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Microfluidic Chips for Detecting the t(4;14) Translocation and Monitoring Disease during Treatment Using Reverse Transcriptase-Polymerase Chain Reaction Analysis of IgH-MMSET Hybrid Transcripts

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Diagnosis platforms incorporating low-cost microfluidic chips enable sensitive, rapid, and accurate genetic analysis that could facilitate customized therapies tailored to match the vulnerabilities of any types of cancer. Using *ex vivo* cancer cells, we have detected the unique molecular signature and a chromosomal translocation in multiple myeloma. Multiple myeloma is characterized by IgH rearrangements and translocations that enable unequivocal identification of malignant cells, detected here with integrated microfluidic chips incorporating genetic amplification via reverse transcriptase-polymerase chain reaction and capillary electrophoresis. On microfluidic chips, we demonstrated accurate and versatile detection of molecular signatures in individual cancer cells, with value for monitoring response to therapy, detecting residual cancer cells that mediate relapse, and evaluating prognosis. Thus, testing for two clinically important molecular biomarkers, the IgH VDJ signature and hybrid transcripts signaling the t(4;14) chromosomal translocation, with predictive value in diagnosis, treatment decisions, and monitoring has been efficiently implemented on a miniaturized microfluidic system. ***(J Mol Diagn 2007, 9:358–367; DOI: 10.2353/jmoldx.2007.060149)***

In multiple myeloma (MM), a cancer characterized by extensive, complex chromosomal abnormalities, recurrent translocations involving the immunoglobulin heavy chain gene on chromosome 14 are among the most frequent translocations, being found in about 60% of myeloma patients[1] and many myeloma cell lines.[2] The t(4;14)(p16;q32) reciprocal translocation, which results in aberrant regulation of genes on both chromosomes 4 and 14, has been particularly well studied.[3,4] As a result of the t(4;14) translocation, the fibroblast growth factor receptor 3 (FGFR3) gene in chromosome 14 is overexpressed, whereas on chromosome 4, the *MMSET* gene is overexpressed.[4] Multiple breakpoints on chromosome 4 have been identified within the *MMSET* gene,[3] with *REII-BP* as a likely target gene.[1] In addition, each MM patient is characterized by unique molecular signature, the *IgH VDJ* gene rearrangement, which provides a unique marker to identify all malignant cells in each patient.

Patients having the t(4;14) translocation have reduced survival.[1,5–7] t(4,14) myeloma, characterized by drug resistance and rapid relapse,[8] has been shown to predict for poor response to conventional chemotherapy and to high-dose chemotherapy followed by stem cell rescue.[7] The clinical significance of the t(4;14) translocation suggests that monitoring of all MM patients would enable more informed treatment decisions. Because of the cost and technical complexity of molecular diagnostics, they are not routinely used in most hospitals.

Although fluorescence *in situ* hybridization readily detects the t(4;14) translocation,[9] fluorescence *in situ* hybridization in its current form is time-consuming, uses expensive probes, is labor-intensive, and requires highly skilled personnel to operate and interpret the results. The t(4;14) translocation can be identified using reverse tran-

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scriptase-polymerase chain reaction (RT-PCR) to detect hybrid IgH-MMSET transcripts from the derivative[4] chromosome.[1,3,6,10,11] The integrated RT-PCR and capillary electrophoresis (CE)-based microchip detection method reported here is well suited for automated patient monitoring and could lead to routine usage at, possibly, every clinic visit. We believe this is the first demonstration of an inexpensive microfluidic system for biomarker screening of cancer patients.

Microfluidic chips consist of networks of reservoirs (microliter and submicroliter volumes) and channels (micrometer scale) within which biomolecules can be manipulated in an automated manner using small volume samples and reduced reagent consumption, leading to lower costs and faster testing, for point-of-care patient monitoring. Currently, few microfluidic systems have extensive integration of functionality or validation using clinical samples. Recently, microchip-based clinically relevant assays[12–18] on microfluidic platforms have been demonstrated. Although many bioanalytical processes have been ported onto the microchip platform,[12,19–28] widespread clinical implementation will require effective and appropriate integration of sample processing, genetic amplification, detection, and fluid-handling systems. Ramsey and colleagues,[29,30] Mathies and colleagues,[31,32] Landers and colleagues,[12,33] and other groups[23,34] have demonstrated impressive integrations of PCR-CE systems. However, there are few reports on the integration of reverse transcription onto a microchip PCR platform[16,28,35] and none that include chip-based CE detection. This perhaps reflects the use of a two-step RT-PCR approach that requires considerable manipulation of reagents and reaction products and increases the possibility of contamination, thereby increasing the complexity of the system and the challenges to integration of CE for product detection. Here, we have used a single-step RT-PCR approach, facilitating the integration of RT-PCR and CE on the same chip. Furthermore, testing strategies published to date do not address the need to monitor cancer at the single-cell level,[36,37] a critical issue in clinical management of cancer heterogeneity.

In this work, microfluidic testing shows strong potential for facilitating the design of customized therapies tailored to each cancer, as well as for monitoring the response to therapy and the detection of residual cancer cells that ultimately may lead to relapse. With progress in miniaturization, these devices could become inexpensive tools for routine testing of molecular biomarkers at every clinic visit. With further development, such testing could be completed in minutes. In contrast, fluorescence *in situ* hybridization, the only other molecular approach in clinical practice, requires days. Our microfluidic platforms have been used for PCR thermal cycling and product analysis on-chip,[38,39] detection of gene polymorphisms,[40–43] and detection of viral titers in unprocessed urine from renal transplant recipients[18] (with integrated sample preparation). Overall, on-chip PCR uses small reaction volumes,[38] with analysis of picoliters of PCR product during each microchip CE run at a sensitivity comparable with that of conventional technology requiring 10 to 200 times more sample.[39] Here, we report on the sensitive detection of IgH MMSET hybrid transcripts and transcripts encoding clonotypic IgH VDJ gene rearrangements in bone marrow (BM) and blood cells from patients with t(4;14) MM, in aggregate populations and in individual cells. We have successfully screened patient samples for these clinically important cancer biomarkers on an RT-PCR-CE microfluidic chip, with validation against a conventional "gold standard."

Materials and Methods

Patients

After Institutional Review Board approval and informed consent, blood and BM samples were obtained at diagnosis or relapse from 12 patients with multiple myeloma, including four with the MB4-1, four with the MB4-2, and four with the MB4-3 breakpoints. Blood and BM were processed as previously described.[36] All patients were confirmed as having the t(4;14) translocation as previously described.[1,5]

Microfluidic Chips and Platform

The chips used here are patterned poly(dimethyl)siloxane (PDMS) irreversibly bonded to a glass substrate as previously described.[18] For the initial phase of this study, a two-chip system was used incorporating a three-reservoir chip to perform RT-PCR and analysis using the glass CE chip. The CE chip is a two-layer glass chip from Micralyne (Edmonton, AB, Canada) as described previously.[39] The two-chip approach increased the speed of protocol development and throughput in analysis. Where indicated, an integrated PDMS/glass chip incorporating the architecture of both of these chips (a PCR-CE chip) was used. Glass CE chips were fully reusable,[44] and consistency was monitored using a standard calibration procedure.[45] Thermal cycling was performed using a custom-designed dual-Peltier system (G. Kaigala, J. Jiang, C.J. Backhouse, and H.J. Marquez, manuscript under revision) and other physical subsystems. This chip thermal cycler is controlled using a custom-built software controller that is resident on a microcontroller of the system. Precision in control of temperature in the range of ± 0.1°C at set points is achieved, and temperature ramp rates of $\sim$6°C/second are achieved. On-chip diaphragm micro-pumping and micro-pinch-off valving using robotic arms that takes advantage of the deforming capability of PDMS are used here as previously described,[38] thus ensuring a system that is reusable with no cross-contamination between runs. Dead volume using this micropumping approach is less than 10% of the reaction chamber volume. For the integrated PCR-CE chips, using the diaphragm pumps, fluid from the reaction chamber (after thermal cycling is completed) is dispensed into the output chamber, which is also the input well to the CE section of this integrated chip.