Rapid and label-free nanomechanical detection of biomarker transcripts in human RNA

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Published online: 26 November 2006; doi:10.1038/nnano.2006.134

The availability of entire genome sequences has triggered the development of microarrays for clinical diagnostics that measure the expression levels of specific genes. Methods that involve labelling can achieve picomolar detection sensitivity, but they are costly, labour-intensive and time-consuming. Moreover, target amplification or biochemical labelling can influence the original signal. We have improved the biosensitivity of label-free cantilever-array sensors by orders of magnitude to detect mRNA biomarker candidates in total cellular RNA. Differential gene expression of the gene 1-8U, a potential marker for cancer progression or viral infections, has been observed in a complex background. The measurements provide results within minutes at the picomolar level without target amplification, and are sensitive to base mismatches. This qualifies the technology as a rapid method to validate biomarkers that reveal disease risk, disease progression or therapy response. We foreseee cantilever arrays being used as a tool to evaluate treatment response efficacy for personalized medical diagnostics.

The sequencing of the entire human genome[1,2] left us with the puzzle that we now know the blueprint of what we are made of, but we do not yet have the manual that tells us how we work. Part of the puzzle concerns the regulation activity of individual genes. The cellular control of the amount of functional products and timing of gene activation can, if altered, be responsible for the occurrence of diseases.

DNA microarray technologies are currently widely used to analyse gene expression[3]. However, this approach presumes substantial analyte preconditioning, such as reverse transcription, as well as amplification to generate more target material, and also requires the sample to be labelled with a fluorescent or radioactive tag. In addition to economic and time considerations, the artificial labels and enzymatic steps involved in sample processing[4,5] may perturb the relative abundance and stoichiometry of a transcript (that is, messenger RNA molecules), potentially biasing analysis of gene expression[6,7].

Nanomechanical devices are emerging as label-free and sensitive real-time indicators for events in which two single strands of DNA combine or 'hybridize' to form a single molecule[8–10]. Examples of DNA hybridization events include single nucleotide polymorphisms (SNPs) (refs. 11 and 12), aptamers[13] and other interactions such as lipid–drug binding[14] or receptor–ligand binding[15–18]. Moreover, cantilever-array technology permits a quantitative assessment of the molecules of interest[19,20]. When sequence-specific DNA hybridization occurs between a probe sequence tethered to one side of the cantilever and target DNA in solution, the cantilever mechanically bends owing to a quantifiable change in surface stress[21]. The resulting bending of the cantilever may be detected optically. Parallel differential readout with an *in situ* reference probe is mandatory, otherwise evaluation of the biospecific signal is hampered owing to convolution with external factors such as thermal drift and unspecific interactions (Fig. 1). Nanomechanical transduction of hybridization in combination with on-chip unspecific reference probes has proved to be highly sensitive to single-base mismatches[12], such as SNPs, which make up 90% of all human genetic variations and can have consequences for how humans develop diseases and respond to pathogens, chemicals and drugs. However, to date, cantilever studies[8] and other methods[22] have focused only on the detection of short synthetic oligonucleotides in buffer solution.

We propose that cantilever arrays can be used as a fast and reliable method to assess a patient's condition in those cases where key genes associated with a specific disease have been identified, taking advantage of the results of microarray screening.

Modern drug development includes the monitoring of gene expression and changes of protein abundance after drug administration. The development of drug resistance, or its natural occurrence, for example, are frequently related to these biological processes. Recently, the therapeutic efficacy success of cytokine proteins, such as interferon-α in anticancer and viral therapy, has sparked interest in their influence on gene regulation. In particular, the modulating effects of interferon-α on signalling

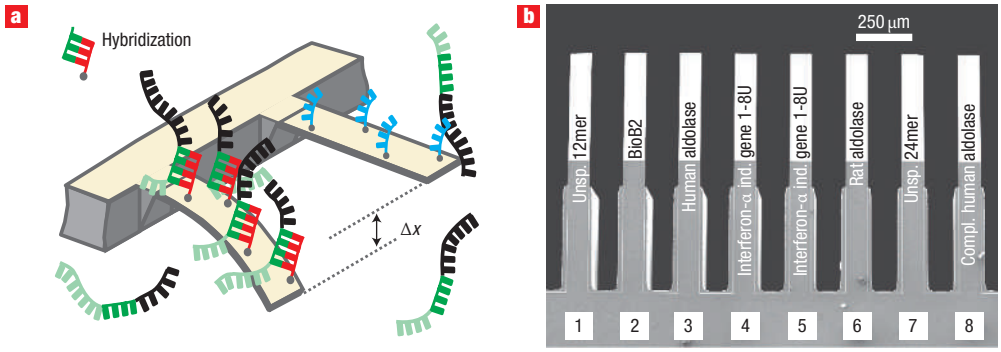

Figure 1 Setup showing sensor and reference cantilevers and the biofunctionalized cantilever array. a, Principle of differential readout using sensor and reference cantilevers. The schematic shows two different capture probes (24mer oligonucleotides, red and blue) tethered to two cantilevers, with hybridization, which induces nanomechanical bending of the sensor cantilever upon binding of long target transcripts in solution (50–100 base pairs). The cantilever that does not respond to injection of genomic material can be used as the *in situ* reference, leading to the differential readout, indicated by Δx. **b**, Biofunctionalized cantilever array. The cantilevers are individually functionalized with different types of thiol-functionalized ssDNA using microcapillaries as described in Table 1. The cantilevers measure 500 μm in length, 100 μm in width and 0.45 μm in thickness. In the experiments the unspecific functionalized cantilevers (alternating sequence of adenine and cytosine residues of either 12 or 24 bases) serve as references.

pathways play an important role in fighting both melanoma[23] and hepatitis C (ref. 24). The transcription of more than 70 genes is directly enhanced by interferon[25–27], of which ~20 genes have been found to be poorly responsive in interferon-α-resistant cells[27] but can be triggered in interferon-sensitive cells. One of these genes encodes the interferon-α-inducible transmembrane protein 1-8U, which plays a role in controlling tumour growth[23]. As interferon therapy is associated with toxic and harmful side effects[28,29], it would be beneficial to develop a rapid method to test in advance if patients will respond to interferon-α treatment, by monitoring the expression of particular mRNA biomarkers, which are indicators of a specific biologic state.

Owing to a significant improvement in biosensitivity (Figs. 2 and 3), we are able to show the detection of messenger RNA of aldolase A in the complex total RNA background. This mRNA is abundantly expressed in every living cell (indicating a housekeeping gene) and serves as a positive internal control for cantilever gene-expression studies. 1-8U gene mRNA expression was analysed in human melanoma cell lines treated with interferon-α. In this experiment the housekeeping gene aldolase A was used for calibration and as reference.

NANOMECHANICAL ANALYSIS OF GENETIC INFORMATION

In contrast with other technologies, cantilever sensors allow precalibration of their mechanical deflection for subsequent normalization and assessment of the experimental data. First, the cantilever array is individually functionalized within microcapillaries (Fig. 1b and Table 1). The array is then mounted in the measurement chamber in a computer temperature-controlled box and stabilized at 23 ± 0.02 °C. It is then flushed with saline sodium citrate (SSC) buffer, pH 7.0, containing 1 M NaCl buffer (that is, running buffer). Prior to the experiments, a calibration test to achieve maximum mechanical homogeneity with the array is performed by applying a heat pulse of 0.7 °C for 30 s with a peltier element mounted directly below the cantilever array (precalibration, Fig. 2a). All of the 450-nm-thick cantilevers deflected approximately 1,600 nm (compressive deflection response), as expected because of their single-sided metal coating, used as substrate for the deposition of the single-stranded DNA-sensitized cantilever interface. The cantilever gold interfaces exhibited a mean roughness of ~0.4 nm on areas of 9 μm^2 and grain sizes of ~30 nm. The smooth interface ensures that all immobilized oligonucleotides with lengths of up to 10 nm are exposed freely to the solution without obstruction by the underlying gold substrate. Only data acquired from cantilevers exhibiting mechanical properties that were identical to within less than 5% peak deflection were considered for evaluation in the experiments (see Fig. 2a). The chamber was allowed to equilibrate again to the setpoint temperature. To ascertain the biological sensitivity of the cantilevers, 12mer oligonucleotides BioB2, complementary to the sequence of one of the cantilevers (Fig. 1b, position 2), were injected at various concentrations. As shown in

Table 1 Probe oligo nucleic acid sequences immobilized on the individual cantilever sensors.

No.	Probe	Sequence	Application
1	Unsp12	5′-HS-$(CH_2)_6$-ACA CAC ACA CAC-3′	Unspecific 12mer reference cantilever; $(AC)_6$
2	Bio-B2	5′-SH-$(CH_2)_6$-TGC TGT TTG AAG-3′	BioB2 cantilever for specificity and picomolar sensitivity check
3	Human aldolase A	(5′ HS-$(CH_2)_6$-GCC CCC CGC TGT CAC TGG GAT CAG 3′	Human aldolase A (base mismatches to the rat aldolase A gene are underlined). Cantilever for genomic human cRNA housekeeping gene detection
4,5	Interferon-α-inducible gene 1-8U	5′-HS-$(CH_2)_6$-GAT GGT TGG CGA CGT GAC CGG GGC-3′	Interferon-α-inducible gene 1-8U cantilever for genomic cRNA gene-induction detection
6	Rat aldolase	5′-HS-$(CH_2)_6$-GCC CCC TGC CGT CCC TGG GGT CAC-3′	Rat aldolase A cantilever for genomic rat cRNA housekeeping gene detection
7	Unsp24	5′-HS-$(CH_2)_6$-ACA CAC ACA CAC ACA CAC ACA CAC-3′	Unspecific 24mer reference cantilever; $(AC)_{12}$
8	Compl. human aldolase	5′-HS-$(CH_2)_6$-GTG ATC CCA GTG ACA GCG GGG GGC-3′	Complementary human aldolase A cantilever for genomic native rat mRNA housekeeping gene detection

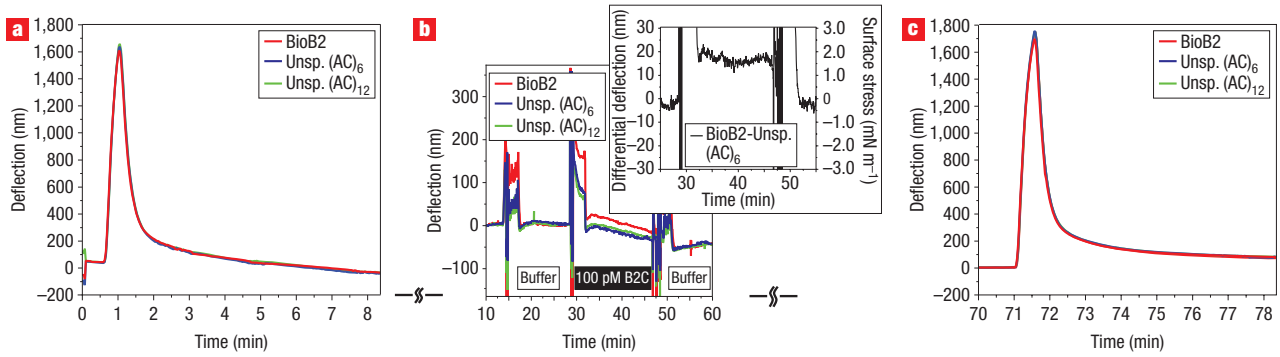

Figure 2 Calibration of the mechanical response and the biosensitivity of the cantilever array sensors. a,**b**, Calibration by application of a heat pulse of 0.7 °C for 30 s (**a**), followed by 100 pM complementary BioB2 cDNA injection (**b**), which results in compressive stress due to hybridization of the specific complementary cDNA with an absolute signal of ~17 nm. Inset: The differential signal is evaluated by subtracting the response of the Unsp. $(AC)_6$-sensitized cantilever from the signal of the BioB2-sensitized cantilever. **c**, Cantilever sensor calibration by applying a heat pulse after the sensitivity-validation experiment. This demonstrates the mechanical stability and integrity of the sensors.

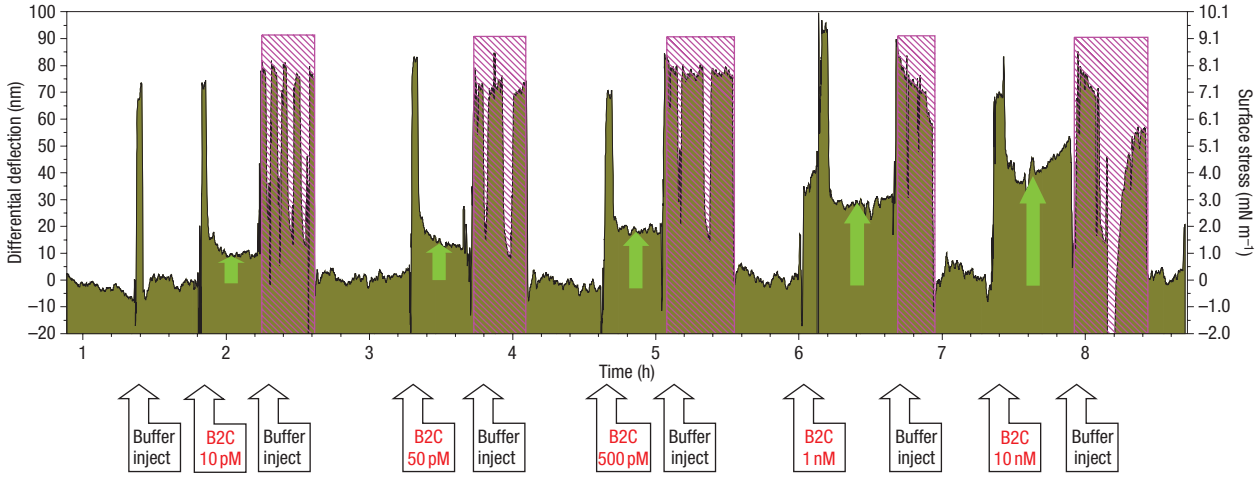

Figure 3 Cantilever response as function of BioB2C concentration. A series of continuous injections of increasing concentrations (10 pM to 10 nM) of complementary ssDNA B2C is shown. During buffer injection the sensors are flexed owing to flow. Dehybridization of the hybridized target (red hatched areas) occurs owing to the short length of the BioB2 oligonucleotides (12mer, see Table 1).

Fig. 2b and Fig. 3, concentrations of 100 pM of complementary ssDNA could easily be detected. Hybridization reactions can occur during the ~3–4 min injection cycles. As reference cantilevers for this calibration, the cantilevers labelled with an alternating sequence of adenine and cytosine residues of either 12 or 24 bases (Fig. 1b, positions 1 or 7) were used. The length of the oligonucleotide BioB2 was selected such that subsequent dehybridization was possible without applying harsh conditions such as 50% formamide or 4 M urea rinsing. The lower detection limit of the current device is about ~10 pM (Figs. 3 and 4). Such a value is comparable to current standard gene-chip technology operating at a limit of 1–6 pM based on spike-in experiments (see, for example, www.affymetrix.com). In this study, we detect the BioB2 hybridization control at 10 pM, which is a sensitivity level sufficient for routine diagnostic tests.

Here, we report the ability to detect specific transcripts without amplification steps in total RNA derived from human or rat cell lines. Using arrays of eight silicon cantilevers, each individually coated with a different sequence of thiolated capture probe ssDNA (Fig. 1b), specific target hybridization events were monitored by cantilever bending as result of changes in surface stress (Fig. 1a). Improvements included exploiting a systematic miniaturization of the cantilever-device geometry together with optimized individual cantilever functionalization using capillary arrays[30]. The dimensions of the cantilever sensor, in particular the thickness of the beam, are known to be critical for surface-stress-sensing applications, as shown by the Stoney[21] equation (equation 1). The change in surface stress, $\Delta\sigma$, defined as the reversible work per unit area required to stretch a pre-existing surface, is related to the deflection of the free end of the cantilever, Δz:

$$\sigma = \frac{Et^2\Delta z}{4l^2(1-\upsilon)} \qquad (1)$$

where t is the thickness of the cantilever, l is the length (500 μm), E is Young's modulus (130 GPa) and υ is the Poisson ratio of

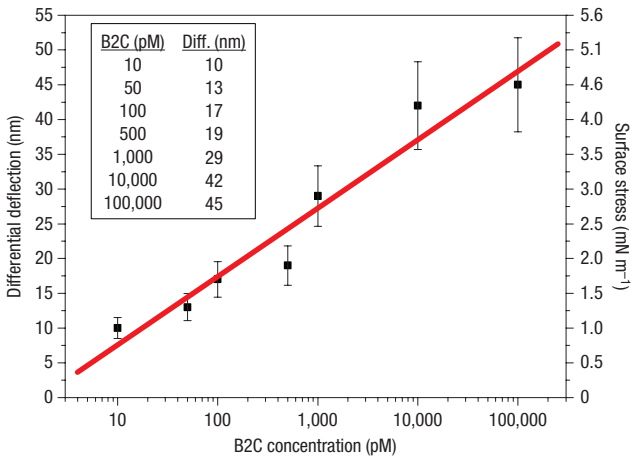

Figure 4 Differential nanomechanical response of cantilevers during specific hybridization. Compressive stress response versus concentration dependence. The error bars represent standard deviation obtained from four measurements.

silicon (0.28). A systematic redesign of the cantilever-deflection measurement device, mainly by scaling down the thickness of the cantilever beams, led to much smaller label-free detection limits in the low picomolar range (Fig. 4). Differential readout, that is, measuring the difference in the readouts of the sensor and the reference oligonucleotide-functionalized cantilever responses, was found to be essential for sequence-specific detection, as the absolute bending signal can be influenced by temperature, changes in refractive index and unspecific reactions occurring on the non-biofunctionalized silicon interface of the cantilever or non-functionalized gold sites. The repetitive adenosine–cytosine probe DNA sequence on the reference cantilever was found to be insensitive to random genetic sequences, and thus served as a good internal reference. Control probe sequences were chosen to have the same length as the probe DNA under investigation. Upon injection of the 100 pM solution of complementary target BioB2C, a robust deflection signal of about 20 ± 4 nm was observed with the BioB2-functionalized cantilever, corresponding to a compressive stress change $\Delta\sigma$ of $\sim 2 \pm 0.6$ mN m^{-1} (Fig. 2b, inset). It should be emphasized that the cantilevers are placed orthogonally to the flow so that an efficient streaming and mixing occurs during sample injection. On each cantilever, the coating procedure immobilizes about 10^9 oligonucleotide probe molecules, which is at least 1,000-fold higher when compared with established commercial microarray platforms. This could reduce the annealing time of the target nucleic acid molecules by the order of tens of minutes. The high density of probes immobilized on the surface of the cantilever leads to a physical steric crowding during hybridization and is the predominant cause of the nanomechanical signal[8].

LABEL-FREE GENE FISHING IN TOTAL RNA

Taking advantage of picomolar sensitivity to analyse physiological samples, our studies were extended to the detection of gene expression. Initial investigations focused on the detection of the RNA sequence that encodes for the glycolytic enzyme aldolase, involved in fructose 1,6-biphosphate metabolism and abundantly expressed in every living cell. The aldolase housekeeping gene therefore served as a positive control for cantilever gene-expression studies. We first used complementary RNA (cRNA) from rats to measure aldolase mRNA levels for sensitivity assessment. Prior to the measurements, the cRNA was randomly fragmented to 50–100 bases in length to better match the probe length. Figure 5a shows the nanomechanical differential deflection detection of rat-aldolase mRNA. Before injection, the cantilevers were equilibrated in running buffer (SSC, 1 M NaCl) until a stable baseline deflection signal of all eight cantilevers was observed. Then, 5-μg fragmented total rat cRNA (600 nM) was injected for 2–5 min. The flow in the liquid cell was stopped, and sequence-specific hybridization between target rat cRNA and the rat-aldolase probe DNA immobilized on the cantilever was monitored. As shown in Fig. 5a, the rat-aldolase probe signal increased, corresponding to a downwards bending of the cantilever, reaching a stable equilibrium signal of approximately 100 ± 7 nm after 20–30 min. This bending signal corresponds to a compressive surface stress $\Delta\sigma$ of $\sim 10 \pm 0.7$ mN m^{-1}. The differential response between the reference 24mer and a second reference probe-coated cantilever BioB2 showed some change in signal (red curve). In gene-chip applications it has been shown previously that some crosstalk from prokaryotic control transcripts such as BioB (EMBL # J04423) with eukaryotic probes occurs. We observed comparable crosstalk with prokaryotic probe sequences (Fig. 5a, red curve), therefore we systematically chose the $(AC)_6$, $(AC)_{12}$ functionalized cantilevers (Fig. 1b) as an *in situ* reference when performing experiments in a complex background.

To evaluate the specificity of the technique, the response of a cantilever coated with the human aldolase oligonucleotide was investigated with rat and human total cRNA. The rat and human aldolase probe sequences differ by four bases. As a result, injection of rat cRNA gives no significant differential response of the cantilever coated with the human aldolase probe, which confirms the sequence specificity of the cantilever technique (Fig. 5b). The expression of aldolase in human cells was then investigated using fragmented cRNA from cultured melanoma ME15 cells[23] (see Methods). Figure 5c shows that a differential signal of 60 ± 3 nm ($\Delta\sigma \approx 4 \pm 0.2$ mN m^{-1}) results due to specific hybridization to the human aldolase probe (aldolase concentration approximately nanomolar). These experiments demonstrate that individual human transcripts in a complex mixture of cRNA can be specifically detected using cantilever sensor arrays.

To investigate the performance of cantilever arrays in complete human RNA, we detected human aldolase mRNA in fragmented RNA, directly isolated from cells without any sample amplification or selection. Note that cRNA and native RNA are complementary to each other; therefore, the sensor cantilever in these experiments was functionalized with the complementary probe sequence (Fig. 1b, position 8). The response in Fig. 5d shows that upon injection of total human RNA, a bending signal of 55 nm is generated, corresponding to compressive $\Delta\sigma \approx 3$ mN m^{-1}. As additional control, the *in situ* reference cantilevers also exhibited no significant response (red curve). This is the first direct demonstration of differential mRNA gene expression detection in real eukaryotic samples (total RNA) without amplification or labelling.

RAPID DETECTION OF GENE EXPRESSION

The investigations described above demonstrate the high sensitivity of cantilever arrays for detecting the expressed mRNA of the aldolase gene in universal reference rat RNA, human melanoma cells and, finally, total universal human reference RNA.

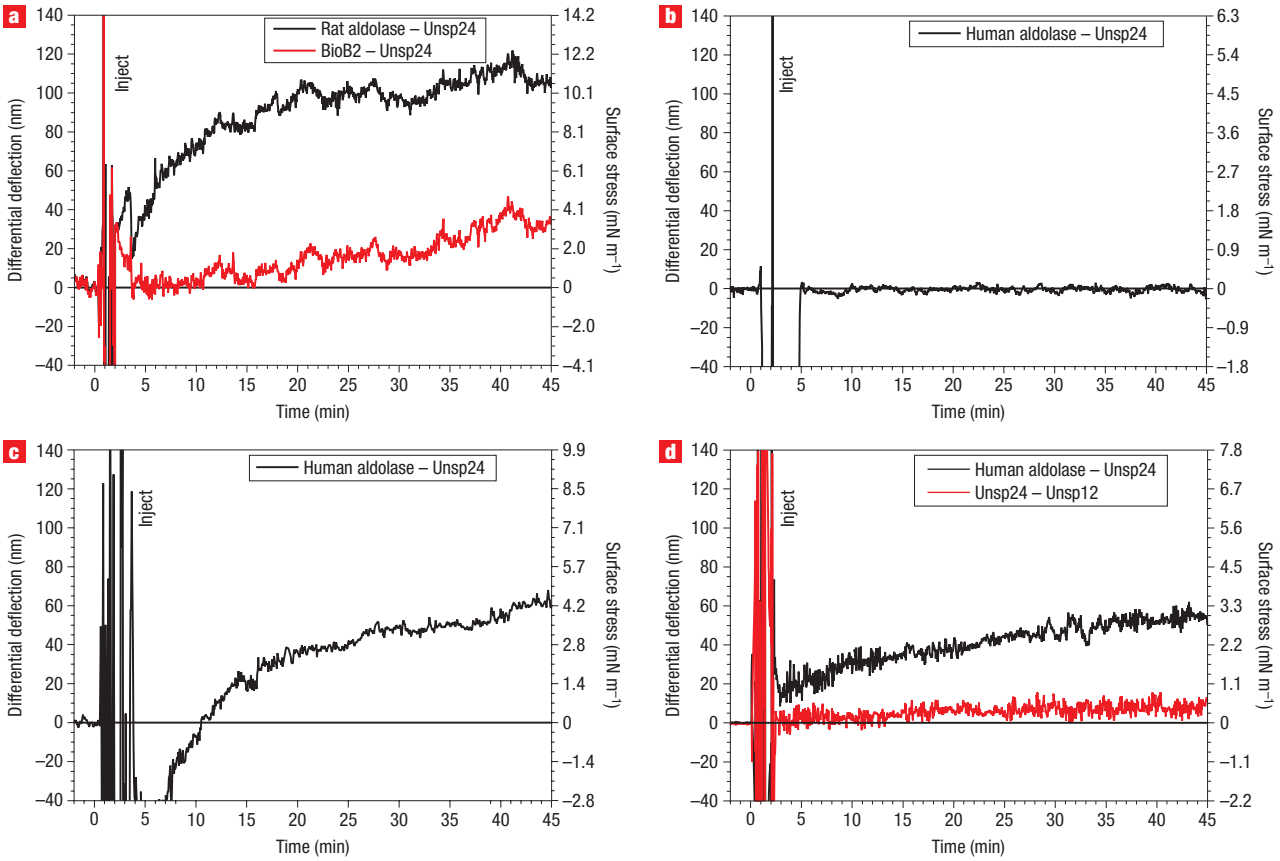

Figure 5 Differential gene fishing in a complex genomic background. a, Injection of fragmented cRNA from rat. Rat aldolase cantilever response is shown as the black line. This is the absolute bending of the rat aldolase functionalized cantilever minus the *in situ* reference (Unsp24). Crosstalk of the prokaryotic sensor is shown as the red line in the eukaryotic background. **b**, Human aldolase A cantilever response. **c**, Human aldolase A signal upon injection of human cRNA (600 nM, 5 μg, ~50 base pairs). **d**, Gene fishing in complete RNA. Detection of individual transcripts (human aldolase A RNA, black line) in nonamplified, nonlabelled fragmented native RNA by compressive stress that builds up during hybridization. The slightly higher noise level is due to an increased amount of RNA (addition of rRNA, tRNA and miRNA in total RNA samples). An internal reference measurement, $(AC)_{12}$ minus $(AC)_6$, is shown as the red line.

The mRNA detection sensitivity is sufficient for biomarker tracking, because only abundant transcripts that are significantly modulated in response to drug treatment are considered. We have chosen an interferon-α inducible gene because its expression is well characterized and understood in conventional microarray platform technology. RNA was extracted from 10^7 cells of a human melanoma cell line. Our studies were extended from the reference aldolase A as a housekeeping gene, which is always turned 'on', to the interferon-α-inducible gene 1-8U in human melanoma ME15 cells. The 1-8U gene expression has been well characterized in ME15 cells[23]. It can be turned on by treatment with interferon-α, inducing a sixfold upregulation (ME15+), or tuned off in the absence of treatment (ME15−). Figure 6 shows the differential nanomechanical bending response of a cantilever coated with the 1-8U capture probe minus the reference unspecific $(AC)_{12}$ 24mer to injections of cRNA fragments from ME15− (Fig. 6a) and ME15+ human melanoma cells (Fig. 6b). In addition to 1-8U probes, cantilever arrays were also coated with the aldolase A probe, which served as further control and internal reference for our gene-expression studies. Figure 6a shows the cantilever response induced by hybridization of complementary mRNA from non-treated cells (ME15−) to the probe sequence on the cantilever interface. As expected, only the aldolase-A-coated cantilever reveals a differential signal (~35 nm), which is comparable to the results given in Fig. 5, and the 1-8U probes give no signal as the gene is not expressed. In contrast, the cytokine-treated ME15+ cells express the 1-8U gene, and transcripts thereof are readily detected. Figure 6b shows a clear 35-nm differential bending signal of the 1-8U-sensitized cantilever, corresponding to a compressive $\Delta\sigma \sim 3.2$ mN m^{-1}. The levels of the aldolase A mRNA served (nanomechanically detected) as internal reference and control. The differential nanomechanical responses of the sensor recognizing the housekeeping gene in these experiments measure ~35 nm and 45 nm, respectively (black lines in Fig. 6a,b). The aldolase gene is a constitutively expressed housekeeping gene at the level of 8,000–10,000 relative fluorescence units (RFU), which is commonly used for calibration in microarray experiments. The nanomechanical differential response of ~20% variability is in the same range as the biological variability. In contrast, 1-8U is normally not expressed (<100 RFU), but interferon-α induces transcription of the gene to levels that are comparable and in the range of aldolase, which has been described previously[27] and is confirmed here with an independent approach. This 1-8U gene was chosen because its expression level correlates in clinical studies with drug response. These findings show that differential cantilever-array detection is indeed competitive with microarray technology, and that it is possible to

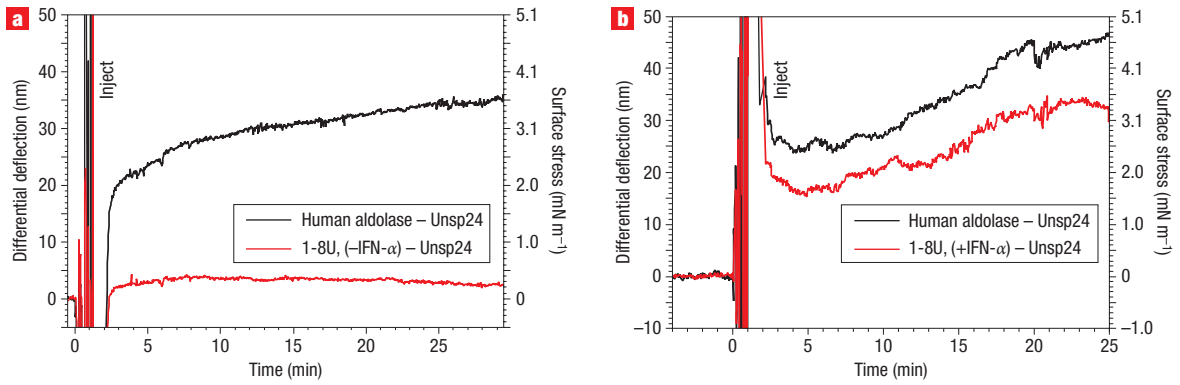

Figure 6 Nanomechanical measurement of the upregulation of a gene. a, Non-interferon-α-treated cell culture (ME15−) left an inactive (normal) 1-8U gene (red line) and showed no cRNA hybridization response, whereas the housekeeping aldolase A gene cRNA (black line), which serves as an internal control, is readily detected. **b**, Label-free gene fishing of an interferon-induced gene. The red line (ME15+) indicates the response of the cantilever coated with an interferon-α-sensitive human 1-8U gene fragment. The black line represents the differential mechanical response of the cantilever sensitized with the human aldolase A oligonucleotide.

measure differential gene expression after drug treatment, which is an important prerequisite for the application of this technology in the area of multiparallel biomarker detection.

CONCLUSIONS

In the present study, we demonstrate fast amplification-free and label-free detection of marker genes that are upregulated to a high expression level upon drug exposure. We detect triggered marker genes in a genomic background and foresee cantilever arrays being used as a tool for fast detection of expression of significant marker genes in the field of personalized medical diagnostics. The AFM-based technology[31] is not considered as a competitor to current gene-chip applications, but rather is a complementary approach focusing on selected diagnostics of biomarkers with robust expression features that have been established and validated by standard techniques such as RT-PCR or microarrays. Such customized chips do not target low and medium copy number genes. In normal complete human RNA samples, as used in this study, the 1-8U gene is not expressed and therefore baseline mRNA levels would be recorded. The sensitivity level of the device is now ~10 pM and is directly comparable with conventional gene-chip technologies where the detection limit is 1–6 pM, applying fluorescent probes. Other label-free techniques such as SPR (ref. 32) are not capable of measuring small target molecules like 12 mers that are easily detected with the cantilever-array technique. Nanowire sensors[33] show sensitivity down to the fM range, although this is in a uniform background where there is no competition from other comparably binding molecules. In contrast, the cantilever array device presented here enables parallel and simultaneous measurements with an *in situ* biological specific reference sensor and internal calibration.

This is the first demonstration of nanomechanical analysis of multiple differential gene expression without sample amplification or labels. State-of-the-art microfabrication allows the use of two-dimensional arrays of cantilevers in large numbers[34]. However, routine diagnostic mRNA detection tests for pathogens require only a small number of parallel assays. This number may be increased to a few hundred probes once disease biomarkers become available for standard analysis.

Scaling cantilever arrays further will enhance the sensitivity and response time. Fully integrated in a microfluidic environment, this technique will lead to unprecedented possibilities for mobile point-of-care applications, representing a new generation of personalized disease diagnostics to assess drug efficacy.

METHODS

TOTAL RNA MATERIAL

Rat cRNA was derived from universal rat reference RNA (isolated from a panel of rat cell lines; Stratagene). Total human RNA was obtained from Stratagene (universal human reference RNA). To obtain the interferon-inducible target gene, total RNA material was isolated from cells/tissues of ME15+ and ME15− cell lines grown at Hoffmann-LaRoche. Melanoma cell lines were cultured in the presence or absence of IFN-α2a. Cultured melanoma cells were harvested by scraping, and total cellular RNA was subsequently extracted[22]. A total of 10 μg of each sample was reverse-transcribed to obtain cDNA; cRNA was transcribed *in vitro* by RNA polymerase and randomly fragmented down to 50–100 bases. The cRNA synthesis by RNA polymerase increases the mRNA by a factor of 30 and not in an exponential manner as in PCR reactions; with regard to total RNA this would be a factor of ~1,000.

PROBE DESIGN

All unmodified and thiol-modified (5′-HS–$(CH_2)_6$) single-strand oligonucleotide probes were obtained from Microsynth. Prior to use, 100 μl of 50-μM-thiol-modified ssDNA was extracted three times with the same volume of reagent-grade ethyl acetate (Fluka) to remove thiol-protecting 0.1 mM DDT, dried under vacuum at 40 °C for 4–5 h using a SpeedVac pump (TeleChem International). The concentrations of the purified thiol-modified ssDNA were determined by UV light absorption at 260 nm by UV-VIS spectroscopy (Hewlett-Packard, 8453A), and calculated on the basis of the optical density of single-stranded DNA.

PREPARATION OF SOLUTIONS

All solutions were prepared using 18 MΩ cm^{-1} Nanopure water. The solutions for the functionalization of the cantilevers consisted of purified thiol-modified ssDNA dissolved at a concentration of 40 μM in 50 mM triethyl ammonium acetate (TEAA) buffer (Fluka). The solution used for hybridization was prepared at a concentration of 600 nM from RNA samples of melanoma cell lines (ME15+, ME15−) in sodium-buffered saline citrate (SSC) with 1 M NaCl (Fluka).

CANTILEVER ARRAYS COATED WITH ssDNA OLIGONUCLEOTIDES

Silicon cantilever arrays consisting of eight identical cantilevers each were fabricated by the Micro- and Nanomechanics group at the IBM Zurich Research Laboratory. The cantilevers are 500 μm long, 100 μm wide and 0.45 μm thick at an array pitch of 250 μm. The spring constant is

0.004 N m^{-1}. Prior to use, cantilevers were cleaned twice using piranha (30% H_2O_2:H_2SO_4 = 1:1) for 20 min, followed by cleaning in a solution consisting of 30% H_2O_2:H_2O:32% NH_3OH = 1:1:1 for 10 min. Each step was followed by rinsing with Nanopure water (18 MΩ cm^{-1}) and ethanol (p.a. grade, Fluka) and drying on a hot plate at 75 °C. Subsequently, a 2-nm-thick titanium layer and a 20-nm-thick gold layer were deposited onto one side of cantilever using an electron double-beam evaporator (BOC Edwards). The evaporation rates of titanium and gold were 0.033 and 0.065 nm s^{-1}, respectively and yielded straight cantilever bars. During gold deposition a maximum temperature of ~60 °C was measured at the level of the cantilever.

ssDNA IMMOBILIZATION AND HYBRIDIZATION

Each of the freshly prepared gold-coated cantilever arrays was incubated for 20 min using an array of microcapillaries to facilitate covalent binding of thiol-modified oligonucleotides onto the cantilevers' gold-coated surface. Each microcapillary was filled with a different solution of 10 μM thiol-modified probes. The immobilized array was subsequently washed using 50 mM TEAA, Nanopure water, SSC with 1 M NaCl, and finally stored in 5×SSC with 1 M NaCl at 4 °C.

The functionalized ssDNA array coated with eight different oligonucleotide probes, including references, was mounted in the liquid chamber and exposed to the hybridization buffer containing 1 M NaCl for equilibrium at 23 °C. The sample fluid was pulled through the chamber at a rate of 300 μl min^{-1} using a programmable syringe pump (Genie-220, Parkland Scientific). Efficient mixing was facilitated by switching between two outlets by means of an external three-way valve. A control experiment using BioB2 complementary target solution at a concentration of 100 pM confirmed that specific hybridization was established. Thereafter, the chamber was cleaned by flushing the chamber with 2–3 ml of SSC. Subsequently, the interferon-inducible RNA samples ME15− and ME15+ were injected (concentration of 600 nM in 1 M NaCl of SSC). Each injection was followed by cleaning steps consisting of 2 ml SSC with 1 M NaCl, 1 ml SSC with 0.1 M NaCl and 1 ml 4M urea for complete dehybridization.

Experiments carried out with cantilevers that were functionalized with long spacer molecules as used in conventional gene-chip technology exhibited no sensitivity and provided no nanomechanical signal upon ssDNA hybridization[35].

Radiolabelling experiments reveal that the footprint of one radioactively labelled thiolated ssDNA on the functional cantilevers is approximately 3.24 nm^2. In other experiments cantilevers were functionalized with thiolated ssDNA (as used throughout this paper). Only 10% of these surface-grafted molecules were then able to hybridize with radiolabelled complementary ssDNA from solution[8].

Received 13 June 2006; accepted 30 October 2006; published 26 November 2006.

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Acknowledgements

This paper was supported by the Swiss National Center of Competence in Research (NCCR) 'Nanoscale Science', the Swiss National Science Foundation (grant no. 3152A0-1059531 to M.H.) and by the ELTEM Regio network. The support of IBM Research GmbH as a research partner of the NCCR and the Cleven-Becker foundation is acknowledged. We thank I. Redwanz (RCMG) for cell culture and RNA isolation work.

Author contributions

U.C., C.G. and M.H. conceived and designed the experiments, J.Z. performed the experiments, J.Z., H.P.L. and A.B. functionalized the cantilever arrays, J.Z., F.H., H.P.L and M.H. analysed the data, and W.G. programmed the hardware and software of the instrumentation. All authors discussed the results and commented on the manuscript.

Competing financial interests

The authors declare that they have no competing financial interests.