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ORIGINAL RESEARCH

Proteome array identification of bioactive soluble proteins/peptides in Matrigel: relevance to stem cell responses

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Abstract Matrigel and similar commercial products are extracts of the Engelbreth-Holm–Swarm sarcoma that provide a basement-membrane-like attachment substrate or gel that is used to grow cells on or in, respectively. To ascertain further what proteins may be present in Matrigel, besides its major basement-membrane constituents, an analysis of the expressed liquid of gelled Matrigel was performed using proteome array technology. Among the growth factors/cytokines assayed, high positive detection was found for IGFBP1, IGFBP3, LIF, platelet factor 4, P*l*GF-2, and VEGF; moderate reactivity was found for cyr61, IGFBP2, IGFBP6, IL-1ra, and NOV; and low, but detectable, responses occurred for aFGF, IL-13, IL-23, M-CSF, and VEGF-B. Among the chemokines assayed, high positive detection was found for MIG and serpin E1; moderate reactivity was found for IP-10, MCP-1, and MCP-5, and low, but detectable, responses occurred for CXCL16, I-TAC, and MIP-1α. Among the other biologically active proteins assayed, high positive detection was found for adiponectin, C5a, endocan, lipocalin-2, sICAM-1, MMP-3, and TIMP-1; moderate reactivity was found for C-reactive protein, coagulation factor III, endoglin, endostatin/collagen XVIII, endothelin-1, ICAM-1, MMP-9, osteopontin, pentraxin-3, and RANTES; and low, but detectable, responses occurred for fetuin A, MMP-8, pentraxin-2, RBP4, resistin, and TIMP-4. The study found several growth factors, chemokines, and biologically active proteins not previously identified in Matrigel, and this may have significance to the interpretations of observed cellular responses when cells are grown on or in Matrigel.

Keywords Cell culture · Extracellular matrix · Matrigel · Protein array

Abbreviations

ADAMTS1	A disintegrin and metalloproteinase with thrombospondin motifs 1
AgRP	Agouti-related protein; a.k.a. the protein product of the agouti-related transcript (ART)
ANGPT-L3	Angiopoietin-like 3
BLC	B lymphocyte chemoattractant; a.k.a. CXCL13; B-cell-attracting chemokine 1 (BCA-1)
CRP	C-reactive protein
C5a	Complement component 5a

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CCL	Chemokine (C-C motif) ligand - #
CXCL	Chemokine (C-X-C motif) ligand- #
Cyr61	Cysteine-rich protein 61, a.k.a. IGFBP-10
DLL4	Delta-like ligand 4
DPPIV	Dipeptidyl peptidase IV; a.k.a., cluster of differentiation 26 (CD26)
EGF	Epidermal growth factor
ESM-1	Endothelial cell-specific molecule-1; a.k.a. endocan
FGF-1	Fibroblast growth factor-1; a.k.a. acidic FGF (aFGF)
FGF-2	Fibroblast growth factor-2; a.k.a. basic FGF (bFGF)
FGF-7	Fibroblast growth factor-7; a.k.a. keratinocyte growth factor (KGF)
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte-macrophage-colony-stimulating factor
HB-EGF	Heparin-binding EGF-like growth factor
HGF	Hepatocyte growth factor
I-309	a.k.a., CCL1 and T-cell activation-3 (TCA-3)
ICAM-1	Intercellular adhesion molecule-1; a.k.a. CD54
IFN-γ	Interferon-gamma
IGF	Insulin-like growth factor-1 and -2
IGFBP	Insulin-like growth factor binding-protein-1, -2, -3, -5, and -6
IL	Interleukin-1α, -1β, -1ra, -2 thru -7, -10 thru -13, -16, -17, -23, and -27
IP-10	Interferon-inducible protein-10; a.k.a. CXCL10 and cytokine responsive gene-2 (CRG-2)
I-TAC	Interferon-inducible T-cell alpha chemoattractant; a.k.a. CXCL11
KC	Keratinocyte-derived chemokine; a.k.a. CXCL1 and growth-related oncogene alpha (GROα)
LIF	Leukemia inhibitory factor
MCP-1	Monocyte chemotactic protein-1; a.k.a. CCL2 and junctional epithelium chemokine (JE)
MCP-5	Monocyte chemotactic protein-5; a.k.a. CCL12
M-CSF	Macrophage-colony stimulating factor; a.k.a. CSF-1
MIG	Monokine induced by gamma-interferon; a.k.a. CXCL9
MIP	Macrophage inflammatory protein-1α; a.k.a. CCL3; -1β/CCL4; -2/CXCL2
MMP	Matrix metalloproteinase-3, -8, -9, and -14
NOV	Nephroblastoma overexpressed gene; a.k.a. CCN3 and IGFBP9
TSG-14	Tumor necrosis factor-stimulated gene-14; a.k.a. pentraxin-3
Pref-1	Preadipocyte factor 1, a.k.a. DLK-1 (delta-like protein-1)
RAGE	Receptor for advanced glycation endproducts
RANTES	Regulated on activation, normal T-cell expressed and secreted; a.k.a. CCL5
RBP4	Retinol-binding protein-4
PAI-1	Plasminogen activator inhibitor-1, a.k.a. serpin E1
PDGF	Platelet-derived growth factor A-chain homodimer (PDGF-AA), PDGF-BB and PDGF-AB
PD-ECGF	Platelet-derived endothelial cell growth factor
P*l*GF-2	Placenta growth factor 2
Serpin E1	Serine protease inhibitor, clade E, member 1; a.k.a. plasminogen activator inhibitor type 1 (PAI-1)
Serpin F1	Serine protease inhibitor, clade F, member 1; a.k.a. pigment epithelium-derived factor (PEDF)
SPARC	Secreted protein acidic and rich in cysteine
SDF-1	Stromal cell-derived factor-1; a.k.a. CXCL12
TARC	Thymus and activation-regulated chemokine; a.k.a. CCL17
TIMP	Tissue inhibitor of metalloproteinases-1 and -4
TNF-α	Tumor necrosis factor-alpha
TREM-1	Triggering receptor expressed on myeloid cells 1
VEGF	Vascular endothelial growth factor

Introduction

Matrigel, or similar products sold as Cultrex or EHS matrix, is a basement-membrane-like matrix extracted

from the Engelbreth-Holm–Swarm (EHS) mouse sarcoma (Kleinman and Martin 2005). It is primarily used for the in vitro culture of cells, either as an aid to cell attachment and growth or as a 3D biological gel in which cells are suspended and grown (Schuetz et al. 1988; Miyazaki et al. 2002; Kleinman and Martin 2005; Talbot et al. 2010; Nguyen-Ngoc and Ewald 2013). The EHS tumor is propagated in vivo and the extracellular matrix-like material extracted from it is mainly comprised of laminin (~60 %), collagen IV(~30 %), nidogen (~5 %), the heparan sulfate proteoglycan perlecan (~3 %), and entactin (~1 %) (Orkin et al. 1977; Kleinman et al. 1982, 1986). In addition, however, Matrigel has been found to contain various other biological components including MMP-2, MMP-9, urokinase [urokinase-type plasminogen activator (uPA)], tissue-type plasminogen activator, amylase, transferrin, and clusterin (Dirami et al. 1995; Gillette et al. 2003; Kleinman and Martin 2005).

Growth factors have also been identified in Matrigel. It was shown to contain transforming growth factor beta (TGFβ), EGF, IGF-1, FGF-2, PDGF, and nerve growth factor (Vukicevic et al. 1992; BD Biosciences Matrigel Product Data Sheet). Because of the various biological effects of these growth factors on a wide array of cell types, attempts were soon made to reduce their concentration, and "growth-factor reduced" Matrigel products are commercially available (Vukicevic et al. 1992; BD Biosciences). More recently, large scale proteomic analyses of Matrigel have been reported in an effort to qualitatively identify the less abundant proteins/peptides contained in it (Hansen et al. 2009; Hughes et al. 2010). These efforts identified the known extracellular matrix components that comprise the bulk of Matrigel and also over one-thousand other proteins. However, nearly without exception the other proteins identified were cellular proteins that are not secretory in nature, i.e., intracellular and membrane component proteins (Hansen et al. 2009; Hughes et al. 2010). What biologically active proteins/peptides that can be reproducibly found in Matrigel, lot-to-lot, is otherwise unreported or unknown.

In an attempt to broaden the knowledge of what biologically active proteins Matrigel contains, we have analyzed the liquid component of Matrigel (centrifrugally expressed from gelled Matrigel) using commercially available mouse specific proteome arrays that purport to define the expression of 106 separate proteins in a semi-quantitative way. The results show that Matrigel contains many more biologically active proteins than has previously been reported, and their potential influences on cells in culture, particularly, embryonic stem cells (ESC) and induced pluripotent stem cells (iPCS), is discussed.

Materials and methods

Matrigel basement-membrane-matrix liquid component preparation

Four separate lots of Matrigel basement membrane matrix (catalog no. 356234) were obtained from BD Biosciences (Bedford, MA, USA). After thawing on ice, two 900 µl aliquots of Matrigel were gelled at 37 °C in two 1.5 ml ultracentrifuge tubes (Beckman Coulter, Inc.; Danvers, MA, USA). The supernatants were collected from the compressed gel after centrifugation at 125,000×*g* for 30 min at 4 °C. The gel was then subjected to a second centrifugation at 125,000×*g* for 30 min at 4 °C, and supernatants were again collected and combined with those from the first centrifugation. From each 1.8 ml sample of Matrigel approximately 1.4 ml of total supernatant could be collected.

Proteome antibody array analysis

Semi-quantitative protein analysis of four independent lots of Matrigel supernatant, diluted 1:3, was performed according to the manufactures instructions on three protein antibody arrays that detect a total of 106 mouse growth factors, chemokines, extracellular matrix factors, and other biologically active proteins (R&D Systems, Inc., Minneapolis, MN, USA; Cat. No. ARY013, ARY006, and ARY015). The arrays' capture antibodies (antibodies to the specified proteins), and positive and negative controls, are printed in duplicate (the results therefore being the average of two reactions) on nitrocellulose membranes and four membranes in total are included. The arrays' chemiluminescent autoradiographs (Fig. 1) were measured by densitometry, corrected for background density, and expressed relative to each array's positive controls (ImageQuant TL Software, GE Healthcare, Piscataway, NJ, USA).

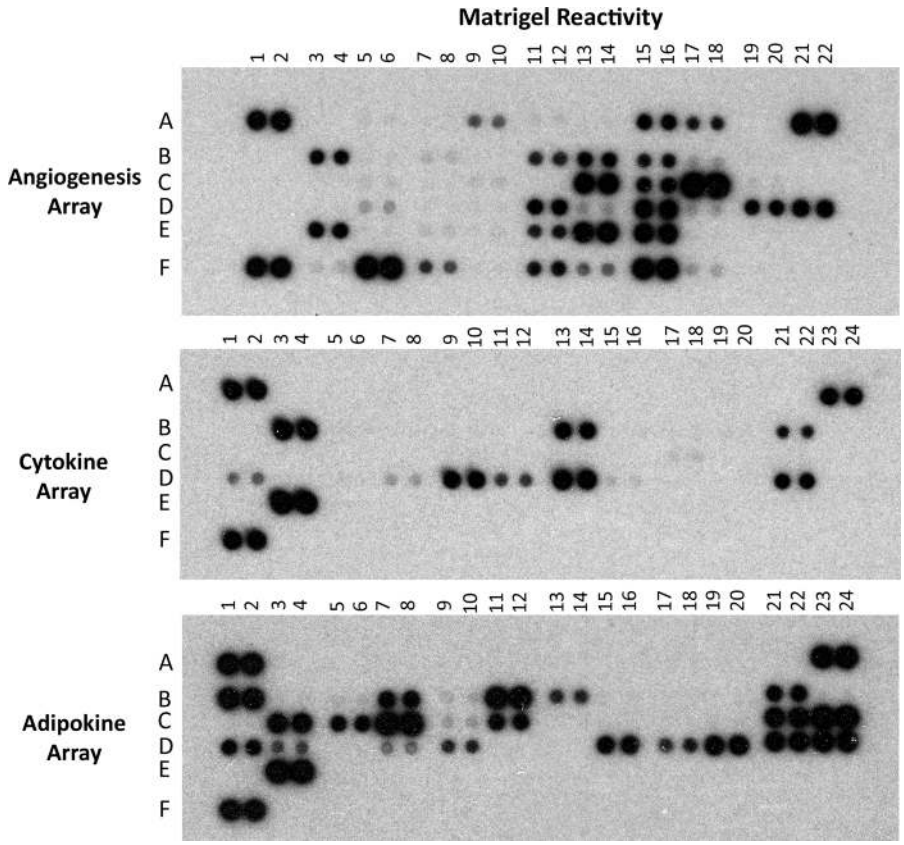

Fig. 1 Representative reactivity of the three proteome arrays after exposure to a 1:3 dilution of gelled-Matrigel liquid-extract. Positive controls are positioned in the corners of each array, and a single negative control is positioned in the lower right corner of each array (R&D Systems, Inc.)

Results

Semi-quantitative protein analysis of four separate lots of Matrigel was performed and representative array results are shown in Fig. 1. The densitometry values ± their standard deviation are listed in Table 1 by array designation ("angiogenesis", "cytokine", or "adipokine") for a total of 106 mouse growth factors, chemokines or other biologically active proteins. The coordinates of each target protein are listed in the first column of Table 1, and their specific locations on the arrays are also available on-line from R&D Systems, Inc.

The strongest detection signals (densitometry value ≥ 50) among the growth factors/cytokines assayed were found for IGFBP-1, IGFBP-3, LIF, platelet factor 4, P*l*GF-2, and VEGF. A moderate positive response (densitometry values approximately between 20 and 49) was found for cyr61, IGFBP-2, IGFBP-6, IL-1ra, and NOV. Low, but detectable, responses occurred for aFGF, IL-13, IL-23, M-CSF, and VEGF-B (densitometry values approximately between 5 and 19). The chemkines assayed by the arrays showed high positive detection for MIG and serpin E1 while moderate reactivity was found for IP-10, MCP-1, and MCP-5, and low, but detectable, responses occurred for CXCL16, I-TAC, and MIP-1α. Among the other biologically active proteins assayed, high positive detection was found for adiponectin, C5a, endocan, lipocalin-2, sICAM-1, MMP-3, and TIMP-1 while moderate reactivity was found for C-reactive protein, coagulation factor III, endoglin, endostatin/collagen XVIII, endothelin-1, ICAM-1, MMP-9, osteopontin, pentraxin-3, and RANTES, and low, but detectable, responses occurred for fetuin A, MMP-8, pentraxin-2, RBP4, resistin, and TIMP-4.

Discussion

The proteome array results indicated the consistent presence of numerous secreted/soluble proteins present in four independent lots of commercially obtained EHS tumor extract, i.e., Matrigel (BD Biosciences).

Table 1 Protein array densitometry of Matrigel

Array no.	Angiogenesis array	Mean	SD
A1, A2, A21, A22, F1, F2	Positive control	100.00	17.50
F19, F20	Negative control	1.01	1.02
A5, A6	ADAMTS1	0.39	0.57
A7, A8	Amphiregulin	0.12	0.54
A9, A10	Angiogenin	6.51	2.06
A11, A12	Angiopoietin-1	0.80	0.43
A13, A14	Angiopoietin-3	0.30	0.51
A15, A16	Coagulation factor III	36.60	5.60
A17, A18	CXCL16	18.31	7.42
B3, B4	Cyr61	22.18	8.34
B5, B6	DLL4	0.69	0.26
B7, B8	DPPIV	1.76	1.07
B9, B10	EGF[a]	0.41	0.46
B11, B12	Endoglin/CD105	22.07	3.44
B13, B14	Endostatin/Collagen XVIII	38.36	6.22
B15, B16	Endothelin-1	29.04	6.73
B17, B18	FGF-1/aFGF	5.45	2.28
B19, B20	FGF-2/bFGF[a]	1.49	0.79
C3, C4	FGF-7/KGF	0.65	0.49
C5, C6	Fractalkine/CX3CL1	1.11	0.39
C7, C8	GM-CSF	0.72	0.83
C9, C10	HB-EGF	1.65	0.35
C11, C12	HGF	2.62	1.15
C13, C14	IGFBP-1	125.98	21.66
C15, C16	IGFBP-2	62.86	11.38
C17, C18	IGFBP-3	231.04	33.77
C19, C20	IL-1α	2.92	1.16
C21, C22	IL-1β	1.18	1.04
D3, D4	IL-10	1.03	0.26
D5, D6	IP-10/CXCL10	3.70	1.25
D7, D8	KC/CXCL1/GROα	0.85	0.29
D9, D10	Leptin	0.89	0.31
D11, D12	MCP-1/CCL2/JE	44.18	17.68
D13, D14	MIP-1α/CCL3	12.02	4.06
D15, D16	MMP-3 (pro/mature form)	100.87	35.72
D17, D18	MMP-8 (pro form)	10.61	3.68
D19, D20	MMP-9 (pro/active form)	36.99	6.05
D21, D22	NOV/CCN3	57.01	14.70
E3, E4	Osteopontin	33.68	4.38
E5, E6	PD-ECGF	1.65	0.44
E7, E8	PDGF-AA	1.90	0.26
E9, E10	PDGF-AB/PDGF-BB[a]	1.32	0.37
E11, E12	Pentraxin-3/TSG-14	23.18	6.39
E13, E14	Platelet factor-4/CXCL4	123.71	20.97

Table 1 continued

Array no.	Angiogenesis array	Mean	SD
E15, E16	P/GF-2	112.56	30.26
E17, E18	Prolactin	2.79	1.33
E19, E20	Proliferin	1.85	1.26
F3, F4	SDF-1/CXCL12	3.34	0.92
F5, F6	Serpin E1/PAI-1	160.49	8.97
F7, F8	Serpin F1/PEDF[b]	12.37	2.05
F9, F10	Thrombospondin-2	1.28	0.52
F11, F12	TIMP-1	27.11	4.28
F13, F14	TIMP-4	9.23	3.17
F15, F16	VEGF	142.52	29.32
F17, F18	VEGF-B	4.67	1.09
Array no.	Cytokine array	Mean	SD
A1, A2, A23, A24, F1, F2	Positive control	100.01	17.32
F23, F24	PBS (negative control)	1.39	1.24
B1, B2	BLC/CXCL13	2.05	0.97
B3, B4	C5a	127.99	16.76
B5, B6	G-CSF	1.75	0.52
B7, B8	GM-CSF	0.56	0.40
B9, B10	I-309/CCL1	0.67	0.48
B11, B12	Eotaxin/CCL11	0.48	0.65
B13, B14	sICAM-1/CD54	96.60	19.23
B15, B16	IFN-γ	1.43	0.20
B17, B18	IL-1α	1.81	0.87
B19, B20	IL-1β	1.96	1.43
B21, B22	IL-1ra	43.89	23.18
B23, B24	IL-2	1.57	1.11
C1, C2	IL-3	1.52	0.95
C3, C4	IL-4	3.37	1.31
C5, C6	IL-5	0.55	0.12
C7, C8	IL-6	0.56	0.39
C9, C10	IL-7	2.12	0.75
C11, C12	IL-10	1.10	0.77
C13, C14	IL-13	5.60	2.85
C15, C16	IL-12 p70	1.21	0.65
C17, C18	IL-16	7.14	6.71
C19, C20	IL-17	1.56	1.25
C21, C22	IL-23	4.92	3.17
C23, C24	IL-27	1.52	1.50
D1, D2	IP-10/CXCL10	22.86	13.55
D3, D4	I-TAC/CXCL11	7.06	1.83
D5, D6	KC/CXCL1/GROα	1.92	0.52
D7, D8	M-CSF/CSF-1	4.39	1.42
D9, D10	MCP-1/CCL2/JE	95.71	18.61

Table 1 continued

Array no.	Cytokine array	Mean	SD
D11, D12	MCP-5/CCL12	20.07	2.49
D13, D14	MIG/CXCL9	145.30	39.04
D15, D16	MIP-1α/CCL3	4.73	1.90
D17, D18	MIP-1β/CCL4	1.64	1.09
D19, D20	MIP-2/CXCL2	2.09	1.51
D21, D22	RANTES/CCL5	89.25	52.54
D23, D24	SDF-1/CXCL12	2.21	1.37
E1, E2	TARC/CCL17	3.98	1.59
E3, E4	TIMP-1	241.36	27.62
E5, E6	TNF-α	2.34	0.54
E7, E8	TREM-1	0.92	0.72
Array no.	Adipokine array	Mean	SD
A1, A2, A23, A24, F1, F2	Positive control	100.00	17.58
F23, F24	PBS (negative control)	1.65	3.17
B1, B2	Adiponectin	92.55	6.89
B3, B4	AgRP	1.72	0.83
B5, B6	ANGPT-L3	1.89	0.61
B7, B8	C-reactive protein	43.67	10.55
B9, B10	DPPIV	2.88	1.67
B11, B12	Endocan/EMS-1	106.74	31.06
B13, B14	Fetuin A	19.82	7.28
B15, B16	FGF acidic	0.73	0.56
B17, B18	FGF-21	0.05	0.13
B19, B20	HGF	0.30	0.20
B21, B22	ICAM-1	44.00	8.32
B23, B24	IGF-1[a]	2.33	1.39
C1, C2	IGF-2	3.48	1.55
C3, C4	IGFBP-1	72.15	8.38
C5, C6	IGFBP-2	40.94	6.24
C7, C8	IGFBP-3	134.18	11.20
C9, C10	IGFBP-5	4.87	3.32
C11, C12	IGFBP-6	47.42	19.39
C13, C14	IL-6	1.18	0.92
C15, C16	IL-10	1.48	0.78
C17, C18	IL-11	0.76	0.48
C19, C20	Leptin	1.95	1.00
C21, C22	LIF	94.83	16.67
C23, C24	Lipocalin-2	142.33	8.73
D1, D2	MCP-1/CCL2/JE	30.51	11.74
D3, D4	M-CSF//CSF-1	18.73	3.69
D5, D6	Oncostatin M	1.72	0.80
D7, D8	Pentraxin-2	7.35	3.27
D9, D10	Pentraxin-3/TSG-14	16.33	10.58

Table 1 continued

Array no.	Angiogenesis array	Mean	SD
D11, D12	Pref-1/DLK-1	1.13	0.66
D13, D14	RAGE	0.63	0.45
D15, D16	RANTES/CCL5	46.70	22.04
D17, D18	RBP4	17.01	5.61
D19, D20	Resistin	63.66	15.75
D21, D22	Serpin E1/PAI-1	91.93	13.82
D23, D24	TIMP-1	119.32	16.48
E1, E2	TNF-α	2.66	0.94
E3, E4	VEGF	124.05	10.27

[a] Proteins previously reported in Matrigel as detected by immunoassay

[b] Proteins previously reported in Matrigel as detected by mass spectroscopy

The results also highlight the apparent absence of several dozen other secreted/soluble proteins in Matrigel (Table 1). Despite recent proteomic analyses of Matrigel employing mass spectroscopy (Hansen et al. 2009; Hughes et al. 2010), the immunoassay analysis presented here identified many secreted/soluble proteins not previously identified in Matrigel, and did so in a semi-quantitative manner. Many of the newly identified proteins have various and well described effects on cell growth, differentiation, or maintenance in general. Because of the wide interest in stem cell biology and the frequent use of Matrigel in various in vitro stem cell assays, some discussion of the result in this context is exemplary and pertinent (Xu et al. 2001; Philp et al. 2005; Kleinman and Martin 2005; Ma et al. 2008; Uemura et al. 2010).

Under the category of growth factors/cytokines, the proteome arrays identified relatively high levels of IGFBP-1 (mean score of 72 and 125 units on separate arrays; relative to the arrays internal negative and positive controls), IGFBP-2 (41 and 63 units on separate arrays), IGFBP -3 (134 and 231 units on separate arrays), and IGFBP-6 (47 units), LIF (95 units), platelet factor-4 (124 units), and P*l*GF-2 (112 units). Insulin-like growth factor binding proteins sustain and mediate the action of IGF-1 and IGF-2, and IGF-1 signaling was found to be necessary for maintenance of human ESC (hESC; Wang et al. 2007). Also in a stem cell context, IGFBP-3, which had the highest response of the IGFBPs detected in Matrigel, is involved in various stem cell processes including vascular endothelial cell differentiation from hematopoietic endothelial precursor cells (Chang et al. 2007), inhibition of neural progenitor cells proliferation (Kalluri and Dempsey 2011), and modulation of liver regeneration from the hepatic stem cell compartment (Steiger-Luther et al. 2010). Besides the detection of IGFBP-1, -2, -3, and -6 with the proteome array, preliminary ELISA data also indicated that Matrigel contains >1 ng/ml IGFBP-4 (unpublished data). Thus, in using Matrigel, it should be understood that it will probably have effects on IGF-1/IGF-2 signal activation. Leukemia inhibitory factor is a key factor in maintaining the undifferentiated state of mouse ESC (mESC; Pease et al. 1990). It's presence in Matrigel, therefore, could have significant effects on assessments of mESC growth and differentiation that should be taken into consideration when using Matrigel and mESC together (Greenlee et al. 2005; Zhou et al. 2010; Massumi et al. 2012). Platelet factor-4 (PF4) is a marker of megakaryocytes and has angiostatic effects (Strieter et al. 1995; Pick et al. 2013). Its relatively high levels in Matrigel might affect hematopoietic differentiation and vasculogenesis from ESC (Gerecht-Nir et al. 2003). Conversely, P*l*GF-2 is a positive factor for angiogenesis and endothelial cell proliferation via its binding to the VEGF receptor, and its presence in Matrigel would also be expected to influence Matrigel-based stem cell assays involving blood cell formation and vasculogenesis (Zhou et al. 2013). Finally, VEGF itself was detected as a high responder, and again, would mean that Matrigel could, in and of itself, affect ESC hematopoiesis and vasculogenesis, and hematopoietic stem cell differentiation, growth or survival (Nakayama et al. 1998; Gerber et al. 2002; Gerecht-Nir et al. 2003).

Chemokines that were indicated to be at high levels in Matrigel by the proteome array results were MIG (145 units) and serpin E1 (160 and 92 units in separate arrays), and some others, MCP-1 (96, 44, and 30 units in separate arrays) and MCP-5 (20 units), were detected at lower levels. These and other chemokines are being found to play important roles in stem cell biology. For example, it was recently shown that MCP-1 (a.k.a. CCL2) stimulated core ESC inducing factors Klf4, Nanog, Sox2, and Tbx3, and, that in conjunction with LIF, maintains pluripotency in mESC and mouse induced pluripotent stem cells (miPSC; Hasegawa et al. 2011). Other reports indicated chemokine participation in stem cell-mediated angiogenesis and cardiogenesis (Chamberlain et al. 2011; Tamura et al. 2011; Bronckaers et al. 2013; Lee et al. 2013).

High positive detection was found for adiponectin (92 units), C5a (127 units), endocan (106 units), lipocalin-2 (142 units), sICAM-1 (97 units), MMP-3 (101 units), and TIMP-1 (241, 119, and 27 units from three separate arrays) in Matrigel. The elevated levels of these functionally diverse proteins in Matrigel may be caused by the effects of the Matrigel-source-tumor on the host mouse's physiology as it grows in the body. That is, these proteins, with the exception of adiponectin, are inflammation related and tissue integrity/remodeling related. Similarly, the other disparate proteins found in Matrigel at moderately high levels, i.e., C-reactive protein (44 units), coagulation factor III (37 units), endoglin (22 units), endostatin/collagen XVIII (38 units), endothelin-1 (29 units), ICAM-1 (44 units), IL-1ra (44 units), MMP-9 (37 units), osteopontin (34 units), pentraxin-3 (23 and 16 units in separate arrays), and RANTES (89 and 47 units in separate arrays) are also involved with inflammation and tissue integrity/remodeling. Be that as it may, some of these proteins can have profound effects on ESC maintenance, growth, and differentiation. For example, matrix remodeling by metalloproteinases (MMP) can support self-renewal of ESC, presumably by mobilizing pluripotency factors sequestered in the surrounding cell matrix (Przybyla et al. 2013). Another example is the potentiating role of MMP-3 in cardiac muscle differentiation in ESC embryoid bodies (Hong et al. 2010). Finally, it is interesting to note the recent report highlighting a connection between the activation of innate cellular inflammatory processes and its enhancement of nuclear reprogramming (Lee et al. 2012). Here, activation of toll-like receptors (TLR), particularly TLR3, led to epigenetic remodeling that render a cell's chromatin more accessible to reprogramming factors and higher reprogramming efficiency. Although very speculative, some of the downstream inflammatory effector molecules linked to TLR activation, and that are found in Matrigel, such as C-reactive protein, endothelin-1, ICAM-1, IL-1ra, pentraxin-3, and RANTES might have a similar effect on nuclear reprogramming. What is sure, however, is that the presence of these inflammatory and cell–matrix remodeling proteins in Matrigel should be taken into account in biological assays using Matrigel because of their wide spread effects on a variety of cell types (Albini et al. 1987; Draper et al. 2004; Kleinman and Martin 2005; Lo et al. 2012).

Some previously reported growth factor components of Matrigel were not detected by the proteome arrays, i.e., FGF-2, IGF-1, PDGF, and EGF. This may reflect a factors low level in Matrigel, i.e., $\sim$1 pg/ml for FGF-2 and $\sim$3–12 pg/ml for PDGF (Vukicevic et al. 1992; BD Biosciences), and the limits of detection of the proteome array. However, proteome array sensitivity would not seem to explain the lack of detection for EGF and IGF-1 since these factors were previously reported to be in Matrigel in nanogram amounts; 3–4 ng/ml for EGF and 6–7 ng/ml (Vukicevic et al. 1992) or even 15 ng/ml for IGF-1 (BD Biosciences). We have previously noted some dissimilar results when comparing proteome array results to the results obtained from commercially available ELISA when measuring growth factors in conditioned cell culture medium (Talbot et al. 2012).

Other apparent anomalies may also be present in the results from the proteome arrays. Across the three arrays, some of the same proteins were targeted on different arrays. In comparing these instances, there were some pronounced differences in the resulting signal, e.g., TIMP-1, IGFBP-1 and -3, and RANTES (Table 1). The small differences between the arrays' positive controls do not explain the wide differences found for these and a few other duplicated proteins on the arrays. This would suggest an inconsistency of the array proteome technology or that separate arrays are using different capture antibodies for the same protein target. Whether this variation is a quality control issue or illustrates the semi-quantitative nature of the proteome array data, this indicates that the data presented here need independent verification by alternative and more quantitative protein detection methods.

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