Supplemental information

An Integrated Microfluidic Platform for Detection of Ovarian Clear Cell Carcinoma mRNA Biomarker FXYD2

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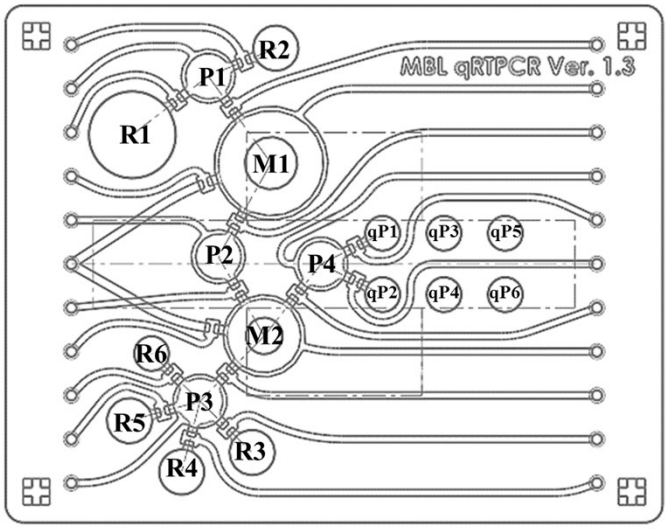

Figure S1. The microfluidic chip for mRNA detection with microcomponents coded for interpretation of Table S1. R1, R2, R3, R4, R5 and R6 are reservoirs for PBS, lysis buffer, Buffer A, Buffer B, Tris-HCl, and water, respectively; P1-4 are micropumps for transporting liquids; M1 and M2 are the micromixer/micropumps in zones 1 and 2, respectively; qP1 and qP2 are the qRT-PCR chambers for the target and reference gene, respectively. The qP3 and qP5 wells were loaded with the negative and positive controls for FXYD2 qPCR, respectively. Meanwhile, the qP4 and qP6 wells were preloaded with the negative and positive controls for GAPDH qPCR, respectively.

Table S1. The actuation procedures of the on-chip detection protocol

Protocol	Steps and duration (min)	Reagents	Chamber/micro-component	Volume
1. Reagent pre-loadings		PBS	R1	400 µL
		Lysis buffer	R2	100 µL
		Buffer A	R3	100 µL
		Buffer B	R4	100 µL
		Tris buffer	R5	100 µL
		ddH_2O	R6	50 µL
		Oligo-dT beads	M2	
		FXYD2 qRT-PCR mixture	qP1	10 µL
		GAPDH qRT-PCR mixture	qP2	10 µL
2. Cancer cell capture	a. Sample loading	200 µL PBS-resuspended ascites + 20 µL aptamer-coated beads	M1	220 µL
	b. Sample mixing (20)	220 µL cells and bead mixture	M1	220 µL
	c. Bead capture (3)	220 µL cells and bead mixture	M1	220 µL
	d. Supernatant removal (1)	220 µL cells and bead mixture	M1 ➔ W	220 µL
	e. PBS washing	20 µL PBS	R1 ➔ P1	20 µL
	f. PBS washing	20 µL PBS	P1 ➔ M1	20 µL
	g. Repeat e & f 9 times (1)	20 µL PBS		
	h. PBS washing (1)	200 µL PBS with beads	M1	200 µL
	i. Bead capture (3)	200 µL PBS with beads	M1	200 µL
	j. Supernatant removal (1)	200 µL PBS with beads	M1	200 µL
	k. Repeat e-j once (6)			
3. Cell lysis	a. Lysis buffer addition	20 µL Lysis buffer	R2 ➔ P1	20 µL
	b. Lysis buffer addition	20 µL Lysis buffer	P1➔ M1	20 µL
	c. Repeat a & b 4 times (1)			
	d. Gentle mixing (5)	100 µL Lysis buffer	M1	100 µL
	e. Bead capture (3)	100 µL Lysis buffer	M1	100 µL
	f. Lysate transfer to second mixer	20 µL Lysate	M1 ➔ P2	20 µL
	g. Lysate transfer to second mixer	20 µL Lysate	P2 ➔ M2	20 µL
	h. Repeat e-g 4 times (1)	20 µL Lysate		
4. mRNA extraction	a. Lysate and oligo-dT bead mixing (15)	100 µL Lysate + oligo-dT beads	M2	100 µL
	b. Bead capture (3)	100 µL Lysate + oligo-dT beads	M2	100 µL
	c. Supernatant removal (1)	100 µL Lysate + oligo-dT beads	M2 ➔ W	100 µL
	d. Buffer A addition	20 µL Buffer A	R3 ➔ P3	20 µL
	e. Buffer A addition	20 µL Buffer A	P3 ➔ M2	20 µL
	f. Repeat d & e 4 times (1)			
	g. Buffer A washing (1)	100 µL Buffer A + oligo-dT beads	M2	100 µL
	h. Bead capture (3)	100 µL Buffer A + oligo-dT beads	M2	100 µL
	i. Supernatant removal (1)	100 µL Buffer A + oligo-dT beads	M2 ➔ W	100 µL

	j. Buffer B addition	20 µL Buffer B	R4 ➔ P3	20 µL
	k. Buffer B addition	20 µL Buffer B	P3 ➔ M2	20 µL
	l. Repeat j & k 4 times (1)			
	m. Buffer B washing (1)	100 µL Buffer B	M2	100 µL
	n. Bead capture (3)	100 µL Buffer B + oligo-dT beads	M2	100 µL
	o. Supernatant removal (1)	100 µL Buffer B + oligo-dT beads	M2 ➔ W	100 µL
	p. Tris buffer addition	20 µL Tris buffer	R5 ➔ P3	20 µL
	q. Tris buffer addition	20 µL Tris buffer	P3 ➔ M2	20 µL
	r. Repeat p & q 4 times (1)			
	s. Tris buffer washing (1)	100 µL Tris buffer	M2	100 µL
	t. Bead capture (3)	100 µL Tris buffer + oligo-dT beads	M2	100 µL
	u. Supernatant removal (1)	100 µL Tris buffer + oligo-dT beads	M2 ➔ W	100 µL
	v. Water addition	20 µL ddH_2O	R6 ➔ P3	20 µL
	w. Water addition (1)	20 µL ddH_2O	P3 ➔ M2	20 µL
5. qRT-PCR preparation	a. Mix oligo-dT beads (1)	20 µL ddH_2O + oligo-dT beads	M2	20 µL
	b. Transfer to qRT-PCR chambers	20 µL ddH_2O +oligo-dT beads	M2 ➔ P4	20 µL
	c. Transfer to qRT-PCR chambers (1)	20 µL ddH_2O + oligo-dT beads	P4 ➔ qP1 & qP2	20 µL
6. qRT-PCR	a. qRT-PCR (140)		qP1, qP2, qP3-qP6	20 µL

Supplemental videos

The link for the video showing the liquid transport in the microfluidic chip.

Supplemental methods

Cell culture

Cells were sub-cultured every 3 to 4 days with 10 mL of culture media in a sterile petri dish (100 mm x 15 mm) as follows. First, old media was removed, and the cells were washed with sterile 1x PBS (pH 7.4, cat: #10010023, Gibco, USA). Then, 1 mL of trypsin (TFS) was added to detach cells for 5 min. The detached cells were pelleted at 300 ×g for 5 min, resuspended in 1 mL of 1X PBS, and ~10^4 cells were inoculated in fresh media at 37°C with humidified 5% CO_2. TOV-21G cells (ATCC, USA) were maintained in 10 mL of a 1:1 mixture of MCDB 105 media (M6395; Sigma-Aldrich) containing a final concentration of 1.5 g/L sodium bicarbonate and Medium 199 (M5017; Sigma-Aldrich) containing a final concentration of 2.2 g/L sodium bicarbonate. The base media was supplemented with 10% fetal bovine serum (FBS; TFS). OVCAR-3 cells (National Cancer Institute #0507709, USA) were maintained in RPMI-1640 cell culture media (Gibco) with 10% FBS. N(E6+E7) cells were provided by NCKUH and cultured in Keratinocyte-SFM 1x (17005042; Gibco). For media preparation, 500 mL of basal media were mixed with 25 mg bovine pituitary extract (BPE) and 2.5 µg human recombinant epidermal growth factor (EGF).These two supplements both were provided with the basal media.

Preparation of aptamer-coated nano-magnetic beads (NMBs)

A previously screened aptamer (21G-2)[31] against TOV-21G cells was conjugated to the surface of AllMag^{TM} NMBs (PM3-020, So-Fe Biomedicine, China) through N-(3-

dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)/N-hydroxysulfo-succinimde sodium salt (NHS) activation. First, 200 µL of NMB (10 mg/mL, 1.4 x 10^{11} beads/mL) were washed twice with 500 µL of 10 mM 2-(N-morpholino)-ethanesulfonic acid (MES) buffer with 0.05% Tween-20 (MEST; pH 6.0) and resuspended in 100 µL of 10 mM MES. Then, 200 µL of 5 mg/mL EDC and 200 µL of 5 mg/mL NHS (H52795, Alfa Aesar, USA) were added to the washed NMB in 500 µL of MES buffer. The carboxyl groups on NMB were activated via EDC/NHS and incubated at 37°C with continuous rotation at 150 rpm for 30 min. Afterwards, the NMBs were washed twice with 500 µL of MEST buffer. Then, 500 µL of 1 µM aptamer were added, and the mixture was incubated for 3 hr at 37°C (150 rpm) to generate aptamer-NMB complexes. The complexes were separated from unreacted reagents by an external magnet (DynaMag™, TFS), with the supernatant then decanted. The collected conjugates were washed twice with 1 mL of 1x PBS with 0.05% Tween-20 (pH 7.4), resuspended in 2 mL of 1x PBS at a final concentration of 1 mg/mL, and stored at 4°C.

Oligo-dT beads for mRNA capture

According to the manual provided by the manufacturer, the mRNA binding capacity of oligo-dT beads used within the Dynabeads™ mRNA DIRECT™ Purification Kit (TFS) is that 1 mg beads can bind to 2 µg mRNA. After calculation, it was then estimated that 5 µL of oligo-dT beads for each reaction could be sufficient to capture 50 ng mRNA. From the same manual, it also reported that the amount of mRNA for one mammalian cell is about 1-5% of total RNA which is about 10-30 pg each cell. Furthermore, the total RNA was reported to be about 10 pg, and the mRNA was 0.1 pg for a mammalian cell (Tang, F., Lao, K. & Surani, M. *Nat Methods* 2011, **8**, S6–S11). When assuming the highest amount mRNA for each cell, for the highest cell number (10^4) in each reaction, it was calculated that the mRNA was about 15 ng. Therefore, the amount of oligo-dT beads should be sufficient in most cases for capturing the mRNA from lysed cells.