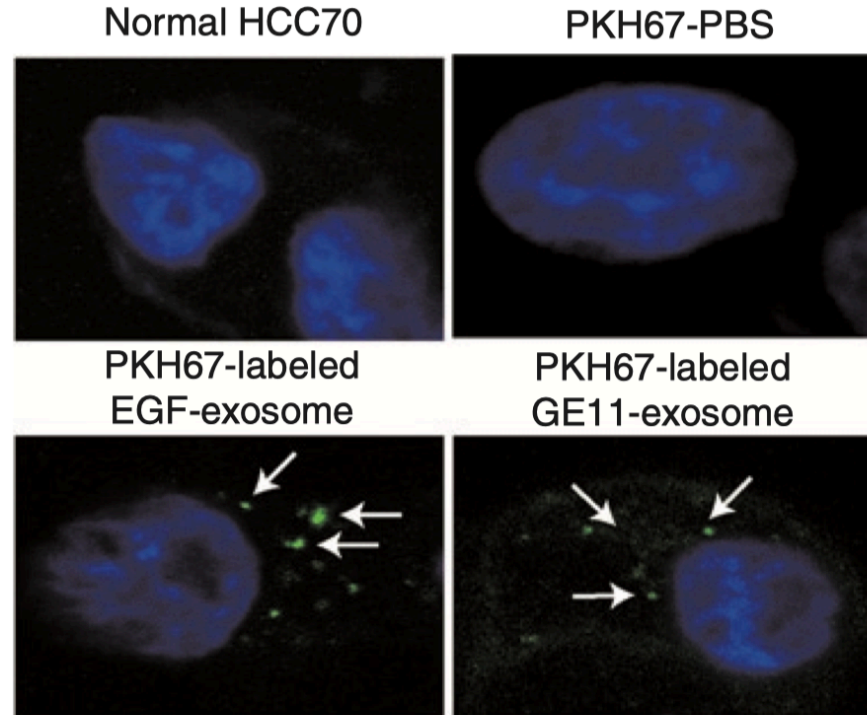
EGFR
expression
~
exosome-
binding



Material & methods

- labeled exosomes detected intracellular

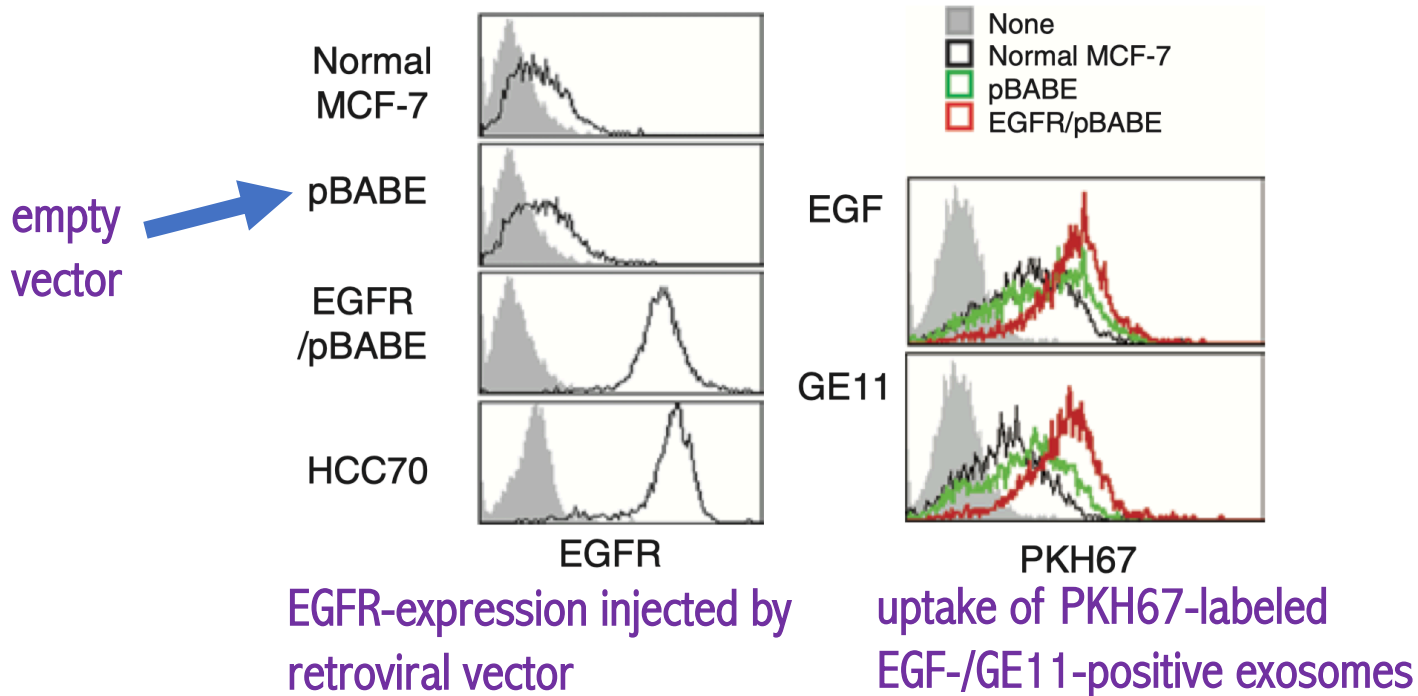
(confocal fluorescence microscopy)



Material & methods

- uptake of modified exosomes into recipient cells

(EGFR-dependent mechanism)

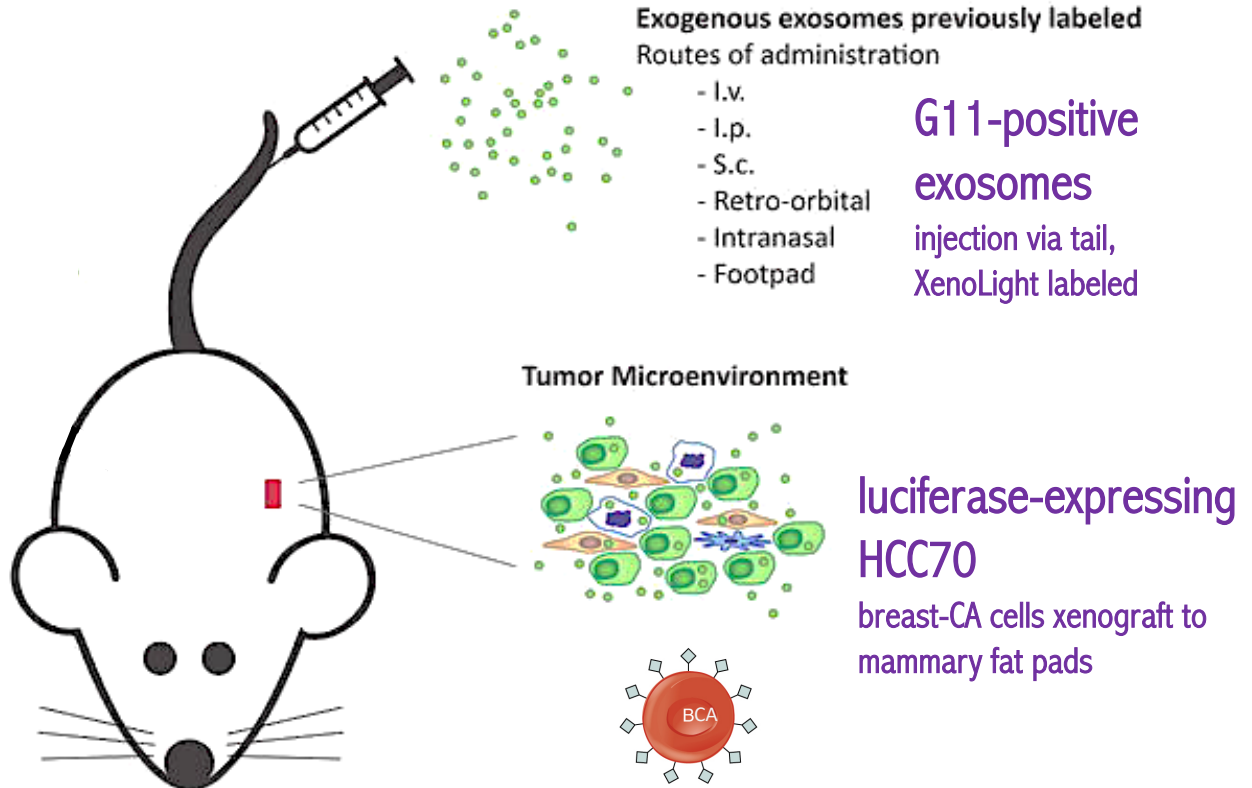


Material & methods

- GE11-exosomes bind tumor cells in vivo

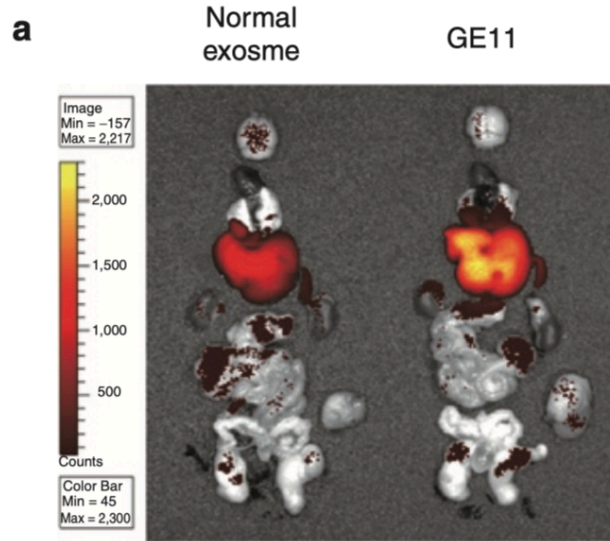
(in-vivo)

RAG2^{-/-}

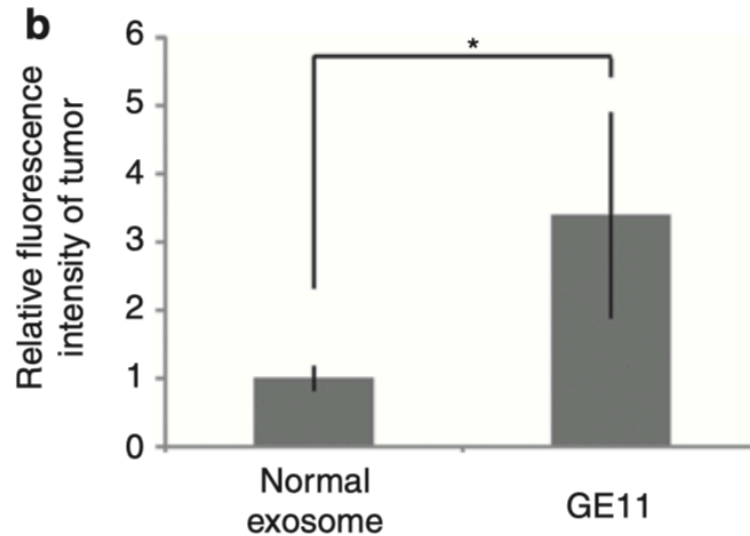


Material & methods

- GE11-exosomes bind tumor cells in vivo (in vivo imaging system IVIS)



detection of migration
of fluorescently
labeled exosomes

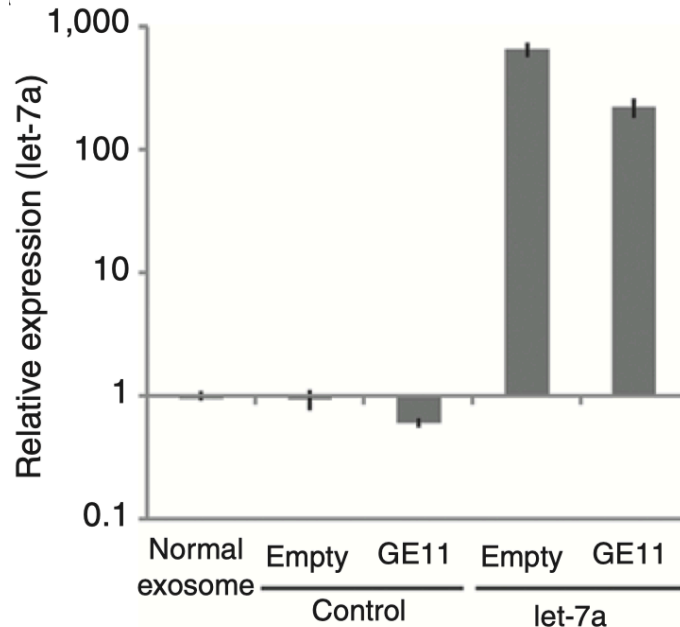


normal \ll GE11

Material & methods

- GE11-exosomes (miRNA-loaded) inhibit tumor growth
- let-7a (or control miRNA) introduced into GE11-/control-exosomes (lipofection/transfection)

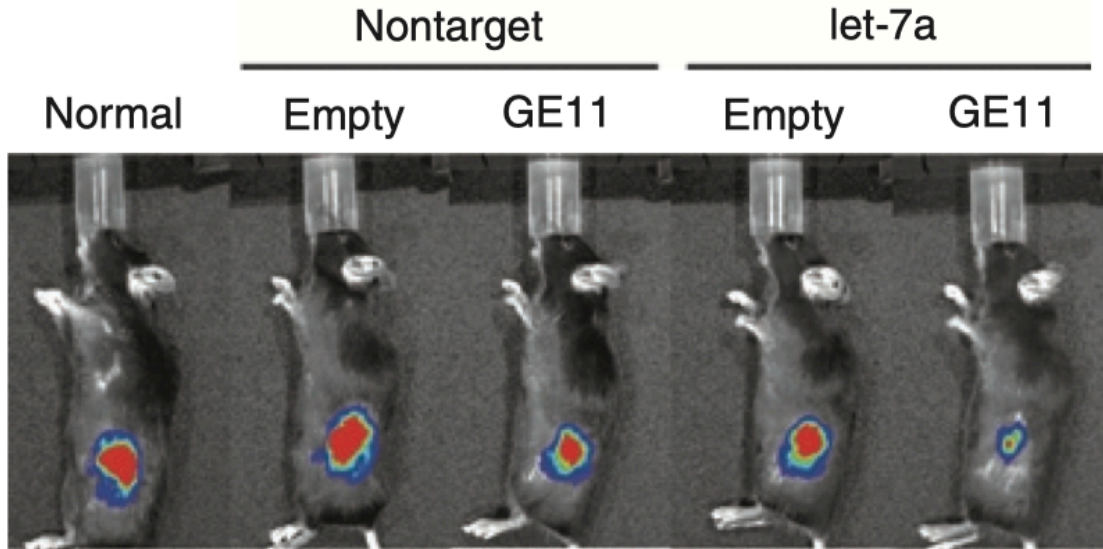
HEK293 G11-expressing cells transfected with let-7a, exosomes purified from supernatant



Amount of loaded miRNA determined in exosomes (PCR)

Material & methods

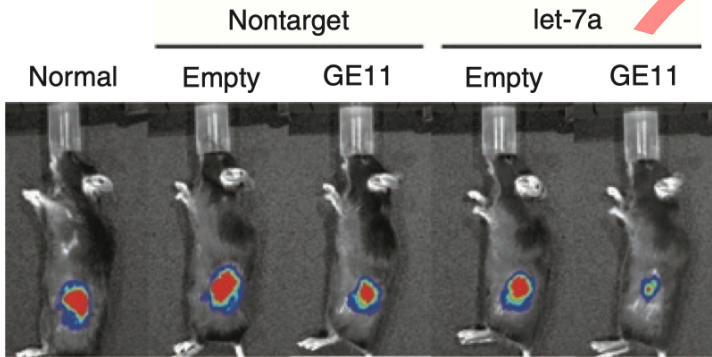
- GE11-exosomes (miRNA-loaded) inhibit tumor growth



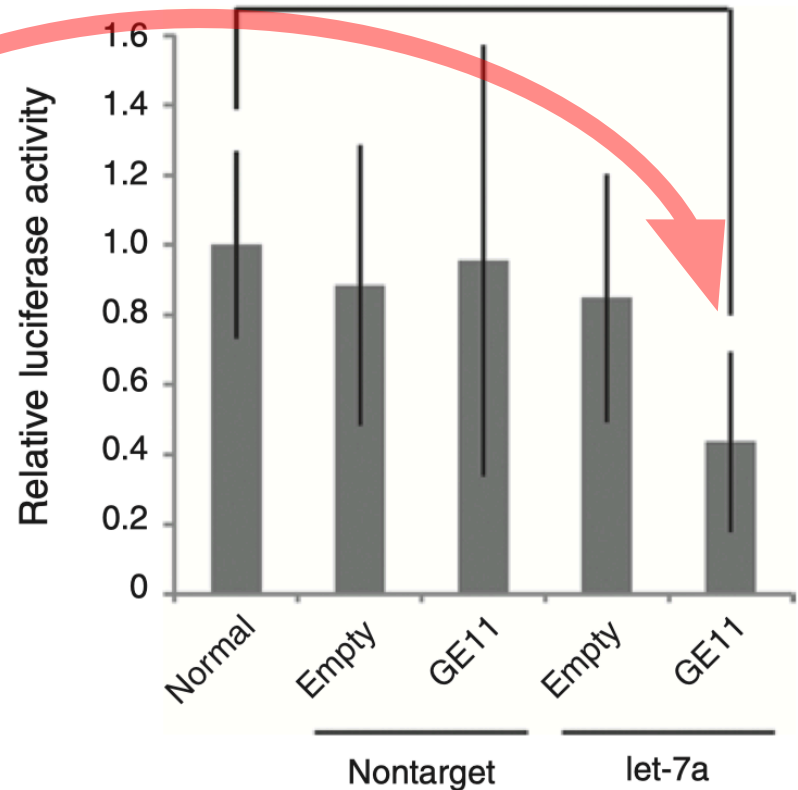
post injection imaging,
signal monitoring by IVIS

Material & methods

- GE11-exosomes (miRNA-loaded) inhibit tumor growth



post injection imaging,
signal monitoring by IVIS



discussion:

PROs:

- miRNA replacement = **NEW** tumor suppressor strategy
- exosomes = natural carriers of miRNA
 - target EGRF-expressing CAs
 - GE11-exosomes do not stimulate EGFR signaling (cell proliferation)
- let7a-containing GE11-positive exosomes inhibit tumor growth
 - potential tumor suppressor (HMGA2 expression ↓)

Cons:

- exosomes as drug delivery largely unknown