Single cell array based toxicity assay for personalized medicines

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Statement of Purpose: Cancer is the uncontrolled growth of cells coupled with malignant invasion and metastasis. Although anticancer drugs had passed stringent screening processes that involve cell lines, animals and individuals, chemo-therapy can fail often for reasons that are unknown but likely due to genetic variation and subtle differences in physiological background. A personalized way that is tailored to each patient's tumor can improve outcomes, and avoid unnecessary treatment. But, sequencing each patient or performing genetic test is labor intensive, and not always feasible due to high cost. Instead of trying to make ideal drugs that are applicable to all cancer patients, the therapeutic effects of existing agents can be tested on tumor cells from each patient at clinical site before drug could be administrated. But, subjecting tumor tissue from each patient to a battery of in vitro screening tests as those used in industry is simply too expensive, and time- and resource-demanding to be realistic. A variety of in vitro toxicity assays has been developed to assess drug effect at cellular levels; the use of existing technology to assess the effect of drugs on tumor tissues is limited for two reasons: (1) Tumor is heterogeneous, and population based toxicity assays cannot differentiate tumor responses masked in statistical averages. (2) Most existing toxicity assays are limited by low throughput and low statistical power, and labor-intensive scoring, and are not suitable for accurate rapid assessments of multiple drugs. (3) Existing assays are expensive, and cannot be used at point of care.

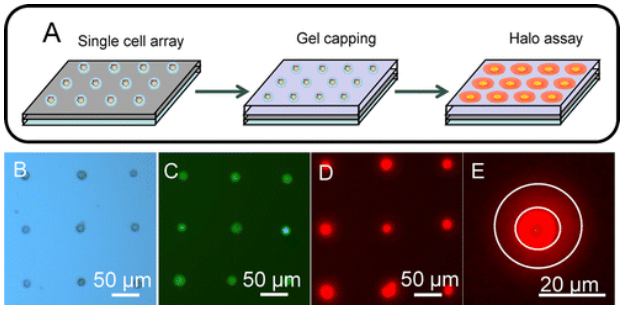

Fig. 1 (A) Single cell halo assay; (B) optical image of single cell array; (C) fluorescence image of single cell array in gel after live/dead assay; (D) fluorescence image of cell array after single cell halo assay; (E) an enlarged halo image from single cell.

Method: We had created a novel single cell array based DNA damage assay (i.e., HaloChip) by combining a halo assay and the state-of-the-art microfabrication techniques, in which surface micro patterns on substrate are used to trap single cells through electrostatic attraction (Fig. 1A). After exposing to drug, cells are patterned and embedded inside an agarose gel, followed by fast alkaline halo assay to quantify DNA damage. Fig. 1B shows an optical image of a single cell array generated on a silicon substrate vial in an electrostatic interaction. The viability of patterned cells is tested with live/dead assay, where EthD-1/Calcein AM stains viable cells to a green. Fig. 1C shows arrayed cells inside the gel are alive after 3 h. Cells are lysed with 0.3 M NaOH for 30 min at room temperature and stained with 10 μg/mL EB for 10 min. During this period, DNA in treated cells diffuses out of the nucleus to form a larger circle relative to the compact nucleoid. Fig. 1D shows a fluorescent image of a gel covered array of HeLa cells treated with 10 μM etoposide for 2 h. The fluorescence intensity is proportional to the amount of DNA damage. Owing to clear boundary, symmetric shapes of halos and nuclei and non-overlapping nature of adjacent cells/halos, nuclear diffusion factor (rNDF) can be accurately derived.

Results: LNCaP and HeLa cell lines are used to test the ability of single cell halo assay to "predict" the response of three clinically approved anticancer drugs (CPT-11, VP-16, and doxorubicin). Fig. 2 shows rNDFs of LNCaP and HeLa cells under normoxic (2A, 2B) and hypoxic conditions (2C and 2D) treated with three drugs at different concentrations. The selected concentrations are close to clinical drug concentrations. Each experimental point is averaged from at least 50 cells, and the results are expressed as mean ± standard deviation using OriginPro 8.5. When drug concentration increases from 0 to 50 μM, rNDFs of LNCaP cells increase from 0 to 5.2, and those of HeLa cells increase from 0 to 5.9 at normal conditions; those of LNCaP cells increase from 0 to 4.4, and those of HeLa cells increase from 0 to 4.1 at hypoxic conditions. The results show that drug effect is reduced at hypoxic conditions. CPT-11 induces more DNA damage in LNCaP cells than doxorubicin and VP-16; VP-16 induces more DNA damage in HeLa cells than CPT-11 and doxorubicin. Thus, LNCaP cells and HeLa cells are more susceptible to CPT-11 and VP-16, respectively. These results suggest that single cell halo assay can differentiate DNA damage level at hypoxic and normoxic conditions after exposure to drug. Single cell halo assay is potential to be a low cost point-of-care platform for personalized screening of therapeutics at clinics.

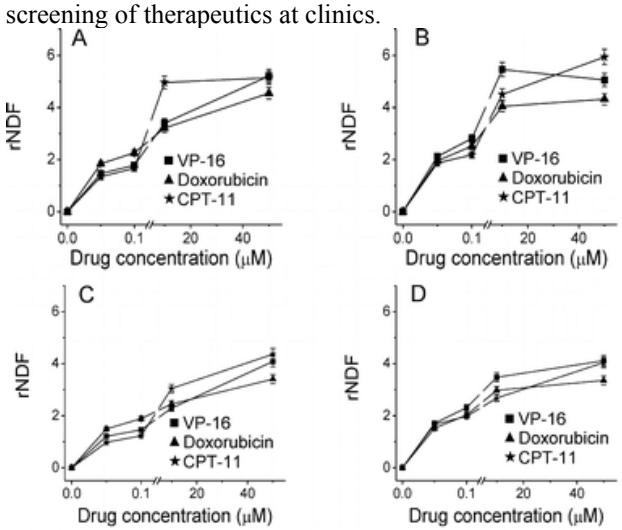

Fig. 2 Drug concentration dependent DNA damage under hypoxia and normoxia. NDF values of LNCaP (A) and HeLa (B) cells treated with three drugs at normoxic conditions, and those of LNCaP (C) and HeLa (D) treated at hypoxic conditions.