nano-tera.ch
swiss scientific initiative in health / security / environment systems

PATLiSci 2 RTD 2013

FNSNF

Diagnosis of HER2 amplification in biopsies from breast cancer using nanomechanical biosensors

F. Huber[1], H. P. Lang[1], M. Plodinec[2], Ch. Gerber[1], and E. Meyer[1]

SWISS NANOSCIENCE INSTITUTE
EINE INITIATIVE DER UNIVERSITÄT BASEL UND DES KANTONS AARGAU

[1] Swiss Nanoscience Institute, University of Basel, Klingelbergstrasse 82, CH-4056 Basel

[2] Biocentre, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel

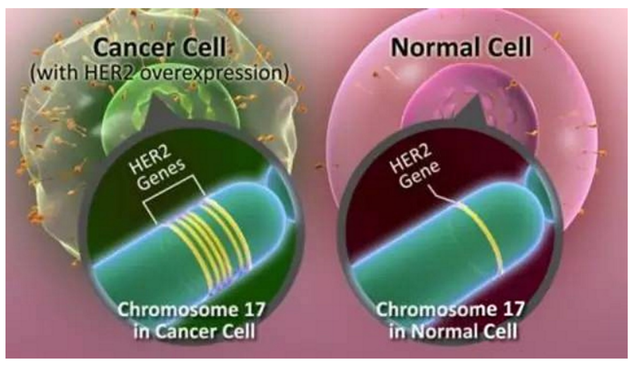


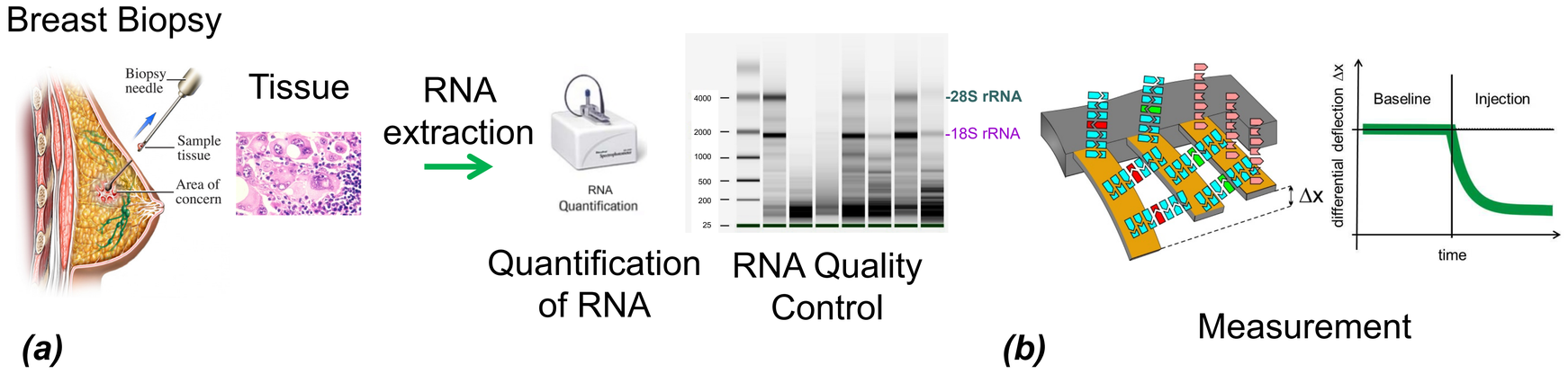
Fig. 1. HER2 gene amplification in a cancer cell vs. normal cell (from http://www.knowpathology.com.au).

Abstract

Breast cancer is the most frequent women's cancer. In about 25% of breast cancers, the HER2 (human epidermal growth factor receptor 2) gene is amplified, which results in overexpression. Patients with HER2 over expressing tumors have poorer prognoses than other types of breast cancer. Nowadays a targeted therapeutic antibody (trastuzumab) for HER2 positive tumors is available for treatment. An important task is to identify the patients who test positive for HER2 overexpression (HER2 positive) as the treatment may have severe adverse effects like cardiotoxicity. Patients tested HER2 negative can not benefit from trastuzumab treatment. We suggest a novel method using nanomechanical microcantilever arrays to do the analysis.

Procedure

Fig. 2. (*a)* From biopsy to diagnostics. Total RNA is extracted from a biopsy, concentration is determined and a RNA quality control is performed employing electrophoresis. An important criterion is if 18S (violet) ribosomal RNA (rRNA) und 28S rRNA (green) bands are visible. **(*b)*** Hybridization of target RNA and subsequent bending of cantilevers produces a deflection signal.



Results

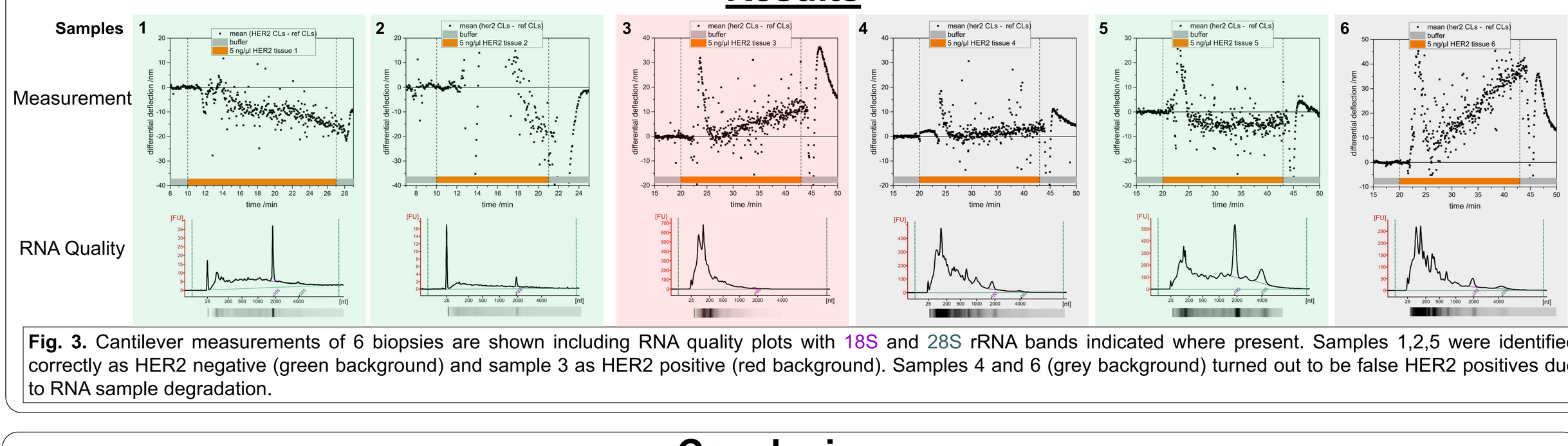


Fig. 3. Cantilever measurements of 6 biopsies are shown including RNA quality plots with 18S and 28S rRNA bands indicated where present. Samples 1,2,5 were identified correctly as HER2 negative (green background) and sample 3 as HER2 positive (red background). Samples 4 and 6 (grey background) turned out to be false HER2 positives due to RNA sample degradation.

Conclusions

Sample Number	Diagnosis	RNA yield. (µg)	RNA conc. (ng/µl)	Cantilever responses	HER2 status	Prediction
1	Invasive ductal carcinoma	10.2	339	-20 nm	negative	negative
2	Invasive ductal carcinoma	0.558	18.6	-20 nm	negative	negative
3	Ductal carcinoma in situ	1.96	65.49	+10 nm	positive	positive
4	Highly differentiated lobular carcinoma (**degraded RNA sample**)	1.92	64.14	+5 nm	negative	positive
5	Invasive ductal carcinoma	2.81	93.51	-5 nm	negative	negative
6	Invasive ductal carcinoma (**degraded RNA sample**)	1.61	53.82	+40 nm	negative	positive

Table 1 shows the summary of a pilot clinical study with six samples: total RNA extracted from tissue. HER2 positive (**Mutant**) overexpressing sample 3 and negative (**Wild Type**) with normal expression levels (samples 1,2,4,5,6). Samples (5,6) turned out to be false positives (in grey) due to degradation of the RNA samples.

In conclusion of these experiments we state that we can characterize mammary carcinomas using nanomechanical microcantilever arrays. Better quality total RNA is required to increase the reliability of the method to exclude false positives. The sensitivity has been improved so that samples with a concentration of less than 5 ng/µl and a total yield of less than 600 ng could be measured. The sensitivity for HER2 detection is by a factor of four better than previous BRAF experiments which required 20 ng/µl as the lowest concentration.