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Rapid HER2 biomarker test for breast cancer

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Abstract

Breast cancer is the most frequent cancer of women. In some breast cancers, the HER2 (human epidermal growth factor receptor 2) gene is amplified and over expressed. Patients with HER2 over expressing tumors have poorer prognoses than other types of breast cancer. Recently progress has been made and a targeted therapeutic antibody (trastuzumab) for HER2 positive tumors is available for treatment. The ability to reliably identify patients who might benefit from trastuzumab treatment is not only important for clinical reasons such as positive clinical effects as well as the possibility of severe adverse events like cardiotoxicity. At the moment two types of tests are performed to assess the HER2 status. The first one relies on immuno histochemistry (IHC) where a specific antibody against HER2 is used to investigate the overexpression of HER2. The second method is employing fluorescence in situ hybridization (FISH) for this purpose. We propose to use our nanomechanical microcantilever arrays to do the analysis in parallel.

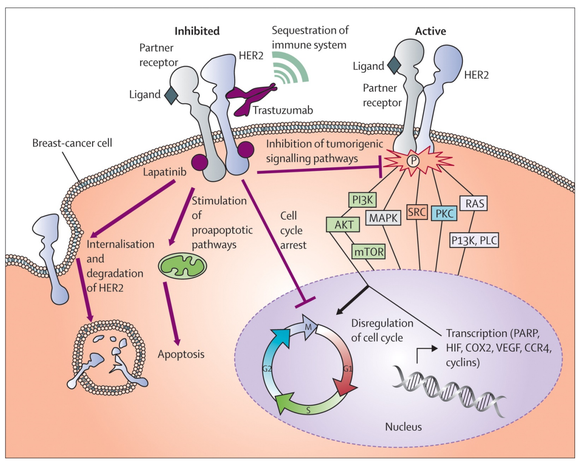

Fig. 1. Cellular pathways involved in HER2 signal transduction, growth regulation and trastuzumab binding site.

Method

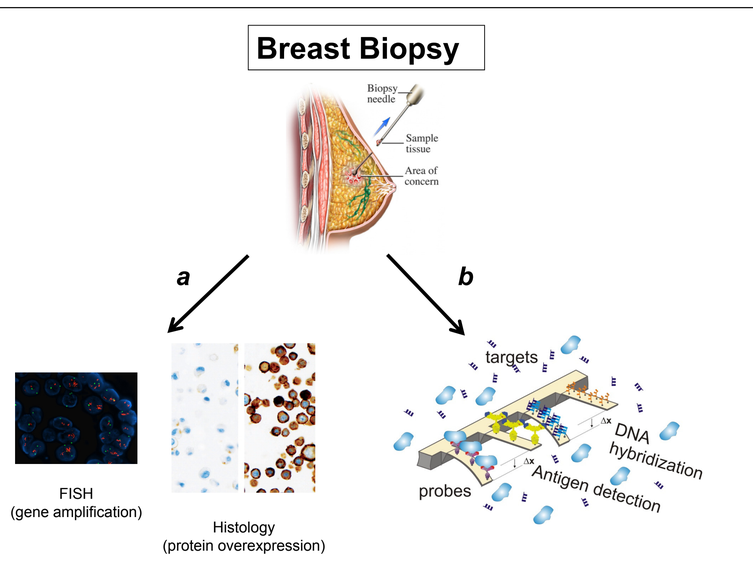

Fig. 2. ***a*** represents the standard procedures where two different methods are employed. ***b*** shows the new method where gene amplification and protein overexpression will be assessed in the same experiment.

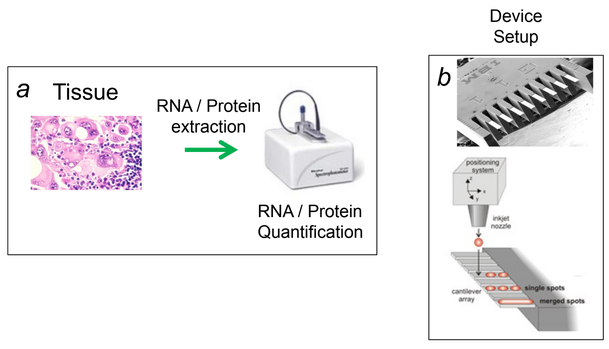

Fig. 3. ***a*** RNA or proteins are extracted from a biopsy and quantified. ***b*** Meanwhile a microfabricated array of eight silicon cantilevers is modified with polyethylene glycol to reduce nonspecific adsorption and a Ti/Au layer for covalent binding of thiol modified Probes. Thiolated oligonucleotides or antibodies are supplied individually to cantilevers using an inkjet spotter to create a sensitive layer for molecular recognition.

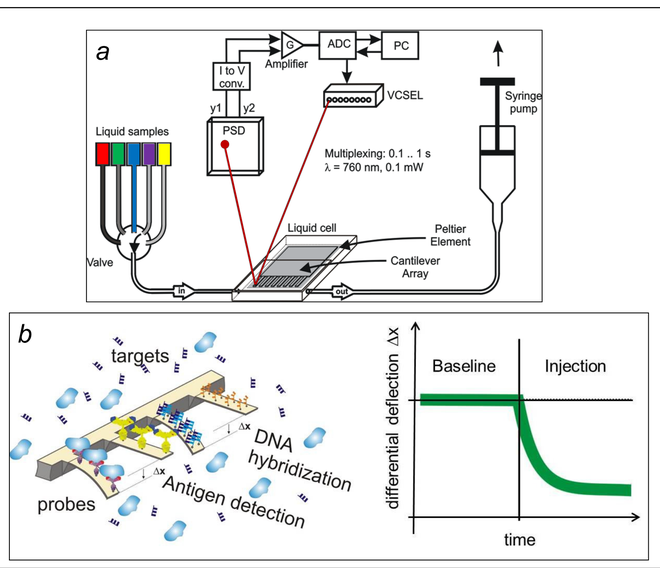

Fig. 4. ***a*** The cantilever array is mounted in a liquid cell. An array of eight vertical cavity surface emitting lasers (VCSELs) and a position sensitive detector (PSD) are used for optical beam deflection measurements of the bending ***b*** of each cantilever separately in a time-multiplexed way.

Results

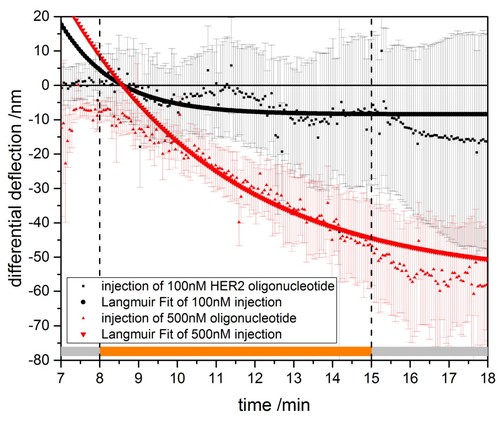

Fig. 5. Shows pilot experiments to investigate cantilever responses to binding of HER2 complement oligonucleotides. The red curves (raw data, with standard deviation and Langmuir fit) show the response to the injection of 500 nM oligonucleotide and the black curves indicate the response to the injection of 100 nM oligonucleotide. Here we see that the experiment is linear in this range of concentrations. The equilibrium differential deflection is 8 nm for the 100 nM samples and 56 nm for the 500 nM samples.

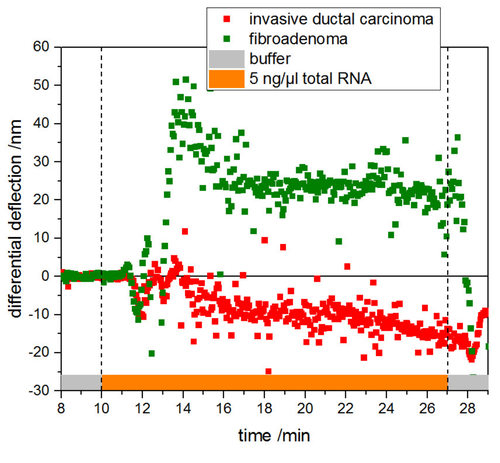

Fig. 6. Total RNA from two different tumors are measured. The green curve corresponds to a benign fibroadenoma and the red curve corresponds to a dangerous invasive ductal carcinoma. The two samples can be clearly distinguished.

Conclusions

-The work shows that nanomechanical microcantilevers are able to identify HER2 .

-The lowest concentration at 100 nM corresponds to 2 ng/µl oligonucleotide. The lowest concentration in our measurements with total RNA from tissue samples is in the same range.

-We successfully performed experiments with total RNA extracted from tissue samples. We were able to distinguish a benign fibroadenoma from a potentially fatal invasive ductal carcinoma which requires a more aggressive therapy.