Published in final edited form as:

Autoimmun Rev. 2011 April ; 10(6): 331–335. doi:10.1016/j.autrev.2010.12.002.

Autoantibodies to tumor-associated antigens as biomarkers in cancer immunodiagnosis

Weihong Liu^{a,b,*}, Bo Peng^b, Yumin Lu^{b,c}, Weijia Xu^a, Wei Qian^b, and Jian-Ying Zhang^{b,c,*}

^a Department of Clinical Laboratory Technology, Dalian Municipal Central Hospital, Dalian, Liaoning 116033, China

^b Department of Biological Sciences, University of Texas at El Paso, 500 West University Avenue, El Paso, TX 79968, USA

^c College of Public Health and Henan Key Laboratory of Tumor Epidemiology, Zhengzhou University, Zhengzhou, Henan 450001, China

Abstract

Cancer sera contain antibodies that react with a unique group of autologous cellular antigens called tumor-associated antigens (TAAs), and therefore these autoantibodies can be considered as reporters from the immune system, to identify authentic TAAs involved in the malignant transformation. Once a TAA is identified, different approaches would be used to comprehensively characterize and validate the identified TAA/anti-TAA systems that are potential biomarkers in cancer immunodiagnosis. In this manner, several novel TAAs such as p62 and p90 have been identified in our previous studies. p62, a member of IGF-II mRNA binding proteins (IMPs), is an oncofetal protein absent in adult tissues, the presence of anti-p62 autoantibodies relates to abnormal expression of p62 in tumor cells. p90 was recently characterized as an inhibitor of the tumor suppressor PP2A (protein phosphatase 2A), and an autoantibody to p90 appears in high frequency in prostate cancer. The present review will focus on the recent advances in studies mainly associated with these two novel TAAs as biomarkers in cancer immunodiagnosis.

Keywords

Autoantibody; Tumor-associated antigen; p62/IMP2; p90/CIP2A; Immunodiagnosis; Cancer

1. Introduction

Many studies demonstrated that the immune system can recognize the antigenic changes in cancer cells, and further develop autoantibodies against these cellular antigens that have been generally called tumor-associated antigens (TAAs). Therefore, these cancer-associated autoantibodies might be considered as “reporters” from the immune system, to identify the antigenic changes in cellular proteins involved in the transformation process [1–3]. There has been a growing interest in using serum autoantibodies against TAAs as biomarkers in cancer immunodiagnosis. The major reason is that these antibodies are generally absent, or present in very low titers, in normal individuals and in non-cancer conditions [3]. Their persistence and stability in the serum of cancer patients is an advantage over other potential

markers, including the TAAs themselves, some of which are released by tumors but rapidly degrade or are cleared after circulating in the serum for a limited time [4]. Furthermore, the widespread availability of methods and reagents to detect serum autoantibodies facilitates their characterization in cancer patients and assay development.

The definition of what constitutes a TAA is a major issue in the field of cancer immunodiagnosis. It is erroneous to include all cellular antigens identified by autoantibodies in cancer sera as TAAs since some autoantibodies may exist in conditions that pre-date malignancy. This was particularly evident in several studies of subjects with liver cancer where serial serum samples were available several years before malignancy when these subjects had conditions such as chronic hepatitis and liver cirrhosis [5–11]. Autoantibodies to cellular components were readily detected by Western blotting during the pre-malignant conditions of chronic hepatitis and liver cirrhosis but the interesting observation was that coincident with or closely preceding the clinical detection of liver cancer, novel autoantibodies were detected in Western blotting and by immunofluorescence assay. In cases where the novel antigen–antibody systems were characterized, many antigens turned out to be cellular components that have been described to be aberrantly expressed in cancer. Failing to recognize the likelihood of pre-malignancy circulating antibodies would result in the inclusion of many antigens erroneously as TAAs, especially if serum drawn at one time point from a cancer subject was used to characterize the antigens since this might include both cancer-related and unrelated antigens. Our work to identify authentic TAAs has been influenced by several observations. Some of the cellular proteins identified by cancer autoantibodies were initially of unknown function but eventually were shown to be involved in tumorigenesis pathways. The identification and characterization of two novel TAAs p62 and p90 are examples of this kind of studies. This review will focus on the recent advances in studies mainly associated with p62 and p90 as biomarkers in cancer immunodiagnosis.

2. p62, a member of IGF-II mRNA binding protein family

As shown in Fig. 1, while analyzing a group of sera from hepatocellular carcinoma (HCC) patients originating from China, it was observed in Western blotting that a number of these sera were reactive with a protein which blotted in the region of 62 kDa [11]. This 62 kDa protein appeared to be expressed in high abundance in a T24 (bladder carcinoma) cell line and in a HepG2 (liver cancer) cell line, whereas the expression was low in a MOLT-4 (T-lymphocyte) cell line. Serum from a patient with high antibody titer to the 62 kDa protein was used to immunoscreen a T24 cDNA expression library and ultimately, a full-length clone was isolated. When the nucleotide sequence for p62 was first identified, it was shown to be a novel unreported gene. Of great interest was the findings that the deduced amino acid sequence of p62 was highly homologous to a family of mRNA binding proteins such as human Koc (k-homolog protein overexpressed in cancer) [12] and chicken ZBP1 (β -actin mRNA zipcode-binding protein) [13]. This family of proteins has distinct structural characteristics, with a consensus RNA binding region called the RNA recognition motif in the N-terminal region and four hnRNP K homology (KH) domains in the C-terminal half of the proteins. A feature which appears to be possessed in common by all members of this family is that they are mRNA binding proteins (see Table 1).

While our paper was in press, Nielsen et al. reported on a family of three proteins that bind to the 5'-untranslated region of leader 3 IGF-II mRNA, which is developmentally regulated and expressed in the fetus but undetectable in adult tissues [14]. These IGF-II mRNA binding proteins (IMPs), which they called IMP 1, 2 and 3 all contained the same RNP consensus sequence and KH motifs. IGF-II mRNA binding by p62 and Koc (IMP2 splice variant and IMP3, respectively) is especially interesting with regard to the possible relationship to cancer. IGF-II has been shown to be overexpressed in many cancers and one

of the earliest demonstrations was its overexpression in human hepatocellular carcinoma [15,16]. An inherited disorder called the Beckwith–Wiedemann Syndrome is associated with IGF-II overexpression, and the syndrome consists of organomegalies and tumors in different organs [17,18]. There have also been transgenic models of IGF-II overexpression and these have resulted in carcinogenesis in the transgenic animals [19–21]. In hepatitis B virus transgenic mice, chronic hepatocellular injury led to HCC in some animals, and in examining for abnormalities in structure and expression of a large number of oncogenes and tumor suppressor genes, including ras, myc, fos, abl, src, Rb, and p53, only IGF-II overexpression was found [22]. Most proteins from the IMP family have been shown to have some possible relationship to malignancy. p62/IMP2 was characterized as a TAA, and antibody to p62/IMP2 was present in 21% of patients with HCC but not in the precursor conditions chronic hepatitis and liver cirrhosis [11]. Our subsequent study has demonstrated that the expression of p62 is developmentally regulated, and expressed in fetal, but not in adult liver [23]. Koc/IMP3 had been shown to be transcriptionally overexpressed in cancer [12]. A murine homologue of this family of proteins (CRD-BP/IMP1) had been shown to bind to the c-myc mRNA coding region and had the property of protecting c-myc mRNA from endonucleolytic cleavage [24,25]. More recent studies demonstrated that other members from the IMP family are also involved in cancer. For example, VICKZ, β -actin mRNA binding proteins (identical to ZBP proteins) help to facilitate the dynamic cell surface morphology required for cell motility, and may play an important role in colorectal carcinoma metastasis [26].

3. p90, an inhibitor of the tumor suppressor protein phosphatase 2A (PP2A)

During studies which identified p62/IMP2, it was noticed that antibody to an intracellular protein of approximately 90 kDa was detected with similar frequency (Fig. 1). Sera with anti-p90 antibody also localized to the cytoplasm [10], and were detected by immunohistochemistry in fetal mouse liver but not in adult liver [27]. Full-length cDNA encoding p90 was successfully isolated from a T24 expression library and comprised a sequence coding for a protein of 905 amino acids with predicted molecular mass of 102 kDa [27]. There were no distinct polypeptide motifs, and search of GenBank databases at that time did not generate any significant alignment to established gene products. Limited analysis showed that anti-p90 antibody was present in 21/160 (13.1%) in HCC, 3/91 (3.3%) in gastric cancer and 1/20 (5.0%) in esophageal cancer. Gastric cancer tissue also confirmed cytoplasmic expression of p90. The function of p90 remained unknown until 2007 [28] when Juntilla et al. reported that p90 possessed oncogenic activity by inhibiting the tumor suppressor PP2A (protein phosphatase 2A). PP2A is a widely conserved serine/threonine phosphatase and recent studies have identified target molecules for which dephosphorylation is critical for tumor suppressor activity of this protein [29,30]. Dephosphorylation of oncogenic transcription factor c-myc is a critical pathway for PP2A tumor suppressor activity. PP2A is a trimeric holoenzyme, with a scaffold subunit (A subunit), a catalytic subunit (C subunit) and several different regulatory B subunits. The B subunits determine the substrate specificity of the PP2A holoenzyme. The mechanism whereby PP2A exerts its tumor suppressor function on c-myc is by dephosphorylation at serine 62 of myc. Juntilla et al. [28] looked for endogenous cellular proteins which could be co-purified with the A subunit and found a protein which turned out to be p90. They showed that p90 was an inhibitor of the tumor suppressor function of PP2A and that this was related to p90 binding c-myc and inhibiting dephosphorylation of S62 by PP2A. This and other studies showed that p90 overexpression enhanced or induced transformation of several tumorigenesis models. They proposed calling p90, cancerous inhibitor of PP2A (CIP2A). They also found that p90/CIP2A was overexpressed in low-passaged cell lines from head and neck squamous cell carcinoma. Studies from other groups also demonstrated that p90/CIP2A is associated with

human breast cancer aggressivity [31], and its immunopositivity and expression can be a predictor of survival for some subgroups of gastric cancer patients [32].

In addition to autoantibodies to p90 and p62 that were identified in sera from HCC patients, a third autoantibody against a 50 kDa cellular protein also appears in high frequency in these sera (see Fig. 1). Unfortunately, this 50 kDa protein was not isolated. Recently, we have examined autoantibodies in a group of HCC sera using immunofluorescence assay and Western blotting, and found that 26 (17.1%) sera were identified by Western blotting analysis to contain antibodies against an unknown 50 kDa cellular protein (unpublished data). No reactivity with the 50 kDa protein was detected in 64 chronic liver disease sera and in 30 normal human sera. Subcellular fractionation of HCC cells (HepG2) showed that the 50 kDa protein was predominant in the cytoplasm, and was not found in the nuclear fraction. The analysis using immunohistochemistry on HepG2 cells confirmed that the sera with antibodies to the 50 kDa antigen appeared to have cytoplasmic staining patterns. Further identification of this unknown cellular protein using two-dimensional gel electrophoresis (2DE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) showed that several proteins of approximately this molecular size were identified, including heterogeneous nuclear ribonucleo protein F (46 kDa), beta-actin-like protein 2 (42 kDa), KRT18 Keratin (48 kDa), isocitrate dehydrogenase (47 kDa), TUFM Tu translation elongation factor (50 kDa), alpha-enolase (48 kDa) and 3-ketoacyl-CoA thiolase (42.5 kDa). Further study to determine which, if any, might be authentic TAA is still underway.

4. Autoantibodies to p62/IMP2 and p90/CIP2A as biomarkers in cancer

As described above, p62/IMP2 was isolated and characterized as a TAA. An interesting feature about the IMP family of proteins is that koc/IMP3 was found to be overexpressed in pancreatic and other types of cancer in a large scale screen for genes which were differentially expressed in cancer specimens [12]. Also, CRD-BP/IMP1 was reported to be overexpressed in breast cancer [33]. Although the latter two studies did not look for autoantibodies to IMP1 or IMP3, we have found that autoantibodies to all three members of the IMP family can be detected in a variety of cancers [34,35] and that they bind to different determinants on these proteins [34] in spite of their high degree of similarity and identity in protein and nucleotide sequence. The credibility of the IMP proteins as TAAs can be assigned to their binding partner, IGF-II mRNA. Full-length recombinant polypeptides of the three IMP proteins were expressed and used as antigens in Western blotting, ELISA and immunoprecipitation for the detection of autoantibodies. Sera from 546 patients with 11 different types of cancer were analyzed. As shown in Table 2, autoantibodies to IMP1 were detected in 13.0%, p62 in 13.6% and Koc in 13.0%, significantly different from the control populations consisting of normal subjects and autoimmune disease patients ($p<0.01$). The data also showed that when the presence of antibody to any one of these three antigens was considered, the cumulative frequency was raised to 28.0% (153/546) for the total group of cancer patients. Our data suggest that the combination of antibodies might acquire higher sensitivity for diagnosis of cancer. The mechanisms by which these proteins elicit autoimmune responses remain to be elucidated. In a subsequent study, we found that the frequency of autoantibodies to p90 and p62 was 30.8% (41/133) and 22.6% (30/133) in prostate cancer patients, respectively, which was significantly higher than that in benign prostate hyperplasia (BPH) patients and normal individuals [36]. p90 and p62 appeared to be the most reactive antigens in prostate cancer. When adding p90 and p62 together with the other two IMP proteins (IMP1 and Koc/IMP3) as a mini-array of four TAAs in the detection of prostate cancer, the data show the stepwise increase in cumulative antibody frequency in prostate cancer versus normal controls. These four TAAs were sufficient to raise antibody frequency to 70.0% versus 10.6% in normal controls ($p<0.01$). Actually, we still do not know why prostate cancer patients have preferential immune responses to p62 and p90, but

this phenomenon may suggest a structural and/or a functional linkage between these two cytoplasmic proteins.

5. Conclusions

The mechanisms underlying the emergence of autoantibodies and the regulation of their production are not completely understood. Some reports have suggested that this immune response is due to a self-immunization process linked to increased immunogenicity of TAAs during malignant transformation [37]. Although this research area is advancing, a number of fundamental questions remain unresolved. One of the most extensively studied TAAs is p53, a tumor suppressor protein. Autoantibodies to p53 in cancer were first reported in 1982 [38], and since then there have been numerous reports confirming and extending this finding [39]. The types of cellular proteins that induce autoantibody responses are quite varied and include oncogene products such as HER-2/neu [40], onconeural antigens [41], differentiation-antigens such as tyrosinase and the cancer/testis antigens [42], and anti-apoptotic proteins such as survivin [43], LEDGF [44], etc. A highly informative study showed that in lung tumors containing several types of p53 gene mutations, including missense, stop codon and frameshift mutations, only the missense p53 mutations, with overexpression of a protein that altered function and increased protein stability, correlated with autoantibody production [45]. In the case of p62/IMP2, a fetal protein absent in adult tissues, the presence of autoantibodies relates to abnormal expression of p62 in tumor cells [11,23]. The immune systems of certain cancer patients appear to sense these aberrant tumor-associated proteins as foreign antigens and have the capability to respond by producing autoantibodies [4]. Thus, tumor-associated autoantibodies might be regarded as reporters identifying aberrant *de novo* or dysregulated cellular mechanisms in tumorigenesis [1,3]. In recent years, the potential utility of TAA–autoantibody systems as early cancer biomarker tools to monitor therapeutic outcomes or as indicators of disease prognosis has been explored. The practical value of the autoantibody responses to p62, p90 and other TAAs might be further determined by a prospective study in different types of cancer. The establishment of more precise time lines to determine when autoantibodies to these TAAs appear as early predictors of cancer and whether anti-TAA antibody expression varies with progression or response to treatment also warrant further study.

Acknowledgments

We thank Dr. Eng M. Tan (The Scripps Research Institute, La Jolla, California, USA) for his support. This work was supported in part by NIH grants (2S06GM008012, 5G12RR08124 and CA56956), and also by a grant from the National Natural Science Foundation of China (30872962).

References

1. Tan EM. Autoantibodies as reporters identifying aberrant cellular mechanisms in tumorigenesis. *J Clin Invest.* 2001; 108(10):1411–5. [PubMed: 11714730]
2. Old LJ, Chen YT. New paths in human cancer serology. *J Exp Med.* 1998; 187(8):1163–7. [PubMed: 9547328]
3. Tan EM, Zhang J. Autoantibodies to tumor-associated antigens: reporters from the immune system. *Immunol Rev.* 2008; 222:328–40. [PubMed: 18364012]
4. Anderson KS, LaBaer J. The sentinel within: exploiting the immune system for cancer biomarkers. *J Proteome Res.* 2005; 4(4):1123–33. [PubMed: 16083262]
5. Imai H, Ochs RL, Kiyosawa K, Furuta S, Nakamura RL, Tan EM. Nucleolar antigens and autoantibodies in hepatocellular carcinoma and other malignancies. *Am J Path.* 1992; 140(4):859–70. [PubMed: 1314027]

6. Imai H, Nakano Y, Kiyosawa K, Tan EM. Increasing titers and changing specificities of antinuclear antibodies in patients with chronic liver disease who develop hepatocellular carcinoma. *Cancer*. 1993; 71(1):26–35. [PubMed: 8380118]
7. Imai H, Chan EKL, Kiyosawa K, Fu X, Tan EM. Novel nuclear autoantigen with splicing factor motifs identified with antibody from hepatocellular carcinoma. *J Clin Invest*. 1993; 92(5):2419–26. [PubMed: 8227358]
8. Imai H, Kiyosawa K, Chan EKL, Tan EM. Autoantibodies in viral hepatitis related hepatocellular carcinoma. *Intervirology*. 1993; 35(1–4):73–85. [PubMed: 8407252]
9. Zhang JY, Zhu W, Imai H, Kiyosawa K, Chan EKL, Tan EM. De-novo humoral immune responses to cancer-associated autoantigens during transition from chronic liver disease to hepatocellular carcinoma. *Clin Exp Immunol*. 2001; 125(1):3–9. [PubMed: 11472419]
10. Zhang JY, Wang X, Peng XX, Chan EKL. Autoimmune responses in Chinese hepatocellular carcinoma (HCC). *J Clin Immuno*. 2002; 22(2):98–105.
11. Zhang JY, Chan EKL, Peng XX, Tan EM. A novel cytoplasmic protein with RNA-binding motifs is an autoantigen in human hepatocellular carcinoma. *J Exp Med*. 1999; 189(7):1101–10. [PubMed: 10190901]
12. Mueller-Pillasch F, Lacher U, Wallrap C, Micha A, Zimmerhackl F, Hameister H, et al. Cloning of a gene highly expressed in cancer coding for a novel KH-domain containing protein. *Oncogene*. 1997; 14(22):2729–33. [PubMed: 9178771]
13. Ross AF, Oleynikov Y, Kislauskis EH, Taneja KL, Singer RH. Characterization of a β -actin mRNA zipcode-binding protein. *Mol Cell Biol*. 1997; 17(4):2158–65. [PubMed: 9121465]
14. Nielsen J, Christiansen J, Lykke-Andersen J, Johnsen AH, Wewer UM, Nielsen FC. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. *Mol Cell Biol*. 1999; 19(2):1262–70. [PubMed: 9891060]
15. Su TS, Liu WY, Han SH, Jansen M, Yang-Fen TL, Peng FK, et al. Transcripts of the insulin-like growth factors I and II in human hepatoma. *Cancer Res*. 1989; 49(7):1773–7. [PubMed: 2466561]
16. Cariani E, Lasserre C, Seurin D, Hamelin B, Kemeny F, Franco D, et al. Differential expression of insulin-like growth factor II mRNA in human primary liver cancers, benign liver tumors, and liver cirrhosis. *Cancer Res*. 1988; 48(23):6844–9. [PubMed: 3180092]
17. Eggenschwiler J, Ludwig T, Fisher P, leighton PA, Tilghman SM, Efstratiadis A. Mouse mutant embryos overexpressing IGF-II exhibit phenotypic features of the Beckwith–Wiedemann and Simpson–Golabi–Behmel syndrome. *Genes Dev*. 1997; 11(23):3128–42. [PubMed: 9389646]
18. Sun FL, Dean WL, Kelsey G, Allen ND, Reik W. Transactivation of Igf2 in a mouse model of Beckwith–Wiedemann syndrome. *Nature*. 1997; 389(6653):809–15. [PubMed: 9349812]
19. Rogler CE, Yang D, Rossetti L, Donohoe J, Alt E, Chang CJ, et al. Altered body composition and increased frequency of diverse malignancies in insulin-like growth factor-II transgenic mice. *J Biol Chem*. 1994; 269(19):13779–84. [PubMed: 7514593]
20. Bates P, Fisher R, Ward A, Richardson L, Hill DJ, Graham CF. Mammary cancer in transgenic mice expressing insulin-like growth factor II (IGF-II). *Br J Cancer*. 1995; 72(5):1189–93. [PubMed: 7577466]
21. Schirmacher P, Held WA, Yang D, Chisari FV, Rustum Y, Rogler CE. Reactivation of insulin-like growth factor II during hepatocarcinogenesis in transgenic mice suggests a role in malignant growth. *Cancer Res*. 1992; 52(9):2549–56. [PubMed: 1568224]
22. Pasquinelli C, Bhavani K, Chisari FV. Multiple oncogenes and tumor suppressor genes are structurally and functionally intact during hepatocarcinogenesis in hepatitis B virus transgenic mice. *Cancer Res*. 1992; 52(10):2823–9. [PubMed: 1316229]
23. Lu M, Nakamura RM, Dent ED, Zhang JY, Nielsen FC, Christiansen J, et al. Aberrant expression of fetal RNA-binding protein p62 in liver cancer and liver cirrhosis. *Am J Pathol*. 2001; 159(3):945–53. [PubMed: 11549587]
24. Doyle GA, Betz NA, Leeds PF, Fleisig AJ, Prokipcak RD, Ross J. The c-myc coding region determinant-binding protein: a member of a family of KH domain RNA-binding proteins. *Nucleic Acids Res*. 1998; 26(22):5036–44. [PubMed: 9801297]

25. Leeds P, Kren BT, Boylan JM, Betz NA, Steer CJ, Gruppuso PA, et al. Developmental regulation of CRD-BP, an RNA-binding protein that stabilizes c-myc mRNA in vitro. *Oncogene*. 1997; 14(11):1279–86. [PubMed: 9178888]
26. Vainer G, Vainer-Mosse E, Pikarsky A, Shenoy SM, Oberman F, Yeffet A, et al. A role for VICKZ proteins in the progression of colorectal carcinomas: regulating lamellipodia formation. *J Pathol*. 2008; 215(4):445–56. [PubMed: 18535985]
27. Soo Hoo L, Zhang JY, Chan EKL. Cloning and characterization of a novel 90 kDa ‘companion’ auto-antigen of p62 overexpressed in cancer. *Oncogene*. 2002; 21(32):5006–15. [PubMed: 12118381]
28. Juntilla MR, Puustinen P, Niemela M, Ahola R, Arnold H, Böttzauw T, et al. CIP2A inhibits PP2A in human malignancies. *Cell*. 2007; 130(1):51–62. [PubMed: 17632056]
29. Arroyo JD, Hahn H. Involvement of PP2A in viral and cellular transformation. *Oncogene*. 2005; 24(52):7746–55. [PubMed: 16299534]
30. Janssens V, Goris J, Van Hoof C. PP2A: the expected tumor suppressor. *Curr Opin Genet Dev*. 2005; 15(1):34–41. [PubMed: 15661531]
31. Côme C, Laine A, Chanrion M, Edgren H, Mattila E, Liu X, et al. CIP2A is associated with human breast cancer aggressivity. *Clin Cancer Res*. 2009; 15(16):5092–100. [PubMed: 19671842]
32. Khanna A, Böckelman C, Hemmes A, Juntilla MR, Wiksten JP, Lundin M, et al. MYC-dependent regulation and prognostic role of CIP2A in gastric cancer. *J Natl Cancer Inst*. 2009; 101(11):793–805. [PubMed: 19470954]
33. Doyle GA, Bourdeaux-Heller JM, Coulthard S, Meisner LF, Ross J. Amplification in human breast cancer of a gene encoding a c-myc mRNA binding protein. *Cancer Res*. 2000; 60(11):2756–9. [PubMed: 10850408]
34. Zhang JY, Chan EKL, Peng XX, Lu M, Wang X, Mueller F, et al. Autoimmune responses to mRNA binding proteins p62 and Koc in diverse malignancies. *Clin Immunol*. 2001; 100(2):149–56. [PubMed: 11465943]
35. Zhang JY, Casiano C, Peng XX, Koziol J, Chan EKL, Tan EM. Enhancement of antibody detection in cancer using a panel of recombinant tumor-associated antigens. *Cancer Epidemiol Biomarkers Prev*. 2003; 12(2):136–43. [PubMed: 12582023]
36. Shi FD, Zhang JY, Liu D, Rearden A, Elliot M, Nachtsheim D, et al. Preferential humoral immune response in prostate cancer to cellular proteins p90 and p62 in a panel of tumor-associated antigens. *Prostate*. 2005; 63(3):252–8. [PubMed: 15538718]
37. Sheever MA, Disis ML, Bernahrd H, Gralow JR, Hand SL, Huseby ES, et al. Immunity to oncogenic proteins. *Immunol Rev*. 1995; 145:33–59. [PubMed: 7590829]
38. Crawford LV, Pim DC, Bulbrook RD. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. *Int J Cancer*. 1982; 30(4):403–8. [PubMed: 6292117]
39. Soussi T. p53 antibodies in the sera of patients with various types of cancer. A review. *Cancer Res*. 2000; 60(7):1777–88. [PubMed: 10766157]
40. Disis ML, Pupa SM, Gralow JR, Dittadi R, Menard S, Cheever MA. High-titer HER-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer. *J Clin Oncol*. 1997; 15(11):3363–7. [PubMed: 9363867]
41. Keene JD. Why is Hu where? Shuttling of early response gene messenger RNA subsets. *Proc Natl Acad Sci USA*. 1999; 96(1):5–7. [PubMed: 9874760]
42. Stockert E, Jager E, Chen YT, Scanlan MJ, Gout I, Karbach J, et al. A survey of humoral immune response of cancer patients to a panel of human tumor antigens. *J Exp Med*. 1998; 187(8):1349–54. [PubMed: 9547346]
43. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med*. 1997; 3(8):917–92. [PubMed: 9256286]
44. Daniels T, Zhang J, Gutierrez I, Elliot ML, Yamada B, Heeb MJ, et al. Antinuclear autoantibodies in PCa: immunity to LEDGF/p75, a survival protein highly expressed in prostate tumors and cleaved during apoptosis. *Prostate*. 2005; 62(1):14–26. [PubMed: 15389814]
45. Winter SF, Minna JD, Johnson BE, Takahashi T, Gazdar AF, Carbone DP. Development of antibodies against p53 in lung cancer patients appears to be dependent on the type of p53 mutation. *Cancer Res*. 1992; 52(15):4168–74. [PubMed: 1322237]

Take-home messages

- Cancer sera contain antibodies that react with a unique group of autologous cellular antigens called tumor-associated antigens (TAAs), and these autoantibodies can be considered as reporters from the immune system, to identify and characterize authentic TAAs such as p62 and p90 that are involved in the malignant transformation.
- p62, a member of IGF-II mRNA binding proteins (IMPs), is an oncofetal protein absent in adult tissues, and the presence of antip62 autoantibodies relates to abnormal expression of p62 in tumor cells.
- p90 was recently characterized as an inhibitor of the tumor suppressor PP2A (protein phosphatase 2A), and autoantibody to p90 appears in high frequency in prostate and liver cancers.
- Autoantibodies to p62 and p90 might be used as biomarkers in the immunodiagnosis of cancer.

Fig. 1.

Reactivity of three HCC sera in Western blotting against whole cell extracts from MOLT-4, T24, and HepG2 cell lines. Lanes 1, 5, and 9 were normal human sera. One HCC serum (lanes 2, 6, and 10) showed strong reactivity with a 90-kDa protein in MOLT-4 cells and reacted weakly with a 62-kDa protein in MOLT-4 cells and T24 cell extracts. A strong reaction with the 62-kDa protein was detected with HepG2 cell extracts, together with a strong reaction with a 50-kDa protein. The second serum (lanes 3, 7, and 11) and the third one (lanes 4, 8, and 12) demonstrate other types of reactions. These representative data demonstrate that HCC sera are heterogeneous in their antibody repertoires and that different cell lines apparently have different expressions of 90-, 62-, and 50-kDa proteins.

Table 1

p62 and IMP family of proteins.

Acronym (reference)	Reported properties
Human p62 [11]	Identical to human IMP-2. A splice variant missing 43 amino acids between KH2 and KH3 domains. An autoantigen in HCC.
Human Koc [12]	hnRNP <u>K</u> homology protein <u>o</u> verexpressed in cancer. Identical to human IMP-3.
Chicken ZBP1 [13]	Chicken β -actin mRNA zipcode-binding protein. Identical to chicken VICKZ1.
Human IMP-1 [14]	<u>I</u> GF-II <u>m</u> RNA binding protein. Binds to 5' UTR of the leader 3 fetal IGF-II mRNA. Human homolog of mouse CRD-BP.
Human IMP-2 [14]	Binds to 5' UTR of leader 3 fetal IGF-II mRNA. Splice variant of p62.
Human IMP-3 [14]	Binds to 5' UTR of leader 3 fetal IGF-II mRNA. Identical to human Koc.
Mouse CRD-BP [24,25]	Murine c-myc <u>c</u> oding region instability <u>d</u> eterminant <u>b</u> inding protein. Shields c-myc mRNA from endonucleolytic cleavage. Homolog of human IMP-1.

Underlined letters represent the acronyms used by various authors to describe the proteins in column one.

Table 2
Frequency of autoantibodies to p62 and two other IMPs in diverse malignancies.

Cancers	No. of tested	IMP1	p62/IMP2	Koc/IMP3	Any one of three antigens
HCC	65	10 (15.4)**	8 (12.3)**	9 (13.8)**	20 (30.1)**
Gastric	91	15 (16.5)**	8 (8.8)*	17 (18.7)**	28 (30.8)**
Esophageal	77	12 (15.6)**	15 (19.5)**	11 (14.3)**	22 (28.6)**
Colorectal	45	6 (13.3)*	5 (11.1)*	4 (8.9)*	10 (22.2)**
Breast	64	5 (7.8)	5 (7.8)	9 (14.1)**	14 (21.9)**
Lymphoma	44	6 (13.6)*	4 (9.1)*	6 (13.6)**	12 (27.3)**
Lung	56	4 (7.1)	12 (21.4)**	5 (8.9)	19 (33.9)**
Pharyngeal	32	4 (12.5)*	6 (18.8)**	5 (15.6)**	11 (34.4)**
Uterine	27	5 (18.5)**	6 (22.2)**	1 (3.7)	9 (33.3)**
Ovarian	22	1 (4.5)	2 (9.1)*	2 (9.1)*	2 (9.1)
Thyroid	23	3 (13.0)*	3 (13.0)**	2 (8.7)	6 (26.1)**
Total	546	71 (13.0)**	74 (13.6)**	71 (13.0)**	153 (28.0)**
NHS	82	2 (2.4)	1 (1.2)	1 (1.2)	3 (3.7)
SLE	62	2 (3.2)	1 (1.6)	1 (1.6)	2 (3.2)
SS	41	0	0	0	0

Cutoff value: "Mean+3SD" of NHS.

p value relative to NHS:

* p<0.05;

** p<0.01.

Abbreviation: NHS, normal human sera. SLE, systemic lupus erythematosus; SS, Sjögren's syndrome.