Supplementary Materials: Cell Line Secretome and Tumor Tissue Proteome Markers for Early Detection of Colorectal Cancer: A Systematic Review

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Supplementary Material File S1: Data sources and search strategy

for PubMed

"Colorectal neoplasm" OR "colon neoplasm" OR "colonic neoplasm" OR "Rectal Neoplasm" OR "colorectal cancer" OR "colon cancer" OR "colonic cancer" OR CRC OR "Colorectal tumor" OR "colon tumor" OR "colonic tumor" OR adenoma
AND
"Cell Line, Tumor"[Mesh] OR "Tumor Microenvironment"[Mesh] OR secretome OR Tissue
AND
"Proteins"[Mesh] OR Proteome OR Proteomics
AND
"Diagnosis" [Mesh] OR sensitivity OR specificity OR AUC OR "area under the curve"
AND
Blood OR Plasma OR Serum
NOT
Therapy OR Treatment OR Progression

As on 18.07.2017-
Pubmed: 1498 records

for Web of Science

TOPIC: (Colorectal neoplasm OR colon neoplasm OR colonic neoplasm OR Rectal Neoplasm OR colorectal cancer OR colon cancer OR colonic cancer OR CRC OR Colorectal tumor OR colon tumor OR colonic tumor OR adenoma)
AND
TOPIC: (Cell Line OR Tumor OR Tumor Microenvironment OR secretome OR Tissue)
AND
TOPIC: (Proteins OR Proteome OR Proteomics)
AND
TOPIC: (Diagnosis OR sensitivity OR specificity OR AUC OR area under the curve)
AND
TOPIC: (Blood OR Plasma OR Serum)
NOT
TOPIC: (Therapy OR Treatment OR Progression)

As on 18.07.2017-
Web of Science: 865 records

Table S2. Stage specific diagnostic performance

First author, Year [Ref]	Number (N)							Sensitivity						Specificity					
	Ad	AA	0	I/A	II/B	III/C	IV/D	A	AA	I/A	II/B	III/C	IV/D	A	AA	I/A	II/B	III/C	IV/D
			Early			Late				Early		Late				Early		Late	
Broll, 2001 [56]				41	27	30	24												
Nam, 2005 [66]																			
Alberthsen, 2006 [60]				15	46	38	20					78						77	
Roessler, 2005 [57]			3	33	23	21	23												
Wu, 2008 [35]				27	72	60	40												
Wu, 2008 [36]				23	90	86	81												
Babel, 2009 [50]				6	14	20	12												
Kim, 2009 [47] S100A8 S100A9 CEA	11			14	23	21	19			32 40 21						95 95 100			
Yoneda, 2009 [41]				40	51	40	26			43	61	68	92						
Ji, 2010 [45]	10			14	37	39	10												
Kijanka, 2010 [62]																			
Tagi, 2010 [40]			6	28	25	27	19			58	44	63	82			80	80	80	80
Xie, 2010 [27] TFF3 GDF15													 74 83						 91 82
Xue, 2010 [28]				68		76													
Babel, 2011 [51]				6	11	16	17												
Fan, 2011 [38]				10	25	35	16			71		71							
Hamelin, 2011 [61]				27	29	29	27												
Han, 2011 [21]																			
Kuo, 2011 [39]				25		34				80		88							
Matsubara, 2011 [42]				12	5	8	1												
Weiss, 2011 [59]					15	18	26												
Barderas, 2012 [52]				6	11	16	17			88						83			
Fijneman, 2012 [63] CHI3L1 CEA	20	22			1	4	1	 25 5	 55 5					 89 10 0	 89 100				
Hosono, 2012 [44]	62							93						82					
Ladd, 2012 [65]				4	13	12	3												
Lee, 2012 [48]				34	83	70	32				47					87			
Rodriguez-P, 2012 [54]				6	13	5	7												
Yao, 2012]30]	14																		

Barderas, 2013[53]				6	7	12	15												
Ji, 2013 [22]	45			102	110	109	84												
Wang, 2013 [25] Kininogen-1 CEA				14	63	37	29			70 39		73 35				66 86		66 86	
Jiang, 2014 [23] DC-SIGN DC-SIGNR CEA CA19-9				5	68	44	69			81 49 19 10						56 93 92 95			
Niewiarowska, 2014 [64]				1	4	38													
Shin, 2014 [46]	11			68	68	65	27												
Sole, 2014 [55]	23			9	27	34	10												
Toiyama, 2014 [43]				48	55	54	38				54					93			
Xue, 2014 [29]				20	56	26	18												
Chiang, 2015 [37]				61		59													
Lin, 2015 [49]				6	9	22	8												
Qiao, 2015 [24]																			
Surinova, 2015 [58]				43	58	49	52												
Taguchi, 2015 [67]	60			11	19	21	9	35		47		50		95		95		95	
Zhang, 2015 [33]																			
Fan, 2016 [31]																			
Wang X, 2016 [26]																			
Wang Y, 2016 [32]					7	9													
Xie, 2016 [34]																			

Abbreviations: Ad- Adenoma, AA- Advanced adenoma, ACA- Adenocarcinoma. Note- Stages I/II/III/IV as per UICC classification of tumors and stages A/B/C/D as per AJCC classification.

Table S3. Function and location of proteins as identified from UniProt database **[**19**]**.

Protein	Molecular Function	Biological Function	Location	Cellular component
A1AT	serine protease inhibitor, glycoprotein binding	Acute phase, Blood coagulation, Hemostasis	secreted	endoplasmic reticulum, extracellular matrix
ACVR2B	ATP binding, growth factor binding, protein serine/threonine/tyrosine kinase activity	activation of protein kinase activity	cell membrane	cytoplasm
Adipophilin	development and maintenance of adipose tissue	lipid storage and long-chain fatty acid transport	membrane	endoplasmic reticulum, cytosol
AK1	Kinase, Transferase	ATP-binding, Nucleotide-binding	cytoplasm	extracellular exosome
ANGPTL2	receptor binding	multicellular organism development	secreted	extracellular space
AOPJ75				
AZGP1	antigen binding, glycoprotein binding	antigen processing and presentation, cell adhesion	secreted	extracellular exosome
α-defensin 6	Antibiotic, Antimicrobial, Defensin, Fungicide	antimicrobial humoral immune response	secreted	extracellular region
BAC85857				
BST2	metalloendopeptidase inhibitor activity, protein homodimerization activity, RNA binding	Antiviral defense, B-cell activation, Immunity, Innate immunity	golgi apparatus	extracellular exosome
CA 19-9	glycoprotein	tumor marker		
CEA	glycoprotein	tumor marker		
CELA1	Hydrolase, Protease, Serine protease, metal-ion binding	inflammatory response, elastin catabolic process	secreted	extracellular space
CELA2	Hydrolase, Protease, Serine protease, metal-ion binding	cornification	secreted	extracellular space
CHI3L1	Antimicrobial, carbohydrate binding, extracellular matrix structural constituent	Apoptosis, Inflammatory response	secreted	cytoplasm, extracellular space
Clusterin	Chaperone	Apoptosis, Complement pathway, Immunity, Innate immunity	secreted	cytoplasm, extracellular matrix
COL10A1	metal ion binding	cartilage development, collagen catabolic process	secreted	extracellular matrix
COL3A1	calcium binding, metal ion binding, integrin binding	aging, cell-matrix adhesion, collagen catabolic process	secreted	extracellular matrix
COL6A3	Protease inhibitor, Serine protease inhibitor	Cell adhesion	secreted	extracellular matrix
CP	chaperone binding, copper ion binding, oxidoreductase	Copper transport, Ion transport, Transport	secreted	extracellular exosome
CRMP2	Developmental protein	Differentiation, Neurogenesis	cytoplasm	cytoskeleton, extracellular exosome
CTRL	Hydrolase, Protease, Serine protease	digestion, proteolysis	secreted	extracellular space
CTSD	Aspartyl protease, Hydrolase, Protease		lysosome, melanosome	extracellular exosome
Cystatin SN	Thiol protease inhibitor	detection of chemical stimulus involved in sensory perception of bitter taste	secreted	extracellular space
DC-SIGN	Host cell receptor for virus entry, Receptor	Adaptive immunity, Cell adhesion, Endocytosis, Host-virus interaction, Immunity, Innate immunity	cytoplasm	extracellular exosome
DC-SIGNR	Host cell receptor for virus entry, Receptor	Adaptive immunity, Cell adhesion, Endocytosis, Host-virus interaction, Immunity, Innate immunity	cytoplasm	extracellular exosome
DK	soluble regulator of keratinocyte differentiation	cornified envelope assembly	secreted	extracellular exosome
Protein	**Molecular Function**	**Biological Function**	**Location**	**Cellular component**
EFEMP2	calcium ion binding, extracellular matrix structural constituent		secreted	extracellular exosome

ESM-1	hepatocyte growth factor receptor binding	Angiogenesis	secreted	extracellular region
FGFR4	Kinase, Receptor, Transferase, Tyrosine-protein kinase	ATP-binding, Nucleotide-binding	secreted	endoplasmic reticulum, cytoplasm
GDF15	Cytokine activity, Growth factor/transforming growth factor beta receptor binding	BMP signaling pathway, cell-cell signaling, cell development	secreted	extracellular exosome
GRN	Cytokine activity, Growth factor activity		secreted	endoplasmic reticulum, extracellular exosome
GTF2i	DNA-binding	Transcription, Transcription regulation	cytoplasm	nucleus, cytoplasm
HMGB1	DNA-binding	Adaptive immunity, Autophagy, Chemotaxis, DNA damage, DNA recombination, DNA repair, Immunity, Inflammatory response, Innate immunity	cytoplasm	cell surface, extracellular exosome
HNP1	Antibiotic, Antimicrobial, Defensin, Fungicide	Antiviral defense	secreted	extracellular exosome
HNP2	Antibiotic, Antimicrobial, Defensin, Fungicide	Antiviral defense	secreted	extracellular exosome
HNP3	Antibiotic, Antimicrobial, Defensin, Fungicide	Antiviral defense	secreted	extracellular exosome
HSP60	Chaperone, Hydrolase, ATPbinding	Host-virus interaction	mitochondrial matrix	cytoplasm, mitochondrion
ICLN	RNA-binding	cell volume homeostasis	cytoplasm	nucleus, cytoplasm
IGFBP2	Growth factor binding	Growth regulation	secreted	extracellular exosome
Kininogen-1	Protease inhibitor, Thiol protease inhibitor,, Vasodilator	Blood coagulation, Hemostasis, Inflammatory response	secreted	extracellular exosome
LAMB1	extracellular matrix structural constituent	cell adhesion	secreted	extracellular matrix
LASS5	DNA-binding, Transferase	Lipid biosynthesis, Lipid metabolism, Sphingolipid metabolism	endoplasmic reticulum	nucleus
LRG1	transforming growth factor beta receptor binding	brown fat cell differentiation, neutrophil degranulation	secreted	extracellular exosome
Mac-2BP	5cavenger receptor activity	cell adhesion	secreted	extracellular matrix
MAPKAPK3	Kinase, Serine/threonine-protein kinase, Transferase	ATP-binding, Nucleotide-binding	nucleus, cytoplasm	nucleus, cytoplasm
MAPRE1	cadherin binding Source, identical protein binding	Cell cycle, Cell division, Mitosis	cytoplasm	Cytoplasm, Microtubule
MRC1	Host cell receptor for virus entry, Receptor	Endocytosis, Host-virus interaction	cell membrane	endosome
MST1/STK4	Kinase, Serine/threonine-protein kinase, Transferase	Apoptosis	cytoplasm	nucleus, cytoplasm
NHSL1		motor neuron migration		membrane
NNMT	Methyltransferase, Transferase	methylation	cytoplasm	cytoplasm
p53	Activator, DNA-binding, Repressor	Apoptosis, Biological rhythms, Cell cycle, Host-virus interaction, Necrosis, Transcription, Transcription regulation	cytoplasm	nucleus, cytoplasm
PAI-1	Protease inhibitor, Serine protease inhibitor	angiogenesis, cellular response to lipopolysaccharide	secreted	extracellular exosome
PEDF	serine-type endopeptidase inhibitor activity	aging, cell proliferation	secreted	melanosome, extracellular exosome
Protein	**Molecular Function**	**Biological Function**	**Location**	**Cellular component**
PIM1	Kinase, Serine/threonine-protein kinase, Transferase	Apoptosis, Cell cycle	cytoplasm	cell membrane
PLSCR1	DNA-binding	Antiviral defense	cell membrane	extracellular exosome
PON1	Hydrolase	aromatic compound catabolic process, carboxylic acid catabolic process, cholesterol metabolic process	secreted	extracellular space

PRDX2	Antioxidant, Oxidoreductase, Peroxidase	cellular response to oxidative stress	cytoplasm	cytoplasm
S100A8	Antimicrobial	Apoptosis, Autophagy, Chemotaxis, Immunity, Inflammatory response, Innate immunity	secreted	cytoplasm, extracellular space
S100A9	Antimicrobial, Antioxidant	Apoptosis, Autophagy, Chemotaxis, Immunity, Inflammatory response, Innate immunity	secreted	cytoplasm, extracellular space
SEC61β	epidermal growth factor binding	Protein transport, Translocation, Transport	cytosol	endoplasmic reticulum
sE-cadherin	Cell adhesion molecule binding	Cell adhesion	cell membrane	golgi apparatus
SERPINA3	Protease inhibitor, Serine protease inhibitor	acute-phase response, inflammatory response	secreted	extracellular exosome
SERPINI1	Protease inhibitor, Serine protease inhibitor	central & peripheral nervous system development	secreted	extracellular exosome
SNP29		Autophagy, Cilium biogenesis/degradation, Protein transport, Transport	cytoplasm	cell membrane
Spondin-2	Antigen binding	Cell adhesion	secreted	extracellular space
SREBF2	Activator, DNA-binding	Cholesterol metabolism, Lipid metabolism, Steroid metabolism, Sterol metabolism, Transcription, Transcription regulation	cytoplasm	endoplasmic reticulum, cytoplasm
STOML2	cardiolipin binding, GTPase binding, receptor binding		cell membrane	cell membrane, cytoskeleton
SULF1	Hydrolase	Apoptosis	endoplasmic reticulum	cell surface
TCF3	DNA-binding	Differentiation, Neurogenesis, Transcription regulation	nucleus	cytoplasm
TFF3		defense response, wound healing	secreted	cytoplasm, extracellular matrix
TIMP1	Growth factor, Metalloenzyme inhibitor, Metalloprotease inhibitor, Protease inhibitor	aging, cartilage development, cell activation	secreted	extracellular exosome
TNF-R1	Receptor	Apoptosis, Host-virus interaction	secreted	golgi apparatus
TRFM	iron ion binding	Ion transport, Iron transport, Transport	cell membrane	cell membrane
TRY2	hydrolase, protease, Serine protease, calcium-ion binding	anti-microbial humoral response, digestion	secreted	extracellular space
TSLC1	Developmental protein	Apoptosis, Cell adhesion, Differentiation, Immunity, Spermatogenesis	extracellular exosome	cell membrane
TUBB5	GTPase activity and binding	cell division, cell process	cytoplasm	cytoplasm
VEGF	Developmental protein, Growth factor, Heparin-binding,	Angiogenesis, Differentiation	secreted	extracellular space
ZNF346	RNA-binding	positive regulation of apoptotic process	nucleus	cytoplasm, nucleus
ZNF638	Activator, DNA-binding, RNA-binding	Transcription, Transcription regulation	nucleus speckle	nucleus, cytoplasm
ZNF700	DNA-binding	Transcription, Transcription regulation	nucleus	nucleus, cytoplasm

Table S4. QUADAS-2 risk of bias assessment of the studies [18].

Study	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference StandarD	Flow and Timing	Patient Selection	Index Test	Reference Standard
Broll, 2001 [56]	☹	?	☹	?	?	☺	?
Nam, 2005 [66]	☹	?	?	?	☺	☺	?
Albrethsen, 2006 [60]	☹	☹	?	?	☺	☺	?
Roeßler, 2005 [57]	☹	☹	☹	☹	☹	☺	?
Wu, 2008 [35]	☹	☹	☹	?	?	☺	?
Wu, 2008 [36]	☹	☹	☹	?	?	☺	?
Babel, 2009 [50]	☹	?	☹	?	?	☺	?
Kim, 2009 [47]	☹	☹	☹	?	?	☺	?
Yoneda, 2009 [41]	☹	☹	☺	☹	?	☺	☺
Ji, 2010 [45]	☹	☹	☹	?	☺	☺	?
Kijanka, 2010 [62]	☹	☺	☺	☺	☺	☺	☺
Tagi, 2010 [40]	☹	?	?	?	☹	☺	?
Xie, 2010 [27]	☹	?	☹	?	☺	☺	?
Xue, 2010 [28]	☹	☹	☹	?	☺	☺	?
Babel, 2011 [51]	☹	☺	☹	?	?	☺	?
Fan, 2011 [38]	☹	?	☹	?	☺	☺	?
Hamelin, 2011 [61]	☹	?	☹	?	?	☺	?
Han, 2011 [21]	☹	☹	☹	?	☺	☺	?
Kuo, 2011 [39]	☹	?	☹	?	?	☺	?
Matsubara, 2011 [42]	☹	?	☹	?	☹	☺	?
Weiss, 2011 [59]	☹	?	☹	?	?	☺	?
Barderas, 2012 [52]	☹	☺	?	?	?	☺	?
Fijneman, 2012 [63]	☹	☹	☺	☺	☺	☺	☺
Hosono, 2012 [44]	☹	☹	☺	☺	☺	☺	☺
Ladd, 2012 [65]	☺	☹	?	?	☺	☺	?
Lee, 2012 [48]	☹	☹	☺	☺	☺	☺	☺
Rodriguez-P, 2012 [54]	☹	☹	?	?	☺	☺	?
Yao, 2012 [30]	☹	☹	☺	?	?	☺	?
Barderas, 2013 [53]	☹	☹	?	?	?	☺	?
Ji, 2013 [22]	☹	☺	☹	?	☹	☺	?
Wang, 2013 [25]	☹	?	☹	?	?	☺	?
Jiang, 2014 [23]	☹	☹	☹	☹	☹	☺	?
Niewiarowska, 2014 [64]	☹	☹	☹	?	☺	☺	?
Shin, 2014 [46]	☹	?	☹	☹	?	☺	?
Sole, 2014 [55]	☹	☺	☺	☹	☺	☺	☺
Toiyama, 2014 [43]	☹	?	?	?	☺	☺	?
Xue, 2014 [29]	☹	?	?	?	☺	☺	?

Chiang, 2015 [37]	☹	?	☹	☺	☺	☺	?
Lin, 2015 [49]	☹	☹	?	?	?	☺	?
Qiao, 2015 [24]	☹	☹	☹	?	?	☺	?
Surinova, 2015 [58]	☹	☺	☺	☺	☺	☺	☺
Taguchi, 2015 [67]	☹	☺	?	?	☺	☺	?
Zhang, 2015 [33]	☹	☹	☹	?	☺	☺	?
Fan, 2016 [31]	☹	☹	☹	?	☺	☺	?
Wang X, 2016 [26]	☹	☹	☹	☹	?	☺	?
Wang Y, 2016 [32]	☹	☹	?	?	?	☺	?
Xie, 2016 [34]	☹	☹	?	?	?	☺	?

Note: In the above table ☺ represents low risk, ☹ represents high risk and unclear risk is represented as ?

Table S5. PRISMA 2009 Checklist [20].

Section/topic	#	Checklist item	Reported on page #
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	2
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	2, 5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	2, S1
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	S1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	2, 5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	2
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	2
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	2
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	2, 7–9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	-

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7, 27
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	3, 5
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	3, 11–12
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	4-10
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	14–15
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	-
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	SF4
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	-
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	14–15
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15