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Capillary electrophoresis based on the nucleic acid detection in the application of cancer diagnosis and therapy

DongSheng Lian; ShuJin Zhao

Email: papersend1@163.com

Abstract: Cancer is malignant desease that causes many deaths worldwide every year, with most deaths occurring in the middle and advanced stages of cancer. Numerous deaths can be avoided by detecting cancer at an early stage, making early diagnosis and timely therapy critical for cancer treatment.Analyses at the level of nucleic acids rather than phenotypes can eliminate various false positive and negative results,and diagnoses can occur at an earlier stage.Many techniques have been developed for this purpose,including capillary electrophoresis (CE),which has the advantages of high-efficiency,high-speed, high-throughput,automation,cleanliness, and versatility, and CE can be conducted on a microscale or coupled with other separation techniques. These advantages afford this technique the ability to meet the future medical requirements that will undoubtedly call for amassing large numbers of samples for analysis, suggesting that CE may become an important tool for providing data in clinical cancer diagnosis and therapy. This review focuses on CE-based nucleic acid detection as it is applied to cancer diagnosis and therapy, and provides an introduction to the drawbacks and future developments of analysis with CE.

Keywords: cancer; mutation; polymorphism; capillary electrophoresis; diagnosis; therapy

Introduction

Cancer is a disease of great concern because it is the second leading cause of death in the world. Although its occurrence is complicated, molecular studies on carcinogenesis have clearly shown that the malignant transformation of normal to cancer cells relies on genetic alterations of a large number of generic tumour suppressor genes or oncogenes. Oncogenomics, a relatively new subfield of genomics proposed to systematically characterize cancer-associated gene mutations, and has been facilitated by the completion of the Human Genome Projects**[1]**. Pathologists and oncologists now believe that a substantial number of DNA sequence alterations, such as gene

rearrangements, mutations, fusions, amplifications, deletions/insertions, microsatellite instability (MSI), and aberrant methylation patterns are important in the diagnosis and therapy of cancers.

Therefore, analyses at the level of nucleic acids are critical for the diagnoses and therapies of cancers. Many techniques have been developed for this purpose**[2-3]**, including capillary electrophoresis (CE), which has the advantages of high-efficiency, high-speed, automation,cleanliness, and versatility,and CE can be conducted on a microscale or coupled with other separation techniques etc. These advantages afford this technique the ability to meet the future medical requirements that will undoubtedly call for amassing large numbers of samples for analysis **[4-7]**, suggesting that CE may become an important tool for providing data in clinical cancer diagnosis and therapy. So this review focuses on CE based on nucleic acid detection in the application of cancer diagnosis and therapy.However, because of some limitations in the technique, CE has not been widely applied beyond laboratory research use since it was developed more than 30 years ago.Thus, this review focuses on CE-based nucleic acid detection as it is applied to cancer diagnosis and therapy,and offers a brief introduction to the shortcomings and future developments of CE.

1 Application to cancer diagnosis

The diagnostic procedures for cancer based on nucleic acid detection using CE primarily to amplify the target gene in polymerase chain reaction(PCR)-based strategies, such as in multiplex ligation probe amplification (MLPA), single nucleotide primer extension and a chimeric primer-based temperature switch PCR (TSP) strategy, and for detection,such as in heteroduplex analysis with CE (EMMA),temperature gradient CE (TGCE),CE with laser-induced fluorescence (CE-LIF),CE with amperometric detection (CE-AD),and protein-facilitated affinity CE (ProFACE) . Therefore, diagnostic accuration depends on two critical elements: the selection of PCR strategies and the choice of CE detection modes.

1.1 Detection of cancer risk-related gene polymorphisms and mutations

To improve the throughput and efficiency of the tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) for assaying single nucleotide polymorphisms (SNPs), Zhang et al.**[8]** combined T-ARMS-PCR with TSP, using T-ARMS-PCR-CE for amplicon

separation and identification and successfully genotyped four breast cancer-related and two cervical cancer risk–related SNPs. The most significant and well characterized genetic risk factors for breast or ovarian cancer are germline mutations in the BRCA1 and BRCA2 genes, which markedly increase breast cancer risk and suggest that polymorphisms in these genes are logical candidates in seeking to identify low penetrance susceptibility alleles. To evaluate the association of several SNPs in these genes with breast cancer risk, Maleva et al.**[9]**initiated a screen for BRCA1/2 gene mutations using direct sequencing, the single nucleotide primer extension method and MLPA analysis, all detected with CE,the results indicated that the polymorphisms rs8176267 in BRCA1 and N372H in BRCA2 showed an association with breast cancer risk. Caux-Moncoutier V et al.**[10]** developed EMMA to screen point mutations and large rearrangements of BRCA1 and BRCA2 in 1,525 unrelated patients. Their results demonstrated that EMMA represented a valuable short-term and midterm option for many diagnostic laboratories requiring an easy, reliable, affordable,rapid and sensitive analysis for a large number of genes.

There is a controversy regarding the association between prostate cancer and the length of the CAG repeats at the 5' end of exon 1 of the androgen receptor (AR) gene. To evaluate the potential effect of short CAG repeats on the androgen receptor gene in prostate cancer risk, Madjunkova et al.**[11]** determined the CAG repeat length in patients with prostate cancer,with benign prostatic hyperplasia (BPH) Patients, and in the general population using PCR-CE.They found that reduced CAG repeat length may be associated with increased prostate cancer risk.

Point mutations in mitochondrial DNA (mtDNA) are found at a high frequency in a variety of human tumours.To determine the biological effects of mtDNA mutations in tumours, Jandova et al.**[12]** screened the tumour mtDNA for single-nucleotide changes using TGCE and found that a mutation hot spot (9821insA) in the mitochondrial encoded tRNA arginine locus was discovered in approximately one-third of premalignant and malignant skin tumours. The biochemical analysis revealed that this specific mtDNA mutation might alter cellular biochemistry, supporting the development of keratinocyte neoplasia. Xu et al.**[13]** investigated the frequency and pattern of mutations in the D310 region in mtDNA using CE,the results of their experiments showed although D310 alterations appeared unrelated to the progression of synchronous ductal carcinoma in situ, they were observed in histologically normal cells adjacent to tumour,which suggested a

field of genetically altered cells and could represent a potential marker for the clonal expansion of premalignant breast cancer cells.

DNA methylation is a fundamental biological mechanism that plays a key role in the epigenetic regulation of gene expression during development of disease. DNA methylation patterns are highly dysregulated in cancer, leading to inactivation of tumour suppressors and activation of oncogenes, thus contributing to tumorigenesis. Therefore, understanding the biological function of DNA methylation will require the development of sensitive analytical methods allowing the detection and quantification of all types of DNA methylation patterns. CE-LIF with a limit of detection for 5-hydroxymethyl-2'-deoxycytidine (5 hmdC) of 0.45 amol, which is equivalent to approximately to one 5 hmdC per 4,000 normal nucleotides (0.025%) using 1 μg of DNA as the matrix, could be analysed in one run for both the methylation and hydroxymethylation of cytosine **[14]**. CE-LIF for the quantification of global adenine methylation in DNA, which yielded a limit of detection for N(6)mdA of 280 pM (1.4 amol) ,could also be applied to the analysis of various DNAs**[15]**. Bisulfite restriction analysis (COBRA)-CE-LIF was used to screen for the degree of DNA methylation in cancer, and the results showed that DNA methylation was eliminated after the cells were treated with an anti-cancer drug (5'-aza-2'-deoxycytidine), suggesting that COBRA-CE-LIF is a potentially useful and cost-effective tool for cancer diagnosis or prognosis based on the heterogeneity of a patient's DNA **[16]**.A novel bisulfite-microfluidic temperature gradient capillary electrophoresis(bisulfite-μTGCE) platform for gene methylation analysis was developed **[17]**, that could identify methylated genomic (g) DNA at a detection limit of 7.5pg, and could distinguish a methylation level as low as 0.1%. Methylation-specific PCR-CE **[18]** was used to investigated the epithelial membrane protein 3(EMP3) promoter hypermethylation status and the results showed that the EMP3 hypermethylation status correlated with 19q13.3 loss and lack of EMP3 expression at protein level.

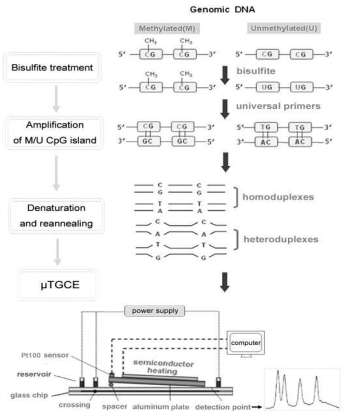

Figure 1. Scheme for detection of CpG methylation by bisulfite-microfluidic temperature gradient capillary electrophoresis (bisulfite-μTGCE)**[17]**. Genomic DNA (gDNA) is first treated with bisulfite to change unmethylated cytosine (C)to uracil (U), followed by universal primer-directed PCR amplification of the differentially methylated fragments, which converts multiple U to thymine (T)mutations into the unmethylated CpGs and does not convert the methylated sites. After denaturation/reannealing to form heteroduplexes, the samples are loaded into the microfluidic chip to undergo automatic sample introduction, μTGCE separation, laser induced fluorescence (LIF) detection, and recording of electrophoregrams. Finally, the methylation status of the sample sequence can be identified according to the electrophoretic peak patterns.

The role of environmental and occupational toxin exposure as a cause or contributing factor for cancer development and progression is incompletely understood. Ellsworth et al. used tissue microdissection-PCR- CE to analyse tumour specimens to better understand cancer causality based on the measurement of the cumulative DNA damage over a defined genomic region **[19]**.

It has been reported that large genomic deletions in the MLH1 and MSH2 genes are a frequent cause of Lynch syndrome in certain populations. Thus, Pérez-Cabornero et al.**[20]** utilized EMMA to identify mutations in the MLH1, MSH2, and MSH6 genes in families with Lynch syndrome and found that more than 16% of the families had pathogenic mutations. 50% were carriers of a novel mutation, and 47% were carriers of a rearrangement. The exon 7 deletion and exon 4 to 8 deletion of MSH2 were new founder mutations. The segregation of a common haplotype and anticipation effects were also observed in these families. These findings may greatly simplify the diagnosis, counselling, and clinical care in suspected Lynch syndrome families.

For enhancing the sensitivity of detecting low-level clinically significant cancer-relevant somatic mutations, Warshawsky et al.**[21]** developed locked nucleic acid PCR-CE to detect FLT3D835/I836 tyrosine kinase domain mutations and insertion mutations in the nucleophosmin 1 gene. Conformation-sensitive capillary electrophoresis (CSCE) for diagnostic mutation scanning with a sensitivity of >99%**[22]**which was indistinguishable from the sensitivity for conventional sequencing by CE and greatly reduced the number of fragments that need to be sequenced, proved to be a highly sensitive and cost-effective technique suitable for routine genetic diagnostic analysis of heterozygous nucleotide substitutions, small insertions, and deletions.

The Ewing sarcoma family of tumours (EFT) is characterized by fusions of the EWSR1 gene on chromosome 22q12 with one of the genes encoding members of the ETS family of transcription factors. Many alternative EWSR1-ETS gene fusions have been encountered, due to variations in the locations of the EWSR1 and ETS genomic breakpoints. The resulting heterogeneity in EWSR1-ETS fusion transcripts may be further increased by the occurrence of multiple splice variants within the same tumour. PCR-CE was used to detect all the EWSR1-FLI1 and EWSR1-ERG fusion transcripts, and the results showed that alternative splicing might frequently affect the process of EFT-associated fusion gene transcription and significantly contribute to the pathogenic role of EFT-associated chromosome translocations, resulting in multiple splice variants within the same tumour **[23]**. The degradation of RNA also presents a significant difficulty in the molecular analysis of possible EWSR1-FLI1 and EWSR1-ERG translocation transcripts in formalin-fixed paraffin-embedded (FFPE) tissues.To overcome this problem,Patócs B et al.**[24]** developed fluorescent multiplex PCR-CE-LIF to detect and identify EWSR1-FLI1 and EWSR1-ERG chimeric transcripts. Their technique had a 60% sensitivity for detecting the most frequent Ewing family of tumour (EFT)-related fusion transcripts and was effective for the diagnosis of EFT in FFPE tissue, especially after defined modifications when the technique proved capable of overcoming the diagnostic difficulties connected with the heterogeneity of the variant translocations in EFT.

For exploring characteristics of DNA damage and TP53 gene mutations in lung cancer, 104 tissue samples from patients with non-small cell lung cancer were studied **[25]**. These TP53 mutations were detected using denaturant gradient gel electrophoresis (DGGE), automated capillary electrophoresis single-strand conformation polymorphism (SSCP-CE) and

sequencing. That study demonstrated that most carriers of G→T transversions also had a high level of bulky DNA adducts in their non-tumorous lung tissue and provided evidence for a high burden of molecular alterations occurring concurrently in the lungs of patients with lung cancer.

To study the causal role of work-related exposure to wood dust in the development of sinonasal cancer, Holmila et al.**[26]**analysed TP53 gene mutations with or without occupational exposure to wood dust using SSCP-CE and direct sequencing. The results showed that TP53 mutations were associated with work-related exposure to wood dust in sinonasal cancer.To investigate a possible association of the TP53 polymorphisms, Arg72Pro and PIN3 (+16bp), with sporadic breast cancer. Arg72Pro polymorphism was detected using a TaqMan assay and PIN3 (+16bp) polymorphism was examined using PCR-CE. The results provided evidence of the association of the TP53 gene polymorphisms Arg72Pro and PIN3 (+16bp) with sporadic breast cancer **[27]**.

Germline copy number variations (CNVs) are known to influence the susceptibility to a wide variety of disorders including cancer predisposing syndromes, autism, schizophrenia, autoimmune disorders, and HIV infection. Somatic CNVs play an important role in cancer progression and their detection is critical in designing efficient therapeutic protocols based on conventional or targeted molecules. Therefore, the development of practical and cost-effective methods to detect CNVs is relevant for molecular diagnostic purposes in research and clinical settings. Thus, a new multiplex PCR-based method for CNV analysis that exploits microfluidic capillary electrophoresis (MCE) through lab-on-a-chip technology (LOC-CNV) was developed for this purpose **[28]**, because of the advantages of LOC-CNV with respect to time, costs, easy adaptation of previously developed multiplex assays and flexibility in novel assay design, which might represent a practical option to evaluate relative CNVs in genomic targets of interest.

Chromosomal translocations and small insertion/deletions occur in specific haematological malignancies and solid tumours. PCR detection of chromosomal translocations and small insertion/deletion mutations is challenging when potential amplicon size varies greatly. Molecular diagnostic laboratories face such difficulties with the BCL2-IGH translocation in follicular lymphoma and with the internal tandem duplication mutation of the FLT3 gene in leukaemia, where breakpoints are widely distributed, mutations may be multiple, signal strength is low, and background noise is elevated.Thus, a strategy based on Δ-PCR-CE was developed to

ensure PCR specificity and identifies individual breakpoints **[29]**. The experimental results also indicated that this strategy could have broader applications for other insertion/deletion and duplication mutations.

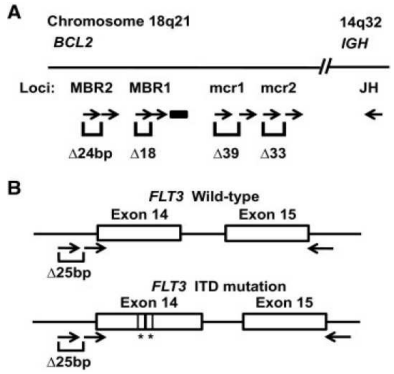

Figure 2.Primer design for detection of *BCL2-IGH* translocation **[29]** (**A**) and internal tandem deletion mutation of the *FLT3* gene (**B**). Sizes are not to scale. **Arrows** indicate primers and lines indicates location of the probe for real-time PCR. The Δ indicates the defined size difference of the paired amplicons for each breakpoint and an asterisk indicates the segment of ITD mutation.

Vitamin D3 is a hormone playing a crucial role in numerous biological processes in the human body, and abundant data relates the level of vitamin D3 with various types of cancer. It has been suggested that SNPs in the vitamin D3 receptor gene might influence both the risk of cancer occurrence and cancer progression.Two SNPs in the vitamin D3 receptor gene were genotyped in minisequencing reactions followed by CE to search for genetic correlations between individual SNPs in this gene and the risk of oral cavity carcinoma**[30]**. The obtained results provided evidence for a genetic association between rs2238135 in the vitamin D3 receptor gene and the occurrence and risk of oral cavity cancer.

Yri OE et al.**[31]** genotyped patients with Hodgkin lymphoma and healthy controls using CE, relating the risk for Hodgkin lymphoma and outcome of chemotherapy treatment to polymorphisms in genes encoding interleukins and metabolizing enzymes.They found that patients with the UGT1A1 TA tandem repeat TA6/6 genotype had a poorer overall survival, and patients above 40 years with the GSTA1 AA genotype had poorer event-free survival after chemotherapy. In patients above 40 years, the IL-10 rs1800890 T-allele was associated with lower

risk for Hodgkin lymphoma. The GSTP1 rs1695 A-allele reduced the risk for HL, and the GSTT1 deleted genotype increased the risk for Hodgkin lymphoma regardless of age.

Glutathione protects against cancer development by detoxifying carcinogens and free radicals and strengthening the immune system. Recently, a GAG-trinucleotide repeat polymorphism in the 5'-untranslated region of the gene for the rate-limiting enzyme of glutathione biosynthesis, γ-glutamine cysteine ligase (GCL), was shown to be associated with lowered GCL activity and glutathione levels in vitro and in vivo. The GAG repeat genotype was determined using PCR-CE to test the hypothesis that this functional polymorphism in GCL is associated with the risk for lung and aerodigestive tract cancers.The result suggested that glutathione synthesis affected the risk of lung and aerodigestive tract cancers, and further implicated a role for oxidative stress in the development of these cancers **[32]**.

The Human androgen receptor contains a highly polymorphic polyglutamine tract encoded by CAG repeats [(CAG)n] in exon 1 of the androgen receptor gene, which had been reported to be inversely correlated with androgen receptor activity. Fluorescently labelled DNA fragments containing (CAG)n were analysed with PCR-CE, and shorter alleles of the (CAG)n in exon 1 of the androgen receptor gene were found to enhance the susceptibility to polycystic ovary syndrome (PCOS)**[33]**.

The CYP19 tetranucleotide TTTA repeat polymorphism was genotyped using PCR-CE to evaluate the association between tetranucleotide TTTA repeat polymorphism in the CYP19 gene and polycystic ovary syndrome.The study showed CYP19 tetranucleotide TTTA repeat polymorphism was an ethnic and racial variant and moderately contributed to the pathogenesis of polycystic ovary syndrome in Han Chinese women **[34]**.

Seeking to unravel the molecular biology of a female patient who in 1985 at the age of 55 was diagnosed with a chronic myeloproliferative neoplasm (MPN) and in whom overt acute myeloid leukaemia (AML) developed in 2005, researchers analysed her DNA and RNA using qPCR and PCR-CGE **[35]**. They found the patient to be positive for the JAK2-V617F mutation throughout the course of disease, whereas a mutation of the nucleophosmin (NPM1) gene emerged at the AML diagnosis and relapse. The 20 year lag between the polycythaemia vera and the AML

added indirect evidence to the growing realization that the leukemic transformation in patients with myeloproliferative neoplasm occurred in a JAK2 wild-type stem cell **[35]**.

Samples were analysed by PCR-CE to explore the association between a functional insertion/deletion polymorphism (-94 ins/del ATTG) in the promoter region of the NFKB1 gene and the risk of advanced ovarian cancer **[36]**. The results suggested that NFKB1-94 ins/del ATTG promoter polymorphism may be associated with increased susceptibility to advanced ovarian cancer.

1.2 Detection of cancer biomarkers

The presence of high plasma levels of free-circulating DNA (cfDNA) in lung cancer patients was reported, leading to an interest in using cfDNA as a potential biomarker for early diagnosis **[37]**, to differentiate patients with asymptomatic tumours from healthy individuals, and to follow-up in people with increased cancer risk. The difference in the concentrations of cfDNA among patients and healthy individuals, was evaluated using various data analysis methods and laboratory procedures, most of which were based on quantitative Real-Time PCR because it is characterized by high accuracy and reproducibility; however, this method is time-consuming and expensive. Chiappetta et al.**[38]** used CE as a new tool to quantify the cfDNA in non-small cell lung cancer (NSCLC) and compared the results with those using Real Time-qPCR.They determined that CE was an effective diagnostic tool to discriminate patients with NSCLC from healthy individuals and suggested a new approach for early detection of NSCLC.

Patients with various cancers reportedly have significantly higher levels of urinary 8-OHdG and 8-NO2 Gua, Hence, 8-OHdG and 8-NO2 Gua have been increasingly considered sensitive biochemical markers of oxidative DNA damage, as well as potential indicators for a wide variety of cancers.Thus, Li MJ et al.**[39]** developed CE with amperometric detection to determine the levels of 8-OHdG and 8-NO2Gua and found that that the urinary levels of 8-OHdG and 8-NO2Gua in cancer patients were significantly higher than those in healthy subjects.

MicroRNAs (miRNAs) are small (~22 nt) regulatory RNAs that have shown promise as tissue- and blood-based biomarkers for cancer classification and prognostication. Because of the important role of miRNAs in gene regulation and disease aetiology, Khan N et al.**[40]** developed

a protein-facilitated affinity CE assay for rapid quantification of miRNA levels in blood serum with a detection limit of 0.5 fM in 1 mL of serum. The sensitivity of the method was comparable to that for existing PCR techniques and could be parallelized to quantitatively detect multiple miRNA-based biomarkers in different biological samples.

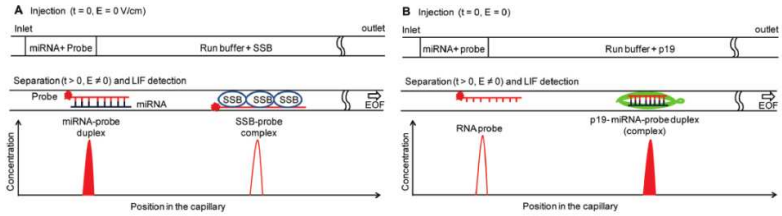

Figure 3.Schematic diagram of the protein-facilitated affinity capillary electrophoresis assay for miRNA detection **[40]**. A mixture of a miRNA and fluorescently labelled probe is injected as a small plug into a capillary and subjected to electrophoresis. (A) When single-stranded DNA binding protein (SSB) is added in the run buffer, it binds only to the DNA or RNA probe and enhances the separation of the miRNA probe duplex from the excess probe. (B) When p19 is present in the run buffer, it binds only to the miRNA RNA probe duplex and shifts it far away from the free probe increasing resolution and sensitivity of the assay.

A novel assay that simultaneously detects multiple miRNAs within a single capillary by combining a tandem adenosine-tailed DNA bridge-assisted splinted ligation with denaturing capillary gel electrophoresis using laser-induced fluorescence was developed **[41]**. This CE-LIF assay not only represented a significant improvement in resolution but also allowed for the detection of multiple miRNAs within a single capillary based on the length differences of specified target bridge DNA. Five miRNAs of Epstein-Barr virus (EBV) were detected in EBV-infected nasopharyngeal carcinoma cells.In addition, the screening of isomer of miRNA (isomiRs) of BART2 was feasible with CE-LIF, indicating that this method is fast, amplification-free, multiplexed and cost-effective, making it potentially applicable to large-scale screening of isomiRs.

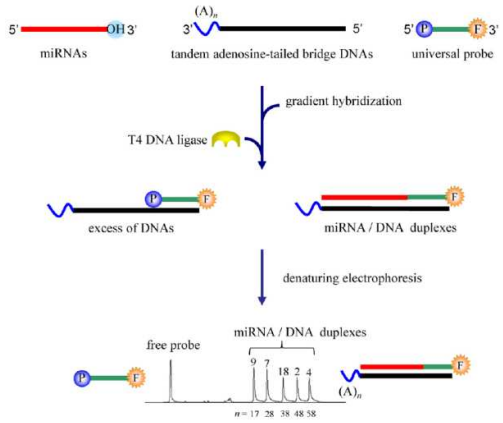

Figure 4.Schematic representation of tandem adenosine-tailed DNA bridge-assisted splinted ligation and capillary electrophoresis for multiplexed microRNA detection **[41]**

Ban E et al.**[42]**developed CE-LIF for determination of low abundance miRNA-499, a biomarker candidate for acute myocardial infarction. The hybridized miRNA-499 was detected in cultured H9c2 cardiomyoblast cells and the analysis of miRNA-499 was completed within 1 h, showing the potential of CE for fast, specific, sensitive and high-throughput analysis of low-abundance miRNAs. A dual-LIF (dLIF) setup combined with CE for microRNA (miRNA) detection was proposed **[43]**, that combined splinted ligation with the fluorescent dye-labeled oligonucleotides and the linear range for the synthetic miRNA was from 1.0 nM to 1.0 pM. Without PCR amplification, CE-dLIF discriminated pre-miR-10b*-transfected cells from hepatocellular carcinoma cells, indicating great potential to provide a rapid comparative assay for miRNAs detection. Parissenti AM et al.**[44]** applied immunohistochemical staining-CE-light microscopy to assess the association of low tumour RNA integrity with response to chemotherapy, revealing the association of the midtreatment tumour RNA integrity with drug dose level and with PCR.These results suggested that tumor RNA integrity might represent an important new biomarker for measuring response to chemotherapy in breast cancer patients.

In order to evaluate the correlation of gene methylation and the clinical outcome of patients with advanced-stage ovarian serous carcinoma, the methylation status of eight candidate genes was first evaluated using methylation-specific PCR-CE to select three potential genes including DAPK, CDH1, and BLU **[45].** The results of the experiments demonstrated that Patients with methylated BLU had significantly shorter progression free survival and overall survival, and that BLU could upregulate the expression of the gene for the apoptotic regulatory protein BAX and

enhance the effect of paclitaxel-induced apoptosis, suggesting that methylation of BLU may be a potential prognostic biomarker for advanced ovarian serous carcinoma **[45]**.

1.3 Detection of cancer-associated virus

Human papillomavirus (HPV) E6/E7 type-specific oncogenes are required for cervical carcinogenesis. Current PCR protocols for genotyping high-risk HPV in cervical screening are not standardized and usually use consensus primers targeting HPV capsid genes, which are often deleted in neoplasia. PCR fragments are detected using specialized equipment and extra steps, including probe hybridization or primer extension. Hence Dictor M et al.**[46]** developed a single-tube multiplex PCR-CE containing type-specific primers to target the E6/E7 genes of two low-risk and 19 high-risk genotypes.Their assay,was shown to be straightforward, robust and reproducible and avoided post-PCR enzymatic and hybridization steps while detecting HPV with high clinical sensitivity in significant HPV-related neoplasia regardless of specimen type.

1.4. Analysis of metabolic profiles of nucleosides

The metabolic profiles of nucleosides in urine are closely related to the pathophysiological status of an organism. Posttranscriptional modifications of RNA (mostly tRNA) in the cell nucleus are responsible for the change in nucleoside levels during malignant disease. Alterations of metabolite profiles in cancers are reportedly expressed primarily by the fold change of the urine levels of most nucleosides. CE was used to examine the profiles of 256 metabolite profiles composed of 19 nucleosides from 160 patients with urogenital tract cancer and 96 healthy controls **[47]**.Metabolite-to-metabolite ratios differed in patients with urogenital cancer compared with the healthy controls. Discrimination of the patients with cancers and the non-cancer healthy subjects was with 76.5% sensitivity and 80.2% specificity, which proved the usefulness of the metabolomics approach in studying urinary nucleoside profiles with high diagnostic potency in urogenital cancers**[47]**.

1.5. Diagnosis based on stored tissue and nucleic samples

The understanding and treatment of human cancer is currently undergoing a major transformation, propelled by the emergence of new genomic technologies, particularly tissue-based RNA and DNA microarrays. This so-called genomic revolution aims to capture the

biology and phenotype of cancer using gene expression profiles and tumour genomic alterations rather than using shared morphologic criteria.These genomic data rely on the availability and quality of tissue repositories. Hence, a prospective institutional review board-approved protocol for the banking of abdominal neoplasms was initiated to investigate the influence of warm ex-vivo ischemia times, storage times, and biobanking protocols on RNA integrity. RNA integrity was determined with microcapillary electrophoresis using the RNA integrity number (RIN) algorithm and with laser-capture microdissection.The results showed that fresh-frozen pancreas tissue banked within a standardized research protocol yielded high-quality RNA in approximately 50% of specimens and could be used for enrichment by laser-capture microdissection.The quality of tissues from the biobank was not adversely impacted by limited variations in warm ischemia times or different storage periods **[48]**.

A large amount of high quality DNA is essential for molecular analysis. The amount of DNA in routine paraffin sections is small. Although the surgical specimens left over from the sampling required for wax embedding and retained in formalin for a long time are a rich source of DNA, they are difficult to extract. Wu S et al. **[49]** designed a microwave-heating method for DNA extraction from such specimens and successfully detected MSI in the DNA samples using Fluorescence multiplex PCR-CE. Indeed, they found significantly higher levels (98.4%) than those obtained from a conventional method (82.3%). Thus,the microwave-heating method is efficient for DNA isolation from long-term formalin-fixed tissue samples. The successful fluorescence multiplex PCR-CE analysis used for such samples may facilitate MSI detection in clinical practice.

Zinellu A et al.**[50]**developed a field-amplified sample injection CE method to measure global methylation in FFPE DNA extracts.Analytes baseline-separated within 8 min with high accuracy proved that this technique was useful for large-scale applications to determine the implications of genomic DNA methylation levels in tumorigenesis.

2 Application for cancer therapy

To the best of our knowledge, most cancers in the middle and advanced stages are difficult to cure. However, because genetic changes occur ahead of the phenotype, therapy based at a genetic

level takes advantage of early discovery and affords a more accurate, targeted therapy.Thus, CE used for nucleic acid detection offers the advantages of high-sensitivity, high-speed and high-throughput and may be applied to effective cancer therapy as follows and discussed below: detecting cancer therapy-related gene polymorphisms and mutations;determining drug therapy influences on the immune system;studying the effects of anticancer agents;aiding in the discovery of new cancer therapy targets; and developing prognostic measures of cancer therapy.

2.1 Detection of cancer therapy-related gene polymorphisms and mutations

Constitutively active Kirsten rat sarcoma viral oncogene (KRAS) mutations involved in various processes of cancer development, render tumour cells resistant to epidermal growth factor receptor (EGFR)-targeted therapies. Mutation detection methods with a high sensitivity may increase the possibility of choosing the correct individual therapy. Zhang H et al.**[51]** established a highly sensitive and efficient microfluidic capillary electrophoresis-based restriction fragment length polymorphism (mCE-based RFLP) platform for low-abundance KRAS genotyping with the combination of mCE and RFLP techniques. The accurate analysis of KRAS statuses in HT29, LS174T, CCL187, SW480, Clone A, and CX-1 colorectal cancer cell lines by mCE-based RFLP was achieved in 5 min with picoliter-scale sample consumption. As low as 0.01% of mutant KRAS could be identified from a large excess of wild-type gDNA, demonstrating that mCE-based RFLP is a sensitive, fast, and cost-effective screening method for KRAS mutations.The successfully detected low-abundance KRAS mutations in such clinical samples will provide more precise individualized cancer therapy.

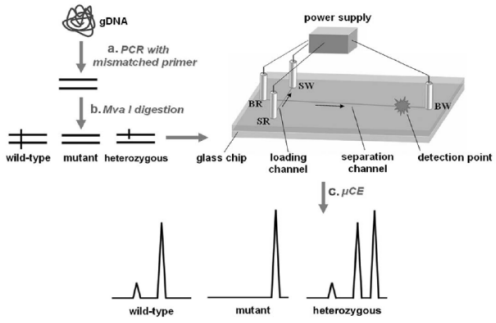

Figure 5.Schematic view of the microfluidic capillary electrophoresis-based restriction fragment length

polymorphism (mCE-based RFLP) platform **[51]**. (a) Mismatched primer PCR. A KRAS gene fragment containing codon 12 was amplified from gDNA with mismatched primer, by which a base substitution was introduced to the amplicon and a Mva I restriction endonuclease recognition site was created for wild-type codon 12. (b) Mva I digestion. The amplicon from wild-type template could be cleaved into two fragments, the amplicon from mutant template could not be digested due to the loss of recognition site, and the amplicon from heterozygous template was halfly digested. (c) mCE. The digested amplicon was loaded into microfluidic chip and separated by CE according to the fragment length. The wild type template resolved into two peaks, the mutant template only showed one peak, and the heterozygous template resolved into three peaks. gDNA, genomic DNA. SR: sample reservoir; BR: buffer reservoir; SW: sample waste reservoir; BW: buffer waste reservoir. R,the direction of fluid flow during sample loading and separation modes.

Alterations in KRAS, BRAF, or mismatch repair (MMR) genes may determine therapeutic response or define a hereditary cancer syndrome. Tumour DNA samples were screened for 7 KRAS mutations and the BRAF p.V600E mutation by fluorescent allele-specific PCR-CE **[52]** The results indicated that this was an accurate and convenient method to assess KRAS and BRAF mutations and that it may also detect mutations not identified by dideoxy sequencing. KRAS mutation status, in conjunction with morphologic or clinical parameters, may be useful in determining whether a tumour should be tested for MSI. MSI testing should not be considered exclusively in right-sided lesions. BRAF analysis may not be useful in rectal adenocarcinomas **[52]**. KRAS mutation-associated pancreatic and ovarian carcinomas often display mucinous differentiation. Xiong J et al.**[53]** investigated KRAS mutations in several morphologic subtypes of endometrial carcinomas with particular emphasis on various degrees of mucinous differentiation using PCR-CE, their results suggested that mucinous carcinoma and endometrioid carcinoma with a significant mucinous component were more likely to be associated with a KRAS mutation.Thus, the potential clinical implications of KRAS mutations may be in the management of recurrent or higher-stage endometrial mucinous tumours that are not responsive to treatment protocols containing EGFR inhibitors. EGFR**[54-55]**, KRAS and phosphatidylinositide-3-kinase catalytic subunit-alpha (PIK3CA) mutations are biomarkers used for the prediction of efficacy of EGFR tyrosine kinase inhibitors (EGFR-TKIs) in advanced non-small cell lung cancer. Genetic testing was performed by a combination of denaturing CE (DGCE) and direct Sanger

sequencing**[56]**.However, the investigators did not observe any role for EGFR, KRAS and PIK3CA mutations in the prediction of EGFR-TKIs efficacy in patients.

A genetic TA repeat length polymorphism in the UGT1A1 promoter affects UDP-glucuronosyltransferase (UGT1A1) expression levels with significant clinical implications. The presence of 7 TA repeats is associated with lowered UGT1A1 expression and the mild hyperbilinrubinemia manifested in Gilbert's syndrome. Furthermore, cancer patients carrying this variant exhibit irinotecan-related toxicity and require lower doses of this chemotherapeutic agent compared with patients carrying the 6 TA repeat allele, which is very common.This,therefore, necessitates the development of a reliable means of detecting TA repeat length in the clinical laboratory to deliver better personalized therapy regimens. Hence, Abou Tayoun AN et al.**[57]** developed and validated a PCR-CE method for accurately determining TA repeat length and guiding irinotecan dosing decisions for cancer patients.

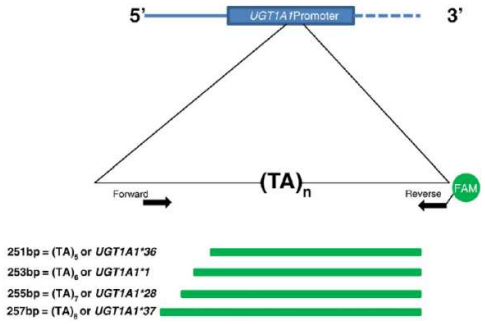

Figure 6.UGT1A1 assay description **[57]**. Primers spanning the TA repeat region were used in which the reverse primer was FAM-labelled at its 5′end. Labelled amplicons (shown in green) were then subjected to capillary electrophoresis and fragment analysis. The TA repeat number was deduced from the obtained fragment sizes.

Mutation-specific inhibitors are being developed for clinical use that target only sub-populations of patients with particular tumour genotypes,making knowledge of the tumour mutation status important for the treatment of cancer. Thus, Lovly CM et al.**[58]** utilized the SNaPshot method (multiplex PCR-multiplex primer extension-CE) to detect the 43 common somatic point mutations in 6 genes (BRAF, NRAS, KIT, GNAQ, GNA11, and CTNNB1) that are potentially relevant to existing and emerging targeted therapies specifically in melanoma.They found that 60% of the melanomas from sites examined throughout the body harboured a tested mutation. Adoption of this genetically-informed approach to the treatment of melanoma already

has shown an impact on clinical trial enrolment and prioritization of therapy for patients.

The DNA repair protein O (6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation in tumours is regarded as the most common predictor of the responsiveness of glioblastoma to alkylating agents. However, MGMT promoter methylation status has been investigated mainly by methylation-specific PCR, which is a qualitative and subjective assay. In addition, the actual enzymatic activities associated with the methylation status of MGMT have not been explored. Thus, Kishida Y et al.**[59]** applied pyrosequencing to quantify MGMT promoter methylation in glioblastomas and MGMT enzymatic activity was assessed using fluorometrically labelled oligonucleotide substrates containing MGMT-specific DNA lesions. CE was used to detect and quantify these lesions. Compared with existing traditional assays, this assay was equally sensitive but less time consuming and easier to perform. The results of their experiments determined that the MGMT promoter methylation values obtained were inversely proportional to the measured enzymatic activity,indicating that the quantification of MGMT methylation represented its enzymatic activity and therapeutic responsiveness to alkylating agents.

Stratifying patients defective in mismatch repair (dMMR) with high microsatellite instability (MSI) in colorectal cancer is of increasing relevance and may provide a more tailored approach to colorectal cancer adjuvant therapy. Morandi L et al.**[60]** studied 340 consecutive colorectal cancers using PCR-CE and their results suggested that metallothionein 1X gene (MT1XT20) represented a sensitive and specific marker for MSI testing and could be included in a complete set of MSI markers for the confident identification of patients with familial or sporadic dMMR in colorectal cancers.

Two single nucleotide polymorphisms (SNPs) of 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, A1298C and C677T, are widely considered to be associated with various neoplasitic disorders. Cheng HL et al.**[61]**established a SSCP-CE method to detect two polymorphisms in the MTHFR gene inpatients with acute lymphoblastic leukaemia (ALL) and attention-deficit/hyperactivity disorder (ADHD). Genotyping results were evaluated in terms of relationships between outcomes for patients with ADHD after ALL chemotherapy and ALL disease. They found that the SSCP-CE method was feasible for detecting mutations of the MTHFR gene.

The identification of somatically acquired tumour mutations is increasingly important in the clinical management of cancer because the sensitivity of targeted drugs is related to the genetic makeup of individual tumours. Thus, mutational profiles of tumours can help prioritize anticancer therapy. Su Z et al.**[62]**developed two PCR-CE based methods(SNaPShot Assay and Triplex Sizing Assay) to detect DNA from more than 40 recurrent mutations in nine genes relevant to existing and emerging targeted therapies in lung cancer.Their results showed that compared with direct sequencing, in which mutant DNA needs to compose 25% or more of the total DNA to easily detect a mutation, the PCR-CE methods could detect mutations in samples in which mutant DNA composed only 1.56% of the total DNA. These results should help accelerate adoption of a genotype-driven approach in the treatment of lung cancer.

The impact of a mutation in the ataxia telangiectasia mutated (ATM) gene in chronic lymphocytic leukaemia treatment outcome had not been examined before. Lozanski G et al.**[63]**studied ATM mutations in 73 patients treated with fludarabine and rituximab. The ATM gene mutation analysis was performed using temperature gradient CE. Their results indicated that truncating ATM mutations were rare in patients with chronic lymphocytic leukaemia .These non-silent variants also had limited impact on progression-free survival and overall survival.

Identification of chromosomal abnormalities is mandatory for classification of acute myeloid leukaemia (AML), and the abnormalities have to be determined quickly, to allow for patient enrolment in multicentre protocols or for selecting therapeutic strategies. Although Rapid AML molecular diagnosis is often difficult to achieve because it is based on numerous different RT-PCR protocols, a new RT-PCR-CE method to simultaneously detect all AML fusion transcripts from six major recurrent translocations was developed **[64]**, that obtained results in less than 24 hours,with a tumor cell detection threshold of 1.5%.

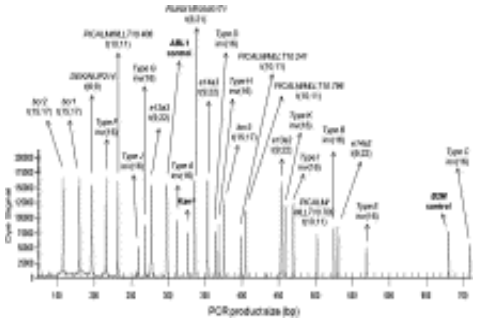

Figure 7. Capillary electrophoresis profile after 26-plex RT-PCR of the multiplex positive control **[64]**. Each peak

corresponds to a specific transcript at a specific size, separated from other peaks by at least 5 bp (observed). Corresponding transcripts and translocations are indicated at the top of each peak. Internal controls are highlighted in bold type. This control was run for each series of patients.

Accumulation of methotrexate polyglutamates (MTX-(Glu)(n)) is a problem in MTX therapy. SNP 452 C>T had been reported to associate with lower catalytic activity and higher accumulation of long-chain MTX-(Glu)(n) in patients treated with high doses of MTX. Cheng HL et al.**[65]** established an SSCP-CE method for detecting SNPs in the the γ-glutamyl hydrolase gene and found it was feasible for SNP screening.Polymorphisms in the 5' regulatory region of the thymidylate synthase gene have been shown to modulate thymidylate synthase expression and are associated with resistance to fluoropyrimidine-based therapies. Thomas F et al.**[66]** genotyped the thymidylate synthase gene 5'-UTR polymorphisms using PCR-RFLP-CE, which allowed identification of a 6-bp insertion in the 3R allele. The allelic frequencies for two uncommon polymorphisms in the TS gene (*TYMS*) promoter enhancer region (TSER) alleles were also found in the study.

Few studies have examined the prognostic implication of kinase domain mutations in patients in the early chronic phase prior to imatinib-based therapy. Carella AM et al.**[67]**analysed the status of mutations in the samples collected at diagnosis from patients in the early chronic phase of chronic myeloid leukaemia (CML) using a direct sequencing (DS)-CE system. They found that kinase domain mutations conferred high-level imatinib resistance in patients with de novo CML and in some patients. This led to disease progression.

2.2 Drug therapy influences on the immune system

The immune systems of patients with multiple myeloma are suppressed by the disease itself,and this immunosuppression can be further exacerbated by standard therapies.An RT-PCR-CE method was established to evaluate the effects of initial chemotherapy and a peripheral blood mobilisation regimen on T-cell population diversity**[68]**.The relative expression of 27 T-cell receptor beta variable(BV) gene families in patients with multiple myeloma undergoing peripheral blood stem cell harvest was analysed. The results showed that the overall BV family usage was restricted and the relative expression of 10 BV families was significantly depressed in patients compared with healthy donors, demonstrating that the preparative regimen

for autologous stem cell transplantation affected the T-cell population in terms of the restriction of its T-cell receptor diversity.

The function of γδ T cells in the post-transplant period is not yet fully understood. Traditional PCR-based techniques (such as PCR- Southern blot analysis or PCR-the third complementarity-determining region spectratyping) established to assess the expression of γ variable (Vγ) and δ variable (Vδ) gene families, were slow and complicated. Thus, Tesarova L et al.**[69]** developed a new PCR-CE method for evaluating the composition of the γδ T-cell population,and applied it to analyse peripheral blood mononuclear cell samples from patients during haemato-oncological treatments. Their results proved that PCR-CE was fast and simple for use as a first screen of the γδ T-cell population composition in tissues of interest.

2.3 Anticancer agents

DNA ladder fragments are regarded as a biochemical hallmark of apoptosis, and selenium compounds inhibit carcinogenesis by increasing apoptosis. Lei M et al.**[70]** applied CE to determine inter-nucleosomal DNA fragmentations induced by xylitol selenite,which is useful in elucidating the anticancer activities of xylitol selenite and other selenium compounds.

Scientists are still pursuing the development of platinum complexes with improved properties regarding side effects and resistance since the anticancer effects of cisplatin were discovered forty years ago. Among these compounds, trans-configured platinum complexes with oxime ligands emerged as a new class distinct from those of established anticancer agents, with features that included different DNA binding behaviour, increased cellular accumulation, and a different pattern of protein interaction. Hence, Bartel C et al.**[71]** studied the reactivity with biomolecules of three novel pairs of cis- and trans-configured acetone oxime platinum(II) complexes and one pair of 3-pentanone oxime platinum(II) complexes. The interaction of the complexes with DNA was studied in cell-free experiments with plasmid DNA (pUC19), in capillary zone electrophoresis with the DNA model 2-deoxyguanosine 5'-monophosphate, and in in vitro experiments showing the degree of DNA damage with the comet assay. Whereas incubation with ciscompounds did not induce degradation of DNA, the trans complexes led to pronounced strand cleavage.

The sequence specificity of a DNA-damaging agent is an essential element in determining the cellular mechanism of action of a drug and a number of DNA-damaging compounds are

widely used as cancer chemotherapeutic agents. The distribution of lesions in a sequence of DNA can give vital clues for determinating the precise mechanism of interaction of the agent with DNA. CE-LIF has high resolution, great sensitivity, high precision, high speed, and is safe and easy to perform in this application **[72]**. Paul M et al.**[73]** utilized a capillary sequencer with LIF to examine the sequence selectivity of cisplatin with purified DNA sequences. The use of this automated machine enabled a high degree of precision for both position and intensity of cisplatin-DNA adducts, and determined that cisplatin strongly formed adducts with telomeric DNA sequences. Nguyen HT et al. **[74]** determined the DNA sequence specificity of cisplatin using CE-LIF, which provided an accurate analysis of cisplatin-DNA adduct formation in a long telomeric repeat DNA sequence. Their results indicated that the 3'-end of the G-rich strand of the telomeric repeat was preferentially damaged by cisplatin and suggested that the telomeric DNA repeat had an unusual conformation. Nguyen HT et al.**[75]** determined the DNA sequence specificity of bleomycin cleavage in telomeric sequences using linear amplification-CE-LIF. They found that the bleomycin DNA sequence selectivity was mainly at 5'-GT dinucleotides, with lesser amounts at 5'-GG dinucleotides. The cellular bleomycin telomeric DNA damage was also compared with bleomycin telomeric damage in purified human genomic DNA and was found to be very similar. These results helped to elucidate the mechanism of action for bleomycin in human cells.

Arsenic trioxide is an effective therapeutic agent for acute promyelocytic leukaemia and other hematopoietic malignancies. Peng CY et al.**[76]** used a high performance capillary electrophoresis (HPCE) assay and found that arsenic trioxide down-regulated the global DNA methylation level in HL-60 cells. They used the combination index method of Chou and Talalay to analyse the interactions between arsenic trioxide and epigenetic therapeutic agents. Their results not only highlighted the possible diversity of the anti-leukaemia mechanisms of arsenic trioxide, but also provided initial guidance for further investigation of leukaemia therapies based on the combination of arsenic trioxide with epigenetic agents.

2.4. Discovery of new cancer therapy targets

Anticancer chemotherapy is strongly hampered by the low therapeutic index of most anticancer drugs and the development of chemoresistance. Therefore, there is a continuous need

for the identification of new molecular targets to selectively hit cancer cells. RNA has been recently validated as a cancer target by the use of different specific ligands or by different agents able to destroy its diverse forms. The ability of several synthetic polyamines to interact and to damage the structure of RNA was evaluated and quantified with MCE.This method allowed for the visualization of the RNA impairment through different electropherograms, the assessment of the RNA integrity number, and the discrimination between the RNA and DNA of these synthetic polyamines **[77]**.

The enzymatic function of the human purine nucleoside phosphorylase (PNP) has important medical implications. Its deficiency in humans leads to an impairment of T-cell function, with no apparent effects on B-cell function. Therefore, T-cell leukaemias and lymphomas can be impaired by designing efficient inhibitors that target PNP. Adenosine deaminase is widely expressed in the intestine, thymus, spleen and other lymphoid and non-lymphoid tissues. Along with a cytosolic localization, adenosine deaminase can be expressed as an ecto-enzyme on the surfaces of lymphocytes and porcine brain synaptic membranes. For evaluating the potential of selective PNP or adenosine deaminase inhibitors as novel therapeutics, for example,as antiviral and antitumour agents, selective inhibitors have to be developed. And simple, efficient, and highly sensitive in-line CE method was developed for the characterization and for inhibition studies of PNP and adenosine deaminase present in membrane preparations of human 1539 melanoma cells **[78]**.

2.5. Prognostic markers in cancer therapy

Interleukin-8 induces tumour angiogenesis independent of the vascular endothelial growth factor pathway through the chemokine C-X-C motif receptor 2 (CXCR-2).Uzunoglu FG et al.**[79]** evaluated germline polymorphisms of these potential therapy targets as prognostic markers for disease free survival and overall survival in surgically treated patients with non-small cell lung cancer using genotyping of CXCR-2 +1208 C>T and +785 C>T, PAR-1 -506 Ins/del and -14 Ivs A>T and ES +4349 G>A with TaqMan assays and with PCR-CE.

A helpful prognostic marker for chemotherapy of gliomas O^6-methylguanine DNA methyltransferase (MGMT) promoter hypermethylation. Mellai M et al. **[80]** investigated MGMT promoter hypermethylation status for a series of 350 human brain tumours using methylation-specific PCR-CE. Their results showed that MGMT promoter hypermethylation was

identified in 37.8% of the studied gliomas and was significantly associated with IDH1/IDH2 mutations in grade II–III tumours, whereas it had a borderline association with 1p deletion in oligodendrogliomas. MGMT hypermethylation was associated with better survival and had favourable prognostic significance.

Mutations in the CCAAT/enhancer-binding protein α (CEBPA) gene appear to be a good prognostic factor in AML.Thus,Fuster O et al.**[81]**established a straightforward and reliable fragment analysis method based on PCR-CE for screening CEBPA mutations, Their method gave a specificity of 100% and sensitivity of 93% with a low detection limit of 1-5% for CEBPA mutations. Their results confirmed that CEBPA mutations could identify a subgroup of patients with a favourable prognosis among those patients having AML with an intermediate-risk karyotype.

In order to evaluate the potential prognostic role of the promoter GTn repeat polymorphism(GTn) of the heme oxygenase-1 gene in benign and malignant pancreatic neuroendocrine tumours（PNET）.Sample DNA was analyzed for GTn using PCR-CE and DNA-sequencing. The results showed that GTn differentiated between benign and malignant PNET and was a strong predictor of tumour recurrence **[82]**.

It is reported that alterations in IKZF1, a transcriptional regulator of B lymphoid development, is associated with very poor outcomes in B-cell progenitor ALL. Therefore, Yang YL et al.**[83]** determined the prognostic significance of IKZF1 deletions in patients with childhood ALL using multiplex quantitative PCR-CE and high resolution melting.The results of their study indicated that detection of IKZF1 deletions upon diagnosis of B-cell progenitor ALL may help to identify patients at risk for treatment failure and that IKZF1 deletions could be incorporated as a new high-risk prognostic factor in future treatment protocols.

3. Limitations and future developments in the application of CE for nucleic acid detection

Many researchers have being attracted to CE analysis since it was developed, especially for the analysis of DNA **[84]**. However, CE has been used mostly in laboratory research rather than in clinical practice for more than 30 years. The application of CE to the analysis of nucleic acids appears to be hindered by six limitations. The first is that CE is a relatively new technique in

analytical chemistry. Genetic diagnostics based on nucleic acid analysis is associated more with medicine and molecular biology. The combination of these two fields requires researchers knowledgeable in both subjects,but many scientists lack such a comprehensive background.The relatively low sensitivity of CE analysis is the second limitation in the field of DNA analysis **[85-86]**. Except the double bond formed by the base group in DNA has a low ultraviolet-vis absorption, and a normal UV detector has a low sensitivity. The intrinsic low detection sensitivity drawbacks of CE, which are the limited light absorption dimension and the restricted sample injection length resulting in small sample injection volumes, also greatly restrain the application of DNA analysis by CE-UV. Consequently, fluorescence detection becomes the solution for sensitivity challenges of DNA analysis used by most researchers. However, this solution demands labeling samples with a fluorescence marker or intercalating dyes**[87]**, unfortunately this procedure is time-consuming,expensive, and most dyes are toxic (such as ethidium bromide [EB]).In addition,the data derived from fluorescence detection electropherograms require integration with those from the more widely used UV electropherograms. Consequently, fluorescence detection has not yet achieved extensive application. Although CE performances have improve greatly over those in the preliminary stages of development, their stability and reproducibility are not as good as those provided by traditional techniques**[88-89]**.Thus,the results derived from CE analyses still require verification by accepted methods like DGGE,direct sequencing,and real-time fluorescence quantitative PCR.This third limitation of CE that the technique is still under development and validation may be the reason that most of the diagnostic methods based on CE are not widely applied to clinical practice.The fourth and fifth limitations are that the equipments and reagents for CE are more expensive than other techniques and that necessary auxiliary equipments pre-existing in the clinic needs to be made compatibles with CE.This is a drawback especially when an automated identification system needs to be developed.The final limitation for the use of CE in clinical applications is that although MCE was developed many years ago, the high-efficiency, high-speed, microscale, automation and high-throughput advantages of this technique are not prominently known because the theory behind MCE is not yet mature and it has a relatively low automation.

On the other hand, the application of CE in clinical practice, has many advantages.The traditional methods for the separation of DNA are slab gel electrophoresis and the various types of

affinity chromatography. Although these techniques enjoy wide-spread use in many laboratories, they suffer some intrinsic systematic disadvantages that have remain unsolved: (1) preparation and separation is time-consuming, taking at least 2 h,is labor-extensive and has a low efficiency; (2) sample elution and post-processing are difficult and often rely on the use of toxic and mutagenic reagents (such as EB and organic solvents),that may harm the health of operator;(3) the waste causes severe pollution. These issues are increasingly urgent when considering future applications in cancer diagnosis and therapy where speediness、safety and effectiveness are compulsory for medical needs. By contrast,the aforementioned shortcomings for analysis using CE may be offset by its higher resolution**[90]**, better sensitivity and reproducibility**[91]**,rapidity,ease of use on a microscale and few harmful reagents.Moreover,CE can complete one detection in as little as a few minutes to dozens of minutes, so that hundreds of detections can be finished in a single day. Even more detections can be acquired using MCE, meeting the need for a large number of clinical samples rapidly analysed.Although the equipments for CE may be relatively expensive, the operational costs are not because the consumed reagents are mainly water,inorganic salt, and a small amount of gel. Thus, after the investment of fixed assets,CE becomes cost-effective and has a great potential for use in the clinical diagnosis.

On the whole, although CE has limitations, many researchers are attracted by numerous advantages.Technique development is a process, and no technique is without limitations. Cures for most cancer pathologies have not yet been found, and an accurate and early diagnosis is essential for successful treatment.Thus,there is great potential for continued development of CE in the application of cancer diagnosis and therapy: The intrinsic drawback of sensitivity in CE may be overcome by combining it with other techniques, such as CE-ICP-MS**[92]**, or sample pre-concentration methods similar to that used in a report on the eletrokinetic stacking of DNA and proteins at an HF etched porous junction may be adopted**[93]**; CE may also be combined with other methods offering additional benefits, such as CE-MS**[94]**; Furthermore,the current advantages of speed,microscale and automation for MCE**[95-102]** can be extended;

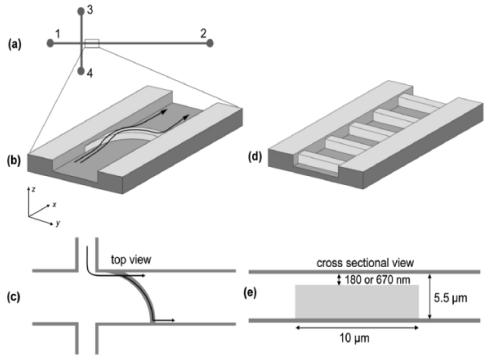

Figure 8. [95] (a) Schematic layout of the microfluidic device (not to scale). All channels are 200 mm wide and 5.5 mm high, except for minicircle purification with width of 100 mm; channels 1, 3 and 4 are 3 mm long, channel 2 is 5 mm long. (b) Central elem8ent of the microfluidic separation device: a bowed ridge is placed in channel 2. The ridge reduces the flow through height to 180 or 670 nm. The black arrows demonstrate the working principle of the separation device. A mixture of two sorts of molecules is continuously injected as a narrow band, close to the left wall. At the ridge, one species passes the nanoslit unhindered, while the other one is deflected. The deflected species is transported along the ridge and continuously migrates further down the channel once it reached the opposite channel wall. (c) Top view of the bowed ridge. The two possible trajectories for separated species are indicated. (d) Scheme of the microstructure used for determining polarizabilities: instead of the bowed ridge, periodically arranged ridges with a period of 30 mm and a total number of 100 are fabricated in channel 2. (e) Cross-sectional view of a single ridge with a flow through height of 180 or 670 nm.

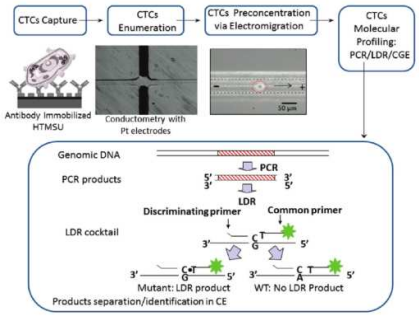

Figure 9. Overview for the Cell Selection, Enumeration, Electrokinetic Enrichment, and Molecular Profiling Strategy Adopted for the Analysis of Low-Abundance CTCs Resident in Peripheral Blood **[98]**

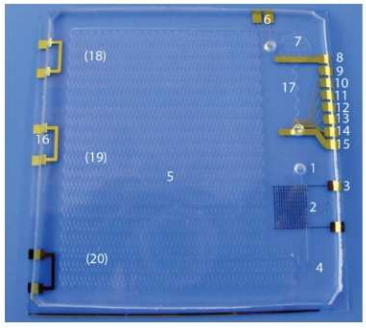

Figure 10.The PCR microdevice showing glass substrate, ITO microheaters, PDMS mold containing microchannels, gold microelectrodes for cell lysis, PCR amplification and CE-AD operation **[100]**.

(1) inlet reservoir for applying sample onto the microchip; (2) Gold interdigitated microelectrodes and (3) contact pad for applying DC potential for electrochemical cell lysis; (4) reservoir for manual extraction of cell lysate from the chip for conventional analysis; (5) microchannels for carrying out 25 cycles of PCR; (6) gold electrodes used for conductometric liquid level sensor used as circuit breaker for syringe pump so that sample flow can be stopped once large reservoir is completely filled with PCR amplicon sample; (7) large reservoir for collecting PCR amplicon sample; (8) and (15) gold electrodes used for applying separation voltage for CE operation; (9–11) optional decoupler electrodes; (12) reference electrode; (13) working electrode; (14) counter electrode; (16) optional resistance temperature detector for feedback temperature control of ITO microheater; (17) spiral CE- microchannel filled with semisolid agarose dissolved in NaOH medium for CE-AD separation of PCR amplicon; (18–20) ITO microheaters on the back side of glass as thermocycler zones for PCR,namely: extension, annealing and denaturation.

In addition, new modes, equipment, concepts and methods which take advantages of CE and meet the practical demands are under development, such as, ELONA-PCR-CE,which is a modified version of MLPA-PCR**[103]**, and a simple modification to FTSS-CE that enhances the limit of detection of cell-line gDNA mixtures from 50%-20% to 5% for G>A transitions and from 50%-5% to 5% for G>C and G>T transversions**[104]**.Additional developments include,a simple, high sensitivity mutation screening using Ampligase mediated T7 endonuclease I and Surveyor nuclease with MCE**[105]**,the PCR-based SNaPshot® assay that combines a single, multiplexed PCR reaction using gene specific primers followed by a single, multiplexed SNaPshot reaction and detection by CE**[106]**, a miniaturized spatial temperature gradient capillary electrophoresis system with radiative heating and automated sample introduction for DNA mutation

detection**[107]**,and cycling temperature capillary electrophoresis (CTCE)**[108]**; Software or programs with improved analyses and integration of data from CE electropherograms are also being established,such as GeneScreen: a program for high-throughput mutation detection in DNA sequence electropherograms **[109]**; In the post-genome era **[110]**,the "omics" era has arrived, with CE poised for use in procedures such as whole-genome sequencing of human cancer **[111]** and the preparation of sequencing libraries **[112]**.

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