
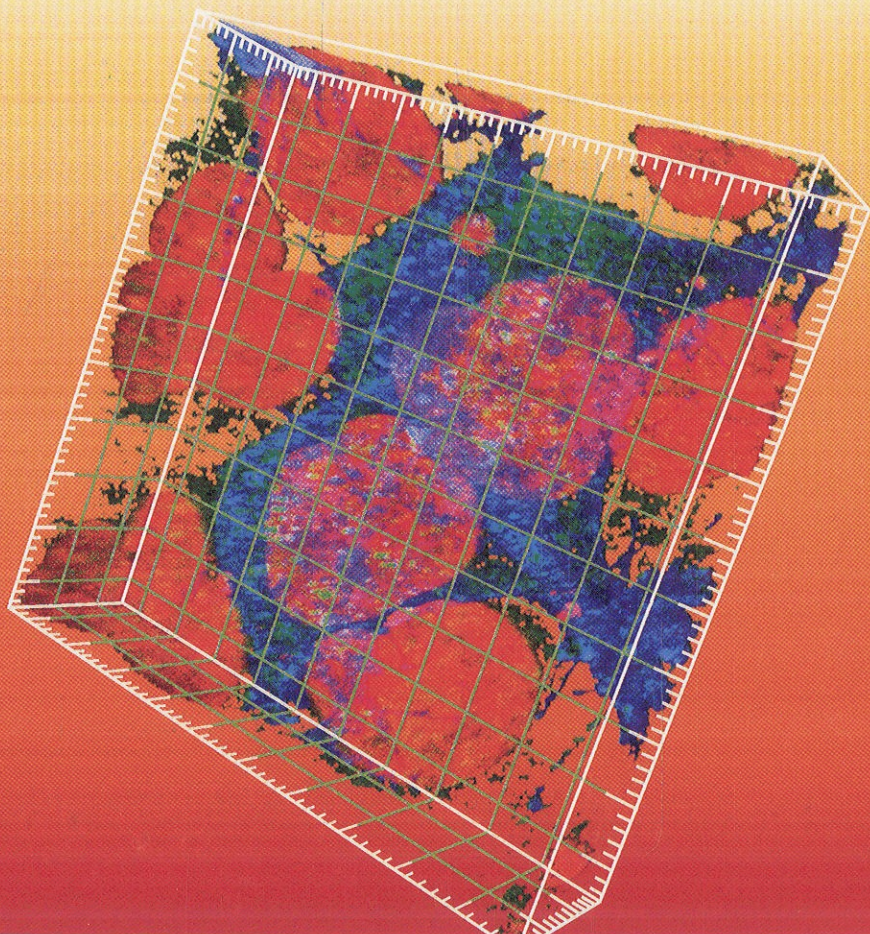


Edited by Olivier Gires
and Barbara Seliger

 WILEY-
BLACKWELL

Tumor-Associated Antigens

Identification, Characterization,
and Clinical Applications



Contents

List of Contributors XV

Part One Tumor-associated Antigens (TAAs): Subclasses of TAAs 1

1 T Cell Antigens in Cancer 3

Annette Paschen

1.1 Introduction 3

1.2 Generation of T-cell Epitopes 4

1.2.1 Subclasses of Tumor-associated T-cell Antigens 5

1.2.1.1 Unique Tumor Antigens 6

1.2.1.2 Cancer Testis Antigens 6

1.2.1.3 Differentiation Antigens 7

1.2.1.4 Overexpressed Antigens 7

1.3 Identification of T-cell Antigens and their Epitopes 8

1.3.1 T-cell Antigens for Cancer Immunotherapy – How are Candidates Selected? 10

1.4 Conclusions 12

References 12

2 Human Tumor Antigens as Targets of Immunosurveillance and Candidates for Cancer Vaccines 23

Olivera J. Finn, Robert J. Binder, Anthony G. Brickner, Lisa H. Butterfield, Robert L. Ferris, Pawel Kalinski, Hideho Okada, Walter J. Storkus, Theresa L. Whiteside, and Hassane M. Zarour

2.1 Introduction 23

2.2 Tumor Antigen Classes 24

2.2.1 Oncofetal Antigens 24

2.2.2 Oncogenes as Tumor Antigens 25

2.2.3 Overexpressed Normal Molecules as Tumor Antigens 26

2.2.4 Cancer-Testis (CT) Antigens 26

2.2.5	Minor Histocompatibility Antigens (mHAggs) as Tumor Antigens	27
2.2.6	Human Melanoma Antigens	28
2.2.7	Human Glioma Antigens and Immunosurveillance in the CNS	29
2.2.8	Heat Shock Proteins, Efficient Carriers of Tumor Antigens	30
2.2.9	Dendritic Cells, Efficient Cross-presenters of Tumor Antigens	31
2.2.10	Spontaneous and Vaccine-induced Immunity and the Tumor Microenvironment	33
2.3	Summary	34
	References	34

Part Two Methods to Detect TAAs 45

3 Humoral Immune Responses against Cancer Antigens: Serological Identification Methods. Part I: SEREX 47

Carsten Zwick, Klaus-Dieter Preuss, Frank Neumann, and Michael Pfreundschuh

3.1	Introduction	47
3.2	The SEREX Approach	48
3.2.1	Identification of Human Tumor Antigens by SEREX	49
3.2.2	Specificity of Human Tumor Antigens	50
3.2.2.1	Shared Tumor Antigens	50
3.2.2.2	Differentiation Antigens	51
3.2.2.3	Antigens Encoded by Mutated Genes	51
3.2.2.4	Viral Genes	52
3.2.2.5	Overexpressed Genes	52
3.2.2.6	Amplified Genes	52
3.2.2.7	Splice Variants of Known Genes	52
3.2.2.8	Cancer-related Autoantigens	52
3.2.2.9	Non-cancer-related Autoantigens	52
3.2.2.10	Products of Underexpressed Genes	53
3.2.3	Significance of Antibodies against SEREX Antigens	53
3.2.4	Reverse T Cell Immunology	53
3.2.5	Functional Significance of Human Tumor Antigens	54
3.2.6	The Human Cancer Immunome	54
3.2.7	Perspectives for Vaccine Development	56
3.3	Conclusions	57
	References	58

4 Humoral Immune Responses against Cancer Antigens: Serological Identification Methods. Part II: Proteomex and AMIDA 63

Barbara Seliger and Olivier Gires

4.1	Introduction	63
4.1.1	A Humoral Response against Self-antigens: The Notion of Tumor-associated Antigens	64

4.2	Implementation of Serum Antibodies: Serological Screening Technologies	65
4.2.1	PROTEOMEX, alias SERPA and SPEARS	66
4.2.1.1	PROTEOMEX Technology and its 'Pros' and 'Cons'	67
4.2.1.2	Candidate Biomarkers Identified by PROTEOMEX	67
4.2.1.3	Implementation of Candidate Biomarkers in the Clinic	68
4.2.2	AMIDA	68
4.2.2.1	Autologous AMIDA	69
4.2.2.2	Allo-AMIDA	69
4.2.3	Advantages and Disadvantages of AMIDA	71
4.3	AMIDA Antigens and Clinical Application	71
4.3.1	Diagnostic TAAs Detected with AMIDA Screening	71
4.3.2	Therapeutic Markers	72
4.4	Conclusions	72
	References	73
5	cDNA and Microarray-based Technologies	79
	<i>Ena Wang, Ping Jin, Hui Lui Liu, David F. Stroncek, and Francesco M. Marincola</i>	
5.1	Introduction	79
5.2	Technical Aspects	80
5.2.1	Handling of Samples and the Need for Consistent Messenger RNA Amplification	81
5.2.1.1	Collection of Source Material and RNA Isolation	82
5.2.1.2	Single Strand cDNA Synthesis	83
5.2.1.3	Double-stranded cDNA (ds-cDNA) Synthesis	84
5.2.2	RNA Amplifications	85
5.2.2.1	Linear Amplification	85
5.2.2.2	PCR-based Exponential Amplification	86
5.2.2.3	Target Labeling for cDNA Microarray using Amplified RNA	88
5.2.2.4	Bioinformatics Tools	89
5.2.2.5	Limitations of Transcriptional Profiling	90
5.2.2.6	Usefulness of Transcriptional Profiling for Antigen Discovery and the Understanding of Tumor–Host Interactions	90
5.3	Summary	93
	References	93
6	Detection and Identification of TAA by SELDI-TOF	103
	<i>Ferdinand von Eggeling and Christian Melle</i>	
6.1	Introduction	103
6.2	SELDI (ProteinChip) Technology	104
6.2.1	The Procedural Method	104
6.2.2	SELDI-MS in TAA Identification	106
6.2.2.1	Tumor Samples	106
6.2.2.2	Body Fluids	106

6.2.2.3	SELDI-MS TAAs and Clinical Potential	107
6.3	Conclusions and Future Perspectives	108
	References	108

Part Three TAAs and Their Usefulness 113

7 Tumor-associated Antigens in Childhood Cancer 115

Uta Behrends and Josef Mautner

7.1	Introduction to Childhood Cancer	115
7.1.1	Incidence, Etiology and Types of Cancer in Children	115
7.1.2	Cure Rates, Treatment Failure and Toxicity	115
7.2	TAA for Pediatric Cancer Therapy	116
7.2.1	Potential Clinical Impact of Childhood TAA and Strategies for their Identification	116
7.2.2	TAA in Childhood Leukemia	117
7.2.3	TAA in Childhood Brain Tumors	119
7.2.4	TAA in Childhood Lymphoma	121
7.2.5	TAA in Pediatric Neuroblastoma	122
7.2.6	TAA in Rhabdomyosarcoma and other Soft Tissue Sarcomas	124
7.2.7	TAA in Osteosarcoma	125
7.2.8	TAA in Tumors of the Ewing Family	126
7.2.9	TAA in Wilms' Tumor	127
7.3	Conclusion	128
	References	129

8 Epigenetically-regulated Therapeutic Tumor-associated Antigens 143

Hugues J. M. Nicolay, Luca Sigalotti, Sandra Coral, Elisabetta Fratta, Alessia Covre, Ester Fonsatti, and Michele Maio

8.1	Introduction	143
8.2	Epigenetics	144
8.2.1	DNA Methylation	144
8.2.2	Histone Post-translational Modifications	145
8.3	TAA	146
8.3.1	Classification of TAA	146
8.3.2	Epigenetically-regulated TAA	146
8.3.2.1	HMW-MAA	146
8.3.2.2	Mucins	147
8.3.2.3	CTA	148
8.4	Perspectives and Conclusion	153
	References	153

9 Cancer Testis Antigens 161

Jonathan Cebon, Otavia Caballero, Thomas John, and Oliver Klein

9.1	Introduction	161
-----	--------------	-----

9.2	Definitions and Classification	162
9.3	Tissue Distribution	163
9.3.1	Normal tissues	163
9.3.2	Tumors	163
9.4	Function	164
9.5	Immunology	166
9.6	Clinical Trials	167
9.7	Conclusions	172
	References	173
10	Rationale for Treatment of Colorectal Cancer with EpCAM Targeting Therapeutics	179
	<i>Patrick A. Baeuerle and Gert Riethmueller</i>	
10.1	Introduction	179
10.2	EpCAM Expression in Colorectal Cancer	181
10.2.1	Frequent and High Level Expression of EpCAM in Primary Tumors of Colon Cancer	181
10.2.2	EpCAM Expression on Colon Cancer Metastases	183
10.2.3	EpCAM Expression on Circulating Colon Cancer Cells	184
10.2.4	EpCAM Expression on Colon Cancer Stem Cells	184
10.2.5	EpCAM Expression on Human Colon Cancer Cell Lines	185
10.3	EpCAM-directed Therapeutic Approaches	185
10.3.1	Clinical Results with Anti-EpCAM Murine Antibody Edrecolomab in Stage II and III CRC	185
10.3.2	Clinical Results with Edrecolomab as a Vaccine for Induction of Anti-idiotypic Response	188
10.3.3	EpCAM Protein as a Vaccine in Colorectal Cancer	189
10.3.4	Adecatumumab, a Novel Fully Human Anti-EpCAM Antibody	190
10.4	Therapeutic Window of EpCAM-directed Therapies	192
10.5	Conclusions	196
	References	198
11	Carcinoembryonic Antigen	201
	<i>Wolfgang Zimmermann and Robert Kammerer</i>	
11.1	CEA Biology	201
11.1.1	CEA Gene Family, Genomic Localization, Protein Structure	201
11.1.2	Evolution	203
11.1.3	Expression	203
11.1.4	Biological Functions	204
11.2	Clinical Relevance of CEA	206
11.2.1	CEA as a Tumor Marker for Prognosis and Post-surgery Follow-up	206
11.2.2	Targeting CEA for Tumor Localization and Therapy	207
11.2.2.1	Animal Models for CEA	207
11.2.2.2	Tumor Localization and Therapy with Anti-CEA Antibodies	207

11.2.2.3	CEA-based Vaccines	209
11.2.3	Immune Monitoring	211
11.3	Conclusion	213
	References	213
12	HER-2 as a Tumor Antigen	219
	<i>Barbara Seliger</i>	
12.1	Introduction	219
12.2	Biology of HER2	219
12.2.1	Features of HER2	219
12.2.2	The Self-antigen HER2 as a Potential Therapeutic Target	220
12.2.3	Determination of HER2 Status in Tumors	222
12.3	General Approaches for Targeting HER2 using Anti-cancer Agents	222
12.4	Active HER2-based Cancer Vaccines	222
12.4.1	HER2-specific Peptides	223
12.4.2	T Cell-mediated Immunity to HER2 in Cancer Patients	224
12.5	Passive Immunotherapy Targeting HER2	224
12.6	HER2 Effects on Immunogenicity: Both Sides of the Coin	225
12.7	Conclusions	226
	References	227
13	Epstein-Barr Virus-associated Antigens	231
	<i>Christoph Mancao and Wolfgang Hammerschmidt</i>	
13.1	Introduction	231
13.2	Functions of EBV Antigens in Latently Infected Cells	232
13.2.1	EBV Nuclear Antigen 1 (EBNA1)	233
13.2.2	EBV Nuclear Antigen 2 and Leader Protein (EBNA2 and EBNA-LP)	233
13.2.3	EBV Nuclear Antigen Family 3 (EBNA3)	234
13.2.4	Latent Membrane Protein 1 (LMP1)	234
13.2.5	Latent Membrane Protein 2 (LMP2)	235
13.3	T-cell Responses to EBV Antigens	236
13.3.1	CD8 ⁺ T-cell Responses	236
13.3.2	CD4 ⁺ T-cell Response	237
13.4	EBV-associated Malignancies	237
13.4.1	Post-Transplant Lymphoproliferative Disease (PTLD)	237
13.4.2	Adoptive T-cell Therapy	238
13.4.3	Burkitt's Lymphoma and Hodgkin's Lymphoma	240
	References	241
14	Human Papillomavirus (HPV) Tumor-associated Antigens	249
	<i>Andreas M. Kaufmann</i>	
14.1	Introduction	249
14.2	HPV-encoded TAA	250
14.2.1	The Proteins of HPV	250
14.2.2	Vaccine Development	253

- 14.2.3 Therapeutic Vaccine Strategies 253
- 14.2.4 HPV-induced Cellular TAA 256
- 14.3 Conclusions and Future Perspectives 256
- References 257

15 Circulating TAAs: Biomarkers for Cancer Diagnosis, CA125 261

Angela Coliva, Ettore Seregni, and Emilio Bombardieri

- 15.1 Introduction 261
- 15.2 Definition and Classification of Mucins 261
- 15.3 Structure of MUC 16 263
 - 15.3.1 Gene and Protein Structure 263
 - 15.3.1.1 Domain I 263
 - 15.3.1.2 Domain II 263
 - 15.3.1.3 Domain III 265
 - 15.3.2 Evolutionary Considerations 266
- 15.4 Distribution of MUC16 266
- 15.5 MUC16 Function in Normal Tissues 266
- 15.6 Release of MUC16: CA125 Tumor Marker 267
 - 15.6.1 History 267
 - 15.6.2 Assay Methods 268
 - 15.6.3 Serum CA125 Levels in Healthy Subjects 269
 - 15.6.3.1 Age 269
 - 15.6.3.2 Menstrual cycle 270
 - 15.6.3.3 Pregnancy 270
 - 15.6.3.4 Race 270
 - 15.6.3.5 Other Factors that may affect CA125 Levels 270
 - 15.6.4 Serum CA125 Levels in Patients with Benign Diseases 270
 - 15.6.5 Serum CA125 Levels in Non-ovarian Cancer 270
 - 15.6.6 Serum CA125 Levels in Ovarian Cancers 270
- 15.7 Clinical Applications 271
 - 15.7.1 Screening 271
 - 15.7.2 Evaluating Pelvic Masses 272
 - 15.7.3 Assessing Prognosis 272
 - 15.7.4 Assessing Response to Therapy 273
 - 15.7.5 Follow-up after Completion of Initial Therapy 274
- 15.8 Novel Markers 274
- 15.9 Conclusions 276
- References 276

Part Four Clinical Applications of TAAs 281

16 Overview of Cancer Vaccines 283

John Copier and Angus Dalgleish

- 16.1 Introduction 283

16.2	Cellular Immune Responses in Tumor Rejection	283
16.3	Selection of TAAs for the Development of Cancer Vaccines	284
16.4	Types of Cancer Vaccine	285
16.4.1	Defined Antigen Approaches	285
16.4.1.1	Peptides	285
16.4.1.2	Recombinant Protein	286
16.4.1.3	DNA	287
16.4.1.4	Viral Vectors	288
16.4.2	Undefined Antigens	289
16.4.2.1	Autologous Tumor Cell Vaccines	289
16.4.2.2	Allogeneic Tumor Cell Vaccines	290
16.4.2.3	Loading DCs with Tumor Preparations	290
16.5	Immune Biomarkers	292
16.6	Problems with the Assessment of Clinical Trials for Cancer Vaccines	292
16.6.1	Biomarkers	294
16.6.2	Schedule and Protocol	294
16.7	Combined Therapies; Synergy with Conventional Approaches	294
16.8	Conclusion	295
	References	296
17	Tumor-associated Antigenic Peptides as Vaccine Candidates	303
	<i>Dhiraj Hans, Paul R. Young, and David P. Fairlie</i>	
17.1	Introduction	303
17.2	Different Types of Antigens	304
17.2.1	Mutation Antigens	304
17.2.2	Shared Tumor-specific Antigens	306
17.2.3	Differentiation Antigens	308
17.2.4	Over-Expressed Antigens	309
17.2.5	Viral Antigens	311
17.3	Conclusions	312
	References	313
18	DNA Vaccines for the Human Papilloma Virus	317
	<i>Barbara Ma, Chien-Fu Hung, and T.-C. Wu</i>	
18.1	Introduction	317
18.1.1	HPV and Cervical Cancer	317
18.1.2	Events in the Progression from HPV Infection to Cervical Cancer	318
18.1.3	HPV Antigens for Vaccine Development	318
18.2	DNA Vaccines for HPV	319
18.2.1	Preventive HPV DNA Vaccines	319
18.2.2	Therapeutic HPV DNA Vaccines	321
18.2.2.1	Importance of DCs in Enhancing DNA Vaccine Potency	322
18.2.2.2	Clinical Progress in HPV DNA Vaccine Development	329

18.2.3	Combined Approaches	331
18.2.3.1	Combined Preventive and Therapeutic HPV DNA Vaccines	331
18.2.3.2	HPV DNA Vaccines in Combination with Other Therapies	331
18.3	Conclusion	332
	References	332
19	Adoptive T-cell Transfer in Cancer Treatment	339
	<i>Andreas Moosmann and Angela Krackhardt</i>	
19.1	Introduction	339
19.2	T Cell-based Therapies	339
19.2.1	Therapy of Virus-associated Cancers with Antigen-specific T Cells	339
19.2.2	T Cells with Specificity for Self-antigens	342
19.2.3	T Cells with Specificity for Alloantigens	343
19.2.4	Engineered T Cells	344
19.2.5	Safety Concerns and Suicide Gene Transfer	345
19.3	Conclusion	346
	References	346
	Index	355