INVITED REVIEW

Translational progress on tumor biomarkers

Hongwei Guo[1,2], Xiaolin Zhou[1,2], Yi Lu[1,2], Liye Xie[1,2], Qian Chen[1,2], Evan T. Keller[3], Qian Liu[4], Qinghua Zhou[5] & Jian Zhang[1,2,3]

1 Key Laboratory of Longevity and Aging-Related Diseases, Ministry of Education, Nanning, China
2 Center for Translational Medicine, Guangxi Medical University, Nanning, China
3 Department of Urology and Pathology, School of Medicine, University of Michigan, Ann Arbor, Michigan, USA
4 Tianjin Key Laboratory of Lung Cancer Metastasis and Tumor Microenvironment, Tianjin Lung Cancer Institute, Tianjin Medical University General Hospital, Tianjin, China
5 Lung Cancer Center, Huaxi Hospital, Sichuan University, Chengdu, China

Keywords
Targeted therapy; translational medicine; tumor biomarkers.

Correspondence
Jian Zhang or Qinghua Zhou, Center for Translational Medicine, Guangxi Medical University, Room 1418, Pharmacology and Biomedical Sciences Building, No.22 Shuangyong Road, Nanning 530021, China.
Tel: +86 771 5300270
Fax: +86 771 5310865
Email: jianzhang008@hotmail.com; zhouqh135@163.com

Received: 22 April 2015;
Accepted 15 June 2015.

doi: 10.1111/1759-7714.12294

Thoracic Cancer **6** (2015) 665–671

Abstract

There is an urgent need to apply basic research achievements to the clinic. In particular, mechanistic studies should be developed by bench researchers, depending upon clinical demands, in order to improve the survival and quality of life of cancer patients. To date, translational medicine has been addressed in cancer biology, particularly in the identification and characterization of novel tumor biomarkers. This review focuses on the recent achievements and clinical application prospects in tumor biomarkers based on translational medicine.

Introduction

For decades, research on life science has made major breakthroughs, including the discovery of stem cells and completion of human genome sequencing, which have great significance in the promotion of medical research progress. However, few basic research findings have actually been applied to clinics for the benefit of patients. A new model of research, translational medicine, has been introduced to fill the gap between basic research and clinical application.[1–3] It is a two-way, open circulation research system, from bench to bedside and from bedside to bench.[4,5] The translational medicine research model has become a strategy direction for the field of biomedical research.

Translational medicine plays an important role in the research of malignant tumors and clinical treatment.[6] Malignant tumors have become the leading cause of death in the Chinese population. Although basic research on tumor biology has broadened our understanding of factors such as occurrence, metastasis, and drug resistance, care of cancer patients is generally by indiscriminate treatment, that is, patients are given the same treatment without fully considering their individual biological characteristics; thus, the general survival rate has not significantly improved in the last 20 years.[7] Tumor biomarkers are of potential use in early cancer diagnosis, anticancer therapy development, and monitoring the response to treatment. We provide a mini-review of recent advances in tumor biomarkers based on translational medicine.

Research development and clinical application of tumor biomarkers

Concepts of tumor biomarkers

Tumor biomarkers are substances present in or produced by a tumor itself or by the host microenvironment in response to the process of tumorigenesis and progression. They cover a

broad range of biochemical entities, such as proteins, hormones, enzymes, and oncogene products. These substances can be found in cells, tissues, or body fluids, and can be qualitatively or quantitatively detected by chemical, immunological, and molecular biological techniques.[8] Tumor biomarkers represent an effective tool for tumor diagnosis, treatment, prognosis, and therapeutic monitoring.[9] The Early Detection Research Network (EDRN) is a large network project in translational research sponsored by the United States National Cancer Institute (NCI). This project mainly focuses on early tumor diagnosis, metastasis and relapse detection, prognosis, and targeted therapy.

Tumor biomarkers in clinical application

As a key to individual medical treatment, research on tumor biomarkers has increasingly gained attention. However, the clinical application of tumor biomarkers is somewhat limited. To date, only 20 types of tumor biomarkers have been used in the clinical setting. Some of these markers are confined to a certain type of cancer, while others exist in two or more types of tumors; however, there is no "universal" tumor marker present in all types of cancer. According to the chemical nature of tumor markers, they can be divided into six types: oncofetal antigens (carcino-embryonic antigens [CEA], alpha fetoprotein [AFP]), carbohydrate antigens (CA125, CA15.3, etc.), enzymes (prostate-specific, neuron specific enolase, etc.), hormones (human chorionic gonadotropin, calcitonin, etc.), proteins (ceruloplasmin, etc.) and genes (P53, V-KI-ras2 Kirsten rat sarcoma viral oncogene homolog [KRAS], etc.). Tumor biomarkers commonly used in the clinical setting are summarized in Table 1.

Table 1 Summary of commonly used tumor biomarkers

Molecular markers	Types of tumors
AFP	Hepatocellular carcinoma, germ cell tumors[10,11]
ALK	Non-small cell lung cancer, anaplastic large cell lymphoma[12,13]
B2M	Multiple myeloma, chronic lymphocytic leukemia[14,15]
BCR-ABL	Chronic myelogenous leukemia[16]
BRAF mutation V600E	Cutaneous melanoma, colorectal cancer[17,18]
β-HCG	Choriocarcinoma, testicular cancer[19]
CA125, HE4, ROMA, OVA1	Ovarian cancer[20]
CA15.3, CA549, MCA	Breast cancer[21–27]
CA27.29, Her-2/neu, ER/PR, uPA, PAI-1, 21-Gene signature, 70-Gene signature	
CA19-5	colorectal cancer[28]
CA19-9, CA50	Pancreatic cancer, gallbladder cancer, bile duct cancer, gastric cancer[29–34]
CA242	gastrointestinal tract cancer, pancreatic cancer[35,36]
CA72-4	Gastric cancer, pancreatic cancer[37,38]
Calcitonin	Medullary thyroid carcinoma[39]
CEA	Breast cancer,[22] pancreatic cancer[29] colorectal cancer[40]
CD20	Non-Hodgkin lymphoma[41]
CgA	Neuroendocrine tumor[42]
DU-PAN-2	Pancreatic cancer, endometrial cancer[43,44]
EGFR, CYFRA 21-1	Non-small cell lung cancer[45,46]
FDP, NMP22, Chromosomes 3, 7, 17, and 9p21	Bladder cancer[47–49]
HAb18G/CD147	Hepatocellular carcinoma[50]
IPO-38	Gastric cancer[51]
KIT	Gastrointestinal stromal tumor, mucosal melanoma[52,53]
KRAS	Colon cancer, non-small cell lung cancer[54,55]
Lactate dehydrogenase	Germ cell tumor[56]
PSA, PAP, PCA3	Prostate cancer[57,58]
Thyroglobulin, galectin-3	Thyroid cancer[59,60]

Latest progress of molecular biomarkers of tumors

Tumor screening and early diagnosis

Conventional tumor biomarkers include tumor antigens and differentially expressed gene products, such as breast cancer 1, early onset (BRCA1) and BRCA2.[61] Researchers from Peking University recently reported that the reproducibility of cancer-specific copy number variation (CNV) offers potential for noninvasive circulating tumor cell (CTC)-based cancer diagnostics.[62] Kinde *et al*. performed whole genome sequencing on cervical secretions for ovarian and endometrial cancer diagnosis, and established suitable routine screening methods for these tumors.[63] Wang *et al*. suggested that Hsp90a is a potential tumor biomarker.[64] Recent discoveries have shown that micro ribonucleic acid (miRNA) is stable in serum and can enter peripheral circulation; thus, circulating miRNA may be used as a biological tumor marker for early diagnosis. Harris *et al*. reported that miR-375 expression level was closely related to the death rate of patients with head and neck cancer, and can, thus, be regarded as a novel tumor biomarker.[65] In 2014, Ribeiro *et al*. proposed that miR-125b might serve as a predictive biomarker for the occurrence of cervical cancer and that MiR-34a might be regarded as a potential biomarker for further development.[66] Kelber *et al*. reported that pseudopodium-enriched atypical kinase 1 (PEAK1) was a novel biomarker for the early prediction of pancreatic cancer.[67] These findings are expected to be applied to early diagnosis and screening if results from a large number of clinical specimens can be validated.

Tumor deterioration biomarkers: Metastasis, recurrence, and drug resistance

Metastasis is one of the primary causes of death in cancer patients. When tumor metastasis occurs, the routine clinical treatment outcome is poor, resulting in high mortality; however, when a tumor is diagnosed, many patients face over-treatment with feasible side effects because there is no optional way to determine whether there is a metastasis. According to statistics, 20~25% of patients diagnosed with lymph node-negative breast cancer will experience tumor metastasis in the 10 years after surgery, but as many as 90% of patients receive postoperative systemic chemotherapy.[68] It is very important to learn how we can more accurately determine the probability of tumor metastasis, and choose relevant treatments with fewer side effects. Early diagnosis of tumors can help patients receive timely and effective treatment. Early diagnosis and prediction of tumor metastasis can also provide more detailed and reliable tumor information for doctors. It may help doctors to decide whether further systemic treatment is needed and choose appropriate clinical treatment after primary tumor resection, in order to improve patients' quality of life and prolong survival.

Some tumor biomarkers may become better predictors of tumor recurrence and metastasis because their abnormal expression often occurs earlier than other detection signs, such as clinical imaging or symptoms. They may also be used to predict the tumor response to different treatments and evaluate prognosis. Dynamic monitoring serums AFP after hepatocellular carcinoma surgery and CEA after colorectal cancer can be adopted for early diagnosis of recurrence and metastasis. Chen *et al*. reported that serum cholinergic muscarinic 2 receptor (CHRM2), family with sequence similarity 5, member C (FAM5C), and promoter hypermethylation of myosin light chain kinase (MYLK) were considered gastric cancer markers, because their serum levels significantly decreased after tumor resection; these findings can be used to evaluate the effect of surgery and prognosis.[69] Recently, Tsai *et al*. observed that a higher serum level of miR-196 correlated with the recurrence of gastric cancer in gastric cancer patients.[70] Budhu *et al*. found that the expression of a 20-miRNA signature had important significance for identifying hepatocellular carcinoma patients who are likely to develop metastases and recurrence.[71] Lu *et al*. also reported that a rise in chemokine CCL2 level might be an important indicator for bone metastasis in prostate and lung cancers.[72–74]

Tumor prognostic biomarkers

The application of molecular markers means that tumor prognosis assessment is no longer confined to clinical pathological parameters. Markers can more accurately assess prognosis by classifying molecular signatures. Mahmoud *et al*. observed that the number of breast tumor-infiltrating $CD8^+$ T lymphocytes was positively correlated with survival period; therefore, it can be considered as an evaluation indicator for the prognosis of breast cancer patients.[75] Winter *et al*. found that pancreatic cancer patients with higher expressions of signal transducer activator of transcription 3 (STAT3), FBJ murine osteosarcoma viral oncogene homolog (FOS), and jun proto-oncogene (JUN) have relatively shorter survival periods. Those with a higher expression of specificity protein 1 (SP1), caudal-type homeobox transcription factor 2 (CDX2), CCAAT/enhancer binding protein alpha (CEBPA) and BRCA1 have relatively longer survival periods. Therefore, these seven genes combined with pathological parameters can accurately classify patients into good and poor prognosis groups, and help to determine whether adjuvant therapy is needed.[76] Lee *et al*. selected 27 proteins related to the prognosis of gastric cancer from 56 genes, based on which patients were divided into two types. Type I tended to be intestinal and early, with a better prognosis than type II; the prognostic accuracy reached 73% or more.[77] Moreira *et al*. reported that neuronal PAS domain protein 3 (NPAS3) drives the progression of human malignant astrocytomas as a tumor suppressor and is a negative prognostic marker for survival.[78] A unique metastatic gene signature enables prediction of tumor relapse in early stage hepatocellular carcinoma patients.[79]

Viral-derived biomarkers of tumor

In recent years, virus-derived DNA, messenger (m)RNA, and proteins, as biomarkers for virus-associated tumors, have been widely used in different clinical applications in the management of tumors, including screening, monitoring, and prognostication. Therefore, the analysis of virus-derived DNA, mRNA and proteins is expected to become an important tool in the management of cancer in the near future.

Epstein-Barr virus (EBV) infection is an important etiology for nasopharyngeal carcinoma (NPC), as the EBV genome can be detected in almost all NPC tumor tissues.[80] Plasma EBV-DNA, when quantitatively analyzed using real-time polymerase chain reaction, has been developed as a biomarker for NPC.[81] In addition, as a result of excellent sensitivity and specificity, plasma EBV-DNA can also be used as a non-invasive biomarker for EBV-positive Hodgkin's lymphoma; serial monitoring could predict response to therapy.[82] Recent research has indicated that autoantibody signatures combined with EBV capsid antigen-IgA (VCA-IgA), as a biomarker panel of NPC, might aid in the screening and diagnosis of NPC.[83] Nishino *et al*. revealed that a high serum level of Epstein-Barr virus–induced gene 3 (EBI3), as an independent prognostic factor, was associated with a poor lung cancer prognosis, suggesting that EBI3 is a potential serum and tissue biomarker, as well as therapeutic target for lung cancer.[84] Epidemiological studies have emphasized that the

human papillomavirus (HPV) is the main etiological factor for cervical cancer and DNA of specific HPV types has been found in almost all cervical cancer biopsies.[85] Attributing to its highly sensitivity, accuracy, and reliability, HPV DNA testing has become a powerful screening tool for the secondary prevention of cervical cancer.[86–89] In addition to high-risk HPV-DNA, the constitutive expression of the viral oncogenes E6 and E7 is another characteristic of cervical cancer.[90] Dürst *et al*. demonstrated that HPV-E6-E7-mRNA could be used as a molecular marker for disseminated cervical cancer in order to predict the risk of recurrence.[91] Moreover, compared with the HPV-DNA test, RNA-based HPV assay was more specific and sensitive for the detection of high-grade pre-cancerous lesions and may be used in primary cervical cancer screening for women 30 years and older.[92]

Tumor biomarkers for targeted therapy

The intervention of some tumor related genes was observed to achieve anti-tumor effects. This so-called "targeted therapy" provides a new method for tumor management. Molecules for targeted therapy can be divided into the following categories: oncogenes and tumor suppressor genes (c-Ras and p53), epidermal growth factor receptor (EGFR), the key protein kinase of signal transduction (PI3K), nuclear transcription factor κB (NF-κB), transmethylase or histone deacetylase (HDAC), and tumor angiogenesis related molecules.[93,94] Targeted therapeutic options include small molecular compounds, antibodies, recombinant virus vectors and small interfering (si)RNAs. For example, the monoclonal antibody trastuzumab interferes with human epidermal growth factor 2 (HER2)/neu over-expression in breast cancer; the small molecule inhibitor imatinib is used for break point cluster/Abelson (BCR/ABL) gene rearrangement in chronic myeloid leukemia, caused by chromosomal translocations; the small molecule inhibitor erlotinib for EGFR-mutated lung cancer; and promyelocytic leukemia (PML)-retinoic acid receptor (RAR) alpha, a fusion protein containing sequences from the PML zinc finger protein and RAR alpha, as a direct drug target in arsenic treatment for acute PML.[95]

Cho-Park and Stellar recently reported an adipose-ribosyl transferase, tankyrase (TNKS), could promote 26S proteasome activity, and a small-molecule inhibitor, XAV939, could inhibit TNKS activity and block proteasome activity, which suggests that a small molecule could be used in the treatment of multiple myeloma.[96] Emerling *et al*. showed that a subset of breast cancers express higher levels of the type 2 phosphatidylinositol-5-phosphate 4-kinases α and/or β (PI5P4Kα and β) and provided evidence that these kinases are essential for growth in the absence of p53. They also indicated that inhibitors of PI5P4Ks could be effective in preventing or treating cancers with mutations in p53.[97] Leprivier *et al*. discovered a protein, eEF2K, which is not important in normal cells but essential in cancer cells; thus, blocking the function of eukaryotic elongation factor-2 kinase (eEF2K) can effectively kill cancer cells without affecting the biological process of normal cells. Therefore, blocking the protein is expected to be significant in the treatment of cancer.[98]

Clinical application prospects of tumor biomarkers

Research on molecular markers of tumors has made great progress in recent years. Tumor biomarkers with potential diagnostic and therapeutic value are accumulating. However, it is important to direct cancer research on diagnosis and treatment toward applying these fundamental research findings to the clinic as soon as possible, and applying novel tumor molecular markers to early diagnosis, targeted therapy, and individualized treatment. Excessive medical treatment should be avoided through the analysis of new tumor biomarkers and appropriate treatments. A large database of clinical specimens to validate new tumor biomarkers is required; therefore, a worldwide EDRN system should be established and a series of standards in the process from tumor biomarker discovery to clinical application should be set.

Conclusion

Translational research on tumor biomarkers has successfully promoted the development of tumor treatment and has brought new hope for cancer patients. As the concept of translational medicine is carried into the field of clinical medicine and basic research, particular emphasis needs to be directed to clinical application, which, in turn, will provide feedback to researchers in order to improve solutions and serve patients.

Acknowledgments

This review was supported by the Natural Science Foundation of China (NSFC) Key Project (81130046); NSFC (81171993; 81272415); and Guangxi Projects of China (2013GXNSFEA053004; 2012GXNSFCB053004; 2013GXNSFBA019177; 1355004-5; 201201ZD004; GZPT13-35; 14122008-22; 11-031-05-K2; KY2015YB057; 14-045-12-K2). The authors thank Drs. Jiejun Fu and Chunlin Zou for helpful discussions and Ms. Xin Huang for editing.

Disclosure

No authors report any conflict of interest.

References

1 Dong E, Hu H, Hong W. Toward a roadmap from translational medicine to medical practice. *Chin Phys Lett* 2013; **4**: 53–62.
2 Wang J, Wang T, Li YS, Zheng YF, Li LR, Wang Q. Research on constitution of Chinese medicine and implementation of translational medicine. *Chin J Integr Med* 2015; **32**: 389–93.
3 Cesario A, Galetta D, Russo P, Margaritora S, Granone P. The role of the surgeon in translational research. *Lancet* 2003; **362**: 1082.
4 Kreeger K. From bench to bedside. *Nature* 2003; **424**: 1090–1.
5 Ledford H. Translational research: The full cycle. *Nature* 2008; **453**: 843–5.
6 Morrow GR, Bellg AJ. Behavioral science in translational research and cancer control. *Cancer* 1994; **74**: 1409–17.
7 Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10–29.
8 Wu L, Qu X. Cancer biomarker detection: Recent achievements and challenges. *Chem Soc Rev* 2015; **44**: 2963–97.
9 Sawyers CL. The cancer biomarker problem. *Nature* 2008; **452**: 548–52.
10 Rich N, Singal AG. Hepatocellular carcinoma tumour markers: Current role and expectations. *Best Pract Res Clin Gastroenterol* 2014; **28**: 843–53.
11 Frazier AL, Hale JP, Rodriguez-Galindo C *et al*. Revised risk classification for pediatric extracranial germ cell tumors based on 25 years of clinical trial data from the United Kingdom and United States. *J Clin Oncol* 2015; **33**: 195–201.
12 Solomon B, Varella-Garcia M, Camidge DR. ALK gene rearrangements: A new therapeutic target in a molecularly defined subset of non-small cell lung cancer. *J Thorac Oncol* 2009; **4**: 1450–4.
13 Gascoyne RD, Aoun P, Wu D *et al*. Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. *Blood* 1999; **93**: 3913–21.
14 Stella-Holowiecka B, Czerw T, Holowiecka-Goral A, Giebel S, Wojnar J, Holowiecki J. Beta-2-microglobulin level predicts outcome following autologous hematopoietic stem cell transplantation in patients with multiple myeloma. *Transplant Proc* 2007; **39**: 2893–7.
15 Delgado J, Pratt G, Phillips N *et al*. Beta2-microglobulin is a better predictor of treatment-free survival in patients with chronic lymphocytic leukaemia if adjusted according to glomerular filtration rate. *Br J Haematol* 2009; **145**: 801–5.
16 Deb P, Chakrabarti P, Chakrabarty S *et al*. Incidence of BCR-ABL transcript variants in patients with chronic myeloid leukemia: Their correlation with presenting features, risk scores and response to treatment with imatinib mesylate. *Indian J Med Paediatr Oncol* 2014; **35**: 26–30.
17 Fu X, Zhang X. BRAF mutation as a potential marker to identify the proximal colon serrated polyps with malignant potential. *Int J Clin Exp Pathol* 2014; **7**: 7319–22.
18 Aksenenko MB, Kirichenko AK, Ruksha TG. Russian study of morphological prognostic factors characterization in BRAF-mutant cutaneous melanoma. *Pathol Res Pract* 2015; **211**: 521–7.
19 Jain P, Cietak KA. Post-term choriocarcinoma with unusually low beta-hCG. *J Obstet Gynaecol* 2008; **28**: 661–2.
20 Nolen BM, Lokshin AE. Biomarker testing for ovarian cancer: Clinical utility of multiplex assays. *Future Oncol* 2013; **17**: 139–46.
21 Banin Hirata BK, Oda JM, Losi Guembarovski R, Ariza CB, de Oliveira CE, Watanabe MA. Molecular markers for breast cancer: Prediction on tumor behavior. *Dis Markers* 2014; **2014**: 513158.
22 Geng B, Liang MM, Ye XB, Zhao WY. Association of CA 15-3 and CEA with clinicopathological parameters in patients with metastatic breast cancer. *Mol Clin Oncol* 2015; **1**: 232–6.
23 Mathelin C, Koehl C, Rio MC. Circulating proteinic biomarkers and breast cancer. *Gynecol Obstet Fertil* 2006; **34**: 638–46.
24 Duffy MJ, McGowan PM, Harbeck N, Thomssen C, Schmitt M. uPA and PAI-1 as biomarkers in breast cancer: Validated for clinical use in level-of-evidence-1 studies. *Breast Cancer Res* 2014; **16**: 428.
25 Calhoun BC, Collins LC. Predictive markers in breast cancer: An update on ER and HER2 testing and reporting. *Semin Diagn Pathol* 2015; pii: S0740-2570(15)00012-X. doi: 10.1053/j.semdp.2015.02.011.
26 Yamauchi H, Nakagawa C, Yamashige S *et al*. Societal cost-effectiveness analysis of the 21-gene assay in estrogen-receptor–positive, lymph-node–negative early-stage breast cancer in Japan. *BMC Health Serv Res* 2014; **14**: 372.
27 Drukker CA, Nijenhuis MV, Bueno-de-Mesquita JM *et al*. Optimized outcome prediction in breast cancer by combining the 70-gene signature with clinical risk prediction algorithms. *Breast Cancer Res Treat* 2014; **145**: 697–705.
28 Andicoechea A, Vizoso F, Alexandre E *et al*. Preoperative carbohydrate antigen 195 (CA195) and CEA serum levels as prognostic factors in patients with colorectal cancer. *Int J Biol Markers* 1998; **3**: 158–64.
29 Reitz D, Gerger A, Seidel J *et al*. Combination of tumour markers CEA and CA19-9 improves the prognostic prediction in patients with pancreatic cancer. *J Clin Pathol* 2015; **68**: 427–33.
30 Sun Z, Zhang N. Clinical evaluation of CEA, CA19-9, CA72-4 and CA125 in gastric cancer patients with neoadjuvant chemotherapy. *World J Surg Oncol* 2014; **12**: 397.
31 Bresadola V, Pravisani R, Terrosu G, Risaliti A. Elevated serum CA 19-9 level associated with a splenic cyst: which is the actual clinical management? Review of the literature. *Ann Ital Chir* 2015; **86**: 22–9.
32 Liu X, Cai H, Wang Y. Prognostic significance of tumour markers in Chinese patients with gastric cancer. *ANZ J Surg* 2014; **6**: 448–53.
33 Xie M, Huang H, Hang J *et al*. Evaluation of the analytical and clinical performances of time-resolved fluoroimmunoassay

for detecting carcinoma antigen 50. *J Immunoassay Immunochem* 2015; **36**: 265–83.
34 Takezako Y, Okusaka T, Ueno H, Ikeda M, Morizane C, Najima M. [Tumor markers for pancreatic and biliary tract cancer.] *Gan to Kagaku Ryoho* 2004; **31**: 1443–6. (In Japanese.)
35 Zhou G, Niu L, Chiu D, He L, Xu K. Changes in the expression of serum markers CA242, CA199, CA125, CEA, TNF-alpha and TSGF after cryosurgery in pancreatic cancer patients. *Biotechnol Lett* 2012; **34**: 1235–41.
36 Jing JX, Wang Y, Xu XQ *et al*. Tumor markers for diagnosis, monitoring of recurrence and prognosis in patients with upper gastrointestinal tract cancer. *Asian Pac J Cancer Prev* 2014; **15**: 10267–72.
37 Goral V, Yesilbagdan H, Kaplan A, Sit D. Evaluation of CA 72-4 as a new tumor marker in patients with gastric cancer. *Hepatogastroenterology* 2007; **54**: 1272–5.
38 Liu P, Zhu Y, Liu L. Elevated serum CA72-4 levels predict poor prognosis in pancreatic adenocarcinoma after intensity-modulated radiation therapy. *Oncotarget* 2015; **6**: 9592–9.
39 Kwon H, Kim WG, Choi YM *et al*. A cut-off value of basal serum calcitonin for detecting macroscopic medullary thyroid carcinoma. *Clin Endocrinol* 2015; **82**: 598–603.
40 Eftekhar E, Naghibalhossaini F. Carcinoembryonic antigen expression level as a predictive factor for response to 5-fluorouracil in colorectal cancer. *Mol Biol Rep* 2014; **41**: 459–66.
41 Lim SH, Levy R. Translational medicine in action: Anti-CD20 therapy in lymphoma. *J Immunol* 2014; **193**: 1519–24.
42 Jensen KH, Hilsted L, Jensen C, Mynster T, Rehfeld JF, Knigge U. Chromogranin A is a sensitive marker of progression or regression in ileo-cecal neuroendocrine tumors. *Scand J Gastroenterol* 2013; **48**: 70–7.
43 Hamada S, Shimosegawa T. Biomarkers of pancreatic cancer. *Pancreatology* 2011; **11**: 14–9.
44 Yasuda M, Saito K, Kobayashi Y *et al*. Serum carbohydrate antigen elevations in endometrial adenocarcinomas: characterization of DU-PAN-2 expression as a tumor marker. *J Obstet Gynaecol Res* 2004; **30**: 59–64.
45 Doroshow JH. Targeting EGFR in non-small-cell lung cancer. *N Engl J Med* 2005; **353**: 200–2.
46 Pujol JL, Molinier O, Ebert W *et al*. CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: Results of a meta-analysis in 2063 patients. *Br J Cancer* 2004; **90**: 2097–105.
47 Kelly JD, Dudderidge TJ, Wollenschlaeger A *et al*. Bladder cancer diagnosis and identification of clinically significant disease by combined urinary detection of Mcm5 and nuclear matrix protein 22. *PLoS ONE* 2012; **7** (7): e40305.
48 Cheng L, Bostwick DG, Li G, Zhang S, Vortmeyer AO, Zhuang Z. Conserved genetic findings in metastatic bladder cancer: a possible utility of allelic loss of chromosomes 9p21 and 17p13 in diagnosis. *Arch Pathol Lab Med* 2001; **125**: 1197–9.
49 Jeong S, Park Y, Cho Y, Kim YR, Kim HS. Diagnostic values of urine CYFRA21-1, NMP22, UBC, and FDP for the detection of bladder cancer. *Clin Chim Acta* 2012; **414**: 93–100.
50 Xu J, Xu HY, Zhang Q *et al*. HAb18G/CD147 functions in invasion and metastasis of hepatocellular carcinoma. *Mol Cancer Res* 2007; **5**: 605–14.
51 Hao Y, Yu Y, Wang L *et al*. IPO-38 is identified as a novel serum biomarker of gastric cancer based on clinical proteomics technology. *J Proteome Res* 2008; **9**: 3668–77.
52 Kang G, Bae BN, Sohn BS, Pyo JS, Kang GH, Kim KM. Detection of KIT and PDGFRA mutations in the plasma of patients with gastrointestinal stromal tumor. *Target Oncol* 2015. doi: 10.1007/s11523-015-0361-1.
53 Slipicevic A, Herlyn M. KIT in melanoma: Many shades of gray. *J Invest Dermatol* 2015; **135**: 337–8.
54 Huang CW, Tsai HL, Chen YT *et al*. The prognostic values of EGFR expression and KRAS mutation in patients with synchronous or metachronous metastatic colorectal cancer. *BMC Cancer* 2013; **13**: 599.
55 Beau-Faller M, Blons H, Domerg C *et al*. A multicenter blinded study evaluating EGFR and KRAS mutation testing methods in the clinical non-small cell lung cancer setting-IFCT/ERMETIC2 Project Part 1: Comparison of testing methods in 20 French molecular genetic national cancer institute platforms. *J Mol Diagn* 2014; **16**: 45–55.
56 von Eyben FE. A systematic review of lactate dehydrogenase isoenzyme 1 and germ cell tumors. *Clin Biochem* 2001; **34**: 441–54.
57 Epstein JI. PSA and PAP as immunohistochemical markers in prostate cancer. *Urol Clin North Am* 1993; **20**: 757–70.
58 Klatte T, Waldert M, de Martino M, Schatzl G, Mannhalter C, Remzi M. Age-specific PCA3 score reference values for diagnosis of prostate cancer. *World J Urol* 2012; **30**: 405–10.
59 Kopczyńska E, Kwapisz J, Junik R, Tyrakowski T. Cellular tumor markers in thyroid cancer. *Pol Merkur Lekarski* 2007; **22**: 295–9.
60 Xue G, Liu J, Huang J *et al*. Detection of galectin-3 in both serum and tissue for early diagnosis of thyroid carcinoma. *Nan Fang Yi Ke Da Xue Xue Bao* 2013; **33**: 1027–30.
61 Kandoth C, Schultz N, Cherniack AD *et al*. Integrated genomic characterization of endometrial carcinoma. *Nature* 2013; **497**: 67–73.
62 Ni X, Zhuo M, Su Z *et al*. Reproducible copy number variation patterns among single circulating tumor cells of lung cancer patients. *Proc Natl Acad Sci U S A* 2013; **110**: 21083–8.
63 Kinde I, Bettegowda C, Wang Y *et al*. Evaluation of DNA from the Papanicolaou test to detect ovarian and endometrial cancers. *Sci Transl Med* 2013; **5**: 167ra4.
64 Wang X, Song X, Zhuo W *et al*. The regulatory mechanism of Hsp90alpha secretion and its function in tumor malignancy. *Proc Natl Acad Sci U S A* 2009; **106**: 21288–93.
65 Harris T, Jimenez L, Kawachi N *et al*. Low-level expression of miR-375 correlates with poor outcome and metastasis while altering the invasive properties of head and neck squamous cell carcinomas. *Am J Pathol* 2012; **180**: 917–28.
66 Ribeiro J, Sousa H. MicroRNAs as biomarkers of cervical cancer development: A literature review on miR-125b and miR-34a. *Mol Biol Rep* 2014; **41**: 1525–31.

67 Kelber JA, Reno T, Kaushal S *et al*. KRas induces a Src/PEAK1/ErbB2 kinase amplification loop that drives metastatic growth and therapy resistance in pancreatic cancer. *Cancer Res* 2012; **72**: 2554–64.

68 Aalaoui-Jamali M, Bijian K, Batist G. Emerging drug discovery approaches for selective targeting of "precursor" metastatic breast cancer cells: Highlights and perspectives. *Am J Transl Res* 2011; **3**: 434–44.

69 Chen L, Su L, Li J *et al*. Hypermethylated FAM5C and MYLK in serum as diagnosis and pre-warning markers for gastric cancer. *Dis Markers* 2012; **32**: 195–202.

70 Tsai KW, Liao YL, Wu CW *et al*. Aberrant expression of miR-196a in gastric cancers and correlation with recurrence. *Genes Chromosomes Cancer* 2012; **51**: 394–401.

71 Budhu A, Jia HL, Forgues M *et al*. Identification of metastasis-related microRNAs in hepatocellular carcinoma. *Hepatology* 2008; **47**: 897–907.

72 Zhang J, Lu Y, Pienta KJ. Multiple roles of chemokine (C-C motif) ligand 2 in promoting prostate cancer growth. *J Natl Cancer Inst* 2010; **102**: 522–8.

73 Lu Y, Cai Z, Xiao G *et al*. Monocyte chemotactic protein-1 mediates prostate cancer-induced bone resorption. *Cancer Res* 2007; **67**: 3646–53.

74 Cai Z, Chen Q, Chen J *et al*. Monocyte chemotactic protein 1 promotes lung cancer-induced bone resorptive lesions in vivo. *Neoplasia* 2009; **11**: 228–36.

75 Mahmoud SM, Paish EC, Powe DG *et al*. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. *J Clin Oncol* 2011; **29**: 1949–55.

76 Winter C, Kristiansen G, Kersting S *et al*. Google goes cancer: Improving outcome prediction for cancer patients by network-based ranking of marker genes. *PLoS Comput Biol* 2012; **8** (5): e1002511.

77 Lee HS, Cho SB, Lee HE *et al*. Protein expression profiling and molecular classification of gastric cancer by the tissue array method. *Clin Cancer Res* 2007; **13**: 4154–63.

78 Moreira F, Kiehl TR, So K *et al*. NPAS3 demonstrates features of a tumor suppressive role in driving the progression of astrocytomas. *Am J Pathol* 2011; **179**: 462–76.

79 Roessler S, Jia HL, Budhu A *et al*. A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. *Cancer Res* 2010; **70**: 10202–12.

80 Chan KC. Plasma Epstein-Barr virus DNA as a biomarker for nasopharyngeal carcinoma. *Chin J Cancer* 2014; **33**: 598–603.

81 Ma B, Hui EP, King A *et al*. A phase II study of patients with metastatic or locoregionally recurrent nasopharyngeal carcinoma and evaluation of plasma Epstein-Barr virus DNA as a biomarker of efficacy. *Cancer Chemother Pharmacol* 2008; **62**: 59–64.

82 Gandhi MK, Lambley E, Burrows J *et al*. Plasma Epstein-Barr virus (EBV) DNA is a biomarker for EBV-positive Hodgkin's lymphoma. *Clin Cancer Res* 2006; **12**: 460–4.

83 Peng YH, Xu YW, Huang LS *et al*. Autoantibody signatures combined with Epstein-Barr virus capsid antigen-IgA as a biomarker panel for the detection of nasopharyngeal carcinoma. *Cancer Prev Res (Phila)* 2015; pii: canprevres.0397.2014. doi: 10.1158/1940-6207.CAPR-14-0397.

84 Nishino R, Takano A, Oshita H *et al*. Identification of Epstein-Barr virus-induced gene 3 as a novel serum and tissue biomarker and a therapeutic target for lung cancer. *Clin Cancer Res* 2011; **17**: 6272–86.

85 Zur Hausen H. Papillomaviruses and cancer: From basic studies to clinical application. *Nat Rev Cancer* 2002; **2**: 342–50.

86 Agorastos T, Chatzistamatiou K, Katsamagkas T *et al*. Primary screening for cervical cancer based on high-risk human papillomavirus (HPV) detection and HPV 16 and HPV 18 genotyping, in comparison to cytology. *PLoS ONE* 2015; **10** (3): e0119755.

87 de Sanjosé S, Ibáñez R, Rodríguez-Salés V *et al*. Screening of cervical cancer in Catalonia 2006–2012. *Ecancermedicalscience* 2015; **9**: 532.

88 Lata K, Bhatla N. HPV testing for cervical cancer screening: Time for a new paradigm. *Natl Med J India* 2014; **27**: 212–3.

89 Costa S, Venturoli S, Origoni M *et al*. Performance of HPV DNA testing in the follow-up after treatment of high-grade cervical lesions, adenocarcinoma in situ (AIS) and microinvasive carcinoma. *Ecancermedicalscience* 2015; **9**: 528.

90 Häfner N, Driesch C, Gajda M *et al*. Integration of the HPV16 genome does not invariably result in high levels of viral oncogene transcripts. *Oncogene* 2008; **27**: 1610–7.

91 Dürst M, Hoyer H, Altgassen C *et al*. Prognostic value of HPV-mRNA in sentinel lymph nodes of cervical cancer patients with pN0-status. *Oncotarget* 2015. doi: 10.18632/oncotarget.4132.

92 Iftner T, Becker S, Neis KJ *et al*. Head-to-head comparison of the RNA-based Aptima® HPV assay and the DNA-based HC2 HPV test in a routine screening population of women aged 30 to 60 years in Germany. *J Clin Microbiol* 2015. doi: 10.1128/JCM.01013-15.

93 Langer CJ. Will FLEX allow us flexibility in the therapy of advanced non-small-cell lung cancer? Insights from the 2008 American Society of Clinical Oncology Meeting. *Clin Lung Cancer* 2008; **9**: 249–51.

94 Gustafson AM, Soldi R, Anderlind C *et al*. Airway PI3K pathway activation is an early and reversible event in lung cancer development. *Sci Transl Med* 2010; **2**: 26ra25.

95 Zhang XW, Yan XJ, Zhou ZR *et al*. Arsenic trioxide controls the fate of the PML-RARalpha oncoprotein by directly binding PML. *Science* 2010; **328**: 240–3.

96 Cho-Park PF, Steller H. Proteasome regulation by ADP-ribosylation. *Cell* 2013; **153**: 614–27.

97 Emerling BM, Hurov JB, Poulogiannis G *et al*. Depletion of a putatively druggable class of phosphatidylinositol kinases inhibits growth of p53-null tumors. *Cell* 2013; **155**: 844–57.

98 Leprivier G, Remke M, Rotblat B *et al*. The eEF2 kinase confers resistance to nutrient deprivation by blocking translation elongation. *Cell* 2013; **153**: 1064–79.

