การทดสอบระดับโมเลกุลของมะเร็ง และการประยุกต์ใช้ทางคลินิก

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บทคัดย่อ

มะเร็งเป็นโรคที่เกิดจากหลายปัจจัยร่วมกันซึ่งรวมถึงปัจจัยทางพันธุกรรมและสิ่งแวดล้อม โรคมะเร็งถือเป็นความเจ็บป่วยที่คุกคามต่อชีวิตโดยเฉพาะในระยะลุกลาม โมเลกุลที่เป็นสาเหตุของความผิดปกติมีความซับซ้อนอันเกิดจากความไม่เสถียรของสารพันธุกรรมในระหว่างการดำเนินโรค การเลือกวิธีวิเคราะห์ที่มีความรวดเร็วและเชื่อถือได้ในการตรวจหาโมเลกุลที่ผลิตจากเนื้องอกหรือโมเลกุลที่เกี่ยวข้องกับโรคมะเร็งถือเป็นสิ่งจำเป็นอย่างยิ่งสำหรับการตรวจคัดกรองมะเร็ง ปัจจุบันการทดสอบระดับโมเลกุลถูกนำมาใช้เพื่อประเมินโรคมะเร็ง ทั้งนี้เนื่องจากช่วยให้สามารถวินิจฉัยโรคได้รวดเร็วขึ้น จึงช่วยลดระยะเวลาในการรอคอยสำหรับการดูแลขั้นพื้นฐาน เนื้อหาบทความนี้มุ่งเน้นไปที่บทบาทและการประยุกต์ใช้ทางคลินิกของการทดสอบระดับโมเลกุลของมะเร็งชนิดต่าง ๆ โดยรวบรวมข้อมูลจากโรคมะเร็งที่พบบ่อยหลายชนิด ได้แก่ มะเร็งเต้านม มะเร็งปอด มะเร็งลำไส้ใหญ่ มะเร็งต่อมลูกหมาก รวมถึงมะเร็งเม็ดเลือดขาว จากข้อมูลชี้ให้เห็นว่าการทดสอบระดับโมเลกุลมีบทบาทสำคัญในการจัดการกับโรคมะเร็ง ทำให้การวินิจฉัยโรคมะเร็งและการจำแนกประเภทของโรคทำได้ง่ายขึ้นโดยเฉพาะอย่างยิ่งเมื่อใช้ร่วมกับข้อมูลทางคลินิกและข้อมูลทางพยาธิวิทยา ผลการทดสอบระดับโมเลกุลหลายชนิดมีผลต่อการตัดสินใจในการรักษาโรคมะเร็ง การทดสอบยังใช้เพื่อประเมินประสิทธิภาพการรักษา นอกจากนี้ข้อมูลทางพันธุกรรมของผู้ป่วยมะเร็งแต่ละรายยังสามารถนำมาใช้สำหรับการประเมินความเสี่ยงในการเกิดมะเร็งของสมาชิกในครอบครัว เพื่อเป็นการเฝ้าระวังเฉพาะบุคคล ผู้ที่ตรวจพบการกลายพันธุ์ของสารพันธุกรรมควรได้รับคำแนะนำแนวทางในการป้องกันโรคมะเร็งและตรวจหามะเร็งอย่างสม่ำเสมอ

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Cancer Molecular Testing and Clinical Applications

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Abstract

Cancer is a multifactorial disease in which both genetic and environmental factors are involved. It is a life-threatening illness especially in the advanced stage. The underlying molecular events are complex due to genomic instability during cancer progression. Rapid and reliable identification of tumor derived or associated molecules are urgently required for cancer screening. Molecular testing is now being used for cancer assessment as it allows early diagnosis, therefore reduces waiting times for the primary care. This review focuses on the roles and clinical applications of molecular testing toward various cancer types. Information from many kinds of common cancers, namely, breast, lung, colorectal, prostate, together with leukemia, were collected. Data suggested that molecular testing plays an important role in cancer management. It makes cancer diagnosis and classification more approachable when combined with clinical and histological data. Several molecular test results have a significant impact on treatment decisions. Those tests, moreover, are used to evaluate treatment efficacy. Additionally, genetic information of individual patients can be used for risk assessment of cancer in their family members. Cancer surveillance including specific early detection programs and prophylactic recommendations are then advised for mutation carriers.

Keywords: Molecular testing, Clinical applications, Cancer

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Introduction

Molecular testing is defined as laboratory tests for analyzing biological markers including genes, proteins as well as other molecules in the samples. In medical usage, molecular biology techniques are applied to check many kinds of clinical specimens such as blood, urine, body fluid and tissue. The techniques can be used to detect a certain change in the molecules that may affect the specific disease development. Molecular testing is not only used for diagnosis but also in monitoring, prognosis and treatment selection aspects. The tests are useful and applicable in a wide range of health conditions including infectious diseases, genetic diseases and cancer. The demand of molecular testing has been increasing continuously during the past decade. Many of the assays are available as routine diagnostics in clinical laboratories.[1-3]

Cancer is known to be a complex disease. Precise risk factor is difficult to evaluate. Both genetic and environmental factors are believed to be involved in cancer initiation and development. Due to genetic alteration during cancer progression, the molecules in cancer cells are changed and thus make them different from normal molecular patterns. Understanding how these molecules contribute to cancer evolution and treatment responsiveness is crucial. Currently, many molecular techniques are well established in routine laboratories. The molecular results have been implicated early in the decision process of cancer patient management. In leukemia, historically, distinguishing between specific types of diseases was time-consuming and difficult. Evolution of diagnostic technology brings the classification more feasible. As shown in previous reports, different types of cancer contain different molecular aberrations.[4-6] In this review, the role of molecular testing in several types of cancer consisting of breast, lung, colorectal, prostate and leukemia are gathered and revealed.

Role of molecular testing in different types of cancer

In the field of cancer, molecular testing is used to evaluate the genetic predisposition to cancer and to study cancer biology in order to enhance our understanding about cancer etiology and progression. Unlike normal tissue, a tumor is genetically heterogeneous due to somatic mutations in rapidly dividing cells. Its progression is an evolutionary process which starts from mutation in a single cell and results in more aggressive cancer clones. A recent study has demonstrated that the mutational patterns are different between baseline and years before cancer diagnosis. The increasing of mutational complexity suggested that premalignant mutation can promote additional mutations and they may finally have a cooperative role in cancer pathogenesis.[7] Since the genetic basis of individuals are different and can cause

different drug responses, information from molecular tests directly affects the clinical management of cancer patients. According to analysis of patient specific molecular alterations and disease relation, molecular testing provides opportunity for patients to approach personalized medicine. Moreover, the molecular testing is playing a role in risk assessment of asymptomatic relatives for developing cancer.[8, 9] Below are the details regarding molecular testing in each type of cancer.

1. Breast cancer

Breast cancer is the most common cancer affecting women worldwide. The incidence is increasing continuously especially in the industrialized countries.[10] Risk factors include behavioral and lifestyle factors such as radiation exposure, smoking, alcohol consumption, pregnancy history, birth control pill usage.[11-13] However, about 5 to 10 percent of breast cancers contain some genetic defects, and are classified as a hereditary cancer. Two important genes associated with breast cancer predisposition are *BRCA1* and *BRCA2*. Women carrying one allele of *BRCA1* mutation are at a high risk for developing breast cancer when compared with the normal individual. *BRCA1* is a multifunctional protein that works with several proteins such as estrogen receptor, cyclin D1 and c-Myc. Its main functions are to repair DNA and maintain genomic stability in cells. Loss of wild-type *BRCA1* alleles or loss of BRCA1 protein activity may lead to breast cancer development.[14] Other molecules such as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) are also claimed to link with breast cancer. Based on expression of these molecules, the tumors are divided into four molecular subtypes which are 1) Luminal A: ER+ and/or PR+, slow proliferation, low grade; 2) Luminal B: ER+ and/or PR+, high proliferation, high grade; 3) HER2-enriched: having HER2 amplification as well as other genes in the same amplicon; and 4) basal-like, ER/PR/HER2-negative (the triple-negative phenotype), showing characteristics of basal-origin.[15]

Identification of molecules underlying specific subtypes helps to decide best practice for breast cancer patients since different molecular characteristics exhibit different phenotypes. From the information described above, ER and PR testing are recommended for all newly diagnosed breast cancer patients and recurrent cases. Hormonal therapy may be considered in hormone receptor-positive group. HER2 detection has been used for therapeutic decision making. In *HER2* amplification and/or overexpression cases, trastuzumab, a humanized monoclonal antibody that blocks directly to the HER2 extracellular domain should be offered. As the drug can reduce recurrence risk and mortality rate in HER2-positive breast cancer, it becomes

one of the most successful HER2 targeted therapy.[15, 16] In a previous report, analysis of primary breast tumors using diverse types of molecular testing, which are genomic DNA copy number arrays, DNA methylation, exome sequencing, messenger RNA arrays, microRNA sequencing and reverse-phase protein arrays, has shown significant molecular heterogeneity in each subtype. The biological features of those subtypes are derived from different subsets of genetic and epigenetic abnormalities. Distinct mutational profiles found in this study may be used as information for new therapeutic target approaches.[17]

Many molecular testing have already been implemented in clinical practice for breast cancer patients. Immunohistochemistry (IHC) is used for detection of steroid receptor (ER/PR) status, HER2 status and Ki67 expression status.[18] Fluorescence *in situ* hybridization (FISH) and chromogenic *in situ* hybridization (CISH) are used for defining *HER2* gene copy number.[19] Although full gene sequencing is the best option for assessment of *BRCA1/2* mutational status, targeted gene sequencing is preferable in the specific founder mutations within certain population groups, for examples 185delAG and 5382insC *BRCA1* mutations and 6174delT *BRCA2* mutation in Ashkenazi Jewish population.[20] Currently, numbers of molecular testing platforms are commercially available to expedite the treatment and improve personalized care. Prosigna, Oncotype DX, MammaPrint, MammaTyper and the NexCourse IHC4 assay are examples of those molecular tests. Several platforms have been developed according to the information from gene expression profiling studies. Some of them have been proposed to be potential prognostic tools and used for chemosensitivity assessment.[21] Additionally, next-generation gene sequencing (NGS) which is a high-throughput technology is also used for elucidating the mutational landscape of breast cancer in a short period of time. It helps improve several aspects of personalized breast cancer treatment including identifying new therapeutic targets and predictive biomarkers for targeted therapy development.[22]

2. Lung cancer

Lung cancer is the disease caused by uncontrolled growth of cells in the lung tissue. The abnormal cells can metastasize out of the lung and interfere with the functions of normal cells, tissues or even organs. Lung cancer is divided into two major types which are small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC).[23] The mortality rate of lung cancer remains high because the large portion of patients are in advanced stage and the effectiveness of current treatments are quite limited. Advanced stage lung cancer patients exhibited low response rate to standard platinum-based chemotherapy and some of those developed chemotherapeutic drug resistance.[24, 25] Somatic molecular alterations, not just environmental drivers, are

found to be linked with lung cancer. Tyrosine kinase domain mutations of epidermal growth factor receptor *(EGFR)* gene and rearrangements of anaplastic lymphoma kinase-1 *(ALK)* gene are often presented.[26, 27] EGFR is a transmembrane protein that is activated by ligands in the epidermal growth factor family. Cytoplasmic tyrosine kinase activity of EGFR is important for DNA synthesis and cell proliferation. Somatic mutations involving EGFR lead to its constant activation which causes uncontrolled cell growth.[25, 26] *EML4-ALK* fusion transcript was detected in almost 7% of NSCLC. The EML4-ALK has been found to promote and maintain the malignant behavior of lung cancer cells.[27, 28]

Many studies have shown that *EGFR* mutation and *ALK* rearrangement carriers are more sensitive to small-molecule tyrosine kinase inhibitors (TKIs). Furthermore, the use of anti-EGFR or anti-ALK leads to reduce the tumor size and extend survival. Those molecules have become important therapeutic targets for the treatment of lung tumors.[25-28] From positive results of drug responsiveness of selective treatment in mutation carriers, molecular testing is now a part of routine diagnosis for lung cancer as it helps select the treatment options.[29]

Several molecular tests implicate in lung cancer diagnosis, therapy and follow-up. A range of strategies have been used for *EGFR* mutation detection including directed sequencing, restriction fragment length polymorphism (RFLP) analysis, size fractionation, allele-specific PCR, and mass spectrometry -based genotyping.[30] In order to analyze *EGFR* copy number, FISH or CISH can be performed. Immunohistochemistry has also been used to evaluate EGFR expression levels. Although high expression of EGFR by IHC may be used as information for selecting monoclonal anti-EGFR treatment, both FISH/CISH and immunohistochemistry are not recommended for prediction of response to TKIs.[31] *ALK* rearrangements can be assessed by FISH, RT-PCR or immunohistochemistry.[23] Recently, molecular testing of other lung cancer-associated genes have been proposed for targeted treatment selection such as *ROS1, BRAF, HER2, KRAS, MET,* and *RET*.[32, 33]

3. Colorectal cancer

Colorectal cancer or colon cancer is a cancer identified in colon or rectum. It is the third most common malignancy worldwide. The most important risk factor for colorectal cancer is increasing age and history of hereditary colorectal cancer syndrome or family history of colorectal cancer in first-degree relative. Increasing risk is found in people over 50 years of age. Less than 5% of colorectal cancers are derived from genetic abnormalities. Individual harboring genetic predisposition such as familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) is prone to develop colorectal cancer.[34, 35] The hereditary

colorectal syndromes have distinct genetic and clinical traits. FAP and attenuated FAP generally result from chromosomal instability from *APC* gene mutation. *MUTYH* mutation is another genetic factor involved in autosomal recessive familial adenomatous polyposis or MYH-associated polyposis (MAP). The HNPCC or Lynch syndrome is usually caused by microsatellite instability (MSI) from DNA mismatch repair (MMR) gene mutations. Molecular genetic testing has been implicated in diagnosis and management of these syndromes. It provides information for genetic counseling for the patients and at-risk family members. Since the syndromes give nearly 100% lifetime risk for cancer endoscopic surveillance and prophylactic surgery to remove colonic polyps are suggested.[36, 37]

In sporadic colorectal cancer, the underlying genetic and molecular pathways involved during cancer progression are chromosomal instability, microsatellite instability, and DNA hypermethylation. Key genes mutated in chromosomal instability pathway include *APC, KRAS,* and *TP53. KRAS* oncogene involves in the mitogen-activated protein kinase (MAPK) pathway. Patients considering anti-EGFR therapy (eg. cetuximab/ panitumumab) should be tested for KRAS status because mutant KRAS results in constitutive MAPK activation thus upstream blockage of EGFR is not effective. Microsatellite instability is related to abnormal DNA mismatch repair system caused by germline mutation in MMR gene or epigenetic inactivation of *MLH1.*[36, 38] Other mutated genes found in *de novo* colorectal cancer are *BRAF, NRAS, PIK3CA* and *PTEN. BRAF* and *NRAS* mutations confer cetuximab resistance while the significance of *PIK3CA* and *PTEN* mutations in anti-EGFR therapy is still controversial. Lately, *HER2* amplification has been found in a small number of patients. Phase 2 trial study showed that combination of HER2-targeted therapies, trastuzumab (anti-HER2 antibody) and lapatinib (HER2 tyrosine kinase inhibitor), is effective in the treatment of KRAS wild type, HER2-positive metastatic colorectal cancer. These data indicate that molecular testing has significant benefits for colorectal cancer patients as it introduces individually tailored treatment options to both familial and sporadic groups.[39, 40]

A wide range of methods have been used for analyzing colorectal cancer-associated molecules as presented in Table 1. Advanced techniques, for example, microarray, proteomics, metabolomics, NGS, miRNA profiling and epigenetics are now proposed to be used for molecular diagnostics of colorectal cancer in the near future.[39]

4. Prostate cancer

Prostate cancer is a malignancy of male reproductive system in which the cancer cells developed in the prostate gland. It is the second common cancer in male following the lung cancer. More than 30% of men manifest

prostate cancer in their fifth decade, and up to 70% of men are diagnosed with prostate cancer at the age of 80 and older. Higher incidence and mortality rates have been observed in more developed regions including Europe and North America comparing to Asia and Africa. However, the mortality-to-incidence ratio (MIR) is shown to be lower in developed countries due to their better health care systems.[34, 41, 42] The primary risk factors of prostate cancer are age, family history and race. The risk is increasing in people who have first-degree relative affected by this type of cancer. For genetic factors, many genes are implicated. Genome-wide association studies (GWAS) have demonstrated that more than 100 single nucleotide polymorphisms (SNPs) are associated with the development of prostate cancer. Pathogenic variants in *BRCA1, BRCA2, HOXB13* as well as *HPC1* increase the lifetime risk of prostate cancer. In addition, *HPC2, MSR1* and *CHEK2* mutations are also identified in familial and sporadic cases.[41, 43-46]

A well-established and sensitive biomarker for prostate cancer is prostate specific antigen (PSA) which is a protein secreted by epithelial cells of prostate gland. Most of prostate cancer cells are not aggressive thus result in high survival rates. Active surveillance by periodic observation of PSA, digital rectal examination (DRE) and prostate biopsy every 1 to 2 years is a cost-effective approach which can preserve the quality of life. Therefore, the surveillance is suggested for low-risk characteristics prostate cancer patients.[47] Exploring the genetic makeup of prostate tumors by molecular testing may facilitate patient classification for personalized medicine.[41] A previous study has shown that *PTEN* loss and *c-MYC* gain contribute to significantly increase genetic instability, and thus can be used as prognostic markers for relapse after prostate cancer radiotherapy. These copy number alterations may cause resistance to PI3K inhibitors hence the use of other regimens should be considered to improve clinical outcomes.[48]

Blood PSA immunoassay is normally used for disease detection and therapeutic monitoring. However, PSA-based screening remains controversial as it confers high rates of overdiagnosis and overtreatment in low-risk tumors.[47, 49] Nowadays advanced molecular profiling is emerged. New aberrations in DNA, RNA, or epigenetic DNA methylation are discovered and being proved to be predictive markers for prostate cancer. A non-coding RNA, prostate cancer antigen 3 (PCA3) had been found to highly express in most of prostate cancers. The PCA3 overexpression is screened by RT-PCR.[50] Copy number alterations of *PTEN* and *c-MYC* are detected by array comparative genomic hybridization (aCGH) or FISH.[48] Interestingly, multigene (panel) tests for prostate cancer-associated gene variants is currently available and being used for prostate cancer screening in some laboratories. Tumor DNA

sequencing is proposed to be a primary method identifying somatic variants in order to select potential treatment.[41]

5. Leukemia

Leukemia is a cancer of the blood cells that are usually initiated in bone marrow and then released into blood circulation. Proliferation of blood cancer cells or leukemia cells in the bone marrow interfere with normal cell growth, so other normal blood cell types tend to be decreased. Leukemia is a subgroup of hematological malignancies which the tumors of lymphoid and myeloid precursors are involved. Four main types of leukemia have been classified, namely, acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL). ALL is the most common hematological malignancies found in children while AML and CLL are more common in adults. The real causes of leukemia are still in controversy. However, several risk factors have been identified such as family history of leukemia, exposure to radiation or carcinogens, previous treatment with chemotherapy, genetic disorders e.g. Down syndrome and smoking.[51-53]

Diverse genetic aberrations are found in leukemia. The abnormality events range from single base-pair substitution to complete chromosomal changes. Chromosomal abnormalities found in leukemia often result in abnormal fusion genes which play an important role in tumorigenesis. Gene fusion can cause the assembly of a new active gene that usually produces much more abnormal proteins, and thus contribute to tumor formation.[51, 54] Numbers of leukemia-associated fusion genes have been elucidated. The first specific genetic alteration of hematological malignancies is *BCR-ABL* fusion gene. It is mostly identified in CML, however can be detected in some ALL cases. The *BCR-ABL* caused by reciprocal translocation of chromosome 9 and 22, t(9;22)(q34;q11). The fusion gene is on chromosome 22 which is later called "Philadelphia chromosome". Due to increased tyrosine kinase activity of BCR-ABL protein, cell cycle is activated while apoptosis is suppressed, and consequently the cells grow uncontrollably. Tyrosine kinase inhibitors (TKIs) are used for treatment of BCR-ABL-positive leukemia. The *BCR-ABL* fusion gene is a hallmark of CML. Detection of Philadelphia chromosome/*BCR-ABL* can be conducted by cytogenetics, FISH or RT-PCR. Recently, real-time-quantitative PCR (RQ-PCR) was developed. *BCR-ABL* detection have been used for diagnosis, treatment selection and disease monitoring.[55-57]

Another most common recurrent cytogenetic abnormality found in leukemia is translocation between chromosome 15 and 17, t(15;17)(q22;q21). This translocation event produces two new fusion transcripts, *PML-RARA* and their reciprocal product *RARA-PML*. *PML-RARA* is a hallmark of acute promyelocytic leukemia (APL) or AML-M3. The PML-

RARA protein blocks myeloid differentiation and that is critical for its pathogenesis. APL with PML-RARA seems to have good responsiveness to all-trans retinoic acid (ATRA) and arsenic trioxide therapy. In addition to conventional cytogenetics, either FISH or RQ-PCR is being recommended for *PML-RARA* assessment due to its sensitivity and rapidity. Detection of *PML-RARA* may apply for diagnosis, treatment as well as monitoring of minimal residual disease (MRD). The other two chromosomal abnormalities found in AML are t(8;21)(q22;q22) and inv(16)(p13.1q22) /t(16;16)(p13.1;q22) that create *RUNX1-RUNX1T1(AML1-ETO)* and *CBFB-MYH11* fusion genes, respectively. The fusion proteins produced by those two genes can disrupt the function of core binding factor (CBF) AML and lead to impaired differentiation. *RUNX1-RUNX1T1* and *CBFB-MYH11* predict favorable outcomes and can be detected by using conventional cytogenetics or FISH. In ALL, t(12;21)(p13;q22) results in *ETV6-RUNX1* fusion. Presenting of *ETV6-RUNX1* is associated with a good prognosis in childhood B-ALL. The fusion gene can be evaluated by FISH or RT-PCR.[55, 56, 58]

Apart from the fusion genes causing by chromosomal abnormalities, some single gene mutations have been shown to be implicated in AML pathogenesis. *FLT3* gene mutations can lead to constitutive activation of tyrosine kinase receptor. Mutation in internal tandem duplication (ITD) of *FLT3* gene, so called *FLT3*-ITD, is reported to be associated with poor prognosis. The clinical significance of *FLT3*-ITD is proposed in AML.[59] Mutations of *NPM1* and *CEBPA* are also clinically established. *NPM1* mutants can be tested by PCR-based assay (exon 12 amplification followed by fragment size analysis), while *CEBPA* can be detected by multiplex PCR or direct sequencing.[60, 61]

Conclusion

Molecular testing is now widely used for both solid cancers and hematologic malignancies assessment due to its time- and cost-effectiveness. The tests gradually replace sophisticated conventional methods, as they provide high sensitivity and specificity with less labor-intensive. Numbers of molecular methods together with their clinical applications in different types of cancer have been explored and summarized (Table 1). The data demonstrated that molecular testing exerts their roles in the major steps of cancer management including diagnosis, treatment and follow-up. Due to the complexity of genetic abnormalities in cancer, for some certain types, the diagnosis has changed from a single gene testing to multi-gene expression profiling. As utilizing of molecular testing for improvement of personalized management is continuously increased, the quality control issues should be considered so that the results can be confidently reported.

Table 1 Methods for detection of cancer biomarkers and their clinical applications[39, 48, 62]

Cancer type	Molecular marker	Detection method	Clinical application
Breast	BRCA1/BRCA2	DS	Screening, Classification, Treatment
	ER/PR	IHC	Classification, Treatment, Prognosis
	HER2	IHC, FISH, CISH	Classification, Treatment
	Ki67	IHC	Classification, Treatment, Prognosis
Lung	EGFR	DS, RFLP, ASPCR, Size fractionation, MS-based genotyping, FISH, CISH, IHC	Treatment, Prognosis
	ALK	FISH, RT-PCR, IHC	Treatment
Colorectal	APC	PTT, DS, MLPA, CSGE, SSCP	Screening, Diagnosis
	MUTYH	DS, T-ARMS-PCR	Screening, Diagnosis
	MSI	Five microsatellite markers analysis (BAT-25, BAT-26, D2S123, D5S346, D17S250)	Screening, Diagnosis
	MMR	IHC, DS, SB, MLPA, SSCP, qPCR, DGGE	Screening, Diagnosis
	KRAS	ARMS, DS, HRM	Treatment
	NRAS	Multiplex PCR, Reflex NRAS testing	Treatment
	BRAF	DS, ARMS, SSCP, RFLP	Treatment
Prostate	PSA	Immunoassays	Screening, Monitoring, Surveillance
	PCA3	RT-PCR	Screening
	HOXB13	DS	Screening
	PTEN	aCGH, FISH	Prognosis
	c-MYC	aCGH, FISH	Prognosis
Leukemia	BCR-ABL	Cytogenetics, FISH, RT-PCR, RQ-PCR	Diagnosis, Treatment, Monitoring
	PML-RARA	Cytogenetics, FISH, RT-PCR, RQ-PCR	Diagnosis, Treatment, Monitoring
	RUNX1-RUNX1T1	Cytogenetics, FISH	Prognosis
	CBFB-MYH11	Cytogenetics, FISH	Prognosis
	ETV6-RUNX1	FISH, RT-PCR	Prognosis
	FLT3	PCR-based fragment size analysis	Prognosis
	NPM1	PCR-based fragment size analysis	Prognosis
	CEBPA	Multiplex PCR, DS	Prognosis

DS: direct sequencing; IHC: immunohistochemistry; FISH: fluorescence *in situ* hybridization; CISH: chromogenic *in situ* hybridization; RFLP: restriction fragment length polymorphism; ASPCR: allele-specific polymerase chain reaction; MS: mass spectrometry; RT-PCR: reverse transcription polymerase chain reaction; PTT: protein truncation test; CSGE: conformation strand gel electrophoresis; SSCP: single strand conformation polymorphism testing; T-ARMS-PCR: tetra-primer amplification refractory mutation system PCR; ARMS: amplification resistant mutation system; HRM: high-resolution melting analysis; SB: Southern blotting; qPCR: quantitative polymerase chain reaction; DGGE: denaturing gradient gel-electrophoresis; aCGH: array comparative genomic hybridization; RQ-PCR: real-time-quantitative polymerase chain reaction

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