Regulators for extracellular vesicle production

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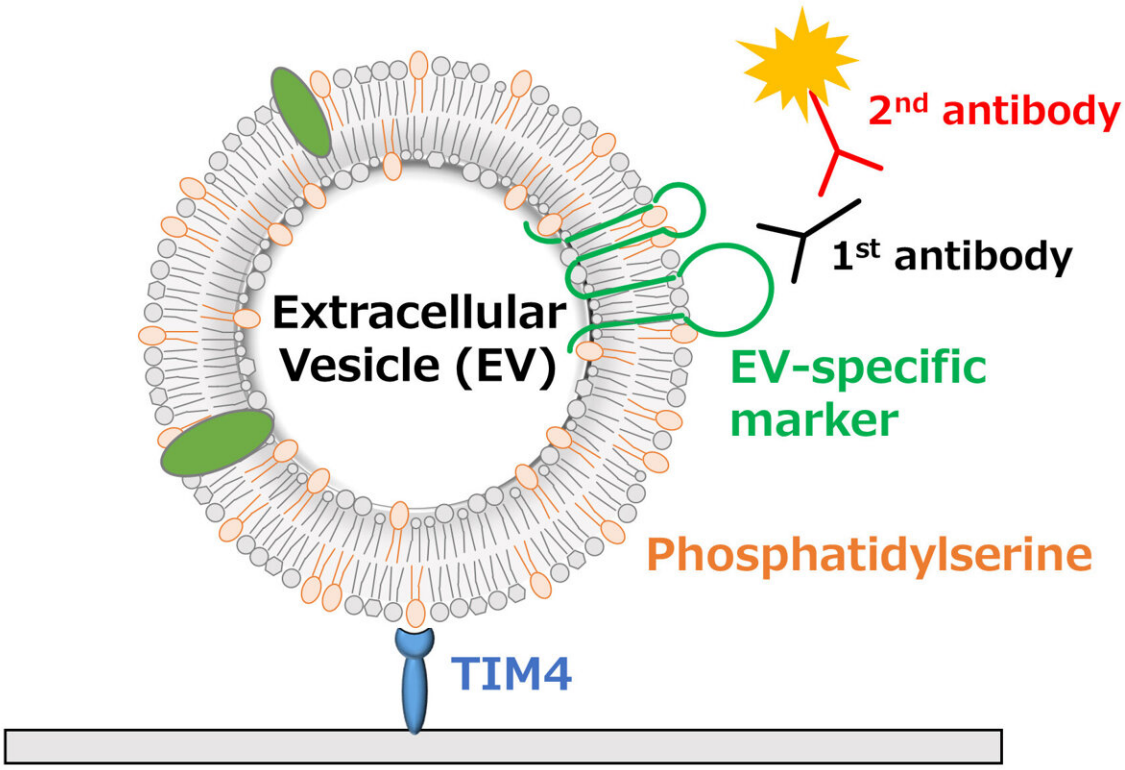

Schematic of an extracellular vesicle (EV) and the screening procedure. An EV is 'captured' by a TIM4 molecule. Then, the captured EV is detected with a primary antibody against an EV-specific marker, and then with a secondary antibody. Credit: Kanazawa University

Biological vesicles are nano-sized containers that transport proteins and

other substances within or between cells. Most cells release so-called extracellular vesicles (EVs), which play important roles in cell-to-cell communication. EVs also are involved in diseases, however—the spreading of a tumor, for example, is sometimes stimulated by particular EVs. For the development of therapies, regulators that can inhibit the secretion of specific EVs, without severe side effects, are therefore needed. On the other hand, EVs can also be employed as therapeutic agents. For example, EVs derived from certain stem cells are known to have a therapeutic effect on damaged tissues. Therefore, regulators that activate EV secretion are also in demand. By screening a large set of biomolecular compounds, Rikinari Hanayama from Kanazawa University and colleagues have now identified 4 potential regulators (1 inhibitor and 3 activators) for EV secretion for a variety of cells.

Central to the scientists' EV regulator identification strategy is a protein called TIM4, which is known to easily bind to a molecule called phosphatidylserine. The latter is present in EVs generated by various cells, and so TIM4 acts as a receptor for the uptake of EVs. Based on this notion, the researchers developed a screening procedure in which more than 1500 candidate EV secretion regulators (inhibitors or activators) were tested.

After the first run of screening, 60 compounds remained as possible regulators. (Potential activators and inhibitors were defined as increasing the secretion of EVs by more than 50% or decreasing it by more than 33%, respectively.) In a second run of screening, the candidate compounds' toxicity to cells was tested, leaving only 24 compounds. In the third, final screening run, the scientists measured the concentration of EV particles by nanoparticle tracking analysis (a method for visualizing and analyzing particles in a liquid). As a result, one inhibitor, called AA2, and three activators were identified.

Hanayama and colleagues tested the effect of AA2 on EV secretion

from several human and mouse cells in vitro, including tumor and non-tumor cells, and observed regulatory effects on the bioactivity of EVs. They also compared the effect of AA2 with slightly different biomolecules, which enabled them to identify the chemical group responsible for the inhibitory effect on EV secretion. This is important for the future development of AA2 derivatives that inhibit EV secretion without simultaneously affecting apoptosis (cell death).

The scientists acknowledge that "there are still several issues to be clarified before [EV regulators] are used for the treatment of EV-related diseases, including the delivery system of EV regulators to target cells, effects on EV secretion from normal cells, and side effects." Nevertheless, the work of Hanayama and colleagues is an important step towards the controlled regulation of the bioactivity of EVs, as it demonstrates the feasibility of "a high-throughput method to detect EVs with high sensitivity and versatility."

The research was published in *Scientific Reports*.

More information: Yunfei Ma et al, Identification of small compounds regulating the secretion of extracellular vesicles via a TIM4-affinity ELISA, *Scientific Reports* (2021). DOI: 10.1038/s41598-021-92860-2

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