Pachinko Biology: Gambling on Single Cells

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***Abstract*-- While stem cells hold great promise as a means of treating otherwise incurable disease due to their characteristic ability to both self-renew and differentiate, they can also play a role in disease. Chronic myeloid leukemia (CML) arises in CD34+ hematopoietic stem, and is known resistant to the tyrosine kinase inhibitors imatinib and dasatinib. Understanding the mechanism which rescue particular fractions of cells from drug-induced death requires analysis at the single cell level. We demonstrate the ability to assay the response of normal and CML stem cells to dasatinib, a small molecule drug approved for treatment of imatinib resistant CML, within a novel microfluidic platform. Dynamic, on-chip three-color viability assays reveal that difference in responses of normal and CML cell to dasatinib are observed even in the early phases of exposure, during which normal cells exhibit a significantly elevated cell death rate as compared to both controls and CML cells. However, even in the CML fraction, dasatinib markedly reduces cell migratory behaviors in all but five percent of cells. This resistant fraction may hold the key to understanding CML persistence following chemotherapeutic assault. This work demonstrates the potential utility of single cell microfluidic platform in stem cell biology.**

I. INTRODUCTION

Possessing the unique ability to both self-renew as well as differentiate into multiple cell phenotypes, stem cells hold enormous potential to one day provide a means of treatment for otherwise incurable diseases such as neurological disorders, diabetes, and cancer. Thus, great importance is laid upon understating the cellular mechanisms which mediate stem cell function. In addition to providing a means to advance medical handling of difficult to treat diseases, this knowledge is also necessary for providing insight into stem cell based illnesses.

Chronic myeloid leukemia (CML) arises from a chromosomal abnormality in CD34+ hematopoietic stem cells. These are primitive cells that can differentiate into any hematopoietic cell depending upon the growth factors to which they are exposed, resulting in the formation of the BCR-ABL oncoprotein. [1, 2] This adult-onset leukemia can be forced into remission using the tyrosine kinase inhibitors, imatinib (Gleevec) and/or dasatinib (Sprycel), although a fraction of primitive, quiescent cells persist despite chemotherapeutic assault. [3] The signaling pathways which enable survival are still unknown, but are of great interest in terms of identifying suitable treatment strategies for CML as well as understanding basic stem cell biology. As such, it is necessary to examine signaling dynamics on the single cell level in order to identify variations in cellular characteristics which protects certain cells, but not others, against drug-induced cell death.

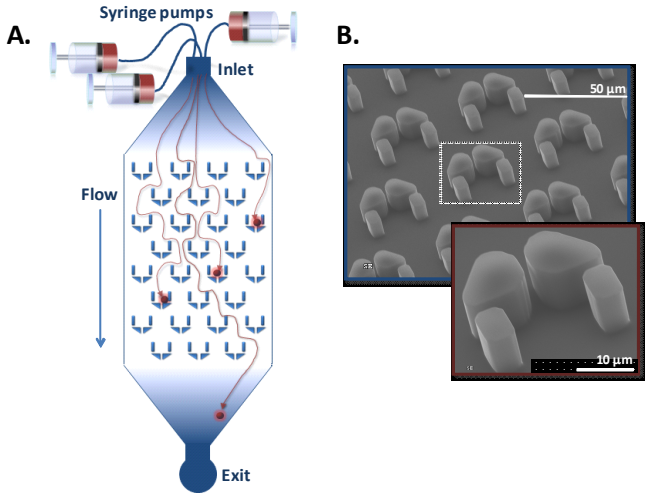

Figure 1. Platform illustrations. (A) Graphic representation of microfluidic device concept, including illustration of cell trapping by hydrodynamic flow. Actual device contain upwards of 440, 18 µm x 18 µm x 10 µm cell traps. **(B)** Scanning electron microscope images of

Microfluidic based cell culture platforms are inherently well-suited for working with populations of rare cell fractions. The microfluidic platform utilized in this paper is based upon one described previously[4, 5] and which is specifically designed to accommodate hundreds of individual, non-adherent cells in parallel via a passive trapping mechanism that functions not unlike capturing balls in a pachinko game. As opposed to bench-top protocols which call for millions of cells per assay, each microfluidic experiment takes place in the microdevice, with a total volume of approximately 20 nl and requires only hundreds of cells and microlitres of reagents. Given the limited quantities of CD34+ hematopoietic stem cells collected from patients, performing experiments using the microfluidic platform not only provides the advantage of single cell resolution, but also the ability to achieve multiple assays on

the same cohort of sampled cells. These attributes make the application of microfluidic technology to study stem cell signaling events as a means of gaining new insight into single cell dynamics.

II. RESULTS AND DISCUSSION

Monitoring stem cell division

The ability to observe stem cell proliferation is significant for two reasons. First, continual proliferation indicates that the microfluidic environment is well-equipped to support long-term cell culture. Indeed, both normal and CML stem cells were observed to proliferate whilst trapped in the device. Viability studies similar to those reported previously [4] were carried out resulting in similar cell survivability rates compared to primary CD4+ T cells, but with the optimal flow rate of 50 to 80 nl/min. Secondly, with regard to basic scientific relevance, stem cells are unique in their ability to both self-renew and differentiate into more specialized cell phenotypes. It is theorized that divisions resulting in differentiated cells may be asymmetric in terms of distribution of cell mass between parent and daughter cell. [6, 7] Using TMRM (tetramethyl rhodamine methyl ester) to label mitochondria and CFSE (carboxyfluorescein succinimidyl ester) to label cytoskeletal elements, largely symmetric divisions were observed in both normal and CML stem cells within the microfluidic device. Future studies will focus upon even more specific markers such as CD34 and Numb for indicating self-renewal and differentiation, respectively.

Dynamic viability assay

Dasatinib treatment has been shown to bypass apoptosis in CML stem cells and cause cell necrosis in all but a primitive, quiescent fraction of cells. [3] To better understand how some cells are able to escape dasatinib-induced cell death, we analyzed individual cells to identify mechanisms which enable their survival.

In order to understand the time course of apoptosis events in arrays of single stem cells, we exposed the cells to cytotoxic reagent. For example, Figure 2 shows CML cells, dynamically labeled with Annexin-V and propidium iodide within the device whilst exposed to 10% DMSO (dimethyl sulfoxide, known to induce apoptosis) in complete media. Further trials included analysis of cells exposed to chemotherapeutic agent, dasatinib.

Normal cells undergo apoptosis in the presence of 150 nM dasatinib as demonstrated by the transition from Annexin-V staining to propidium iodide. After approximately 60 minutes, normal cells begin to die rapidly such that nearly 50% were dead after two hours. CML cells are more resistant as a population to the effects of dasatinib compared to that of normal cells. In fact, the cell viability data for CML cells exposed to dasatinib are indistinguishable from those of control populations within three hours. This apparent resistance to drug effects might be attributed to additional activated survival pathways in the leukemic cells rendering these cells less vulnerable to the effects of the drug. These results suggest that TKI naive CD34+ CML stem/progenitor cells are resistant to the effects of dasatinib from first exposure. The exact mechanisms that underlie this resistance, however, remain under investigation.

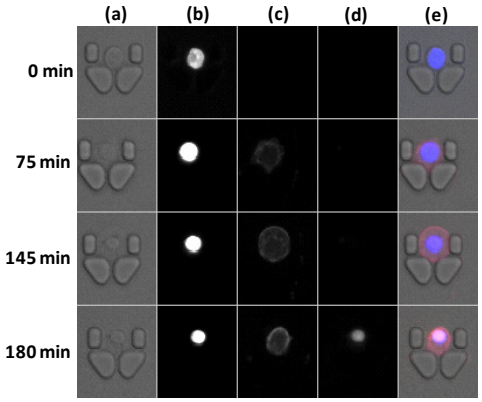

Figure 2. Dynamic viability assay. CML cells (a) exposed to 10% DMSO and (b) Hoescht to label nuclei, (c) annexin-v, (d) and propidium iodide to monitor cell apoptosis and death. Combined fluorescent and bright field images shown in (e).

Analyzing cell motility characteristics

Observation of cells within the device under control conditions revealed that CML cells are highly motile and can exert large mechanical forces in order to migrate against fluid flow (as high as 80 nl/min) and squeeze through gaps as small as 2 μm in diameter. The presence of dasatinib, however, markedly reduces cell motility in CML cells (data not shown). Only five percent of CML cell exposed to dasatinib exhibited highly migratory behavior, defined as actively migrating out of a PDMS trap against fluid flow (not to include random movements within the trap), as opposed to upwards of 30% of cells in the absence of drug. Dasatinib is known to be a potent, dual Abl/Src kinase inhibitor and src kinases are known to play a role in actin organization and enhancing cell motility. [9, 10] Studies have shown that dasatinib specifically interrupts cell motility in other forms of cancer. [11-14] It is likely that a similar process occurs in CML cells; a subject currently under investigation. Whilst these cells are resistant to apoptosis, dasatinib does exert a significant inhibitory effect upon cell motility by blocking src kinase mediated activity. One hypothesis is that the fraction CD34+ CML stem/progenitor cells whose migratory behavior is left uninhibited by dasatinib is capable of migrating to HSC niches within the bone marrow where, in the hypoxic microenvironment, they become deeply quiescent and resistant to the effects of the drug. These cells would then account for the persistent molecular disease and rapid relapse upon drug therapy withdrawal, thus preventing complete remission.

III. CONCLUSIONS

A novel microfluidic platform previously validated for application towards non-adherent cells was applied to study patient-derived, hematopoietic stem cells and their response to dasatinib assault. This body of work details the successful on-chip implementation of dynamic cell proliferation, viability, and motility assays which are otherwise difficult to perform using conventional techniques.

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