There is no doubt that the early diagnosis of tumors is the most effective way to reduce the mortality caused by neoplastic diseases. Pre-symptomatic tumor diagnostics may be considered as secondary prevention by effective screening to identify cancer at a localized and curable stage. In this book methods, possibilities and perspectives of pre-symptomatic tumor diagnostics will be discussed. Because of the heterogeneity of tumors in genes and protein expression as well as in the biological behaviour one approach alone is insufficient for a highly specific, sensitive and cost-effective screening. In four chapters the requirements for an effective screening, the value of conventional "screening" methods (tumor markers, anatomical imaging modalities), and the search for novel biomarkers (tumor associated genes, antigens and autoantibodies) in terms of non-imaging screening as well as novel developments in imaging technologies and endoscopical approaches will be described.

Pre-symptomatic tumor diagnostics is a complex field that needs interdisciplinary research. The societies Gesellschaft für Immundiagnostik e.V. (www.gid.de) and Präsymptomatische Tumordiagnostik e.V. (www.tumornetzwerk.de) co-operate to provide a communication platform - "Innovation Forum Pre-symptomatic Tumor Diagnostics" - that supports complex research projects between clinicians, researchers, and industry to develop and validate new diagnostic technologies and products. This national platform will be turned into an international institution in the future.

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PREFACE

There is no doubt that the early (pre-symptomatic) diagnosis of tumors is the most effective way to reduce the mortality caused by neoplastic diseases. The search in the field of pre-symptomatic tumor diagnostics is very complex and interdisciplinary and should result in highly specific and sensitive as well as cost-effective routine diagnostic programs to identify cancer at a localized and curable stage. Many specialists working in different fields such as immunology, clinical chemistry, molecular biology, genetics, pathology, radiology, and clinical medicine are involved in the research for improving the early diagnosis of tumors. The new book series “Pre-symptomatic Tumor Diagnostics” is designed to provide clinicians and scientists of all fields in oncological research and clinical medicine with reviews and new results on all topics that are relevant for the early diagnosis of cancer. In the 1st volume, possibilities and perspectives of pre-symptomatic tumor diagnostics will be described and discussed. Because of the heterogeneity of tumors in genes and protein expression as well as in the biological behaviour one approach alone is insufficient for a highly specific, sensitive and cost-effective screening. In four chapters the requirements for an effective screening, the value of conventional "screening" methods (tumor markers, anatomical imaging modalities), and the search for novel biomarkers (tumor associated genes, antigens and autoantibodies) in terms of non-imaging screening as well as novel developments in imaging technologies and endoscopical approaches are described. The topics reviewed and discussed in this volume do not represent all fields involved in pre-symptomatic tumor diagnostics. Missing aspects as well as the novel developments will be the focus of the following volumes.

The editors

Michael Bachmann
Karsten Conrad
Werner Lehmann
Ulrich Sack
Chapter 1

TUMOR AND AUTOIMMUNITY
Tumor specific autoimmune responses - Why and when do they develop?

Jian-Ying Zhang¹ and Edward K.L. Chan²

¹Department of Biological Sciences, University of Texas, El Paso, Texas, USA; ²Department of Oral Biology, University of Florida, Gainesville, Florida, USA.

The development of cancer is attributed to many factors such as environmental and genetic contributions leading to gene mutations and dysregulations in the expression of many cellular genes. Overexpressed or abruptly expressed self proteins are released from the continuous turnover of tumor cells and are presented to the immune system of the host. The continuing elevated exposure of these proteins and/or macromolecular complexes, especially with fetal oncoproteins that are not normally expressed in adult tissues, initiates immune response that is manifested as autoantibodies. This scenario predicts that the autoimmune response to be initiated early on as the growing tumor mass is turning over sufficient antigens. The autoimmune response is likely to continue if the presentation of these self antigens persists. This hypothesis provides an explanation how autoantigens in cancer may represent reporters of aberrant or overexpressed proteins participating in the tumorigenesis process. Potential benefits to the host from these autoimmune responses have not been adequately examined. It is possible and likely that a sustained autoimmune response in cancer patients represent a failed response in tumor rejection. More work is needed to address the overall effect of these autoimmune responses to the tumor. This brief overview outlines supporting data from our laboratories.

Identification of tumor-associated antigens (TAAs)

Many studies demonstrated that cancer sera contain antibodies which react with autologous cellular antigens generally known as TAAs [1-3]. In our laboratories, the approach used in the identification of TAAs has involved initially examining the sera of cancer patients using extracts of tissue culture cells as source of antigens in Western blotting and by indirect immunofluorescence on whole cells. With these two techniques, we have identified sera which have high-titered fluorescent staining or strong signals to cell extracts on Western blotting and subsequently use these antibodies to immunoscreen cDNA expression libraries and isolate cDNA clones encoding targeted antigens. In this manner, several novel TAAs including HCC1 [4], p62 [5], p90 [6] and others have been identified. Immunoscreening of cDNA libraries with serum antibo-
ies for identifications of autoantigens is a well-established method and has been used not only to identify TAAs but also antigens in autoimmune diseases [7]. This methodology was the basis of the methods described in SEREX (serological analysis of recombinant mRNA expression libraries) with the difference that cDNA expression libraries constructed from autologous patient tumor were used as substrate in immunoscreening [8]. Subsequent reports using the SEREX technique have shown that the TAAs identified are no different from standard methods using cDNA expression libraries from cell lines derived from different sources, so that there did not appear to be any advantage to using cDNA libraries from autologous patients.

**Overexpression of TAA p62 and p90 in cancer**

Recent data from our laboratory suggest a mechanistic process may be involved in humoral immune responses in certain cancers such as hepatocellular carcinoma (HCC). HCC is unique in that one can follow a cohort of patients with chronic liver disease who will likely progress to develop malignancy over a period of 10 or more years. It has been observed that during transition from chronic liver disease to HCC, novel autoantibodies can appear which are not detected prior to pre-malignant conditions [9,10]. These novel antibody responses may be stimulated by cellular proteins which are involved in carcinogenesis. By immunoscreening an expression library, a RNA-binding protein autoantigen p62 has been identified in HCC and autoantibodies to p62 were found in 21% of a cohort of HCC patients [5]. p62 is a cytoplasmic protein which binds to mRNA encoding insulin-like growth factor II (IGF-II), a growth factor which is known to be overexpressed in HCC and is tumorigenic in transgenic animals. We showed that p62 was aberrantly expressed in 30% of unselected HCC [11] suggesting that p62 could play a role in HCC and other tumors by upregulating expression of growth factor IGF-II in the milieu of other oncogenic factors. A “companion” antigen p90, autoantibodies to which were found associated with anti-p62 responses in the same HCC patient group, was also identified by cDNA expression cloning [6]. Indirect immunofluorescence showed that, like p62, p90 localized to the cytoplasm in cultured cells and mouse fetal, but not adult liver. Among 11 human gastric cancer tissues examined, p90 was overexpressed in 6 (55%). Our data support the working hypothesis that autoantibody production in cancer may be directly linked to aberrant autoantigen expression of p62 and p90 autoantigens.

**TAAs are often tightly linked to tumorigenesis?**

The types of cellular proteins which induce autoantibody responses are quite varied and include oncogene products such as HER-2/neu and ras [12,13], cellular proteins which shield mRNAs from natural physiological degradation
Autoantibodies and tumorigenesis

such as p62 [5] and CRD-BP [14], onconeural antigens in the paraneoplastic disorder syndromes [15], differentiation-antigens such as tyrosinase and the cancer/testis antigens [16]. Factors leading to the production of such autoantibodies are not completely understood but the available data show that many of the target antigens are cellular proteins whose aberrant regulation could lead to tumorigenesis, such as p53, HER-2/neu, and ras, or are proteins whose dysregulation could have tumorigenic potential including mRNA binding proteins such as p62 and cell-cycle control proteins such as cyclin B1 [17,18]. One of the most extensively studied TAAs is p53. Autoantibodies to p53 in cancer were first reported in 1982 [19] and since then there have been numerous reports confirming and extending this finding (reviewed in [20]). A highly informative study showed that lung tumors contained several types of p53 gene mutations including missense, stop codon and frameshift mutations, but it was the missense mutations with overexpression of p53 protein which altered function and increased stability that correlated with antibody production [21]. In the case of p62 which is a fetal protein absent in adult tissues, immunogenicity appears to be related to abnormal expression of p62 in tumor cells [11]. With the onconeural antigens in paraneoplastic neurological disorders, antibody responses are thought to be related to ectopic expression of neuron-restricted cellular proteins in tumor cells [15]. The immune system in certain cancer patients appears to have the capability of sensing these abnormalities and it was proposed that autoantibodies might be regarded as reporters identifying aberrant cellular mechanisms in tumorigenesis [1]. As the detection of antibody immunity to tumor antigens becomes more routine, investigations have evolved to begin to address specific clinical questions such as the role of antibody immunity as a marker for patients exposed to cancer, as a tool to monitor therapy, or as an indicator of disease prognosis [22].

Using a mini-array of multiple TAAs to enhance antibody detection in cancer.

A feature of HCC is that antecedent liver cirrhosis and chronic hepatitis are common precursor conditions and during transition to malignancy some patients develop autoantibodies which were not present during the preceding chronic liver disease phase [9,10,23,24]. A hypothesis which has been proposed is that these antibody responses may be stimulated by cellular proteins which are involved in carcinogenesis. Many investigators have been interested in the use of autoantibodies as serological markers for cancer diagnosis, especially because of the general absence of these autoantibodies in normal individuals and in non-cancer conditions. Enthusiasm for this approach has been tempered by low sensitivity. One of our recent studies showed that detection of autoantibodies in cancer can be enhanced by using mini-array of seven TAAs as target antigens [25-27]. As shown in table 1, the mini-array comprised seven TAAs: c-myc [28], p53 [20], cyclin B1 [17,18], p62 [5,29], Koc [29,30], IMP1 [31] and survivin [32,33]. Antibody frequency to any indi-
individual TAA was variable, and ranges from 10.8 to 24.6 percent in HCC. With the successive addition of TAAs to a final total of 7 antigens, there was step-wise increase of positive antibody reactions up to 56.9 percent in HCC. Compared to HCC, frequency of antibody to any one of these same 7 antigens was 67.9 percent in lung cancer. The fact is that antibodies to any individual antigen such as anti-p53, anti-p62 or anti-c-Myc do not reach levels of sensitivity which could become routinely useful in diagnosis [27]. These data in one way indicate that the combination of antibodies might acquire higher sensitivity for diagnosis of cancer. On the other hand, the data also suggest that in the selection of different antigen-antibody systems, some of the antigens may turn out to be more specific for a certain type of cancer while others may be not. It is conceivable that autoantibody profiles involving different panels or arrays of TAAs might be developed and the results could be useful for diagnosis of certain types of cancer. More recently, we added three other TAAs (p90, cyclin D1 and cyclin A) into our previously used mini-array system for detection of antibodies in prostate cancer. The results showed that the cumulative positive reactions in prostate cancer sera reached 92.5%, significantly higher than in benign prostatic hyperplasia (BPH) and other controls [34].

Although there have been a steadily increasing number of studies describing and characterizing autoantibodies to “cancer-related antigens” in recent years, many of these antigen-antibody systems are not found to be useful in differentiating cancer and normal. For example, we clearly demonstrate that antibodies to three of so-called “cancer antigens” α-enolase, B23 and elongation factor-1γ (EF-1-γ) are not unique to HCC, and suggest that its detection in HCC might be attributed to antibody being already present in the precursor liver diseases such as chronic hepatitis, liver cirrhosis or primary biliary cirrhosis [35]. This study also emphasizes the importance of a comprehensive analysis of antibody responses to putative cancer antigens in different pre-cancer conditions such as chronic hepatitis and liver cirrhosis before conclusions can be made regarding their contribution to HCC. Some of the identified antibody-antigen systems may be unique to cancer while others may not. A comprehensive analysis and evaluation of various combinations of selected antibody-antigen systems will be useful for the development of autoantibody profiles involving different panels or arrays of TAAs, and the results could be useful for the detection of certain types of cancer such as lung cancer, HCC and so on. Our strategy to actively eliminate false-positive TAAs from our mini-array assay is qualitatively different from other approaches published to date.
Table 1. Frequency of antibodies to seven tumor-associated antigens in cancer patients.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>No Tested</th>
<th>c-myc</th>
<th>p53</th>
<th>Cyclin B1</th>
<th>p62</th>
<th>Koc</th>
<th>IMP1</th>
<th>Survivin</th>
<th>Any of 7 Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC</td>
<td>63</td>
<td>16(24.6)**</td>
<td>7(10.8)*</td>
<td>8(12.3)*</td>
<td>8(12.3)*</td>
<td>9(13.8)**</td>
<td>10(15.4)**</td>
<td>7(10.8)*</td>
<td>37(56.9)**</td>
</tr>
<tr>
<td>Gastric</td>
<td>91</td>
<td>14(15.4)**</td>
<td>12(13.2)**</td>
<td>14(15.4)**</td>
<td>8(8.8)*</td>
<td>17(18.7)**</td>
<td>15(16.5)**</td>
<td>9(9.9)*</td>
<td>48(52.7)**</td>
</tr>
<tr>
<td>Colorectal</td>
<td>45</td>
<td>2(4.4)</td>
<td>8(17.8)**</td>
<td>7(15.6)**</td>
<td>5(11.1)*</td>
<td>4(8.9)*</td>
<td>6(13.3)*</td>
<td>2(4.4)</td>
<td>23(51.1)**</td>
</tr>
<tr>
<td>Breast</td>
<td>64</td>
<td>12(18.8)**</td>
<td>5(7.8)</td>
<td>3(4.7)</td>
<td>5(7.8)</td>
<td>9(14.1)**</td>
<td>5(7.8)</td>
<td>5(7.8)</td>
<td>28(43.8)**</td>
</tr>
<tr>
<td>Lung</td>
<td>56</td>
<td>6(10.7)**</td>
<td>9(16.1)**</td>
<td>15(26.8)**</td>
<td>12(21.4)**</td>
<td>5(8.9)*</td>
<td>4(7.1)</td>
<td>6(10.7)*</td>
<td>38(57.9)**</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>321</td>
<td>50(15.6)**</td>
<td>41(12.8)**</td>
<td>47(14.6)**</td>
<td>38(11.8)**</td>
<td>44(13.7)**</td>
<td>40(12.5)**</td>
<td>29(9.0)*</td>
<td>174(54.2)**</td>
</tr>
<tr>
<td>NHS</td>
<td>82</td>
<td>0(0.0)</td>
<td>2(2.4)</td>
<td>2(2.4)</td>
<td>1(1.2)</td>
<td>1(1.2)</td>
<td>2(2.4)</td>
<td>2(2.4)</td>
<td>9(11.0)</td>
</tr>
</tbody>
</table>

Cutoff value: Mean + 3 S.D. of NHS. *p<0.05, **p<0.01. (simplified from reference [27])
References


Autoantibodies and tumorigenesis 53


Acknowledgments
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Autoantibodies as indicators of tumor development

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Autoantibodies (AAB) against various types of tumor associated antigens (TAA) with varying sensitivities and specificities for malignancy have been described. The immunogenicity of TAA mainly depends on the level and kind of expression. Therefore, tumor associated AAB (TA-AAB) can be regarded as reporters identifying aberrant cellular mechanisms in tumorigenesis and may be used as early indicators of tumor development. However, most of the TA-AAB lack sufficient sensitivity and specificity for use as biomarkers in the clinical practice. An optimal combination of highly specific TA-AAB in multiparametric assays as well as the standardization of the AAB analysis is necessary to realise the potential of TA-AAB in the early (pre-symptomatic) diagnosis and monitoring of malignancies.

Introduction

The malignant transformation of cells is a result of altered expression of genes involved in cell growth control and differentiation. Mutations, inherited through the germline or, more commonly, arising in somatic tissues later in life, as well as epigenetic changes that influence the expression and/or function of oncogenes and tumor-suppressor genes drive the development of tumors from the earliest to the last stage [1-3]. Fortunately, the tumor development can be checked by different tumor surveillance mechanisms acting on the level of DNA (repair of damaged DNA sequences), cell proliferation control (programmed cell death) and immune defence [4]. The loss or impairment of such control mechanisms also influence the development and progression of tumors. Therefore, tumor growth may depend on the balance between tumor promoting and tumor controlling factors/mechanisms.

The early detection of cancer that allows an effective and probably curative therapeutic management is most important in clinical oncology. However, current biomarkers often lack specificity and sensitivity for an efficient and cost-effective screening, leading to false results and late detection of malignancies. In theory, all changes in expression, structure and/or function of genes/proteins involved in the tumorigenesis may be markers for pre-symptomatic tumor diagnosis. Different approaches are used to find parameters/biomarkers (single or in combination) for a specific and sensitive diagno-
sis of early cancer stages (see Chapter 2 and 3). Here we describe the potential of tumor associated autoantibodies (TA-AAB) as indicators of cancer development and hence early (pre-symptomatic) tumor diagnosis.

**Anti-tumor immune responses**

It is well known that tumors in humans and animal models may trigger anti-tumor immune responses. Those responses may be differentiated in (1) immune defence mechanisms according to the “immune surveillance” hypothesis, (2) polyclonal activation of naturally occurring autoantibodies / activation of natural defence mechanisms [5], and (3) immune responses against overexpressed or aberrantly expressed self-antigens independent of immune defence mechanisms.

The so-called immune surveillance reflects the immune defence mechanisms that control tumor development mainly at the level of precancerous cells. By this way the immune system protects the organism from the growth of potentially neoplastic cells that are initiated by viruses (e.g., human papilloma viruses, Epstein–Barr and other human Herpes viruses) and perhaps other mechanisms. Besides effects of specific induced immune responses, tumor growth may be modulated by naturally occurring autoantibodies (NOA) that bind tumor cells or components of the immune system [6,7]. NOA are germ-line-encoded polyreactive antibodies probably involved in the “first line defence”, in the clearance of senescent products and in immune regulatory mechanisms. Among the first functions described for NOA was the reactivity with tumor cells [8]. This reactivity is most probably a manifestation of polyreactivity rather than an interaction between a tumor-specific antigen and a corresponding antibody [9]. NOA may have different biological effects ranging from enhancement to surveillance of tumor development [6,7].

Immune reactivity against tumor cells does not always represent an anti-tumor defence mechanism but simply may be a result of an antigen-driven immune response. Regardless of their biological role, autoantibodies (AAB) that specifically recognize antigens of the tumor may have a great potential for the detection of early tumor development. However, only a subset of patients with a tumor type develops a humoral response to a particular antigen, for example p53 [10]. The immunogenicity of a tumor depends on several factors which may be variable among tumors of a similar type. Regarding the (tumor-associated) antigens, the level of expression, post-translational modification, or variations in protein processing, are of great importance (table 1). Furthermore, the specific immune response to a defined antigen depends on the structure of the highly polymorphous MHC molecules.
<table>
<thead>
<tr>
<th>Mechanisms underlying immunogenicity</th>
<th>Cause(s)</th>
<th>Genes/proteins involved</th>
<th>Examples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overexpression of autotumor antigens in malignant tissues</td>
<td>Gene amplification and other mechanisms of enhancement of gene expression (e.g. promoter hypomethylation)</td>
<td>Oncogenes and other antigens involved in cancer development (cell-cycle/mitosis associated proteins, helicases)</td>
<td>p185&lt;sup&gt;HER-2/neu&lt;/sup&gt;, c-myc, L-myc, eIF-4-&lt;sub&gt;γ&lt;/sub&gt;, p160&lt;sup&gt;ROCK&lt;/sup&gt;, Fibulin-1, WT1, CAGE</td>
<td>14,16,20, 28,110-112</td>
</tr>
<tr>
<td>Expression of aberrant autotumor antigens</td>
<td>Point mutations</td>
<td>Oncogenes, tumor suppressor proteins</td>
<td>p53, p53&lt;sup&gt;T&lt;/sup&gt;</td>
<td>15,113</td>
</tr>
<tr>
<td></td>
<td>Truncation</td>
<td>B23 (nucleophosmin) CRT32 (truncated form of calreticulin)</td>
<td></td>
<td>82,114</td>
</tr>
<tr>
<td>Modified expression in tumors (underglycosylation, sialylation)</td>
<td>Aberrant post-translational modifications</td>
<td>Carbohydrate membrane antigens</td>
<td>PEMMUC1, Gangliosides (GM1, GM2, GD2)</td>
<td>115</td>
</tr>
<tr>
<td>Protein modification</td>
<td>Apoptosis-associated proteolytic cleavage</td>
<td></td>
<td>BARD-1</td>
<td>31</td>
</tr>
<tr>
<td>Generation of cross-reactive epitopes</td>
<td>Aberrant expression of an unrelated autoantigen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expression of chimeric proteins</td>
<td>Chromosomal/gene translocations</td>
<td>SSX-2 gene c-aml proto-oncogene (2:5) translocation &gt; hybrid oncopgenic tyrosine kinase</td>
<td>HOM-MEL-40, p21&lt;sup&gt;WAF1/cip1&lt;/sup&gt;, NPM-ALK</td>
<td>13,117,118</td>
</tr>
<tr>
<td>Ectopic expression of autotumor antigens</td>
<td>Gene activation or derepression</td>
<td>Cancer-testis antigens (CTA)</td>
<td>NY-ESO1, MAGE-1, MAGE-3</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Gene activation or derepression or post-transcriptional regulatory mechanisms (?)</td>
<td>Onconeural antigens (ONA)</td>
<td>Hu, R&lt;sub&gt;y&lt;/sub&gt;, Yo, Amphiphysin VGCC</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PGP 9.5, Annexin-I, -II</td>
<td>76,80</td>
</tr>
<tr>
<td>Autotumor antigen secretion by tumor cells and/or release of autotumor antigens by tumor necrosis and cell death</td>
<td></td>
<td>Many intracellular antigens</td>
<td></td>
<td>83</td>
</tr>
</tbody>
</table>
Target antigens of tumor associated autoantibodies

There is growing evidence that antigens that become aberrantly or overexpressed during the transition to malignancy can be targets of the cellular and/or humoral immune response (table 1) under special conditions (e.g. sequence of MHC molecules, pro-inflammatory stimuli). Those antigens may be involved in or the consequence of the tumorigenesis and therefore, immune responses (e.g. autoantibodies) against them can be regarded as tumor associated or even tumor specific. Alterations in at least three groups of genes are responsible for the tumorigenesis: oncogenes, tumor-suppressor genes and stability genes (reviewed in [11]). Examples for autoantibodies against products of those genes are shown in table 2.

Oncogene products. The cellular oncogenes are derived from their normal counterparts, the protooncogenes, by activating mutations or transcriptional activations [12]. Their products include a broad range of factors that promote cancer (e.g., growth factors, growth factor receptors, downstream mediators and modulators of cellular signal transduction pathways, transcriptional regulators). An important example of an anti-receptor immune response is the AAB against the HER-2/neu product, a 185 kDa transmembrane protein with extensive homology to the epidermal growth factor receptor. Anti-HER-2 AAB are found in breast cancer patients in up to 55% [13,14]. The AAB response correlates with HER-2/neu protein overexpression in the patient’s primary tumor, but were also found in women with HER-2/neu negative breast cancer, suggesting an active immunoselection for HER-2/neu negative variants [14]. This possibility is also underlined by the higher frequency and the higher titers of p185HER-2/neu AAB in the early stage of disease [14]. AAB against the p21 ras protein, a member of the GTPase complex whose transforming activity evolves by point mutations, has been found in 32% of patients with colon cancer [15]. Although p21ras is activated by point mutations, most AAB detect epitopes near the carboxyl terminus of the wild-type protein [15]. In addition to these AAB directed against growth factor receptors (p185HER-2/neu) or GTP binding proteins (p21ras), AAB to another group of oncoproteins have been described in patients with solid tumors (colorectal, breast, ovary, lung cancer) and patients with leukemias/lymphomas. These AAB are directed against nuclear regulatory proteins such as myb and myc [17-21]. However, with the exception of AAB to the L-myc protein, these antibodies have been shown to be relatively unspecific for tumors in some studies. Furthermore, the frequencies of c-myc AAB in healthy volunteers and SLE patients varied greatly in the different studies [16-18]. In general, different methods of AAB determinations and differences in the populations studied may account for varying results. The source and purification of autoantigens and the assays used for AAB determination may influence results dramatically.
### Table 2. Autoantibodies against targets that are involved in tumorigenesis

<table>
<thead>
<tr>
<th>Products of oncogenes</th>
<th>Autoantibodies against</th>
<th>Tumor entities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>p185HER-2/neu</td>
<td>Breast cancer (11-55%)</td>
<td></td>
<td>13-16, 19, 21, 118-121</td>
</tr>
<tr>
<td>p21Ras</td>
<td>Colon cancer (32%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>Colorectal (57%) and lung (13.2%) cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-myc</td>
<td>Lung cancer (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myb</td>
<td>Breast (43%), colon (40%) and ovary cancer (33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p145Abl, p210Bcr-Abl</td>
<td>Chronic myelogenous leukemia (50-70%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>csk</td>
<td>Colon (20%), bladder (19%), lung (17%), ovary (10%) and breast (5%) cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALK</td>
<td>Anaplastic large-cell lymphoma (ALCL) (90.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Products of tumor-suppressor genes</td>
<td>p53</td>
<td>Most cancer (3-65%)</td>
<td>10, 28, pp. 92-107</td>
</tr>
<tr>
<td>WT1</td>
<td>Leukemias (AML: 81.3%, ALL: 45.5%, CML: 61.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Products of stability genes</td>
<td>Rad51</td>
<td>Pancreatic cancer (7%)</td>
<td>30,32</td>
</tr>
<tr>
<td>hMSH2</td>
<td>Pancreatic cancer (13.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hPMS2</td>
<td>Pancreatic cancer (8.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibitor of apoptosis proteins</td>
<td>Survivin</td>
<td>Lung (21.6-58.1%), colorectal (8.2%), head and neck (46%), breast (6.3%), and prostate (3%) cancer</td>
<td>34-39</td>
</tr>
<tr>
<td>Livin</td>
<td>Lung (41.3%) and gastrointestinal (47%) cancer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
For example, c-myc AAB were determined with ELISAs using 31-mer c-myc peptides [17] or human prokaryotically expressed recombinant c-myc protein [18], or with immunoblotting using the recombinant protein [16]. Furthermore, variations in the definition of standards of detectability led to different frequencies, as has been shown for p185HER-2/neu AAB in breast cancer patients (11-21%) and in healthy volunteers (0-1%) [14]. But ethnic differences and different influences of endogeneous and exogenous factors in the populations studied may also be relevant for the variation in results. For example, the frequency of p185HER-2/neu AAB is highest in women with premenopausal breast cancer because there is also highest frequency of HER-2/neu protein overexpression [13]. In conclusion, there is a further need for studies of the clinical and biological nature concerning humoral autoimmune responses to oncoproteins such as (a) the evaluation of diagnostic relevance (diagnostic sensitivity and specificity) and the prognostic significance (correlation with the stage of the disease and survival) in defined patient groups using optimized and standardized methods, (b) the search for associations of antibody titers with disease progression or relapse and therapeutic effects, (c) the search for possible mechanisms of AAB induction (correlation with protein overexpression, mutations or presence of oncoproteins in the circulation) and (d) the search for possible effects of AAB on tumor cells.

**Tumor suppressor proteins.** Alterations in tumor suppressor genes that lead to the loss or disturbance of the function(s) of their products are of major importance in tumorigenesis. Mutations may lead to impaired function and cellular accumulation of the protein by different mechanisms such as a prolonged half-life [22] with the potential of the induction of immune responses. The p53 AAB is the most extensively studied humoral autoimmune response in human cancer patients (reviewed in [10,23], see also Montenarh (pp. 92-107). Mutations of the p53 tumor suppressor gene are the most frequently reported gene alteration in human cancers [24,25]. In 1982 it was shown for the first time that p53 may become immunogenic in cancer patients [26]. In the years that followed p53 AAB were detected in sera from a variety of cancer patients in frequencies between 3 and 65 % depending on tumor type and method of antibody detection, whereas the prevalence of such AAB in normal populations was low [10,23,27]. Recently, another humoral anti-tumor suppressor immune response has been described in patients with hematopoietic malignancies, the response against Wilms tumor gene product gene WT1 [28]. IgG anti-WT1 antibodies were found in 27.2-50% of leukemia patients and in 69.6% of patients with myelodysplastic syndromes (MDS) but only in 4.7% of healthy volunteers. The simultaneous production of IgM and IgG antibodies was limited to the patients.

**Products of stability genes.** Stability genes or caretakers encode products that keep genetic alterations to a minimum. Inactivation of those genes leads to a higher mutation rate and, thus, a higher risk of tumor development. The best known examples of cancer genes of this group are the breast cancer associated BRCA genes (see Jandrig, pp. 237-248). AAB against BRCA prod-
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Autoantibodies have not been described so far. Interestingly, AAB have been found against the recombination factor Rad51 and the BRCA associated ring domain BARD1 proteins that are co-localized with BRCA products to areas of damaged DNA supporting a role in DNA repair [29]. Rad51 is highly expressed in human pancreatic adenocarcinoma and AAB were found in 7% of patients but not in healthy volunteers [30]. Anti-BARD1 AAB have been induced during treatment of rats with peritoneal colon carcinomatosis by injections of apoptotic bodies derived from tumor cells and interleukin 2 [31]. It was demonstrated that the humoral immune response was directed against a cleaved form of BARD1 present in apoptotic bodies derived from rat and human colon and mammary carcinoma cell lines. Recently, another autoimmune response to stability genes, the DNA mismatch repair enzymes hMSH2 and hPMS1, has been described in patients with pancreatic cancer [32]. These enzymes are also overexpressed in pancreatic ductal adenocarcinomas.

Inhibitors of apoptosis proteins (IAP). An important mechanism involved in cancer formation is the inhibition of apoptosis, which, by extending the lifespan of cells, favors the accumulation of transforming mutations [33]. Apoptosis pathways are effectively blocked by proteins belonging to the IAP family that directly inhibits caspase and pro-caspase molecules. It has been shown recently that at least two of the known IAPs, survivin and livin, are targets of an autoimmune response in human cancer [34-39]. Survivin is abundantly expressed in fetal tissue and in a variety of human tumors including lung, colon, breast, prostate, pancreatic, and gastric cancer as well as in high-grade lymphomas and neuroblastomas [40-42]. Anti-survivin AAB are described so far in lung, colorectal, head and neck, breast and prostate cancer (table 2). Livin is also highly expressed in cancer, but shows little or no expression in normal tissues [43,44]. Therefore, it was no surprise to find AAB in cancer patients. Contrary to survivin AAB, which were found in all clinical stages, anti-livin AAB were found only in patients with advanced stages [36,37]. It has been speculated that larger expression of livin is needed for induction of AAB responses to livin compared with survivin.

Cancer/testis class of tumor antigens (CTA) are expressed in a variable proportion of a wide range of human tumors, but are silent in most normal tissues except the testis. They were initially identified as targets for cytotoxic T cells (MAGE, GAGE, BAGE) and, later on, uncovered by SEREX (serological analysis of recombinant cDNA expression libraries of human tumors with autologous serum) analysis (reviewed in [45,46]). CTA identified by SEREX elicited an AAB response in tumor patients. Therefore, this methodology leads not only to the detection of new tumor antigens but also to the identification of specific humoral responses which may be used for diagnostic purposes. Stockert et al. were the first who tested a great number of tumor sera for humoral immune response to SEREX-identified tumor antigens, including several CTA, by ELISA with recombinant proteins [47]. They showed that 9.4% of melanoma patients, 12.5% of ovarian cancer patients, 4.2% of patients with lung cancer and 7.7% of patients with breast cancer have AAB against NY-
No AAB were found in 47 patients with NY-ESO-1 negative melanomas, but were present in 53 % patients with NY-ESO-1 positive melanomas, suggesting an autoantigen driven response. No AAB against CTA were found in 70 blood donors [47]. The current determined tumor associated humoral anti-CTA responses are summarized in table 3. With few exceptions those responses are relatively infrequent. NY-ESO-1 AAB seem to be the most frequently observed anti-CTA response. Jäger et al. showed that both NY-ESO-1 autoantibodies and cytotoxic T cells (CTL) against NY-ESO-1 peptides can be present in the same patient [48]. This suggests that the screening for an AAB response may be a simple and effective way to identify concomitantly CTL reactivity.

**Onconeural antigens (ONA)** are normally restricted to the nervous system but are aberrantly expressed in a number of tumors possibly by gene activation or derepression or post-transcriptional regulatory mechanisms. They may then be recognized by the immune system as "foreign" and elicit an autoimmune response causing paraneoplastic syndromes affecting the nervous system. AAB against ONA may detect neuronal nuclear antigens (= anti-neuronal nuclear antibodies ANNA), cytoplasmic antigens of Purkinje cells (= anti-Purkinje cell antibodies APCA), synaptic or retinal proteins (see table 4). In most cases of paraneoplastic syndromes the detection of specific AAB can strongly suggest the presence of a tumor (for review see [49-54]). Anti-Hu positive patients with paraneoplastic encephalomyelopathies (PEM) or subacute sensory neuronopathy (SSN) most often have small cell lung cancer (SCLC) as underlying disease. Similarly, anti-Yo positive patients with paraneoplastic cerebellar degeneration (PCD) often harbor gynaecological neoplasms. Furthermore, Hu, Yo and Ri AAB can be found in lower frequency and at lower titers in SCLC or ovarian cancer patients without neurological diseases [55,56]. Neurological syndromes associated with AAB to ion channel proteins have a lower frequency of tumors as the syndromes associated with Hu, Yo and Ri antibodies. Patients with anti-VGKC positive acquired neuromyotonia have SCLC or thymoma in 20% and only 10-15% of the AchR antibody positive Myasthenia gravis (MG) cases are associated with thymoma. In thymoma associated MG other autoantigens such as ryanodine receptor and titin may also play a pathogenic role [57,58]. Recently, AAB against neuroectodermal antigens, SOX group B and zinc-finger gene of the cerebellum (ZIC)2 proteins, have been found in high frequency of SCLC patients without the presence of paraneoplastic neurologic syndroms [59]. The presence of a strong humoral autoimmune response to SOX1 and ZIC2 proteins without concomitant development of autoimmune neurologic disease and the correlation of anti-SOX1/ZIC2 AAB with prognosis suggest that these antigens could be targets for cancer vaccine strategies [59].
Table 3. Autoantibodies against cancer/testis class of tumor antigens (CTA)

<table>
<thead>
<tr>
<th>CTA family</th>
<th>Autoantibodies against</th>
<th>Tumor entities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAGE-A</td>
<td>MAGE-A1</td>
<td>Lung (4%), colon (12.8%) and ovarian (3.1%) cancer, melanoma (1-36%)</td>
<td>47,122</td>
</tr>
<tr>
<td></td>
<td>MAGE-A3</td>
<td>Colon cancer (8.1%)</td>
<td>123</td>
</tr>
<tr>
<td>CAGE</td>
<td>CAGE</td>
<td>Colon cancer (12%), endometrial cancer (26.7%), melanoma (10%)</td>
<td>124</td>
</tr>
<tr>
<td>XAGE</td>
<td>XAGE-1b</td>
<td>Lung cancer (25%, all adenocarcinoma), prostate cancer (1.6%)</td>
<td>125,126</td>
</tr>
<tr>
<td>SCP</td>
<td>SCP-1 (HOM-TES-14)</td>
<td>Breast (50%) and renal (3.2%) cancer</td>
<td>127</td>
</tr>
<tr>
<td>ESO</td>
<td>NY-ESO-1</td>
<td>Lung (4-20%), colon (6.8%), prostate (8.2-20%), ovarian (12.5%), and breast</td>
<td>47,48,123,126-130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cancer (8%), melanoma (9.4%)</td>
<td></td>
</tr>
<tr>
<td>cTAGE</td>
<td>cTAGE-1</td>
<td>Colon cancer (25%), cutaneous T-cell lymphoma (33%; only stage D2)</td>
<td>131,132</td>
</tr>
<tr>
<td>GAGE-A</td>
<td>Truncated GAGE</td>
<td>Colon cancer (12.5%)</td>
<td>131</td>
</tr>
<tr>
<td>SSX2</td>
<td>SSX2</td>
<td>Colon cancer (2.7%), melanoma (18%)</td>
<td>122,123</td>
</tr>
<tr>
<td>LAGE</td>
<td>LAGE-1</td>
<td>Prostate cancer (15%)</td>
<td>129</td>
</tr>
</tbody>
</table>
Proliferation associated antigens other than oncoproteins. Proteins involved in cell cycle regulation / progression and mitosis, but also other proteins involved in cellular processes that might be increased in unregulated cell growth, may drive autoimmune responses in tumor patients. AAB against nuclear and cytoplasmic cell cycle regulated or regulating proteins and proteins involved in splicing processes and ribosome biosynthesis could be detected in tumor patients. This supports previous observations that AAB responses against intracellular antigens are often directed at molecules involved in cellular biosynthetic or proliferative functions [60].

Cyclins and cyclin-dependent kinases (CDK) are a group of cell cycle regulating proteins acting at different points of the cell cycle progression. They are amplified and overexpressed in many tumors [61-64]. Covini et al. showed that AAB to cyclin B1, cyclin A and CDK2 are present in sera of patients with hepatocellular carcinomas (HCC) in 15%, 1% and 1%, respectively [65]. Furthermore, anti-cyclin B1 antibodies could be found in patients with a higher risk of HCC development, e.g. in patients with chronic hepatitis (in 1 of 70 cases) and cirrhosis (in 3 of 70 cases), suggesting a predictive relevance of these AAB. Recently, AAB against cyclin B1 have been found in frequencies higher than 10% in patients with lung, colorectal, gastric and prostate cancer [38,66]. In prostate cancer also anti-cyclin D1 and anti-cyclin A AAB have been described [38].

Many other AAB against proliferation associated antigens with more or less specificity are present in sera of cancer patients, such as AAB against (1) SG2 nuclear antigen, a member of a novel family of calmodulin binding proteins associated with the serine/threonine phosphatase PPA2 [67,68], (2) the centromere protein F [69], (3) DNA topoisomerase II [70], (4) a novel nuclear autoantigen with splicing factor motifs, provisionally designated HCC1 [71], (5) insulin-like growth factor II (IGF-II) mRNA-binding proteins IMP-1, IMP2 (p62), and IMP-3 (Koc) that regulate the expression of IGF-II [72,73 ], (6) DEAD-box protein 48 (DDX48), also known as eukaryotic initiation factor 4A-like NUK-34 [74], (7) 32 kDa subunit of replication protein A (RPA32) [75], (8) annexins I, II, and XI-A [76,77], AIS gene product p40 [78], only to name a few. The list of cancer autoantigens is growing rapidly: several hundreds have been found via recognition by AAB in cancer patients sera using SEREX [45,46,79; see Kosowski, pp. 78-91 or proteomics-based technologies [74,81-83; see also Klein-Scory, p. 141]. How can we use this potential on humoral autoimmune responses in research and clinical practice?
<table>
<thead>
<tr>
<th>Autoantibodies against</th>
<th>Paraneoplastic syndromes</th>
<th>Associated tumors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ri antigens (NOVA-1, NOVA-2)</td>
<td>Opsoclonus/myoclonus syndrome</td>
<td>Breast cancer Small-cell lung cancer</td>
<td>52</td>
</tr>
<tr>
<td>Yo antigens (cdr34, cdr62-1, cdr62-2)</td>
<td>Paraneoplastic cerebellar degeneration</td>
<td>Breast cancer Small-cell lung cancer Hodgkin's lymphoma</td>
<td>54</td>
</tr>
<tr>
<td>Syaptic vesicle-related protein amphiphasin</td>
<td>Paraneoplastic stiff man syndrome Paraneoplastic encephalomyelitis (LEMS)</td>
<td>Breast cancer Small-cell lung cancer</td>
<td>133</td>
</tr>
<tr>
<td>Protein(s) of the P/Q, N and L type of voltage-gated calcium channels (VGCC)</td>
<td>Lambert-Eaton myasthenic syndrome (LEMS)</td>
<td>Small-cell lung cancer</td>
<td>51,52</td>
</tr>
<tr>
<td>Protein(s) of voltage-gated potassium channels</td>
<td>Acquired neuromyotonia (Isaacs' syndrome)</td>
<td>Small-cell lung cancer Thymoma</td>
<td>51</td>
</tr>
<tr>
<td>Protein(s) of the acetylcholine receptor Cross-reactive titin epitopes Ryanodine receptor</td>
<td>Myastenia gravis</td>
<td>Thymoma or thymic carcinoma</td>
<td>57,58</td>
</tr>
<tr>
<td>Recoverin, a protein of photoreceptor cells</td>
<td>Cancer-associated retinopathy</td>
<td>Breast cancer Small-cell lung cancer</td>
<td>105</td>
</tr>
<tr>
<td>Zinc-finger protein ZIC4</td>
<td>Subacute cerebellar degeneration</td>
<td>Small-cell lung cancer</td>
<td>134</td>
</tr>
<tr>
<td>Zinc-finger protein ZIC2</td>
<td></td>
<td>Small-cell lung cancer</td>
<td>59</td>
</tr>
<tr>
<td>Collapsin response mediator protein-5 (CRMP-5)</td>
<td>Subacute cerebellar degeneration</td>
<td>Small-cell lung cancer</td>
<td>134</td>
</tr>
<tr>
<td>Sry-like high mobility group box (SOX) antigens SOX1 and SOX2</td>
<td></td>
<td>Small-cell lung cancer</td>
<td>59</td>
</tr>
</tbody>
</table>
Relevance of tumor associated autoantibodies

Since AAB response in tumor patients is often associated with aberrant or overexpression of TAA in tumor tissue or serum, most TA-TAA seem to be a result of antigen-driven immune responses like those suggested for autoimmune diseases [60]. Furthermore, it may be concluded that TA-AAB can be viewed as reporters from the immune system revealing the identity of antigens which might be playing roles in the tumorigenic processes [84,85]. Therefore, TA-AAB are important markers for different approaches:

Molecular probes for the identification of novel proliferation associated antigens and pathways [68,71,86-88]. Sera of cancer patients have been shown to be useful reagents for identifying new cellular proteins possibly involved in tumor development. A new cell cycle specific DNA-binding nuclear protein has been identified using autoimmune serum of a patient with bladder and metastatic lung cancer [67,89]. This serum produced a previously undescribed cell cycle-related staining pattern on HEp-2 cells. According to the cell cycle distribution the detected antigen was provisionally named SG2NA (S/G2 nuclear antigen). The centromere protein F is another novel proliferation associated and cell cycle dependent protein detected by autoimmune sera. Casiano et al. identified a centromere protein provisionally designated p330d (doublet polypeptide of 330 kDa), which accumulates in the nuclear matrix during S phase, reaching maximum levels during G2 phase and localized at the centromeres during prophase and metaphase and at the central spindle and midbody regions during anaphase and telophase [86]. The same protein, designated centromere protein F (CENP-F), was identified by Rattner et al. using a serum from a lung cancer patient [90].

Search of new targets for vaccine-based therapies. Most TA-AAB detect antigens that are highly expressed predominantly in tumor cells. Those tumor associated antigens are putative candidates for a tumor vaccination strategy because the B-cell response is often accompanied by a cellular immune response [48].

Biomarkers for the prediction, diagnosis and monitoring of tumors. Despite the large number of TA-AAB detected until now the practical application is limited by the following reasons: (1) The aberrant or overexpression of TAA in tumors is, with few exceptions necessary, but not sufficient for immune activation. Usually, individual TA-AAB are detected in only small numbers (<20%) of patients with cancer depending on the MHC status and other individual factors. Therefore, the diagnostic sensitivity of most TA-AAB is too low for diagnostic screening. (2) Overexpressed genes/proteins are thought to elicit an immune response by over-riding thresholds critical for the maintenance of tolerance [91]. Because proliferation associated antigens may be
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also overexpressed in other hyperproliferative disorders such as autoimmune diseases [92], the AAB response is often not specific for malignancy [17,93,94]. (3) The results regarding sensitivity and specificity of TA-AAB may differ dramatically from study to study due to different methods used for AAB determination, study design and ethnicity of tested subjects. Nevertheless, TA-AAB may have a great potential in the early (pre-symptomatic) diagnosis of cancer, because the autoimmune response does not depend on the size of the tumor.

Tumor associated autoantibodies – biomarkers for the prediction and early diagnosis of cancer?

There is still a need for parameters which are specific for tumors and are detectable in preclinical stages. The ideal tumor marker should be highly sensitive and highly specific for tumors. The tumor sensitivity should be higher than that of other diagnostic methods and the earlier diagnosis should lead to an improvement of therapy. Tumor-associated antigens present in sera of cancer patients (e.g., CEA, NSE, SCC, CA50 etc.) can be useful markers for prognosis and for monitoring cancer therapy but have a limited value for diagnosis, especially for the early diagnosis of cancer. Novel approaches and developments (see Oehr, pp. 26-43 and Chapter 2-4) may improve the diagnostic possibilities but are too expensive for a broader use or for the screening of risk groups. TA-AAB may develop (very) early with respect to tumor formation. Hints for predictive relevance of TA-AAB are given by different observations and approaches:

**TA-AAB are significantly more often detectable in risk groups for cancer development than in healthy volunteers.** A higher risk of tumor development is observed in populations expressing cancer susceptibility genes (hereditary cancer syndromes, see Jandrig, pp. 237-248), populations who are exposed to carcinogenic substances (e.g., uranium miners), and in populations with preneoplastic or cancer predisposing diseases (e.g., Barretts’ oesophagus, chronic liver inflammation or cirrhosis, dermatomyositis, Sjögren’s syndrome). TA-AAB have been described in some of the known risk groups in higher frequencies than in healthy controls: Anti-p53 AAB in uranium miners [95]; anti-p53 AAB in patients with liver cirrhosis and other chronic liver diseases [96]; anti-Crt32 AAB in HBV positive chronic hepatitis [82]; anti-WT AAB in myelodysplastic syndromes [28]; anti-hMSH2 and hPMS1 AAB in patients with dermatopolymerosis [32]; anti-HMdU (5-hydroxymethyl-2’-deoxyuridine) and anti-p53 AAB in otherwise healthy women who had a family history of breast cancer [97,98]. However, the TA-AAB response in these populations does not indicate that all AAB positive subjects will develop cancer, because overexpression of the relevant autoantigens with the potential of AAB induction can be observed also in non-tumorous cells [99]. The AAB
responses rather reflect changes that might be relevant in tumorigenesis and therefore indicate a rising risk of tumor development in those subjects.

**TA-AAB are significantly more often detectable in pre-malignant and early tumor stages than in healthy volunteers:** Anti-RPA32 and anti-p53 AAB are detectable in ductal carcinoma in situ of the breast with early, non-palpable (3-5 mm) lesions [75,100]; Anti-YKL-40 AAB are detectable in early stage of ovarian cancer more often than the conventional tumor markers (65% vs 35% CA125 and 13% CA15-3) [101].

**TA-AAB against neuroectodermal antigens in paraneoplastic syndromes.** Paraneoplastic syndromes are (most often neurologic) disorders (PND), that are caused by a strong immune response against a shared antigen in the tumor and normal host tissues. Because there is a direct relationship between those TA-AAB and paraneoplastic manifestations, the PND specific AAB response is an early indicator of tumor development (table 4). It has been shown that AAB against the neuroectodermal antigens HuD, amphiophysin, recoverin, or enolase precede the diagnosis of cancer in approximately 70% of patients by up to 4 years [102-105].

**Retrospective studies** demonstrate how long TA-AAB are detectable before disease manifestation or the definite diagnosis with conventional methods. Lubin et al. [106] were the first who described that the humoral anti-p53 response may be an early event during tumorigenesis and can be detected before clinical manifestation of the disease. In two retrospective studies Trivers et al. showed that p53 AAB were present months to years before the manifestation of tumors: (1) angiosarcoma of the liver in workers occupationally exposed to vinyl chloride and (2) lung cancer in heavy smokers with chronic obstructive pulmonary disease [107,108]. In a retrospective study on former uranium miners, we could show that anti-p53, anti-NY-ESO-1 and anti-survivin AAB are detectable in sera from patients with lung cancer collected over a period of up to 10 years prior to disease manifestation or confirmed diagnosis [34,95,109]. Anti-RPA32 AAB was shown to be present 18 months before diagnosis of cancer in one patient [75] and in another one anti-IMP1 and anti-IMP3 (Koc) AAB were detectable approximately 8 years before diagnosis of HCC [73].

**Prospective studies.** The follow-up of TA-AAB positive persons is of great importance to show the real risk of cancer development in those subjects. Up to now only one study has been published: Women, healthy at blood donation but who were diagnosed 0.5-6 years later with breast or colorectal cancer exhibited significantly increased anti-HMdU (5-hydroxymethyl-2’-deoxyuridine) AAB over the age-matched controls [97]. Hopefully, further prospective studies of TA-AAB positive persons will show whether defined AAB can be used in the screening for preneoplastic or microinvasive tumor lesions allowing an early diagnosis and an early intervention of cancer.
Conclusions

Of the several hundreds AAB specificities detected in sera of tumor patients so far, more and more have become candidates for introduction in the clinical practice. According to the spectrum of reactivity, two classes of TA-AAB can be differentiated: those associated with specific types of cancer (e.g., anti-HCC1) and those common to a wide spectrum of cancer (e.g., anti-p53, anti-survivin, and anti-RPA32 AAB). For further use TA-AAB should be selected for their specificity regarding malignancies and for their potential clinical application (screening of risk groups, early diagnosis, disease monitoring). If selected for high specificity, for the screening of risk groups, the sensitivities of most TA-AAB are too low. Even the highest frequencies of about 30 to 50% found in lung, colorectal, head and neck cancer (anti-p53, -p21ras) and breast cancer (anti-HER-2/neu) with concomitant high specificity are not sufficient for a screening programme. A combined determination of two or more tumor specific AAB may overcome this problem. Therefore, a further evaluation of the relevance of known AAB specificities as well as the search for novel diagnostically relevant TA-AAB is necessary.

Taken together, the screening for an AAB response in tumor patients may lead to new diagnostic tumor markers and may be a simple and effective way to identify concomitantly CTL reactivity. Furthermore, as "reporters from the immune system" such AAB could be used to elucidate the nature of autoantigens which drive the immune response.

References
70 Karsten Conrad, Dirk Roggenbuck, and Michael Bachmann


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(58) Mygland A, Aarli JA, Matre G, Gilhus NE. Ryanodine receptor (RyR) antibodies are detected in about 50% of patients with myasthenia gravis who have a thymoma. J Neurol Neurosurg Psychiatry 1994;57:843-846.

(59) Vural B, Chen LC, Saip P, Chen YT, Üstüner Z, Gonen M, Simpson AJG, Old LJ, Ozbek U, Gure AO. Frequency of SOX group B (SOX1, 2, 3) and ZIC2 antibodies in Turkish patients with small cell lung carcinoma and their correlation with clinical parameters. Cancer 2005;103:2575-2583.


Autoantibodies as indicators of tumor development


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(121) Bénistant C, Bourgaux JF, Chapuis H, Mottet N, Roche S, Ball JP. The COOH-terminal Scr kinase Csk is a tumor antigen in human carcinoma. Cancer Res 2001;61:1415-1420.


(127) Tureci O, Sahin U, Zwick C, Koslowski M, Seitz G, Pfreundschuh M. Identifica-
Autoantibodies as indicators of tumor development


