



Integrated microfluidic device for drug accumulation measurement on a single MDR transporter-expressing prostate cancer cell isolated among blood cells

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Outline

- Why measuring single cells? We can measure rare cells even though there are only a few of them
- Are the rare cells representative of the whole? High statistical power of the same single cell analysis (SASCA)
- Results confirmed by flow cytometry which analyze thousand of different cells
- SASCA on prostate, lung cancer cells
- SASCA on AML patient cells

Hard to do bulk analysis with a small amount of rare cells



Richard Cote, U Miami

CTC microfluidic chip

20 mm SSFIAC В Chamber 2 Chamber **Chamber 1** Label-free cell isolation 30 mm 30 mm D D based on size difference С Chamber 2 **Cell retention structure** for capturing a single cancer cell

100 µm

Preparation of the mixture of PCa and blood cells







1st PBS washing



50 µm

Separation of the prostate cancer cell among other blood cells



Blood cells only (WBCs +RBCs)



Prostate cancer cells only



2<mark>00 μm</mark>

Prostate cancer cell mixed with blood cells

Isolation of tumor cells from blood cells



Drug accumulation measured in a single PCa cell



F_t: total drug fluorescent intensity **F**_i: intracellular drug fluorescent intensity **F**_e: extracellular drug fluorescent intensity

Drug accumulation in a single 22Rv1cell in the presence of Fumitremorgin C (FTC) as a MDR inhibitor



Trypan blue treatment conducted in Chamber 2 of the CTC chip



Comparison of drug accumulation in captured single prostate cancer cells and in normal white blood cells



500 0 0

1000

2000

3000

Time (second)

4000

5000



DNR: daunorubicin FTC: fumitremorgin C CsA: cyclosporine A **PTX:** paclitaxel Pgp: P-glycoprotein HBSS: Hank's balanced salt buffer

20 µm

f3

20 um

Enhancement of OG-PTX accumulation in single 22Rv1 cells due to ABCG2 and ABCB1 inhibitors



Lung cancer cell study



Time (second)

Beyond cell lines

- AML patient cells from British Columbia Cancer Agency: 10 CR samples and 10 NR samples,
- AML: acute myeloid leukemia
- CR: complete remission
- NR: non-responsive

Assay of AML patient cell samples



















Three types of cells (M:MDR, N:non-B1C1-MDR and W: white blood cells)



DNF

+MK571

+CsA

DNF

+NaN₃

Daunorubicin treatment on NR samples

AML patient data (CR samples: diagnosis and relapse)



DNR: daunorubicin MK571: ABCC1 inhibitor FTC: fumitremorgin C (ABCG2 inhibitor) CsA: cyclosporine A H: Hank's balanced salt buffer

a

С

е

Detection of CD34 expression after drug accumulation measurement 7

Time (second)

Time (second)

Single-Cell Bioanalysis (SCB)

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How many samples?



One sample, one single cell

One sample, 23 single cells

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Supporting slides

Isolation of white blood cells from blood through a Ficoll gradient



Optimization of DNR and OG-PTX concentrations for drug accumulation measurements



Effective concentrations of MDR inhibitors for experiments on the 22Rv1 cells



Enhancement of DNR accumulation in single 22Rv1 cells due to ABCG2 and ABCB1 inhibitors

