OMTN, Volume *19*

Supplemental Information

Binding and Structural Properties of DNA Aptamers with VEGF-A-Mimic Activity

Toru Yoshitomi, Misako Hayashi, Takumi Oguro, Keiko Kimura, Fumiya Wayama, Hitoshi Furusho, and Keitaro Yoshimoto

Supporting information

Binding and structural properties of DNA aptamers with VEGF-A-mimic activity

Toru Yoshitomi[1], Misako Hayashi[1], Takumi Oguro[1], Keiko Kimura[1], Fumiya Wayama[1], Hitoshi Furusho[2], and Keitaro Yoshimoto*[,1,3]

[1]Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo 153-8902, Japan

[2]Chemical General Division, Nissan Chemical Industries, Ltd., 2-10-2 Tsuboi-nishi, Funabashi, Chiba 274-8507, Japan

[3]JST, PRESTO, The University of Tokyo, Komaba 3-8-1, Meguro, Tokyo 153-8902, Japan

*Corresponding author: Keitaro Yoshimoto, Ph.D., Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Meguro, Tokyo 153-8902, Japan

Phone and FAX: +81-3-5454-6580

E-mail: ckeitaro@mail.ecc.u-tokyo.ac.jp

Table S1. *In vitro* selection conditions

Selection		MB	[MB]	[ssODN]	Incubation volume (µL)	Number of washes	Washing volume (µL)
cycle	round		(µg)	(µM)			
1	1	MB1	8.5	0.64	20	3	200
	2	MB2		0.25			
2	3	MB1		0.28			
	4	MB2		0.15			
3	5	MB1		0.17			
	6	MB2		0.21			

Table S2. Abundance distribution of Apt01 families (5′-XGTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCXXXX-3′) at each round.

Selection cycle	Selection target	Number of total reads	Sequence (5′ to 3′)	Ranking	Counting number	Total counting number	Total abundance ratio (%)
1	VEGFR-1	32,984	Not found	-	ND	ND	0
	VEGFR-2	52,245	GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCCC	5th	3	3	0.06
2	VEGFR-1	32,173	GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCCC	2nd	185	287	0.892
			GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCC	5th	90		
			GTCGTGTTTGTTGTTGTTTTCATTTTTTGCGGCC	42nd (tie)	6		
			GTCGTGTTTGTTGTTGTTTTCATTTTTTGCGGCCC	42nd (tie)	6		
			GTCGTGTTTGTTGTTGTTTTCATTTTTGTGGCCC	757th (tie)	1		
			GTCGTGTTTGTTGTTGTTTTCGATTTTTGCGGCC	757th (tie)	1		
	VEGFR-2	34,000	GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCCC	4th	60	90	0.26
			GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCC	8th	29		
			GTCGTGTTTGGTTGTTGTTTCATTTTTGCGGCC	1459th	1		
3	VEGFR-1	22,093	GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCCC	2nd	238	351	1.59
			GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCC	10th	82		
			GTCGTGTTTGTTGTTGTTTTCATTTTTTGCGGCCC	42nd	8		
			GTCGTGTTTGGTTGTTGTTTTCATTTTTGCGGCCC	72nd	5		
			GTCGTGTTTGTTGTTGTTTTCATTTTTTGCGGCC	103rd	4		
			GTCGTGTTTGTTGTTGTTTCATTTTTGCGGCC	278th (tie)	2		
			GTCGTGTTTGTTGTTGTTTTCATTTTGCGGCCC	278th (tie)	2		
			GTCGTGTTTATTGTTGTTTTCATTTTTGCGGCCC	819th (tie)	1		

		GTCGTGGTTTGTTGTTGTTTTCATTTTTGCGGCC	819th (tie)	1		
		GGTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCCC	819th (tie)	1		
		GTCGTGTTTGTTGTTGTTTTCATTTTTGCTGCCC	819th (tie)	1		
		GTCGTGTTGTTGTTGTTTTCATTTTGGCGGCC	819th (tie)	1		
		GTCGTGTTTGTTGTTGTTTTCATTTTGCGGCC	819th (tie)	1		
		GTCGTGTTTGTTGTTGTTTTCGATTTTTGCGGCCC	819th (tie)	1		
		GTCGTGTTTGTTGTTGTTTTTCATTTTTTGCGGCC	819th (tie)	1		
		GTCGTTTTTGTTGTTGTTTTCATTTTTGCGGCCC	819th (tie)	1		
VEGFR-2	24,684	GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCCC	5th	165	266	1.07
		GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGCC	7th	86		
		GTCGTGTTTGTTGTTGTTTTCATTTTTTGCGGCC	51st	5		
		GTCGTGTTTGTTGTTGTTTTCATTTTTTGCGGCCC	65th	4		
		GTCGTGTTTGGTTGTTGTTTTCATTTTTGCGGCCC	98th	3		
		GGTCGTGTTTGTTGTTGTTTTCATTTTTGGCGGCCC	824th (tie)	1		
		GTCGTGTTTGGTTGTTGTTTTCATTTTTGCGGCCT	824th (tie)	1		
		GTCGTGTTTGTTGTTGTTTTCATTTTTGCGGTCC	824th (tie)	1		

Table S3. Abundance distribution of Apt02 families (5′-GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGG XXX-3′) at each round

Selection		Number of total reads	Sequence (5′ to 3′)	Ranking	Counting number	Total counting number	Total abundance ratio (%)
cycle	target						
1	VEGFR-1	32,984	Not found	-	ND	ND	ND
	VEGFR-2	52,245	Not found	-	ND	ND	ND
2	VEGFR-1	32,173	GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGGCC	23rd	14	22	0.068
			GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGGGCC	38th	7		
			GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGCC	772nd	1		
	VEGFR-2	34,000	GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGGCC	14th	18	30	0.088
			GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGGGCC	16th	11		
			GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGCC	1,014th	1		
3	VEGFR-1	22,093	GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGGCC	22nd	18	22	0.10
			GCTGATAGGATGGGTTGTAGGTATAGGGGGGGGCC	819th (tie)	1		
			GCTGATAGGATGGGTTGTAGGTCTAGAGGGGGGCC	819th (tie)	1		
			GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGGGCC	819th (tie)	1		
			GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGGGGC	819th (tie)	1		
	VEGFR-2	24,684	GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGGCC	34th (tie)	7	15	0.061
			GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGGGCC	34th (tie)	7		
			GCTGATAGGATGGGTTGTAGGTCGTAGGGGGGGGCC	824th (tie)	1		
			GCTGATAGGATGGGTTGTAGGTCTAGGGGGGGCC	824th (tie)	1		

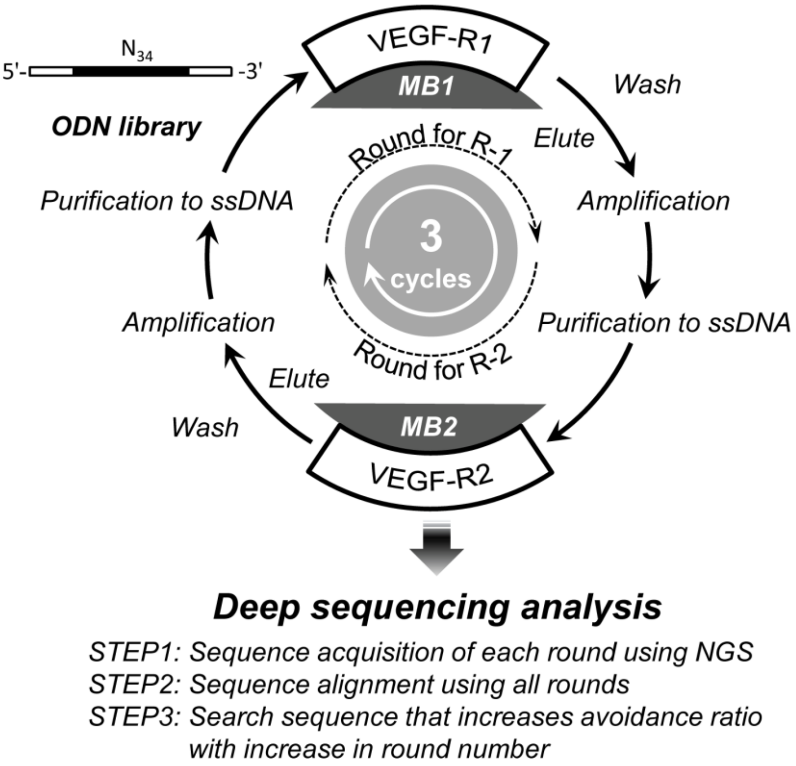

Fig. S1 Schematic of alternating consecutive selection of DNA aptamers using VEGFR-1 and VEGFR-2 immobilized on magnetic beads, referred to as MB1 and MB2, respectively, with deep sequencing analysis.

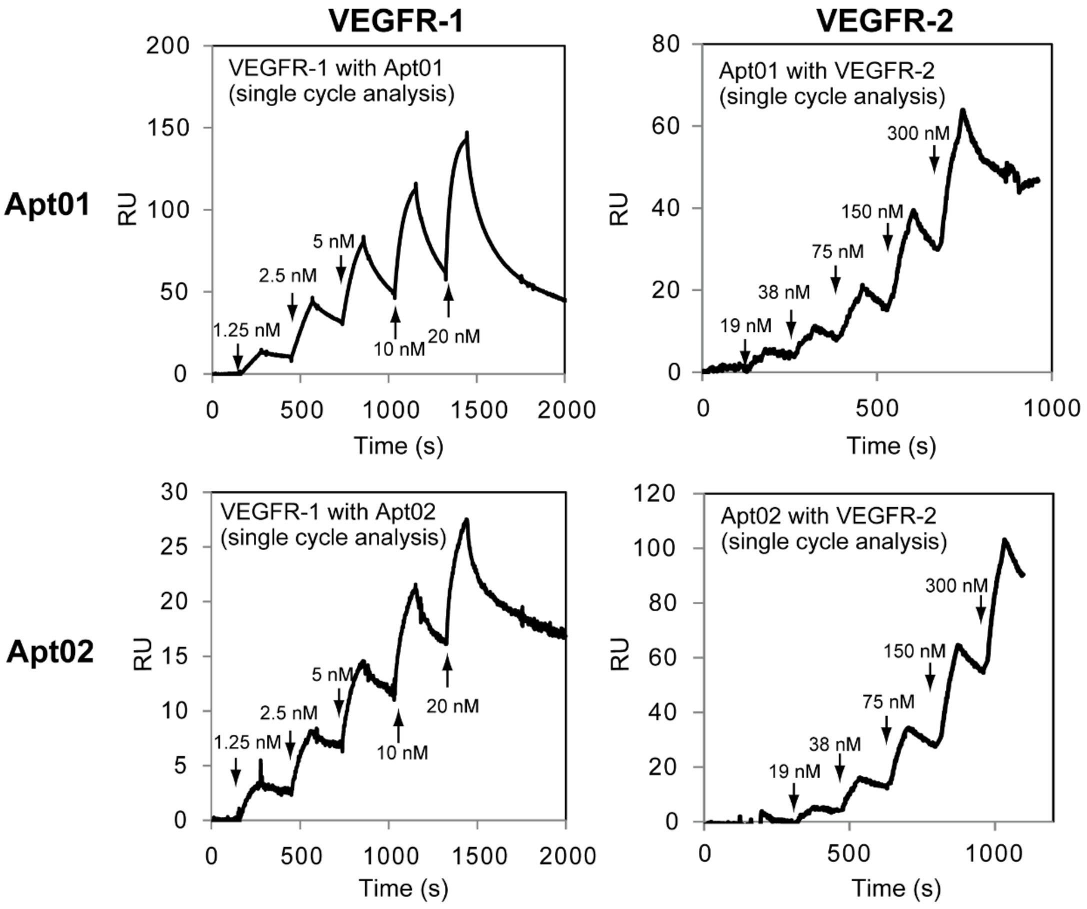

Fig. S2 SPR sensorgrams used to determine the binding affinities according to single-cycle kinetic analysis.

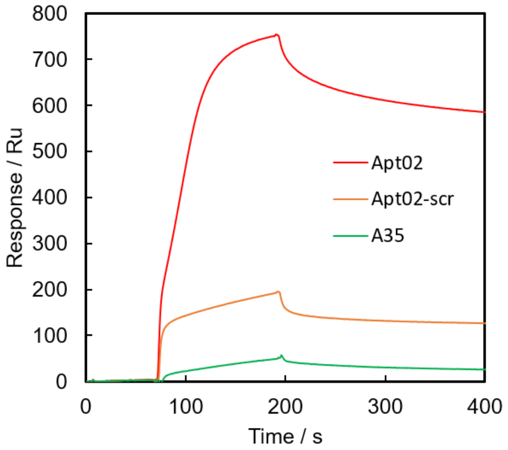

Fig. S3 SPR sensorgrams of Apt02, Apt02-scr, A35 binding to VEGFR-1.

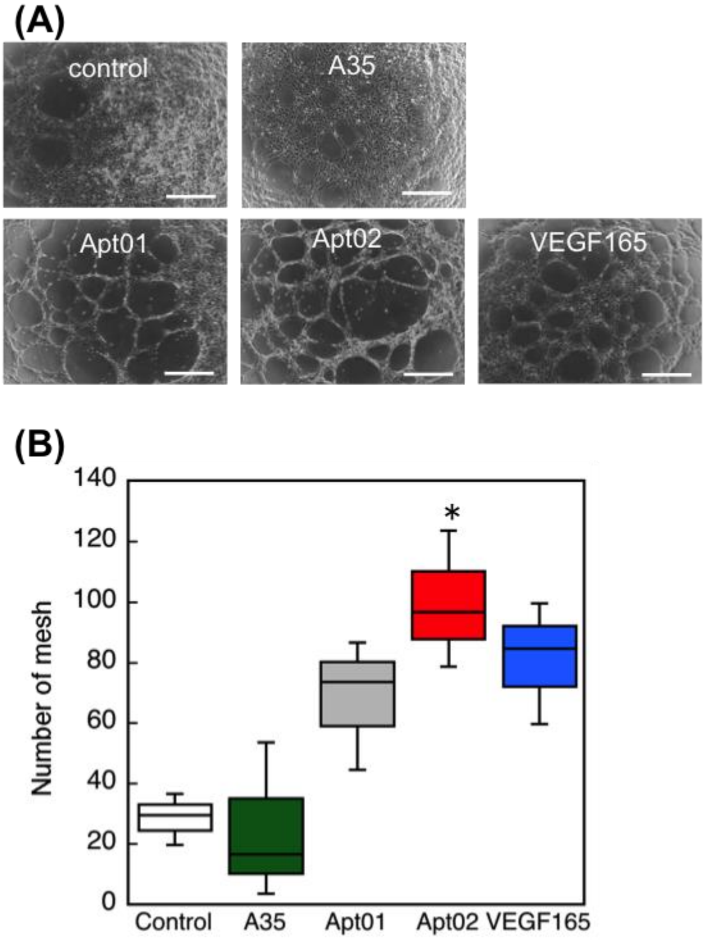

Fig. S4 *In vitro* tube formation assay using human umbilical vein endothelial cells (HUVECs) on a three-dimensional gel consisting of diluted Matrigel. The cells were treated with Apt01 (20 μM), Apt02 (20 μM), A35 (20 μM) or VEGF165 (20 ng/mL, 0.52 nM). (A) Representative images of tube formation of human umbilical vein endothelial cells (HUVECs) on Matrigel at 4 h. Scale bars are 0.5 mm. (B) Number of mesh in the images of the HUVECs networks at 4 h. (n=3 plots). * $P < 0.05$ as compared to control.

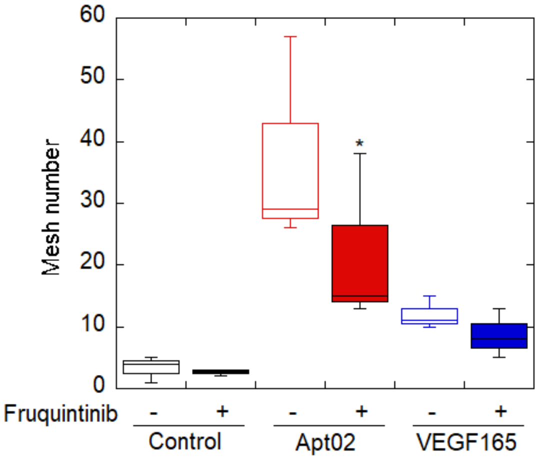

Fig. S5 Inhibition of HUVEC tubule growth by fruquintinib at 5 h. The cells were treated by Apt02 (20 µM), or VEGF165 (20 ng/mL, 0.52 nM) with or without highly selective small molecule inhibitor of VEGFR tyrosine kinases, fruquintinib (10µM). Number of mesh of the HUVECs networks was counted at 5 h. (n=3 plots). * $P < 0.05$ as compared to Apt02-treated group without inhibitor.