2022 Best Research Poster Award

Barwon Health

DEAKIN UNIVERSITY

A fluorescence polarisation-based method for detection and classification of extracellular vesicles using aptamers

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INTRODUCTION

Extracellular vesicles (EVs) hold enormous potentials in cancer liquid biopsy [1]. However, current EV detection methods with high sensitivities require great level of microfabrication and biomedical engineering, whereas simple methods (e.g., ELISA) have high limits of detection, making these technologies difficult to be widely employed in general EV research laboratories and clinical diagnostic settings. Fluorescence polarisation (FP) has been popularly utilized in clinical settings and high-throughput assays [2]. Chemical antibodies (aptamers) with small sizes and extracellular vesicles (EVs) with high volume and mass are expected to be a perfect pair for FP assays. In this work, we developed a method of **fluo**rescence **p**olarisation using **a**ptamers for the **d**etection of **e**xtracellular nanovesicles (FluoPADE) with great applicability in both EV research and clinical diagnostics.

METHOD

Working principle of FluoPADE assays is illustrated in Figure 1. CD63 aptamer and HER2 aptamer were employed in FluoPADE assays working on EVs suspended in buffer and human plasma. EVs produced from three cancer cell lines including HT-29 (colorectal cancer), SKBR3 (breast cancer) and HepG2 (liver cancer) were target EV sources.

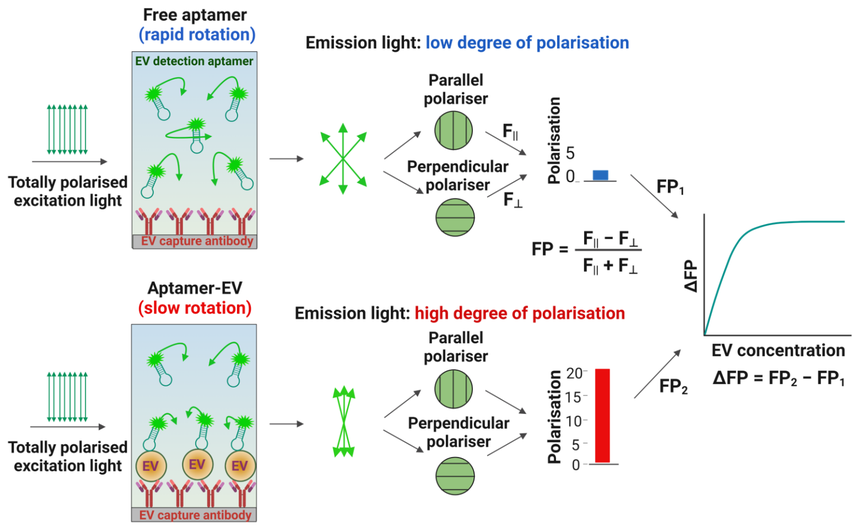


Figure 1. Schematic illustration of working principle of fluorescence polarisation-based cancer EV detection assay using aptamers (FluoPADE assay).

RESULTS

1. Assay design and working strategies

FluoPADE assays consist of three steps including immobilising EVs, adding detection aptamers, and reading the FP signals using a microplate reader (Fig. 2). Cancer-derived EVs can be detected and quantified by either FluoPADE assay 1 using antibodies against cancer biomarkers (EpCAM) as capture antibodies and aptamer against EV marker (CD63, CD63-BP aptamer) as detection aptamer or FluoPADE assay 2 with capture antibodies for EV markers and detection aptamers against cancer biomarker (HER2, HER2-HApt aptamer) (Fig. 2).

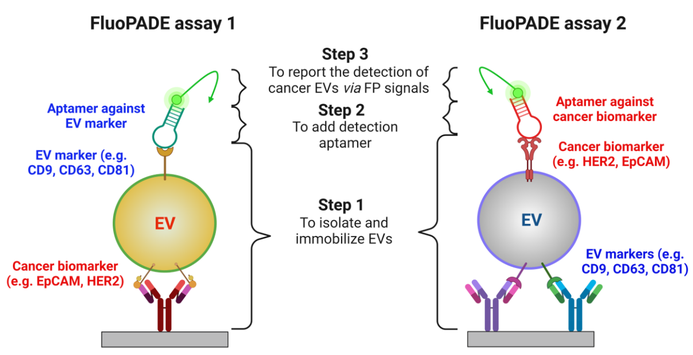


Figure 2. The design of and the steps involved in the FluoPADE assays.

RESULTS

2. FluoPADE assays have great potentials for both EV research and clinical applications with outperformance over fluorescence intensity assays

FluoPADE assays could specifically detect EpCAM$^+$ and HER2$^+$ EVs at low limits of detections and quantify these EVs in wide linear ranges (Table 1).

Moreover, aptamer-based FluoPADE assays could quantify EVs in wider ranges as opposed to respective fluorescence intensity (FI) assays which has been most utilized as a measurement quantity in fluorescence-based quantitative assays (Table 1).

Table 1. Summary of limits of detection (LOD) and linear dynamic ranges of FluoPADE assays and fluorescence intensity (FI) assays

Method	Detection probe	LOD in PBS (EVs/mL)	Linear range in PBS (EVs/mL)	LOD in plasma (EVs/mL)	Linear range in plasma (EVs/mL)
FluoPADE	CD63-BP	5.0×10^6	$5.0 \times 10^8 – 2.0 \times 10^{10}$	5.0×10^7	$5.0 \times 10^8 – 1.0 \times 10^{10}$
FluoPADE	HER2-HApt	3.0×10^7	$5.0 \times 10^8 – 2.0 \times 10^{10}$	5.0×10^7	$8.0 \times 10^7 – 1.0 \times 10^{10}$
FI	CD63-BP	2.0×10^8	$3.0 \times 10^8 – 2.0 \times 10^9$	5.0×10^8	$1.0 \times 10^9 – 1.0 \times 10^{10}$
FI	HER2-HApt	5.0×10^8	$1.0 \times 10^9 – 2.0 \times 10^{10}$	1.0×10^9	$3.0 \times 10^9 – 1.0 \times 10^{10}$
FI	Anti-CD63 antibody	1.0×10^7	$5.0 \times 10^7 – 1.0 \times 10^9$	5.0×10^7	$1.0 \times 10^8 – 2.0 \times 10^9$
FI	Anti-HER2 antibody	1.0×10^9	$2.0 \times 10^9 – 2.0 \times 10^{10}$	3.0×10^9	$5.0 \times 10^9 – 1.0 \times 10^{10}$

Data shown are means ± S.D., n =3.

3. FluoPADE assays are very sensitive

FluoPADE assays are able to detect one biomarker-positive EV in the background of 1000 biomarker-negative EVs (Fig. 3).

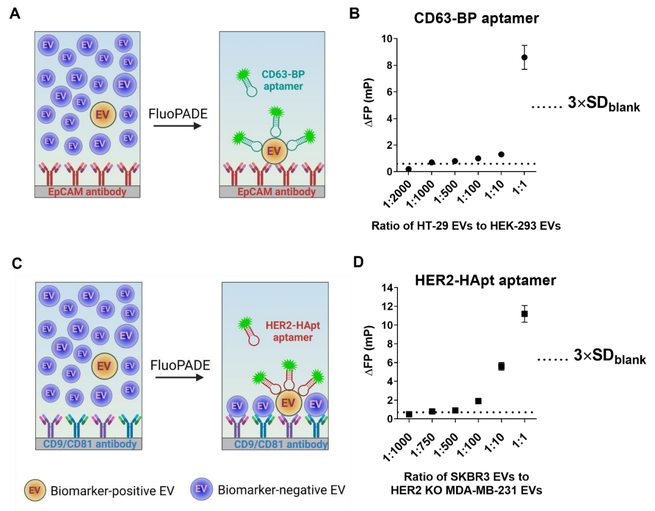
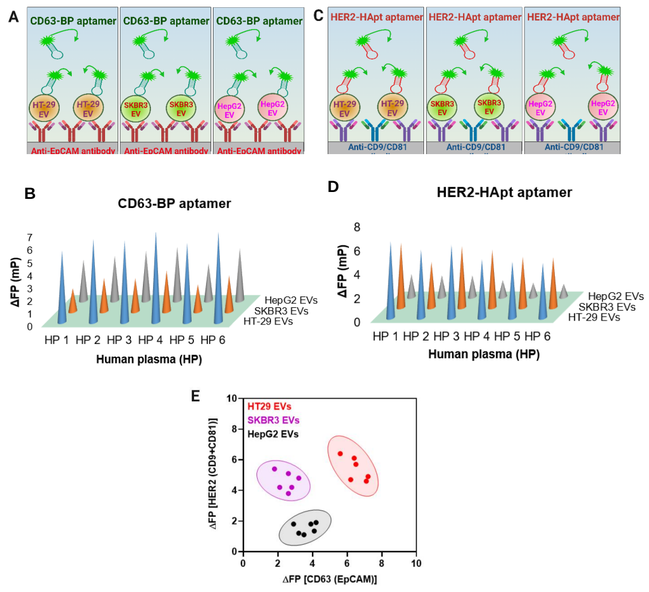


Figure 3. The sensitivity of FluoPADE assays. (**A**), Schematic illustration of the sensitivity assay using CD63-BP aptamer. (**B**), The ΔFP of serially diluted EpCAM-positive HT-29 EVs with EpCAM-negative HEK293 EVs but maintaining the total EV concentration using CD63-BP aptamer. (**C**), Schematic illustration of the sensitivity assay using HER2-HApt aptamer. (**D**), The ΔFP of serially diluted HER2-positive SKBR3 EVs with HER2-negative knockout MDA-MB-231 EVs but maintaining the total EV concentration using HER2-HApt aptamer. Data shown are means ± S.D., n=3.

4. The FluoPADE is capable of differentiating EVs

Dual-parametric analyses of cancer-derived EVs using FluoPADE assays enable the delicate classification of EVs from three different origins of disparate cell types of solid cancer. (Fig. 4).

Figure 4. The FluoPADE is capable of differentiating EVs displaying the same set of biomarkers but from different sources *via* dual analysis of FluoPADE assay. **(A)** and (**C**), Schematic illustrations of FluoPADE assays detecting EVs from three different cancer cell lines spiked into human plasma from 6 individual donors using CD63 aptamer and HER2 aptamer, respectively. (**B**) and (**D**), Differential changes in FP generated in FluoPADE assays illustrated in **A** and **C**, respectively. (**E**), Clustering plot of HT-29 EVs, SKBR3 EVs and HepG2 EVs prepared from ΔFPs using CD63 aptamer as in **B** and ΔFPs using HER2 aptamer as in **D**. Data shown are representative of three independent experiments.



CONCLUSION

FluoPADE assays were successfully developed with novelty, simplicity, specificity, and sensitivity for quantifying and classifying EVs in buffer and human plasma. Collectively, FluoPADE assays hold a great potential for applications in personalised precision oncology.

REFERENCES

[1] Mathieu M, Martin-Jalular L, Lavieu G, Théry C. Nat Cell Biol, 21 (1) (2019), pp. 9-17. [2] Jameson DM, Ross JA. Chem Rev, 110 (5) (2010), pp. 2685-708.