# Role of Exosomes in Squamous Epithelial Cell Biology through ITGAL Signaling

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## INTRODUCTION

**Exosomes:** Nanosized membrane bounded vesicles secreted into the cellular microenvironment by almost all types of cells[1]

- Carry biomacromolecules and efficiently deliver cargo to recipient cells[1]
- Elicit functions and mediate cellular communications[1]

**Integrin Subunit Alpha L (ITGAL):** Binds with a β subunit to form the integrin lymphocyte function-associated antigen-1 (LFA-1) found on leukocytes[2]

- LFA-1 interacts with intercellular adhesion molecules (ICAMs) 1 through 3 and enables leukocyte intercellular adhesion[2]

**Clinical Significance:**

- ICAM-1 is expressed by squamous cell carcinoma and is correlated to a cancer's invasive potential through effects on proliferation, invasion, and cytokine production activities[3]
- Higher levels of ICAM-1 expression are a negative prognostic marker in some carcinomas[4]

**Knowledge gap:** The biodistribution of exosomes and the role of ITGAL-expressing exosomes in squamous cancers, including head and neck squamous cell carcinoma (HNSCC), should be determined.

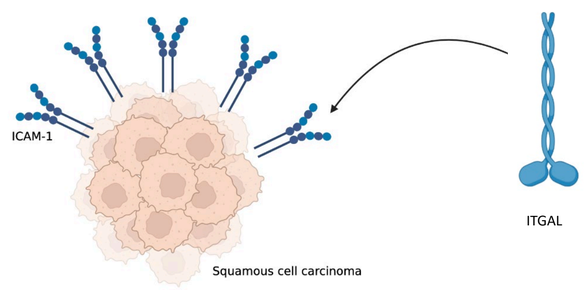


**Figure 1:** Interaction of ITGAL with ICAM1 in squamous cancers

## OBJECTIVES

**Objective #1:** To establish the methodology and study the biodistributions of exosomes

**Objective #2:** To Identify the role of ITGAL-ICAM1 signaling mediated via exosomes in squamous cancers

## METHODS & MATERIALS

Isolate exosomes form HEK cells
ExoQuick

Characterization of exosomes
NanoSight

Exosome and Cell Lysate Isolation and Quantification
BCA assay

Western Blot
Exosomal markers (CD63, CD81)

Exosome Labeling and Injection into Mice
Label with ExoFECT and inject into 12 mice

Organ Harvesting and Biodistribution Imaging
Fluorescence signaling was analyzed to detect the biodistribution of the exosomes

Study the role and biodistributions of exosomes and signaling of ITGAL expressing exosomes in squamous cancers including HNSCC

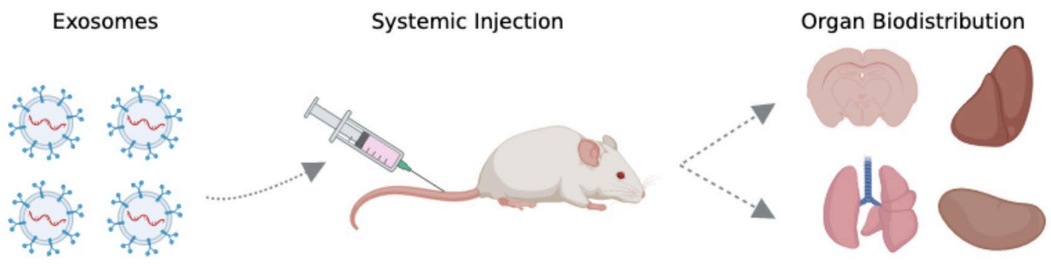


**Figure 2: Procedure overview of in vivo mice studies.** Exosomes have been labeled with ExoFECT. A total of 200ug of each group of exosomes was injected into 6 mice. After serial intervals the mice were imaged using an IVIS imager before organ removal. Immediately after the full mouse imaging, the organs were harvested and imaged using an IVIS imager.

## RESULTS

### Western Blot analysis of Exosomes and HEK 293T Cells

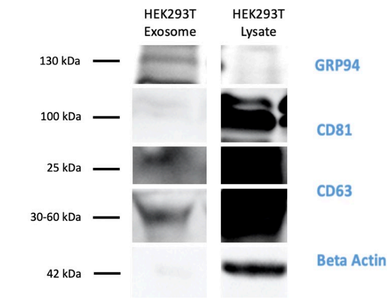


**Figure 3:** Western Blot using isolated exosomes and cell lysates of control HEK293T cells and exosomes. The presence of exosomal markers CD81, CD63 in exosomes were observed.

### *In Vivo* Exosome Biodistribution Studies

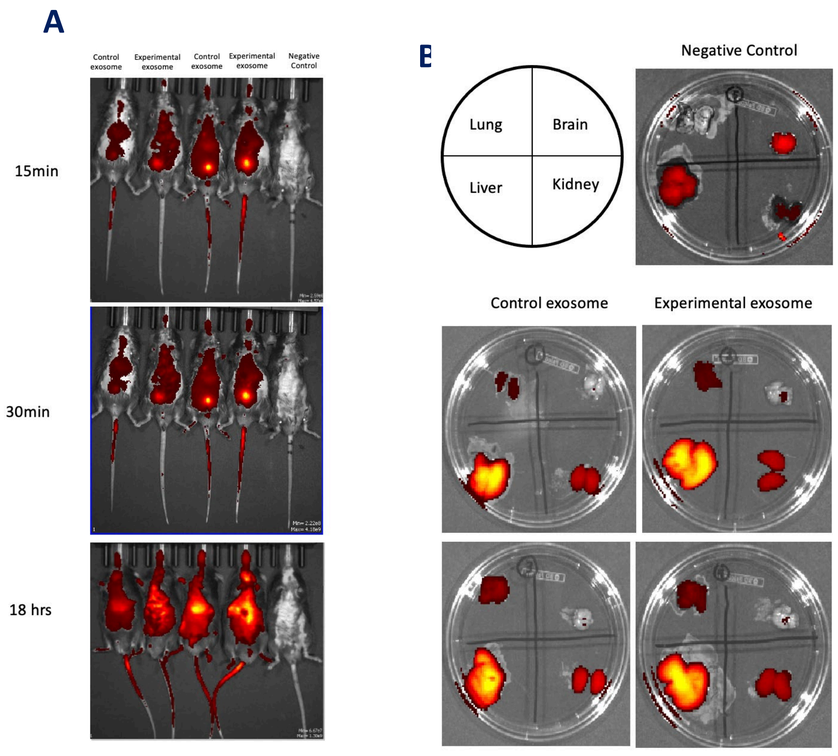


**Figure 4:** Demonstration of *in vivo* biodistribution studies of exosomes. (A) IVIS imaging of mice at 15 minutes, 30 minutes and 18 hours post-exosome injection. (B) IVIS imaging of the lung, brain, liver and kidney post organ harvesting. Indicating increased absorption in the liver and rapid uptake of exosomes in the tissue.

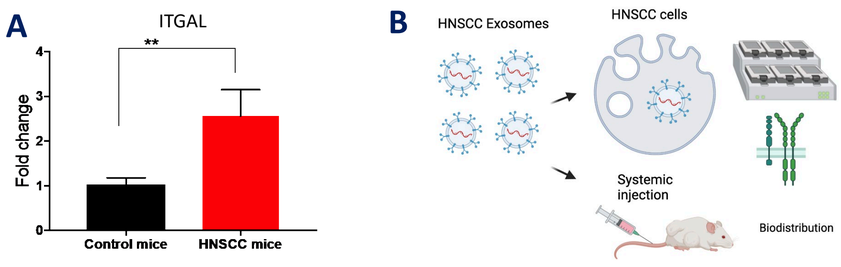


**Figure 5:** (A) Analysis of ITGAL expression in exosomes isolated from plasma of mice with and without head and neck squamous cell carcinoma showed that there is an increase in the level of ITGAL in circulating exosomes (n=5 per group) ($P<0.01$). (B) In our future studies, we will study the biodistribution and the role of exosome mediated ITGAL signaling and its interactions with ICAM-1 in squamous tumors.

## DISCUSSION

- The western blot and our quality control experiments demonstrated that our purification methods yielded pure exosomes.
- Our biodistribution studies demonstrated that adequate labeling and imaging of exosomes post-injection into mice can be achieved in both control and experimental groups.
- Our in vivo studies showed that exosomes have rapid uptake in organs.
- We found a increase in the levels of ITGAL exosomes in circulation of mice with head and neck cancer and we will identify the role of ITGAL-ICAM1 signaling in pathogenesis of head and neck cancer.
- Our biodistribution and functional studies will be followed up to identify the role and biodistribution of HNSCC exosomes and their role in ITGAL-ICAM signaling.

## CONCLUSIONS

Identification of signaling and biodistribution on exosomes can have clinical impact in both diagnostics and therapeutics.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Zaborowski, M. P., Balaj, L., Breakefield, X. O., & Lai, C. P. (2015). Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study. Bioscience, 65(8), 783–797. https://doi.org/10.1093/biosci/biv084
2. Gene [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – . Gene ID: 3683, Homo sapiens ITGAL integrin subunit alpha L, protein; [cited 2022 03 10]. Available from: https://www.ncbi.nlm.nih.gov/gene/3683
3. Usami, Y., Ishida, K., Sato, S., Kishino, M., Kiryu, M., Ogawa, Y., Okura, M., Fukuda, Y., & Toyosawa, S. (2013). Intercellular adhesion molecule-1 (ICAM-1) expression correlates with oral cancer progression and induces macrophage/cancer cell adhesion. International journal of cancer, 133(3), 568–578. https://doi.org/10.1002/ijc.28066
4. Sökeland, G., Schumacher, U. The functional role of integrins during intra- and extravasation within the metastatic cascade. Mol Cancer 18, 12 (2019). https://doi.org/10.1186/s12943-018-0937-3

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