Molecular characterisation of drug-tolerant persisters cells to overcome chemotherapy induced relapse in breast cancer


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Background
The majority of primary breast cancers respond to chemotherapeutic intervention. However, years or even decades later many women suffer from relapse, which gradually lose drug sensitivity until ultimately resistance develops. To prevent these, we aim to investigate the few cancer cells, which withstand chemotherapy, without being resistant a priori. These cells are called ‘drug tolerant persister cells’ (DTPs).

Hypothesis
Chemotherapeutic drug treatment bequeaths a rare surviving quiescent persister cell population, which represents a lurking reservoir of surviving cells. These cells eventually give rise to relapsing tumours and potentially constitute the causative factor for drug resistance.

Aims

AIM 1: In vivo identification and tracking of DTPs

AIM 2: Morphological characterisation of DTPs

AIM 3: Molecular and functional characterisation of DTPs

AIM 1: The successful generation of a polyclonotic lentiviral construct expressing the AkaLuc luciferase and the fluorescent reporter protein mCherry (mCA) allowed the transduction of murine breast cancer organoids (KB1P) resulting in the mCA-KB1P model for the non-invasive intravital detection and tracking of rare DTP cells in living mice.

AIM 2: Live cell imaging pilot studies of the DTP cell phase showed dynamic changes of the motility and morphology of DTPs and recorded their reawakening after a dormancy period of 15 days. We generated a cell line with fluctuency and cytostasis, which enables in vitro DTP tracking and cell size determination of DTP cells. This will allow the morphological characterisation and classification of different DTP cell subpopulations.

AIM 3: RNA-sequencing of drug-naive, DTP and repopulated cells shows the distinctiveness of the three populations, suggests subpopulations within the DTP cell pool and reveals a transcriptional DTP signature. We validated selected DTP marker genes which will be used to investigate the fate of the DTP cells both in vitro and in vivo. The determination of potential key regulators of the DTP cell phase will help to identify promising drug targets.

Conclusions
Summarising, we have established a non-invasive in vivo imaging tool, which will be used to identify and track DTP cells in living animals. Moreover, our live cell imaging approach revealed the plasticity of the surviving cells after chemotherapy. This will allow sub-classification of DTP cells to identify cells with the potential of re-entering the cell cycle and thereby giving rise to proliferating colonies. The determination of a DTP cell specific transcriptional signature will help to identify potential drug targets and will contribute to unveil the Achilles’ heel of the few surviving DTP cells to eventually overcome chemotherapy induced relapse in breast cancer.

Further sources
http://resources.pinktamer.ch/labi
https://oncology.imperial.ac.uk/Spectrum/CL-Hero.png
https://bowendge.com

Literature cited

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Figure 1: Engraftment of a non-invasive in vivo imaging tool. (A) Human breast cancer organoids are successfully generated in syngeneic transplantation in mCA-Cherry-plated mice. Station plasent shows KB1P organoids expressing the fluorescent reporter, mCA-Cherry. (B) intravital AkaLuc imaging – 3 cells in a plate-based therapy trial. (C) Fluctuating mCA-KB1P organoids retain their original size. Left panel shows a control KB1P-transplanted mouse and four mCA-KB1P-transplanted mice at day 10 after primary fat pad transplantation of 5×106 cells. The live signal was an excellent signal of the substance. Right panel shows the mCA-KB1P-transplanted mice at day 10 after transplantation.

Figure 2: Phenotypic characterisation of DTP cells. (A) HD-IMR-90 human breast cancer cells were treated for 7 days with a 500 nM drug concentration. Surviving cells were transplanted subcutaneously on day 1 to the 0.5 cm drug inoculum. (B) MCF7-KCl cells from a control, untreated condition were treated with 0–500 nM drug concentration. Drug survival above 0.1% is determined by live cell imaging that the cell cycle and starts forming a colony. Images were taken from day 1 to day 19 of drug treatment.

Figure 3: Molecular characterisation of DTPs. (A) RNA-seq analysis identifies transcriptional profiles for DTP and repopulated cells. Up-regulated genes are depicted in red and down-regulated genes are in blue. (B) Immunohistochemistry using anti-mCA and anti-mCherry antibodies reveals spatially distinct expressions of DTP marker genes. mCA-marked subpopulation in the DTP cell phase comprises 3 of 10 human naïve organoids. Each change (FC) values of the DTP survival are shown in each group.