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The Involvement of Outer Membrane Vesicles Secreted by Human Microbiota in Carcinogenesis

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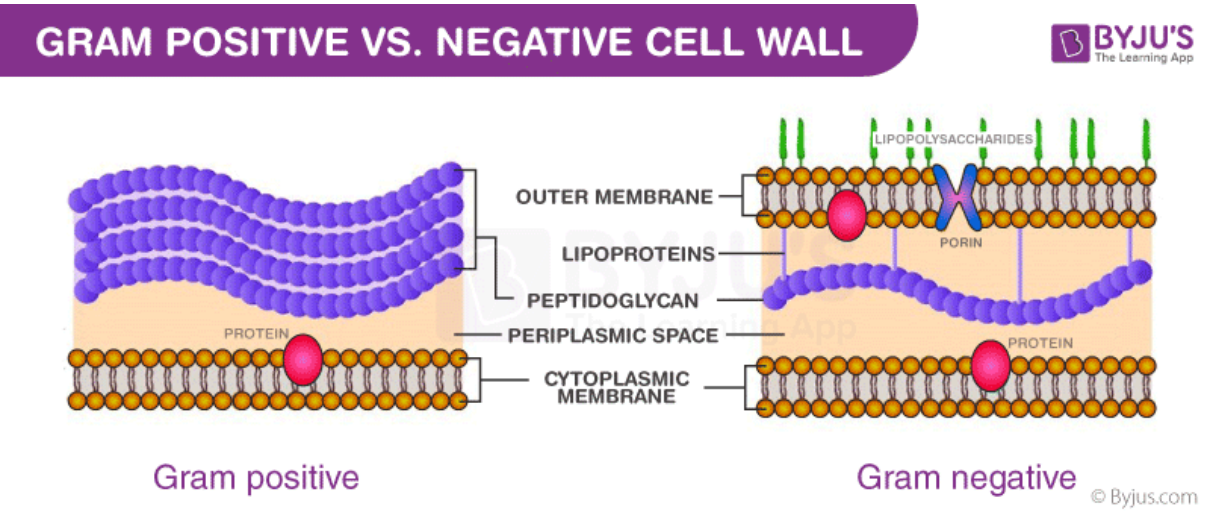
Outline

- ➢ Introduction of bacteria outer membrane vesicles (OMVs)
- Discovery, biogenesis and functions
- ➢ Bacterial OMVs and human carcinogenesis
- OMVs and gastric cancer, colon cancer, and oral cancer
- ➢ Clinical applications of bacterial OMVs

Introduction of Bacteria OMVs

Discovery, biogenesis and functions

What is Bacteria OMVs?



https://byjus.com/biology/composition-of-bacterial-cell-wall/

- Outer-membrane vesicles (OMVs) are spherical buds of the outer membrane filled with outer-membrane lipids, proteins, and periplasmic content, which are commonly produced by **Gram-negative bacteria**.

- The production of OMVs allows bacteria to interact with their environment and mediate diverse functions, including **promoting pathogenesis, enabling bacterial survival during stress conditions and regulating microbial interactions within bacterial communities.**

(Schwechheimer C, Kuehn MJ. *Nat Rev Microbiol*. 2015)

Bacterial OMVs discovery

- OMVs from Gram-negative bacteria were first described in *Escherichia coli* in 1965.

Biochem. J. (1965) **96**, 567

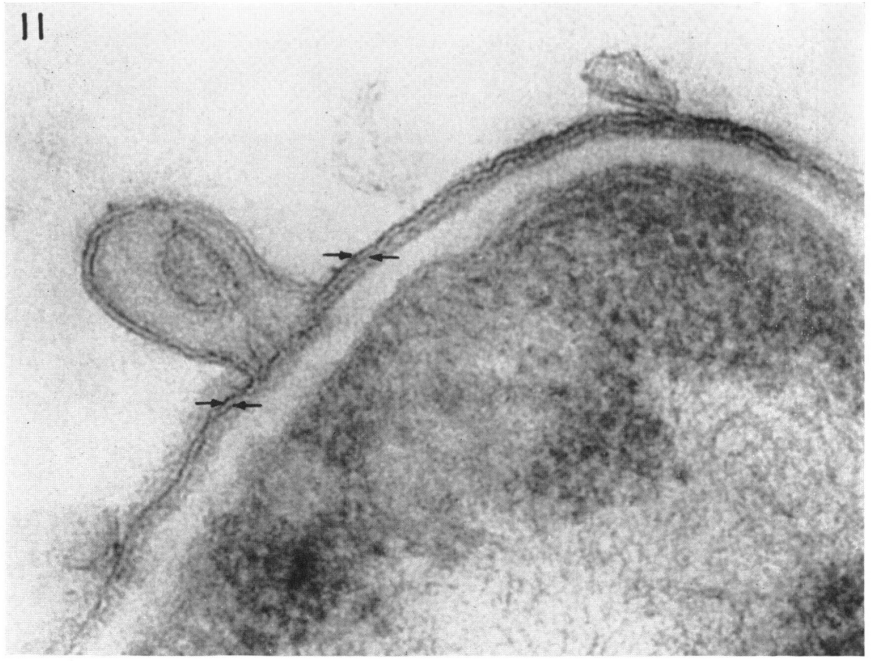
An Extracellular Glycolipid Produced by *Escherichia coli* Grown under Lysine-Limiting Conditions

BY D. G. BISHOP* AND ELIZABETH WORK
Twyford Laboratories, Twyford Abbey Road, London, N.W. 10

(*Received* 31 *December* 1964)

(Bishop DG, Work E. *Biochem J*. 1965)

- Later, Knox and collaborators showed by electron microscopy that these secreted cell-free LPS elements were part of membrane structures and proposed that these vesicles were derived from the outer membrane (OM).



(Knox KW, Vesk M, Work E. *J Bacteriol*. 1966)

Bacterial OMVs discovery

- Despite the increasing evidence of OMV production by bacteria, OMVs were considered mere growth artifacts or cell lysis by-products for several years.
- Later, OMVs were observed in cerebrospinal fluid samples from patients with acute meningitis, suggesting that OMVs were not generated only in lab conditions (DeVoe IW, Gilchrist JE. *J Exp Med*. 1975).

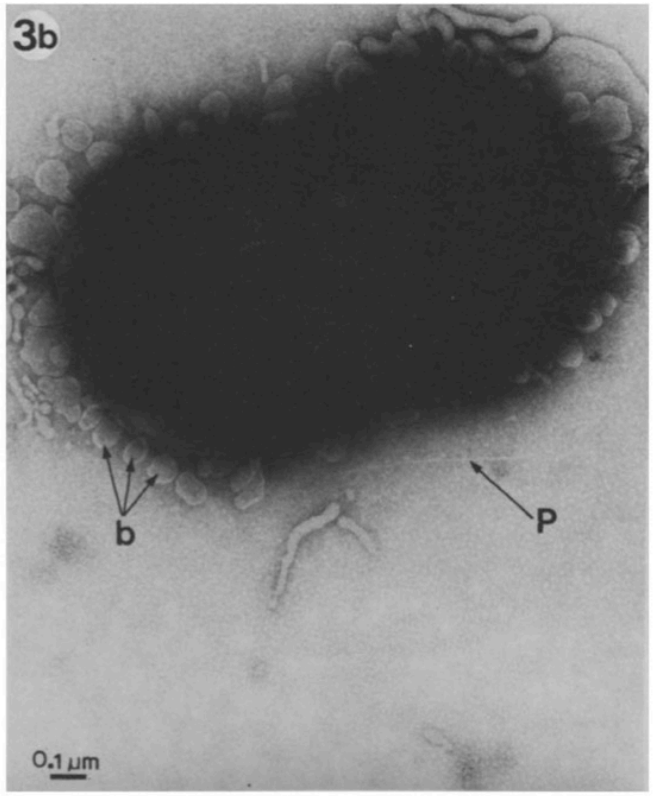
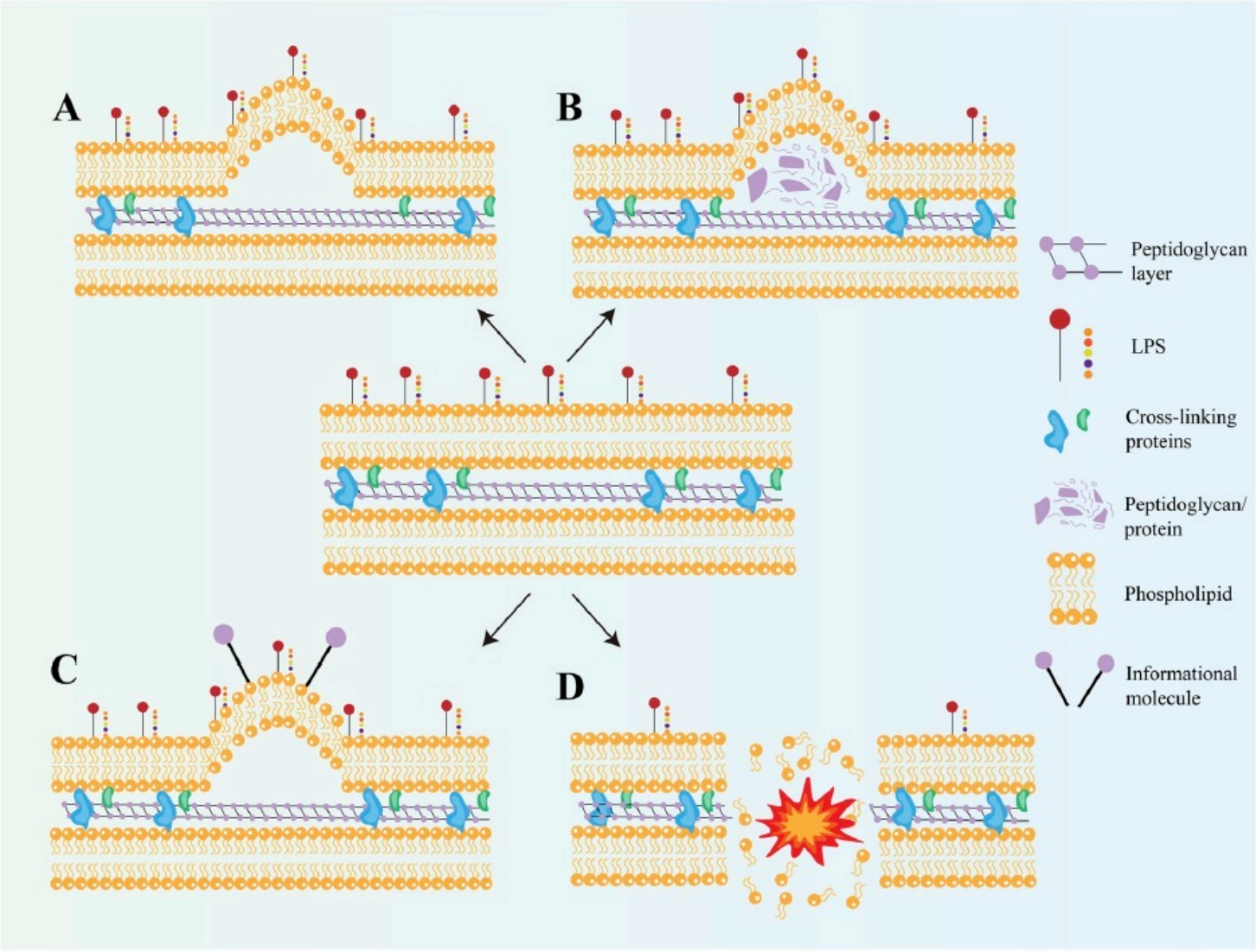


FIG. 3. Meningococcus from second serial culture from (b) cerebrospinal fluid

(DeVoe IW, Gilchrist JE. *J Exp Med*. 1975)

Bacteria OMVs biogenesis



Mechanism of OMVs generation:

(A) Breakage or loss of cross-linking proteins

(B) Excess peptidoglycan or improperly folded proteins accumulate

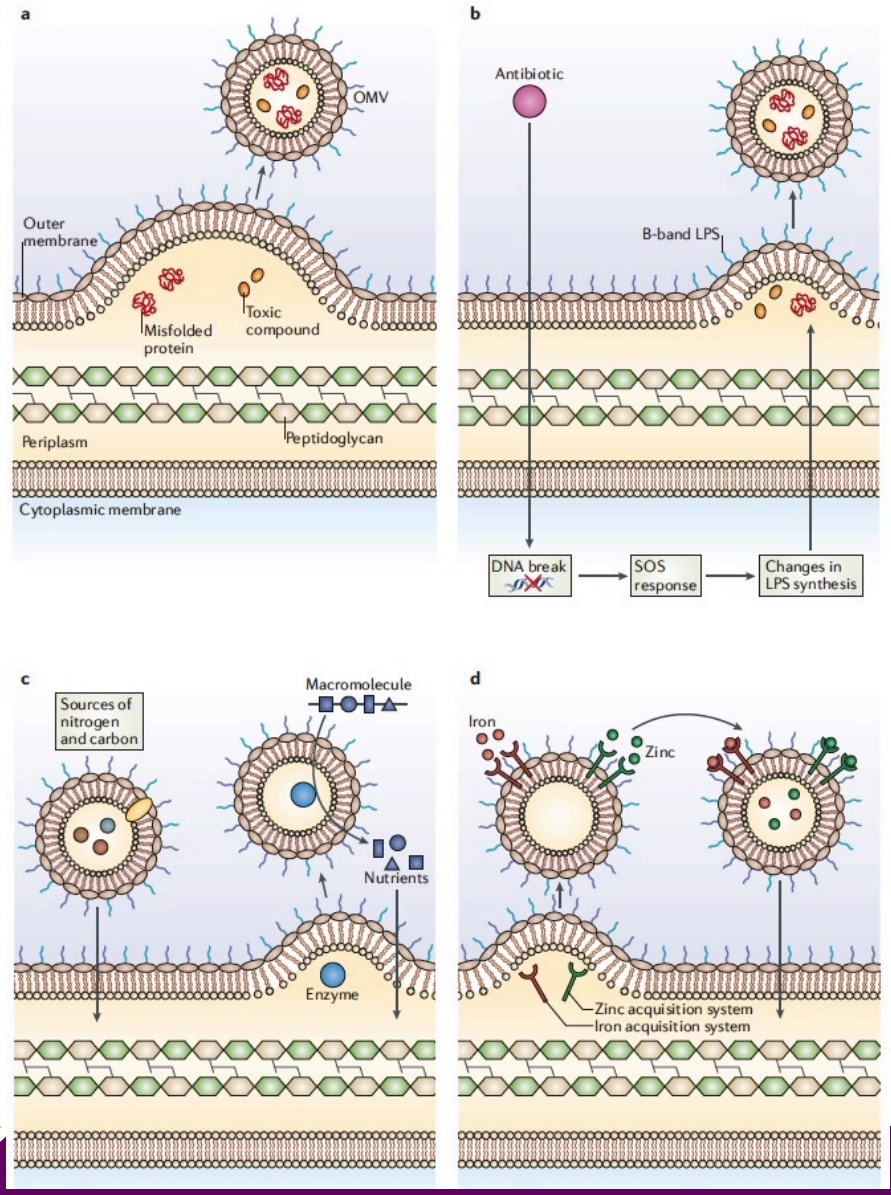
(C) The insertion of foreign signal molecules affects the charge balance of the outer membrane.

(D) The rupture and lysis of the cell

(Li D, Zhu L, Wang Y, Zhou X, Li Y. *Biomed Pharmacother*. 2023)

Bacteria OMVs functions in bacterial physiology

- **a.** Remove toxic compounds, such as misfolded proteins, from bacterial cells under stress conditions.
- **b.** Stress conditions can increase OMV production.
- **c.** Serve as sources of carbon and nitrogen, and can carry and disseminate enzymes.
- **d.** Carry iron and zinc acquisition systems



(Schwechheimer C, Kuehn MJ. *Nat Rev Microbiol*. 2015)

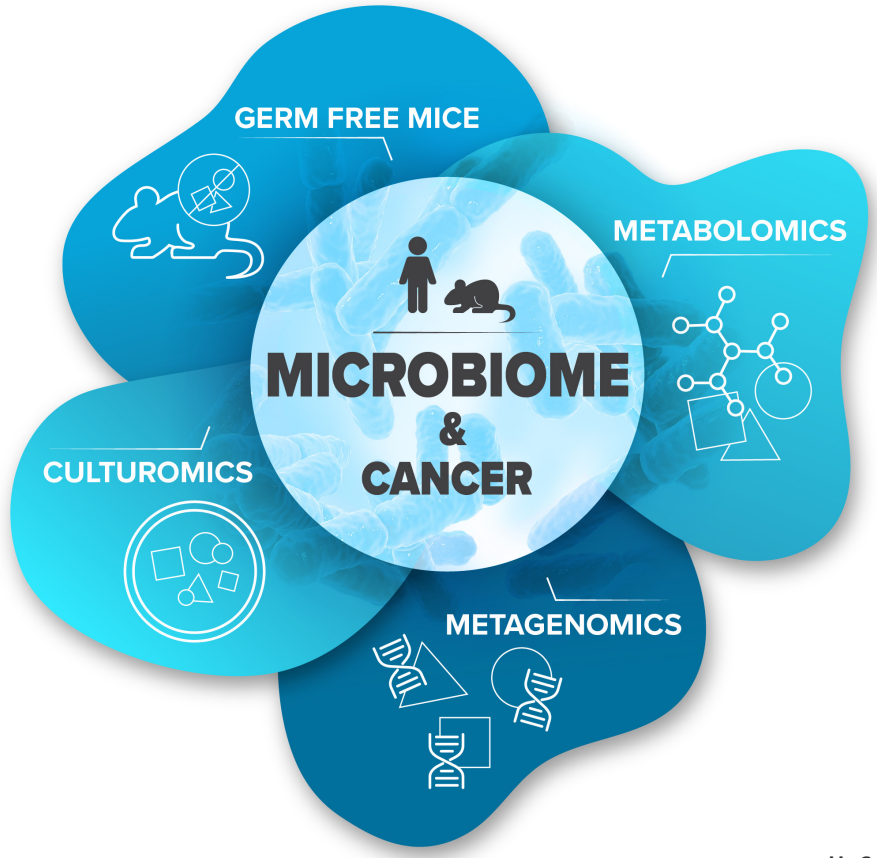
Bacteria OMVs and Carcinogenesis

Oral cancer, Gastric cancer, and Colon cancer

Microbiome & Cancer

- Infectious microorganisms like bacteria, viruses, and fungi are thought to be significant pathogenic factors, accounting for 15% of cancers globally(Yasunaga JI, Matsuoka M. *Cancer Sci*. 2018).

- OMVs have attracted significant attention in cancer research as they serve as important mediators of intercellular communication, capable of transferring signal molecules between bacteria and cells to influence their behaviours and functions.



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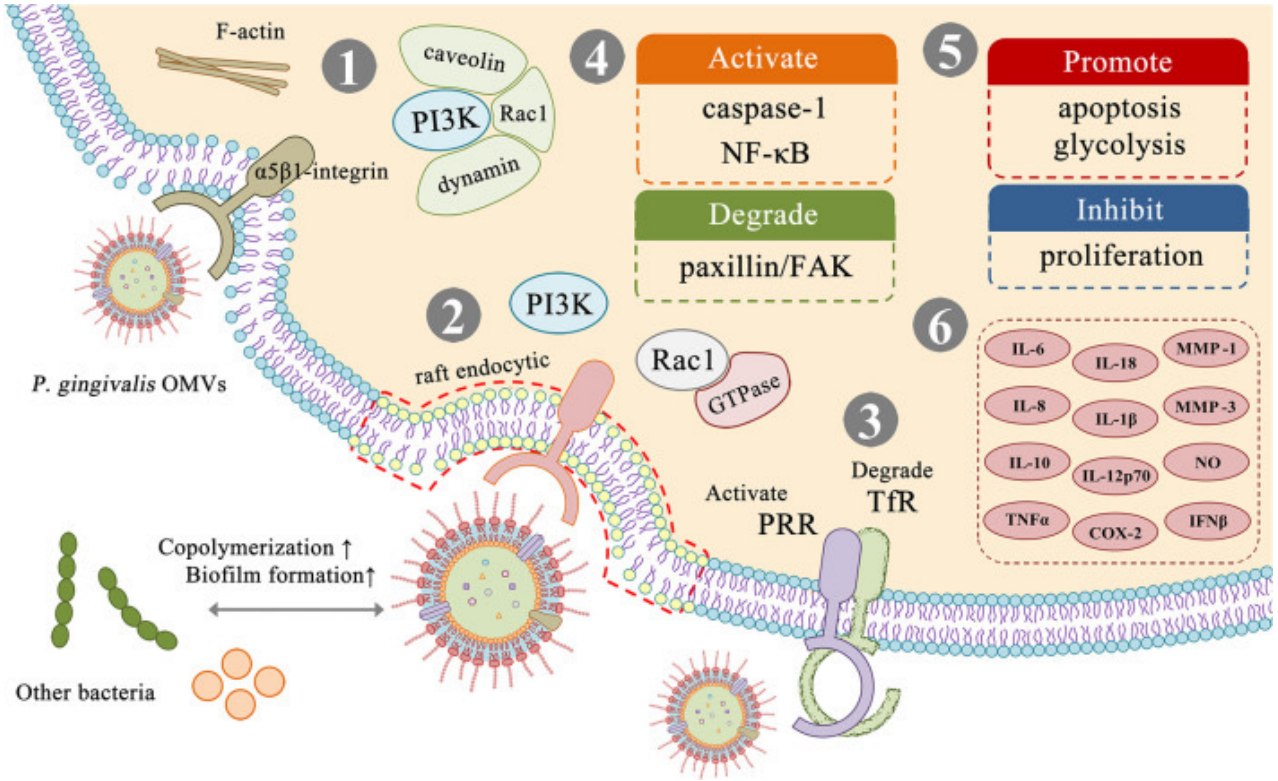
Oral cancer

Porphyromonas gingivalis

- Kamaguchi et al. demonstrated that *P. g* OMVs strongly promote coaggregation between *S. aureus* with oral microorganisms, like *Streptococcus*, *Actinomyces*, and mycelium-type *Candida albicans* (Kamaguchi A, Nakayama K, Ichiyama S, et al. *Curr Microbiol*.2003).

- Grenier found that *P. g* OMVs can mediate the coaggregation between *Treponema denticola* and *Lachnoanaerobaculum saburreum* (Grenier D. *Int J Dent*. 2013).

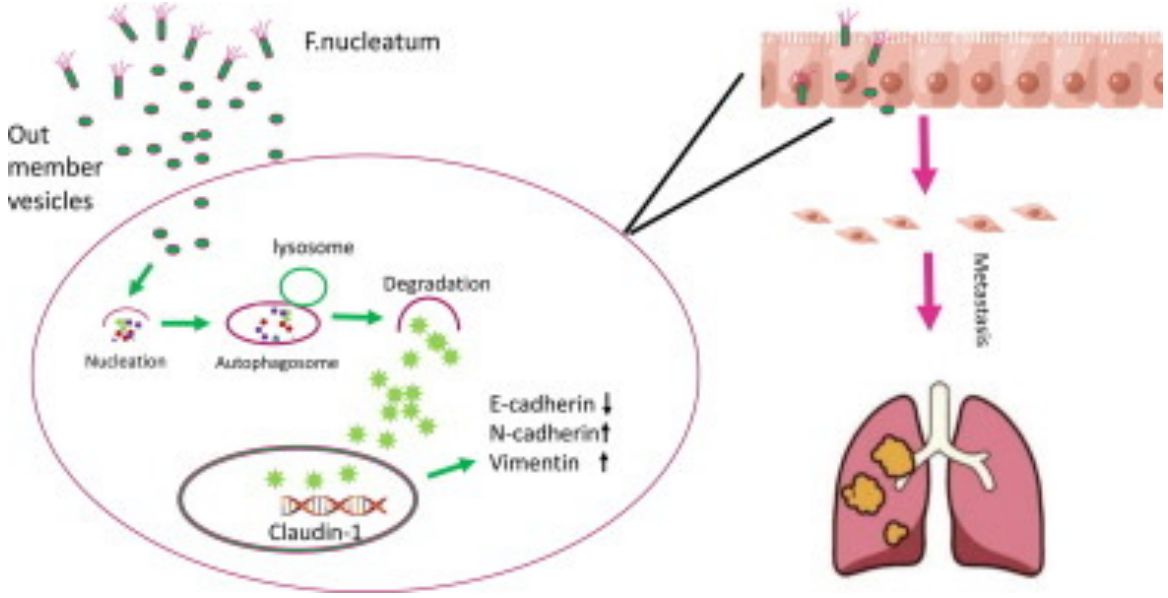


(Zhang Z, Liu D, Liu S, Zhang S, Pan Y. *Front Cell Infect Microbiol*. 2021)

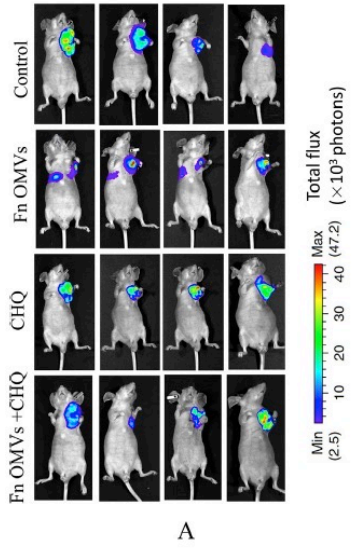
Oral cancer

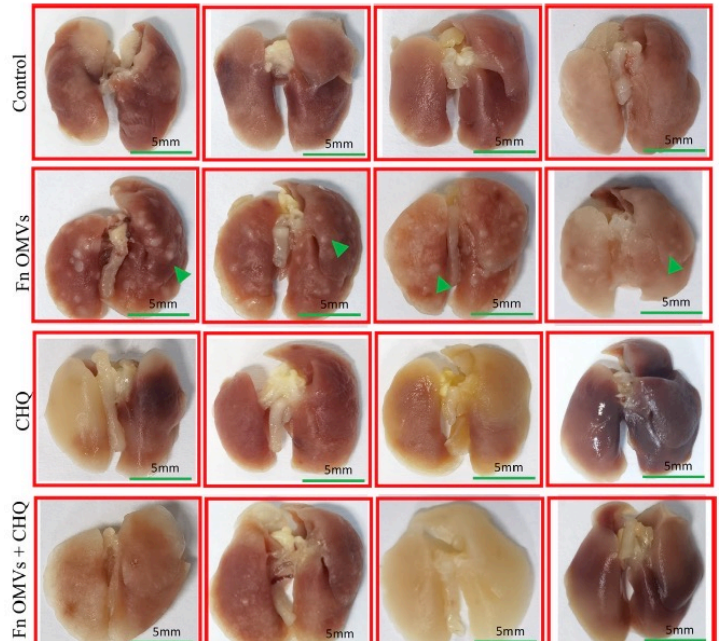
Fusobacterium nucleatum

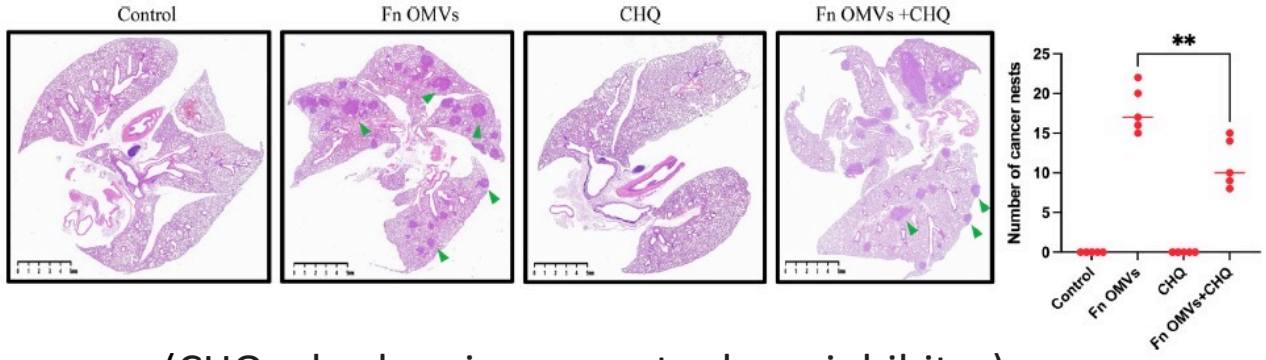
F. nucleatum outer membrane vesicles activate autophagy to promote oral cancer metastasis.



(Chen G, Gao C, Jiang S, et al. *J Adv Res*. 2023)





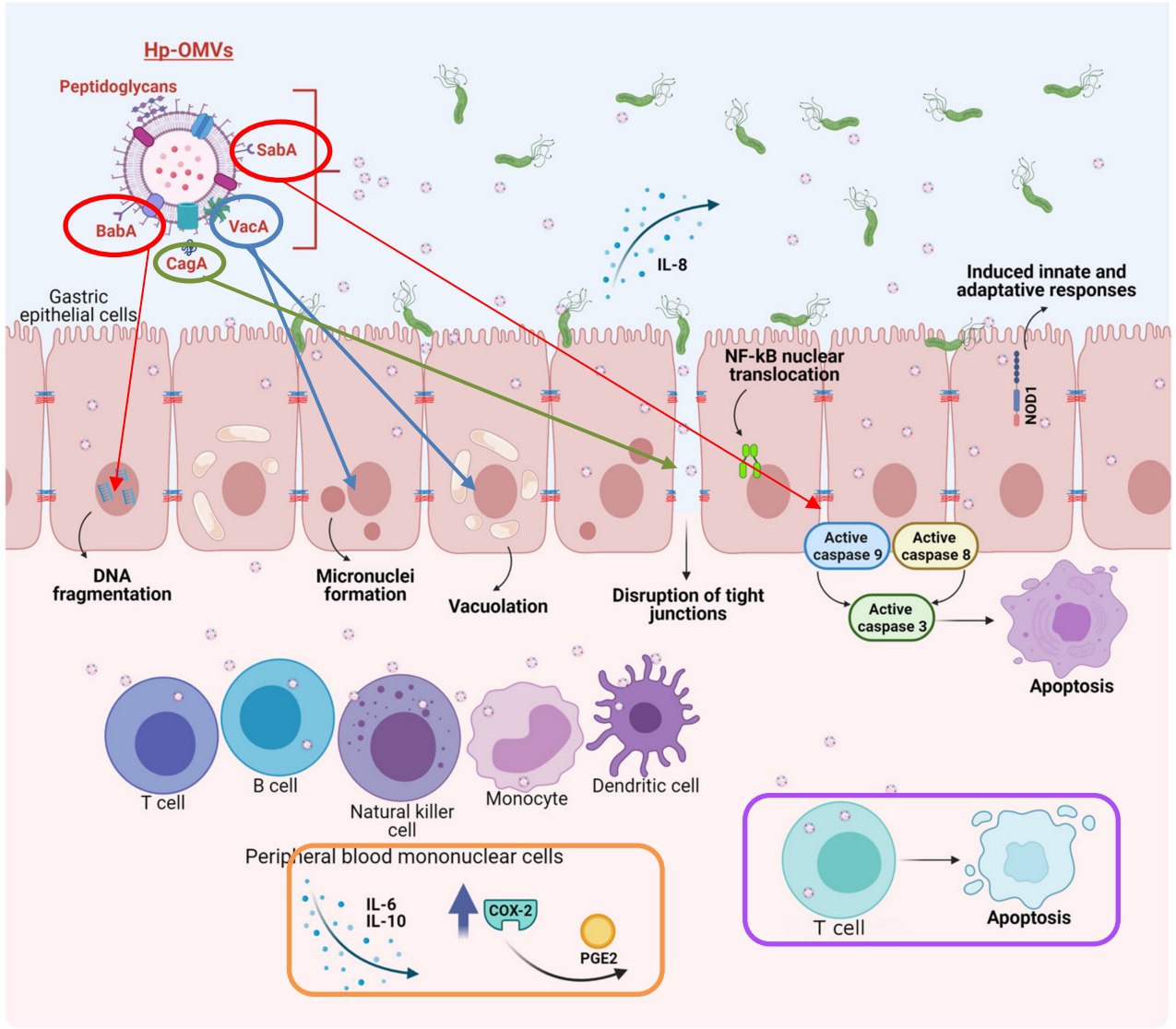


(CHQ, chroloquine, an autophagy inhibitor)

Gastric cancer

Helicobacter pylori

- A common bacterium that infects the gastric mucosa, is considered a major risk factor for gastric cancer.

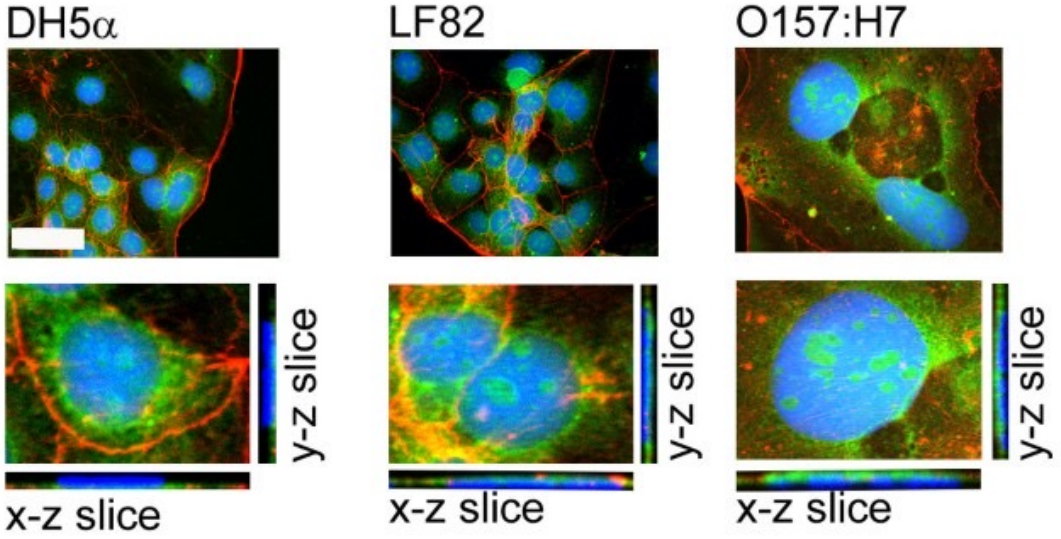


(González MF, Díaz P, Sandoval-Bórquez A, Herrera D, Quest AFG.*Int J Mol Sci*. 2021)

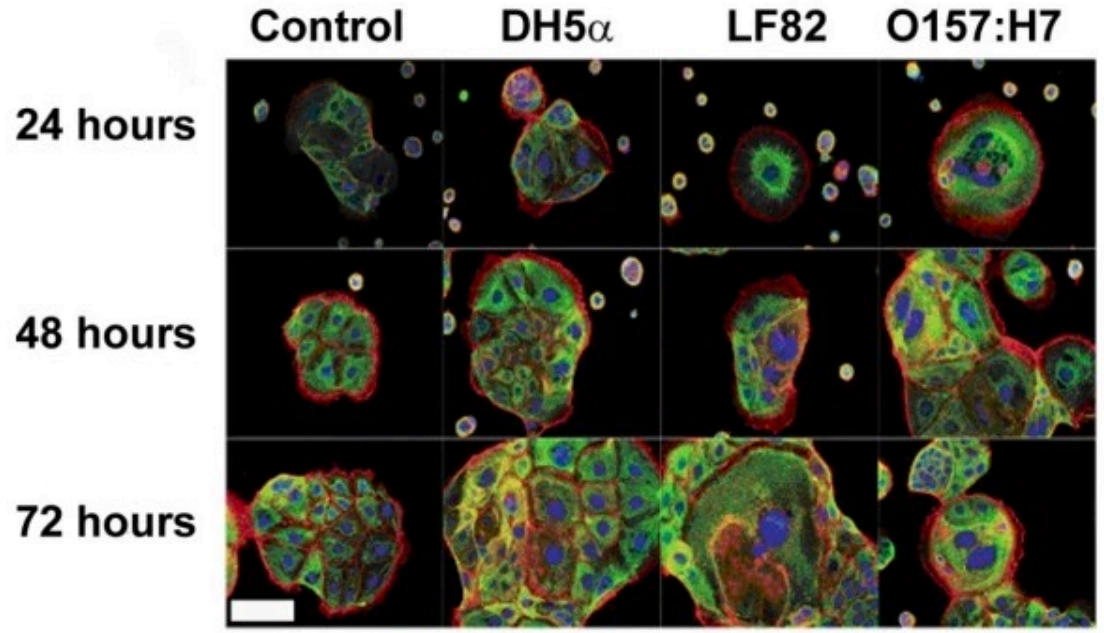
Colon cancer

Escherichia coli

avirulent | adherent-invasive | enterohaemolytic



Fluorescent DiO–labelled OMVs (green), were added to Caco-2 cells for 4 hours before the cells were stained for actin (red) and nuclei (blue).



Immunofluorescence labelling of actin (red), cytokeratin 18 (green), and nuclei blue was used to show that OMVs cause endoreplication in Caco-2 cells over time

They found that OMVs derived from *E.coli* were internalized by Caco-2 cells, increased cell numbers, induced double-stranded DNA breaks, initiated DNA replication, and produced distended multinucleate cells.

(Tyrer PC, Frizelle FA, Keenan JI.*Infect Agent Cancer*. 2014)

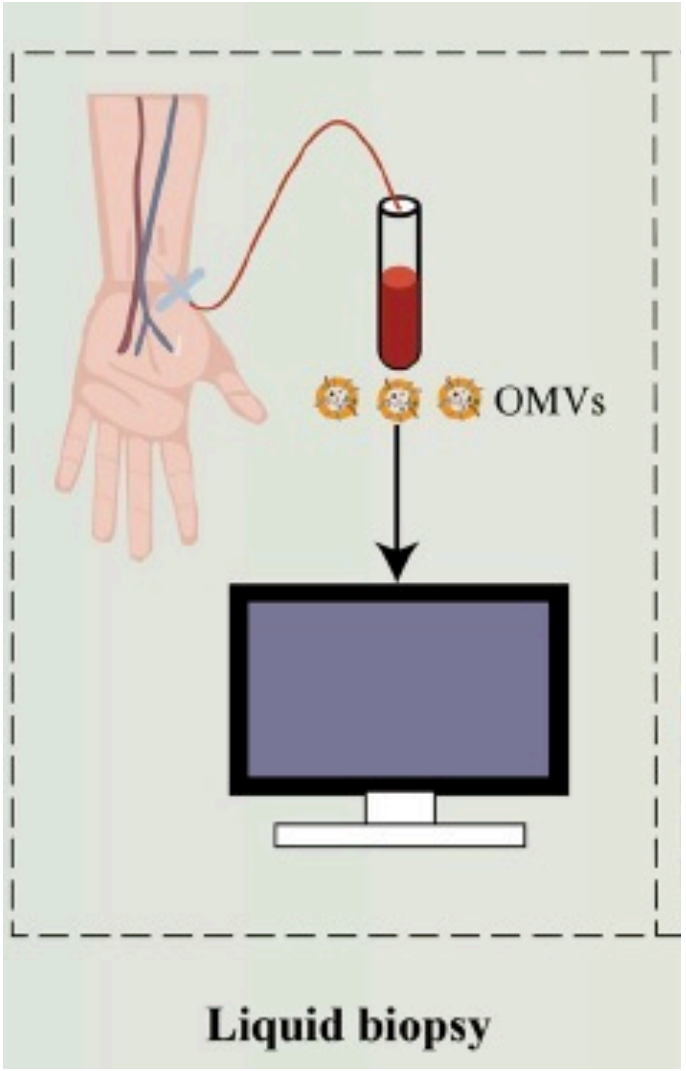
Bacteria OMVs Applications

Biomarkers, Tumor therapies, and Vaccines

Biomarkers

- Cargo that are conserved among a bacterium's OMVs, independent of growth conditions and stage, would serve as ideal biomarkers

- Cargo specific to a given bacterium that would allow for rapid identification and differentiation between bacterial species.

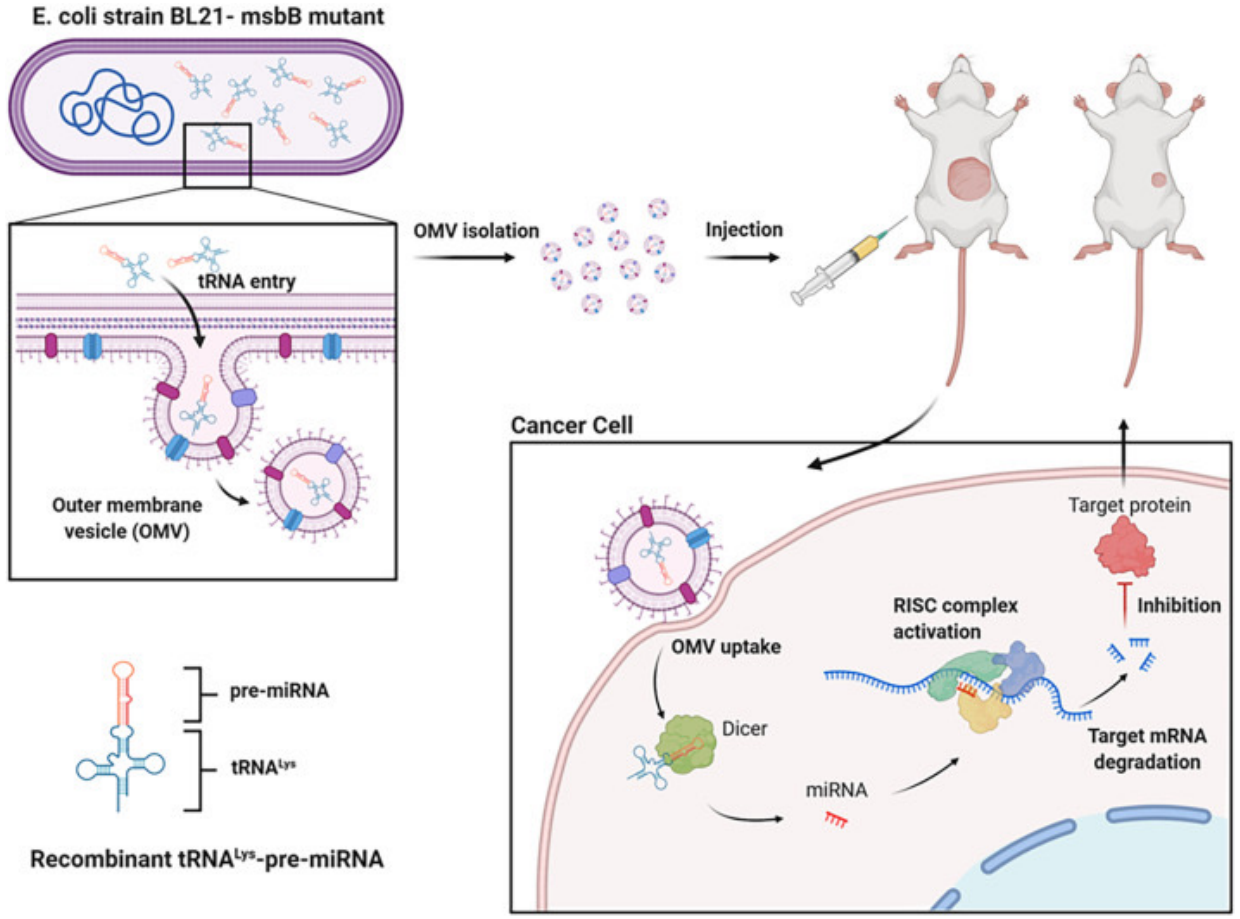


(Li D, Zhu L, Wang Y, Zhou X, Li Y. *Biomed Pharmacother*. 2023)

Anti-tumor therapy

- A cheap and potentially mass-produced method of OMV bacteria self-embedding with pre-miRNA by genetic engineering.

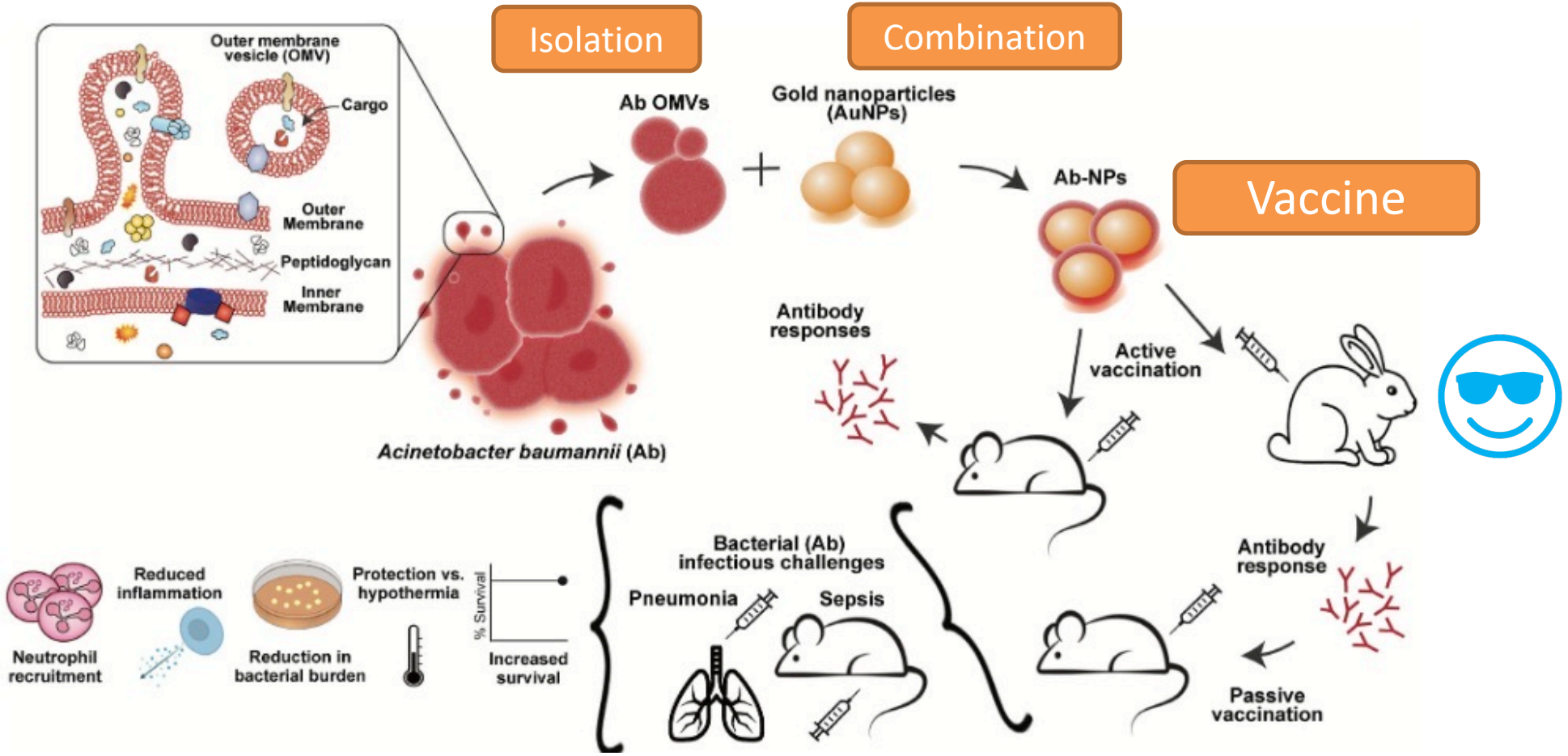
- ✓ They found that OMV can be released and inherit over-expressed $tRNA^{Lys}$-pre-miRNA from mother *E. coli* that is directly used for tumor therapy.
- ✓ The over-expressed pre-miRNA inside OMV could be released and processed into mature miRNAs with the aid of the camouflage of "tRNA scaffold".
- ✓ Moreover, the group *in vivo* treated with targeted $OMV^{tRNA\text{-}pre\text{-}miR\text{-}126}$ obviously inhibited the expression of target oncogenic CXCR4 and significantly restrained the proliferation of breast cancer tissues.



(Cui C, Guo T, Zhang S, et al. *Nanomedicine*. 2022)

Vaccines

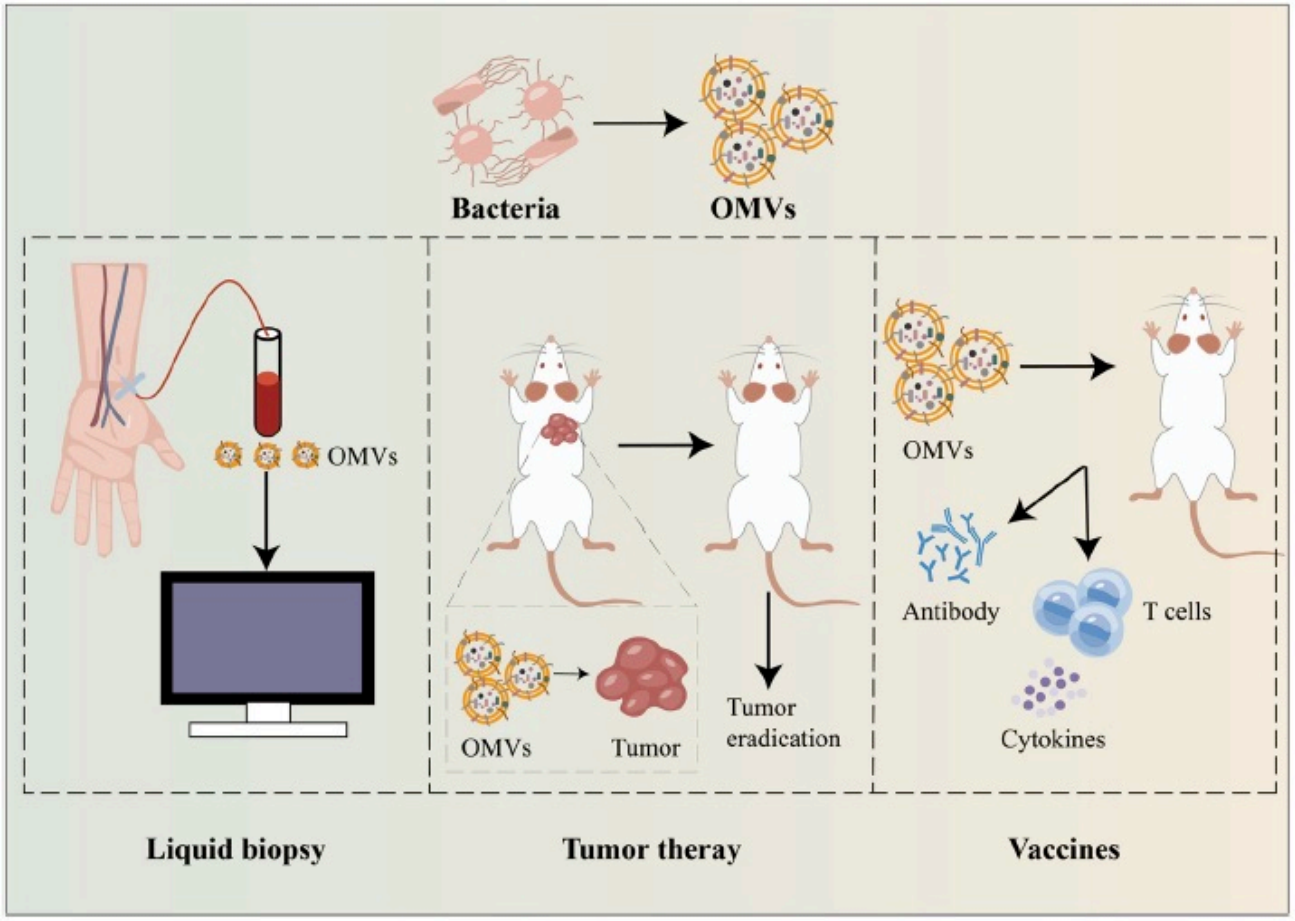
- OMVs-coated nanoparticle vaccine protects against *Acinetobacter baumannii* pneumonia and sepsis.



（Bjanes E, Zhou J, Qayum T, et al. *Adv Nanobiomed Res*. 2023)

Remarks and Future Perspectives

- How does the intracellular material enter into the vesicles in the outer membrane during OMV generation?
- The composition of OMV is complicated, and the specific substances that play a role are yet to be determined.
- Understanding the composition and pathogenic mechanisms of OMVs is crucial for ensuring their safe clinical application, given their pro-inflammatory and potentially pathogenic properties.
- The application of OMVs is still in the laboratory stage, and more efficient methods are needed to control the cost and ensure the efficiency and safety of mass production.



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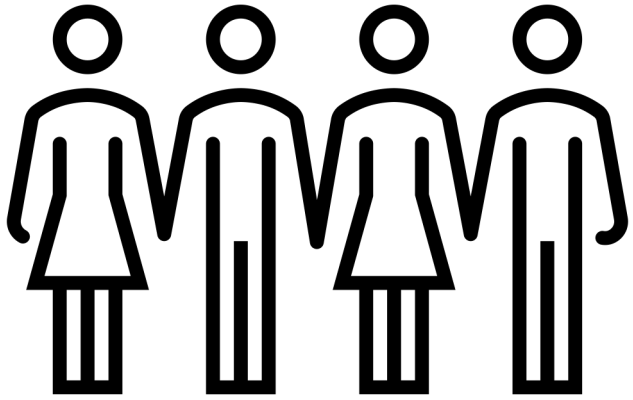

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Q&A