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Chemotherapy impedes *in vitro* microcirculation and promotes migration of leukemic cells with impact on metastasis

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ABSTRACT

Although most cancer drugs target the proliferation of cancer cells, it is metastasis, the complex process by which cancer cells spread from the primary tumor to other tissues and organs of the body where they form new tumors, that leads to over 90% of all cancer deaths. Thus, there is an urgent need for anti-metastasis therapy. Surprisingly, emerging evidence suggests that certain anti-cancer drugs such as paclitaxel and doxorubicin can actually promote metastasis, but the mechanism(s) behind their pro-metastatic effects are still unclear. Here, we use a microfluidic microcirculation mimetic (MMM) platform which mimics the capillary constrictions of the pulmonary and peripheral microcirculation, to determine if *in-vivo*-like mechanical stimuli can evoke different responses from cells subjected to various cancer drugs. In particular, we show that leukemic cancer cells treated with doxorubicin and daunorubicin, commonly used anti-cancer drugs, have over 100% longer transit times through the device, compared to untreated leukemic cells. Such delays in the microcirculation are known to promote extravasation of cells, a key step in the metastatic cascade. Furthermore, we report a significant ($p < 0.01$) increase in the chemotactic migration of the doxorubicin treated leukemic cells. Both enhanced retention in the microcirculation and enhanced migration following chemotherapy, are pro-metastatic effects which can serve as new targets for anti-metastatic drugs.

1. Introduction

Although chemotherapy drugs target and kill malignant cells during cancer treatment, there is emerging evidence that such drugs inadvertently promote metastasis [1–3]. Yet, it is metastasis, a complex multistep process that leads to death in over 90% of cancer cases [4,5]. In spite of the very wide variety of cancers with respect to their molecular biology, pathogenesis and prognosis [6], metastasis occurs in all cancers. Thus, there is an urgent need for anti-metastasis therapy [7]. Incidentally, cellular mechanical properties have been shown to be good markers for metastatic potential of cancer cells [8,9] and can be used for diagnosis of cancer itself [10–12]. Furthermore, chemotherapeutic drugs alter the mechanical properties of cells during cancer treatment [13]. We therefore posed the question: can chemotherapeutic drugs alter cell mechanical properties in ways that might promote metastasis?

This question is important for several reasons. Firstly, the therapeutic index of anti-cancer drugs will have to go beyond immediate cytotoxicity tests to encompass their effects on the cellular mechanome [14,15] since the latter have been shown to influence and determine crucial cell functions such as differentiation [16–18], division [19,20], immune response [21,22] and migration [23–25]. Secondly, the question posits the possibility of having cell mechanical properties as therapeutic targets in the context of the ongoing search for effective anti-metastasis drugs [7,12]. Thirdly, the question is capable of eliciting answers that increase our understanding of metastasis especially the connections between deterministic biochemical approaches (genes and proteins responsible for metastatic genotypes and phenotypes [26]) and stochastic results [27].

To address the question, we considered the fact that the main pathways for circulating tumor cells (CTCs) during metastasis are blood vessels [28,29], and so we developed and validated a

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microfluidic device that mimics the hematogenic microcirculation in terms of attributes such as the presence of tens to hundreds of constrictions as found in microcapillary beds *in vivo*. This device, the microfluidic microcirculation mimetic (MMM) [30,31], enables the advection of cells through 187 capillary-like constrictions, thereby effectively replicating the tortuous journey of cells in microcirculatory compartments such as those of the lungs. Furthermore, disseminating tumor cells (DTCs) must be competent in migration, including the ability to intravasate and extravasate, in order to metastasize and form new tumors [28,29]. Hence, we have used standard migration assays to compare the migratory abilities of chemotherapy treated and untreated cancer cells, in order to assess the metastatic impact of the mechanical changes induced by drugs.

We chose the drugs, daunorubicin (Dauno) and doxorubicin (Dox), both anthracyclines [32], because they are commonly used in the clinic against several kinds of cancers including breast, lung and ovarian cancers, malignant melanomas and leukemia [13,33]. Such anthracyclines are known to cause actin network reorganization leading to stiffening of cells [13]. It has been suggested that such stiffening might contribute significantly to vascular complications by obstructing microcirculation, thereby accounting for leukostasis: the accumulation of leukemia cells in the vasculature of vital organs such as the lungs and the brain, sometimes resulting in respiratory failure and intracranial hemorrhage [13] and death in 69–74% of leukemia patients who develop vascular complications (usually diagnosed via autopsy) [12]. Intriguingly, we find, using our *in vitro* assays, that daunorubicin and doxorubicin impede microcirculation by inducing over 100% increase in the transit times of HL60 leukemic cells. Surprisingly, the chemotactic migration of doxorubicin-treated HL60 cells is significantly ($p < 0.01$) enhanced. The implication is obvious: some chemotherapeutic drugs can indeed alter cell mechanical properties in ways that enhance various steps in the metastatic cascade such as localization for extravasation and then migration.

2. Materials and methods

2.1. Cell culture

The ATCC CCL-240 strain of HL60 cells (a promyelocytic leukemia cell line derived from an acute myeloid leukemia patient [34,35]), which we used, was purchased from ATCC and cultured in an incubator maintained at 95% air; 5% CO_2 and a temperature of 37 °C. The growth medium was RPMI 1640 (11875093, Life Technologies), supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin. Cultures were maintained by the addition of fresh medium, between 1×10^5 and 1.5×10^6 cells/mL. Corning® T-75 flasks and T-25 flasks were used for sub-culturing. This required diluting the culture every 2–3 days. The HL60 cells are non-adherent cells which grow in suspension and therefore mimic the circulatory phase of cancer cells. Moreover, current *in vivo* models of acute myeloid leukemia based on the use of patient samples are not easy to establish and manipulate in the laboratory and so HL60 continues to be used as a robust model cell line for assessing the efficacy of chemotherapeutic agents [36]: hence, our choice of HL60 cells.

2.2. Pharmacological interventions

The main drugs used clinically for anticancer therapy are anthracyclines, platin-based drugs, and various taxanes [3]. These cytotoxic drugs are sometimes the only therapeutic modality for advanced, refractory, and metastatic tumors as well as for those cancers lacking a specific molecular target [3,7]. The first line induction therapies for leukemia involve the use of anthracyclines [13,32] and the major anthracyclines are doxorubicin (or adriamycin) and daunorubicin (or daunomycin). We used doxorubicin (Sigma 4458) at a final concentration of 5 μM [37,38] and daunorubicin (Sigma D8809) at a final concentration of 1 μM [13,32].

2.3. Microfluidic microcirculation mimetic (MMM)

Modeling the capillary constrictions that impose stretches on circulating cells *in vivo*, the MMM is a polymer-based device with inlets and outlets linked to reservoirs (Fig. 1). Unlike existing models of the microcirculation, the MMM device does not involve a network of channels. Rather, it is designed from the point of view of the cell: the cell experiences a series of capillary constrictions (here, 187) as they advect through the vasculature, branched or unbranched. Thus, the MMM is a serial model with a large number of repeating constrictions, allowing us to rapidly measure the transit times of hundreds of cells in less than 10 min, while watching in real time the fate of those cells during the advection. Previously, a precursor of the MMM device (which had only one rectangular constriction) tested the stiffness of monocytes, neutrophils and macrophages in the context of stem cell differentiation [16]. To mimic the advection of cells in capillaries, many of which have constrictions smaller than blood cell diameters, we developed the MMM in four main steps: (1) conceptualization/drawing, (2) printing of photomask, (3) photolithography to produce master moulds and (4) soft lithography for replica moulding. Details have been reported in our recent works [16,30,31] and also included in supplementary information. Additionally, to mimic the pluronan-rich surface of the vascular endothelium, we added Pluronic F-127 acid (final concentration 0.1% with doxorubicin and 1% with daunorubicin) to the cell suspension [39].

2.4. Morphometry, migration assay and viability tests

These are described in the supplemental information including Supplementary Fig. S1.

3. Results

3.1. Chemotherapy leads to morphological changes before cell death

Using phase contrast microscopy and standard image segmentation algorithms, we monitored the morphology of HL60 cells from induction of chemotherapeutic drugs ($t = 0$ h) till significant cell death ($t = 12$ h). In addition to well-known cytotoxic effects of

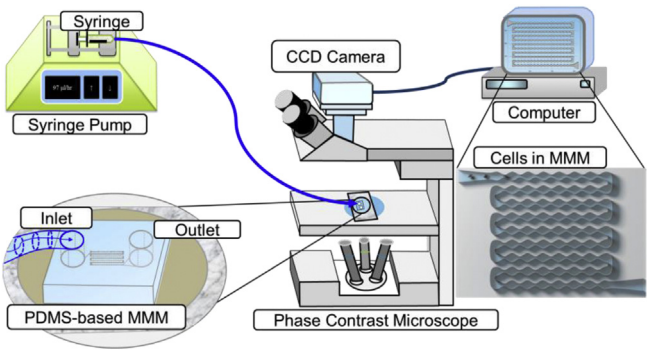

Fig. 1. Schematic of the microfluidic microcirculation mimetic (MMM). A suspension of cells in a syringe is placed on a syringe pump, connected by tubing to the PDMS-based MMM chip and placed on a phase contrast microscope equipped with a CCD camera. The camera is connected to a computer which enables the monitoring and running of the device, data collection and analysis.

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