Expression of Stage Specific Embryonic Antigen-4 (SSEA-4) defines spontaneous loss of epithelial phenotype in human solid tumor cells

Running title: SSEA-4 defines changes in epithelial phenotype of solid tumors

Kavitha Sivasubramaniyan[1†], Abhishek Harichandan[1, 4†], Karin Schilbach[2], Andreas Mack[3], Jens Bedke[4], Arnulf Stenzl[4], Lothar Kanz[1], Gerhard Niederfellner[5], Hans-Jörg Bühring[1*]

[1] University Clinic of Tübingen, Department of Internal Medicine II, Division of Hematology, Immunology, Oncology, Rheumatology and Pulmonology, Tübingen, Germany

[2] University Children's Hospital, Department of Pediatric Stem Cell Transplantation, Tübingen 72076, Germany

[3] Institute of Clinical Anatomy and Cell Analysis, Eberhard Karls University of Tübingen

[4] University Clinic of Tübingen, Department of Urology, Tübingen, Germany

[5]Discovery Oncology, Pharma Research and Early Development, Roche Diagnostics GmbH, Penzberg, Germany

[†] authors contributed equally to this work

*Authors of correspondence: **Dr. Hans-Jörg Bühring, Ph.D.**
University Clinic of Tübingen, Department of Internal Medicine II, Division of Hematology, Immunology, Oncology, Rheumatology and Pulmonology, Otfried-Müller-Str. 10, 72076 Tübingen, Germany
Email: hans-joerg.buehring@uni-tuebingen.de
Ph: +49-7071-2982730
Fax: +49-7071-292730

Suppl. Table 1. Reactivity profiles of existing in-house generated mAbs on different cell lines

	A-172	Caco-2	MCF-7	A431	T47D	HT1080	Calu-3	K562	U-118	DU4478	HC-1	KU812
2E4B4A11	++	-	+ (sub)	+++	-	++	-	-	+++	+	-	++
6D3H7	-	+++	++	+++	+++	++	++	+++	+++	+++	++	+++
7C5G1	+	++	-	+++	+	+	++	-	+++	++	+ (sub)	++
9A3G2	-	++	-	+	+	-	-	-	++	++	-	++
56D5A1A8	-	-	-	+ (sub)	+	-	-	++	+	++	-	++
56D2H5	+	-	-	+++	+++	-	-	++	+	++	++ (sub)	+++
58B1A2	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	-	++
59A3B3	-	++	++	+++	+++	+	++	++	+++	+++	++	+++
BV2A5B6	+++	-	-	+	++	+++	-	-	+++	++	-	++
BV8C2C2	-	-	-	+	++	+	-	++	+	++	+	++
HEK-3D6	+++	++	+++	+++	+++	+	+ (sub)	++	++	+++	-	++
HEK-6D6	-	-	-	++	+++	-	-	-	-	+++	-	+
HEK-8C6	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	+++	+++
HEK4-1A1	+	+	++	++	++	+	+	++	++	++	+	+++
HEK4-2D6	-	-	-	-	++	+	-	-	-	++	-	+++
HEK5-1B3	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	-	+++
HEK5-2B5	-	-	-	-	+++	+	-	++ (sub)	+	++	-	++
HEK7-4D1	-	+++	++	+++	+++	++	++	+++	+++	+++	++	+++
HEK9-2B5	-	++	++	+++	+++	++ (sub)	+	+++	+++	+++	++	+++
HEPB3	++	+	-	++	++	-	-	+	+	+	-	++
HEPC20	-	-	-	+	+	-	-	-	-	+	-	++
W1D6C4	-	+	+	+	+	-	-	-	-	+	-	++
W3D5A9	+	++	++	+	++	-	+	+ (sub)	-	-	-	++
W5C4C5	+++	++	+++	+++	++	++	++	++	+++	-	-	++
W5D3B11	++	+ (sub)	+++	-	++	-	-	-	-	-	-	+
W5C5A8	+	+	++	++	++	-	+	+ (sub)	-	-	-	+
HEK9-3C2	++	++	+++	+++ (sub)	++	-	+	+ (sub)	++	-	-	+
CH1A4D1	-	-	+++	-	+	-	-	-	-	+	-	++
CH2A3B5	-	-	+	-	+	-	-	-	-	-	-	-
CH4D3	-	-	+++ (sub)	-	++	+	-	-	+	++ (sub)	-	-

(sub) reacts only with a subpopulation of cells; + weak reactivity; ++ medium reactivity; +++ strong reactivity.

Suppl. Table 2. Reactivity profiles of newly generated iPS122 derived mAbs on different cell lines

(sub) reacts only with a subpopulation of cells; + weak reactivity; ++ medium reactivity; +++ strong reactivity.

	SW480	SW620	HCT-116	Caco-2	MCF-7	Tcam-2	NT2	NCCIT	2102 Ep	PC3	DU 145	Weri-Rb1	HeLa
IPS-K-1A6G5	-	+	+ (sub)	-	-	++ (sub)	++ (sub)	++ (sub)	++ (sub)	+	++ (sub)	-	-
IPS-K-2B6A8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IPS-K-1D4C6	-	+	++	-	+	+++	+	-	-	+++	+	-	+++
IPS-K-3C1D1	+++	+++	+++	+	+++	+++	+++	-	++	+++	+++	+	+++
IPS-K-3C2D5	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	-	+++
IPS-K-3C4A6	-	+	-	-	+	++ (sub)	+ (sub)	+ (sub)	+ (sub)	(+)	-	-	-
IPS-K-3C5G8	+++	+++	+++	++	+++	+++	+++	+	++	+++	+++	++	+++
IPS-K-4A2A9	-	+	-	-	+	-	-	+	+	+	-	-	-
IPS-K-4A2B8	++ (sub)	++ (sub)	+ (sub)	++ (sub)	++ (sub)	++ (sub)	+++	++	+++	++ (sub)	+ (sub)	-	-
IPS-K-4B5G6	+++	++	+++	(+)	+++	++	+++	-	++	+++	+++	+	+++

Suppl. Table 3. Cross blocking of GMb1 on the binding of SSEA-4 specific antibodies

Blocking glycosphingolipid GMb1 (μM)	% inhibition of test mAb binding	
	MC813-70	IPS-K-4A2B8
0	0	0
15	9.8	0
30	20.3	0
45	24.6	0
60	19.4	0
75	20.2	0

Suppl. Table 4. Generation of tumors by SSEA-4$^{+/-}$ cells in vivo

Cell type	SSEA-4^{+}			SSEA-4^{-}			Unfractionated		
No. of cells	**150,000**	**50,000**	**3,000**	**150,000**	**50,000**	**3,000**	**150,000**	**50,000**	**3,000**
No. of sites of tumor formation	**4/4**	**4/4**	**6/6**	**4/4**	**4/4**	**2/6**	**4/4**	**4/4**	**4/6**

Suppl. Table 5. Commercial antibodies used in the study

Antibody	Source	Catalogue Number
Mouse anti-human CD24-PE	Miltenyi Biotec	130-098-861
Mouse anti-human CD29-PE	BioLegend	303003
Mouse anti-human CD44-FITC	BD Biosciences	555478
Mouse anti-human CD49a-PE	BD Biosciences	559596
Mouse anti-human CD49b-PE	BD Biosciences	555669
Mouse anti-human CD49e-PE	BD Biosciences	555617
Mouse anti-human CD49f-FITC	BD Biosciences	555735
Mouse anti-human CD51-FITC	BD Biosciences	555505
Mouse anti-human CD90-APC	BD Biosciences	561971
Mouse anti-human CD117-APC	BD Biosciences	561118
Mouse anti-human CD133-PE	Miltenyi Biotec	130-098-046
Mouse anti-human CD324-PE	eBioscience	46-3249
Mouse anti-human CD324-APC	Miltenyi Biotec	130-099-723
Mouse anti-human CD326-PE	eBioscience	12-9326
Mouse anti-human CD340-PE	BD Biosciences	340552
Mouse anti-human Tra-1-60-PE	BD Biosciences	560884
Mouse anti-human Tra-1-81-PE	BD Biosciences	560885
Mouse anti-human TNAP-PE	Miltenyi Biotec	130-093-587
Mouse anti-human Trop-2-APC	R&D systems	FAB650A
Mouse anti-human SSEA-3-PE	BD Biosciences	560879
Mouse anti-human SSEA-3-FITC	BD Biosciences	560881
Purified mouse anti-human Caveolin-1	BD Biosciences	611338
Purified mouse anti-human cortactin (p80/85)	Millipore	05-180
Purified mouse anti-human EEA1	Abcam	ab70521
Purified mouse anti-human GM130-Alexa Fluor 488	BD Biosciences	560257
Purified mouse anti-human CD29	BioLegend	303001
Purified mouse anti-human CD49b	BioLegend	359301
Purified mouse anti-human CD49e	BioLegend	328002
Purified mouse anti-human CD49f	BioLegend	313602
Purified mouse anti-human CD51/61	BioLegend	304402
Purified mouse anti-human CD104	BioLegend	327802

Purified mouse anti-human CD107b (LAMP2)	BioLegend	354301
Purified rabbit anti-human Rab11	Abcam	ab3612
Polyclonal Goat anti-mouse immunoglobulin-PE	DAKO	R048001
Polyglobulin (Gamunex 10%)	Talecris Biotherapeutics	G130022
Purified mouse anti-human CD49a	BioLegend	328302
Purified rat anti-human CD49f	BD Biosciences	555734
Alexa Fluor 488 goat anti-mouse IgG2a	Life Technologies	A-21131
Alexa Fluor 488 goat anti-mouse IgG1	Life Technologies	A-21121
Alexa Fluor 488 goat anti-mouse IgG2b	Life Technologies	A-21141
Alexa Fluor 488 goat anti-mouse IgM	Life Technologies	A-21042
Alexa Fluor 488 goat anti-mouse IgG	Life Technologies	A-11029
Alexa Fluor 488 goat anti-rabbit IgG	Life Technologies	A-11034
Alexa Fluor 555 goat anti-mouse IgM	Life Technologies	A-21426
Cy3 Goat anti-mouse IgG	Life Technologies	A10521
Cy3 Goat Anti-rabbit IgG	Life Technologies	A10520

Suppl. Table 6. List of primers for qRT-PCR

Gene symbol	Gene name	Primer sequence	
CLDN7 (IS)	Claudin-7	**F**	GAGCCCTAATGGTGGTCTCC
		R	CACTTCATGCCCATCGTG
CDH1 (IS)	Cadherin 1, epithelial	**F**	CCGCGTCCTGGGCAGAGTGAA
		R	CGGGTGTCGAGGGAAAAATAGGCT
ESRP1 (IS)	Epithelial splicing regulatory protein 1	**F**	TTTTGAATCCACGAGCACTG
		R	CACCATACATCTTTCAAACACTGG
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	**F**	AGCCACATCGCTCAGACAC
		R	GCCCAATACGACCAAATCC
GRHL2 (IS)	Grainyhead-like 2	**F**	GGACAGCACATACAGCGAGA
		R	AGCCCCAACTGAAGCACTC
CDH2 (IS)	Cadherin 2, type 1, N-Cadherin	**F**	CTCCATGTGCCGGATAGC
		R	CGATTTCACCAGAAGCCTCTAC
ST3GAL2 (IS)	ST3 beta-galactoside alpha-2, 3-sialyltransferase 2	**F**	GTCCAGAGGTGGTGGATGAT
		R	CAGCACCTCATTGGTGTTGT

IS : Intron spanning