Development of immuno-conjugates magnetic graphene beads for specific extraction of potential SARS–CoV-2 biomarkers from liquid biopsies

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INTRODUCTION: Liquid biopsy, characterized by minimally invasive detection using biological fluids such as blood, saliva and urine, has proven to be a revolutionary strategy in cancer diagnosis and prognosis prediction (Pisapia P. et al., 2021). Among them, exosomes are essential components of liquid biopsy and can act as carriers of proteins and nucleic acids (Cordaro A. et al., 2020).

AIM: The exosome isolation procedure by ultracentrifugation concentrates a heterogeneous exosome population. Thus, an approach based on coupling liquid biopsies with new miniaturized magnetic beads is used to obtain a specific exosome population. Therefore, this study aims to develop an in vitro exosome-enrichment system for the molecular characterization of the circulating exosomes in the SARS–CoV-2 infection.

MATERIALS AND METHODS: SARS-CoV-2 infection was reproduced in vitro by generating SARS-CoV-2 pseudotyped S particles (SARS-Spp) expressing the spike glycoprotein (Millet J.K. et al., 2016). Quantitative infectivity of SARS-Spp was performed using a luciferase activity assay on VERO cells. Therefore, A549-ACE cells were transduced with SARS-Spp for 48 h, and the released exosomes were purified by ultracentrifugation and characterized by Western blotting of the main target protein of the exosomes. The graphene based MAGnetic Units (MAGU) functionalized at 4°C with CD9 antibody, commonly used as an exosome marker, were incubated with SARS-Spp-transduced A549-ACE-derived exosomes.

RESULTS: The results demonstrate the success of the conjugation protocol of MAGU beads with CD9 antibody. The MAGU-CD9 system can fish in an exosome pool and recover the exosome subtype expressing ALIX on the membrane.

CONCLUSION: Immunoconjugates with magnetic graphene beads can be a valuable tool to search for new disease markers in body fluids using a non-invasive method.

References

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