Perspective: Flicking with flow: Can microfluidics revolutionize the cancer research?

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(Received 8 November 2012; accepted 20 December 2012; published online 31 January 2013)

According to the World Health Organization, cancer is one of the leading causes of death worldwide. Cancer research, in its all facets, is truly interdisciplinary in nature, cutting across the fields of fundamental and applied sciences, as well as biomedical engineering. In recent years, microfluidics has been applied successfully in cancer research. There remain, however, many elusive features of this disease, where microfluidic systems could throw new lights. In addition, some inherent features of microfluidic systems remain unexploited in cancer research. In this article, we first briefly review the advancement of microfluidics in cancer biology. We then describe the biophysical aspects of cancer and outline how microfluidic system could be useful in developing a deeper understanding on the underlying mechanisms. We next illustrate the effects of the confined environment of microchannel on cellular dynamics and argue that the tissue microconfinement could be a crucial facet in tumor development. Lastly, we attempt to highlight some of the most important problems in cancer biology, to inspire next level of microfluidic applications in cancer research. © *2013 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4789750]

I. INTRODUCTION

Cancer is the plague of our time. In 2008 alone, more than seven and half million people died of cancer worldwide, which accounted for nearly 13% of the total deaths that occurred in the same year.[1] Manifestation of the disease is still intriguing to the researchers, while its generic treatment strategy remains an elusive dream. In the beginning, and in the middle of the last century, cancer did not, however, get the required attention of the scientists, which it receives today. All that changed when in 1971, then the President of the United States of America, Richard Nixon, implemented the National Cancer Act and declared "The War on Cancer."[2] Yet, it would take another thirty years, very much to the beginning of this millennium, for cancer research to get organized and to have the first consensus generalized picture of the disease out of its conspicuously diverse appearances. In 2000, to isolate the pharmacologically targetable commonalities among different forms of cancer occurring in virtually all of the human organs, two scientists, Douglas Hanahan and Robert Weinberg, published a review article, titled "The Hallsmarks of Cancer."[3] In this article, they attempted to assort the essential characteristics of cancer that enable the disease to develop and spread over the body, in only six hallmarks.[3] According to the list, successful cancer cells need to sustain perpetually proliferative signaling, evade the inherent growth suppression machinery of the cell, resist the programmed cell deaths as by apoptosis, enable replicative immortality by minimizing senescence, induce angiogenesis (new blood vessel formation) within the tumor mass, and lastly invade the

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surrounding tissue and propagate to distant organs by a process known as metastasis (Greek word meaning "next placement"). With these hallmarks identified and characterized, cancer research was now endowed with a logical framework, and it accelerated unprecedentedly. In the ensuing decade, being bolstered further by the knowledge available from the human genome project, progresses in molecular genetics and advancements in live-cell microscopy techniques have enabled the identifications of signaling pathways that transform the a normal cell mass to a cancerous one. In 2011, Hanahan and Weinberg reviewed the progress in cancer research, a decade after the first hallmarks were published, and added two new hallmarks to the original list—reprogramming of energy metabolism and evading immune destruction, and concluded that the "tumor microenvironment" could be the most critical factor regulating the tumor development.[4] By then, several molecular targets for anti-cancer therapy have been marked.[5–8]

Towards the end of last millennium, similar revolution was also happening in the field of microfabrication and miniaturization. A series of seminal papers from the research groups of George Whitesides and others showed that the fluid mechanics at low Reynolds number regime could be useful to analytical chemistry and biology.[9–11] The field microfluidics was born. Supported by then a newly developed rapid prototyping technique called softlithography, the research in microfluidics advanced enormously, just like the cancer research at the same time.[12–16] In next few years, microfluidics provided a range of microdevices where a single cell could be isolated in a controlled microenvironment. In several such devices, different segments of a cell could be treated with different chemicals, scarcely few, rare cell types could identified from a population, and chemical gradients of different shapes could be created with unparalleled spatiotemporal specificity.[17–21] Such extreme feats of cell manipulation were believed to be impossible under the framework of the conventional, macrofluidic cell culture systems.[22] Availability of this new tool for cell culture and manipulation did not go unnoticed by the cancer researchers, and microfluidics made its entry into cancer biology.[23]

In this article, we will first review (Sec. II) the progress in microfluidic research in presenting *in vitro* cancer models, fabricating detection/diagnostic tools, designing assay systems, and facilitating anti-cancer treatment. We will not, however, limit ourselves to the review only, as there are good articles already available on that subject.[23–25] Rather we will attempt to enumerate most of the current problems in cancer research, in both biochemical and biophysical dimensions, and in some cases, we will even attempt to offer clues, albeit presumptive, on how tools of microfluidics could be used to solve them. To this end, we will first highlight the biophysical aspect of cancer (Sec. III), which is gaining remarkable attention, since both physical and chemical facets of tumor microenvironment are coming out to be crucial in tumor development. Then, we will move on to demonstrate how microfluidic devices present fundamentally different environment to the biological cells than any other existing cell culture system does (Sec. IV). There, we will emphasize on the effect of microconfinement on cellular dynamics, and its implications in oncology. Finally, before concluding on a positive note, we will try to catalog some of the most fundamental and challenging problems in cancer biology (Sec. V), so as to inspire the next generation of microfluidic applications in cancer research.

II. TUMORS ON CHIP

A. Tumor microenvironment and microfluidic cancer models

In the previous decade, conventional concepts in cancer biology have undergone a total revision. Traditionally, tumors were believed to be a pure mass of cancer cells, all of them trying to proliferate at a vigorous rate, sometimes causing the displacement of the surrounding tissue. We now know that a tumor is as complex and diverse as any other normal tissue.[4,26,27] In addition to cancer cells, a typical solid tumor, especially the one that is at advanced stage, may contain cancer-associated fibroblasts, tumor-promoting inflammatory cells, endothelial cells, and pericytes, all contributing to the process of cancer progression (Fig. 1). Even all cancer cells are not alike, but only few of them are the active players of proliferation and tissue invasion.[28–30] This revelation has led biologists to recognize the importance of tumor microenvironment in oncogenesis. We should clarify here that by tumor microenvironment, we mean the

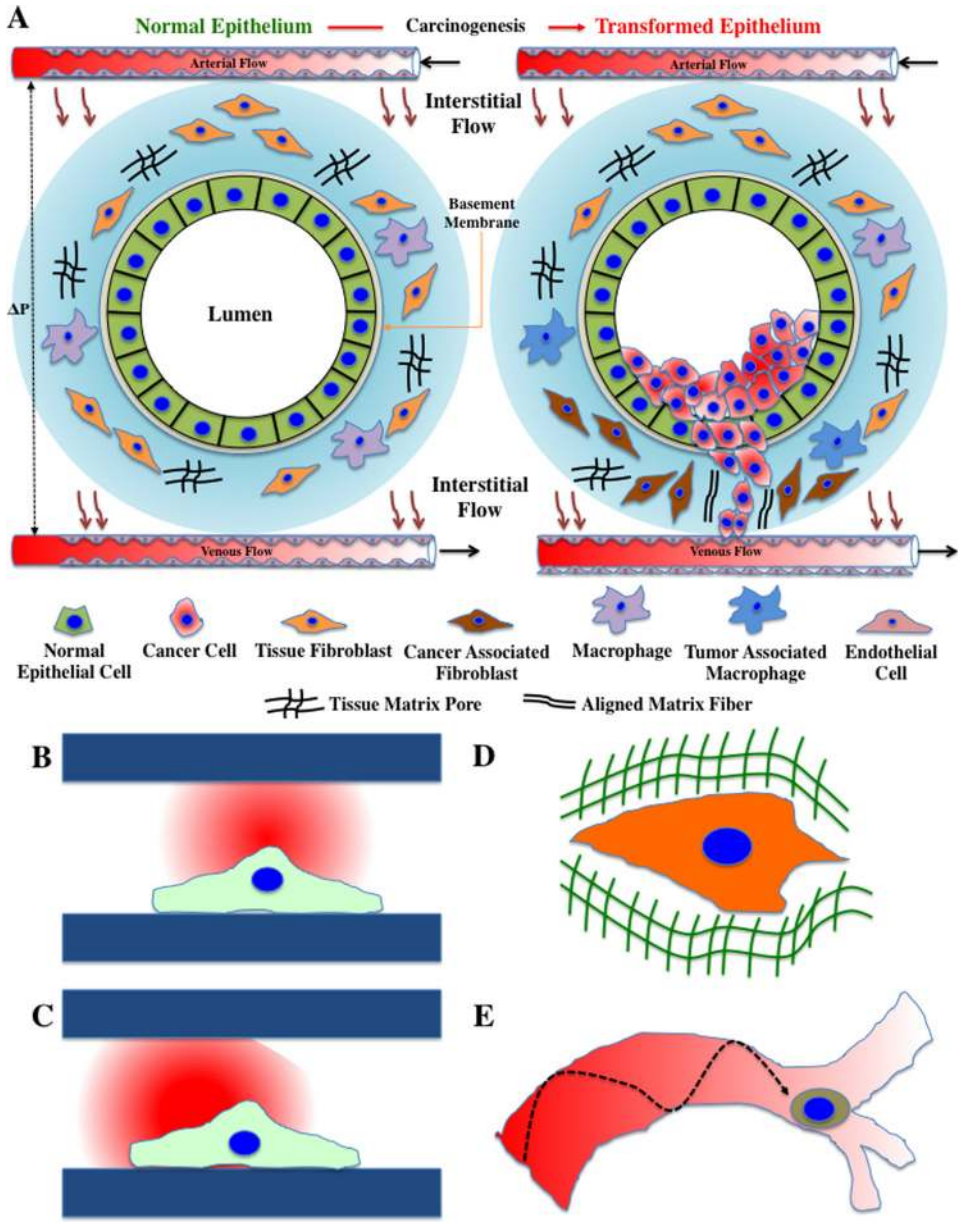

FIG. 1. *Tumor microenvironment and confinement*. (a) Typical architecture of normal and transformed epithelial tissue. Other than the cancer cells, the tumor microenvironment is composed of several non-cancer cell types including cancer associated fibroblasts (CAFs) and tumor associated macrophages (TAMs). These help the cancer cells to become invasive and move towards the blood vessel. Also the interstitial flow, which originates because of the pressure gradient (ΔP) between arterial and venous flow, could facilitate the directional migration of cancer cells. Lastly, either the cancer themselves or the other tumor associated cells can align and bundle the matrix fiber to make a track for the migrating cancer cells. (b) Concentration distribution of secreted growth factor in a confined environment, and (c) the same in the presence of a flow, directed right to left. Even a small magnitude flow can bias the growth factor in one direction, which could lead to autologous cell migration. (d) Migrating cancer cell in a confined tissue environment. (e) Cancer cell within confined environment of circulatory system.

microscopic composition of the tumor including spatial distribution of different cell types, chemical nature of the extracellular matrix, concentration of different secreted proteins (e.g., growth factors, enzymes), and distribution of physical forces. While using conventional cell culture systems, effects of these microenvironmental factors cannot be isolated individually, microfluidics offers novel opportunities to achieve controllable physicochemical conditions. Such is the importance of this progress in understanding of cancer progression and in high-throughput

screening of anti-cancer drugs that a large number of microfluidics based *in vitro* cancer model systems have been proposed in recent years (Fig. 2). For example, researchers have developed a microcell cell culture analog (microCCA) in which metabolism-depended cytotoxicity of anti-cancer drugs can be studied. This device enables multiple cell culture in a three-dimensional (3-D) hydrogel system and therefore, simulates the metabolic functions and multicellular interactions of different organs.[31] For the studies devoted to understand pharmacokinetics and pharmacodynamics of anti-cancer drugs, microCCA could be a useful *in vitro* analytical tool. Other similar studies attempting to mimic the tumor microenvironment have enabled real time monitoring of distribution of drug concentration and cell deaths in a tumor mass.[32–34] Using a microfluidic tumor model, researchers have investigated how macrophage cells invade a breast tumor system, and how this invasion depends on composition of the extracellular matrix environment within the tumor.[35] In breast cancer, the transition of otherwise benign tumor cells to invasive phenotypes is a crucial step towards metastasis, and this process is known to depend on the activity of the fibroblast cells that form the layer beneath the growing tumor.[36] Nevertheless, how exactly fibroblast cells influence the transformation of cancer cells, and what kind of signal

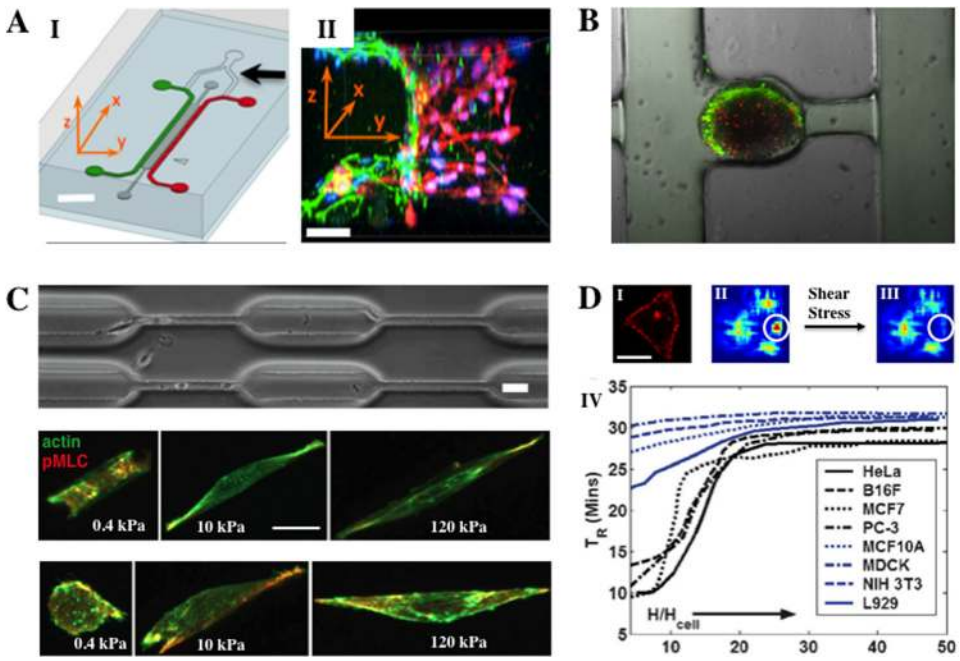

FIG. 2. *Microfluidic devices in cancer research.* (a) Microfluidic Model of Tumor-Vascular interface. I. A 3-D extracellular matrix (ECM) channel (dark grey) separates the tumor channel (red) and the endothelial channel (green). Scale bar is 2 mm. II. A 3-D confocal image showing ECM invasion of the tumor cells and their adherence to the endothelium. Scale bar is 30 μm. Images reproduced with permission from K. Zervantonakis, S. K. Hughes-Alford, J. L. Charest, J. S. Condeelis, F. B. Gertler, and R. D. Kamm, Proc. Natl. Acad. Sci. U.S.A. **109**(34), 13515-13520 (2012). Copyright 2012 National Academy of Sciences, USA. (b) Microfluidic Device for Spheroid Entrapment and Drug Screening. Overlapped differential interference contrast and fluorescence confocal image showing the entrapment of an ovarian cancer spheroid and the distribution of live (green) and dead (red) cells within the spheroid. Cell death occurs mostly at the center. Trap width is 500 μm, while the width of the subsequent narrow neck region is 200 μm. (c) Microfluidic platform to study independent regulation of tumor cell migration by matrix stiffness and confinement. (Top) Phase contrast image of device fabricated from 120 kPa polyacrylamide hydrogel and containing consecutive wide (width = 40 μm) and narrow (width = 10 μm) sections. Scale bar 40 μm. Variation of cell morphology with the changes in matrix stiffness in narrow (middle row) and wide (bottom row) sections. Scale bar is 20 μm. Images reproduced with permission from A. Pathak and S. Kumar, Proc. Natl. Acad. Sci. U.S.A. **109**(26), 10334-10339 (2012). Copyright 2012 National Academy of Sciences, USA. (d). Confinement increases the stress response speed of cancer cells. I. Fluorescence image of a HeLa cell, cultured inside a microchannel, and labeled for membrane lipid rafts. II. Cell-substrate Traction force landscape before the application of shear stress, and III, the same after the application of shear stress. Flow direction is right to left. Cell loses adhesion in upstream section. Scale bar is 10 μm. IV. Confinement, represented by channel height to cell height ratio, decreases the time taken by the cells to respond to shear stress. This decrease is more prominent in cancer cells (top four in the legend) than in normal cells (bottom four). Images reproduced with permission from T. Das, T. K. Maiti, and S. Chakraborty, Integr. Biol. (Camb) **3**(6), 684–695 (2011). Copyright 2011 The Royal Society of Chemistry.

transmission (soluble through fluid medium or physical through intercellular contact—mediated cancer cell-fibroblast interaction) could not be known using the conventional cell culture models. A compartmentalized microfluidic device has been shown to overcome this limitation.[37] Using this device, researchers were able to precisely control the separation between mammary epithelial (from which the breast cancer cells arise) and fibroblast cell populations and clearly show that transition to invasive phenotype requires these two cell types to interact physically.[37] Moreover, in this device, they could also monitor the active modification of extracellular matrix. In future, implications of such compartmentalized devices could be tremendous in studying several others physiologically important interactions between disparate cell types. Very recently, a three-dimensional microfluidic model has been proposed for studying the entry of tumor cells into blood vessels (intravasation), and how this process is influenced by interaction between tumor cells and tumor-associated macrophages (TAMs) (Fig. 2(a)).[38] Macrophages are originally cells of the innate immune system of peripheral tissues where they engulf and thus, clean the debris of dead parasites. They also infiltrate the tumor tissue, but surprisingly, instead of restricting the tumor growth, they actually help the process of tissue invasion and intravasation by supplying the cancer cells with essential growth factors and signaling molecules.[39,40] In the aforementioned microfluidic device, authors attempted to divulge the microscopic dynamics of the vicious cycle that ran between tumor cells and macrophages.[38] They showed that though originally the cells constituting the linings of blood vessels or the endothelial cells form an insurmountable barrier to restrict the intravasation of tumor cells, the presence of macrophages, or the signaling molecule they release, is sufficient to increase the barrier permeability towards successful intravasation. As we will illustrate in detail later in this article, the journey of a cancer cell from its original tissue (e.g., breast) to distal organs of body (e.g., bone and liver) is a multi-step process, each of steps depending critically on the corresponding physicochemical microenvironment and on the interactions among numerous cell types.[41–43] With microfluidic system at our disposal, we are probably just beginning to explore this mostly unfathomed territory in a methodical way.

B. Three dimensional spheroid culture model for drug screening

For *in vitro* cancer models, culturing the cells in three dimensions crucially perturbs the way the cells respond to chemotherapeutic agents.[44] Compared to the cells cultured in conventional two dimensional monolayer, cells grown in three dimensions do not enjoy a homogeneous microenvironment. In a three dimensional culture with feature-radius exceeding 400 μm, concentrations of available oxygen, metabolites, or therapeutic agents form inward gradients while that of carbon-di-oxide or excreted cell waste, or the acidity of extracellular fluid form outward gradient.[45–47] These gradients, and the downstream consequences that follow them, form the basis for the increased drug resistance that may be observed specifically in three dimensional system.[48] Importantly, such gradients do exist within *in vivo* tumor tissues, and they interfere with the efficacy of a chemotherapeutic treatment.[48] Moreover, biochemical nature of cell-cell and cell-extracellular matrix (cell-ECM) contacts and the structural organization of cells differ significantly between two dimensional and three dimensional cultures, which could also be responsible of augmented drug resistance of cancer cells in three dimensional culture.[49,50] Since real tumor tissue is often very complex to analyze, is not available in enough numbers, and histologically varies case-to-case, one of the convenient ways of studying several aspects of it, preserving in dimensional feature, is to create three dimensional aggregate or spheroids of cancer cells (Fig. 2(b)).[51–53] Spheroids are the mimics of avascular (that does not contain blood vessels) solid tumors,[54] and to some extent, they can be used as models for studying increased drug resistance of cancer cells in three dimensions. Moreover, spheroids with sufficiently large radius (>400 μm) show different oxygen, nutrient, and drug concentrations at core and at periphery.[55] High rate of death at core (necrotic core) is also very common for such large spheroids (Fig. 2(b)). While spheroids are useful models to test the efficacy of anticancer drugs in the closest possible approximation of tumor tissues, challenge exists in producing large numbers of spheroids with homogeneous features in high throughput way, often

from small quantity of available cell samples. Spheroids are conventionally produced by culturing cells in hanging droplets, on non-adherent surfaces, in spinner flasks, or in rotary culture vessels.[52,56] All of these methods suffer from low efficiency, low reproducibility, lack of homogeneity among samples, inability to maintain the culture for long time, and incompatibility with real-time imaging and monitoring systems. In recent times, researchers have used microfluidic devices, designed for spheroid culture, to overcome such limitations.[56] Several research groups have developed microfluidic devices with chambers, posts, microbubble array, or microwells to trap cells in cluster, which would eventually grow into spheroids.[57–67] Due to defined geometric features and precisely controlled microenvironmental aspects, spheroids generated in microfluidic devices are more homogeneous in size and characteristics than those generated by conventional methods. Optical transparency of microfluidic devices and the provision for continuous perfusion, on the other hand, have enabled real time monitoring of spheroid growth and response to drug molecules for a considerable period of time. Additionally by co-culturing different tumor associated cells, such as osteoblasts and endothelial cells, along with the cancer cells in a spheroid, researchers have been able to create a model system that bears high similitude with actual tumor microenvironment.[62] Still many challenges remain in this field. For example, microfluidic based spheroid culture devices require passivation of channel surfaces by hydrophobic modification for preventing the cell-surface adhesion that could disrupt the spheroid morphology. Yet, many potential drug molecules have significant absorption affinity to hydrophobic surfaces, and any nonspecific absorption could decrease the effective drug concentration in solution. This problem becomes very critical if sensitivity of cancer cells to small concentration of drug is of prime interest, which is often the case. For this reason, till date, microfluidic spheroid culture devices could not be used in large scale screening of compound library. Any solution to this problem will have big impact for the pharmaceutical industries. Another relevance of testing the drug resistance in spheroid model comes from its internal feature. In tumor tissue, the flow of fluids through tissue matrix, namely, the interstitial flow, has an impact of the transport of drug molecules to cancer cells.[68] While we will discuss about physical and chemical aspects of the interstitial flow in detail later, here we should mention that in tumor tissue, uncontrolled proliferation of cells leads to partial blockage of the flow passages, increasing the flow pressure within the tissue.[69] This pressure then prevents the drug molecules from entering the tissue by convection and reduces the efficacy of the drug.[69,70] Relieving the interstitial pressure by normalizing the tumor vasculature or simply by puncturing the tissue increases the efficacy of chemotherapy many folds.[68] Yet, at present, no spheroid model, conventional or microfluidic, has the provision to account for the effect of interstitial flow on drug delivery and efficacy.

C. Detection, analysis, diagnosis, and treatment

There is a general notion in the cancer research that earlier is the detection of a tumor, better is the treatment outcome. Yet for many cancer types, there is hardly any standardized pathological test for detecting the disease at its early stage and indicating the course of treatment for the best possible outcome (good prognosis). For many others, even if such tests exist, they are not very accurate, being associated with high rate of false positive or undetected cases. There is a prime reason behind this paucity of diagnostic methods. Unlike most of other human diseases, cancer at its early stage rarely (except for few brain tumors) causes any bodily discomfort or shows any sign of its presence. But when cancer cells start metastasizing by migrating up to the nearest blood vessel or lymphatic duct and then riding the circulatory system of the body, the presence of the original tumor could be identified if only we could isolate and characterize the circulating tumor cells (CTCs).[71,72] As compared to regular blood cells such as red blood corpuscle (RBC), white blood corpuscle (WBC), and platelets, CTCs are extremely rare in blood samples. It has been estimated that there could be only one CTC per billion of normal blood cells, even at an advanced stage of cancer.[72] By conventional means, in a blood sample, CTCs are recognized only when they are present in good numbers (that is, when the disease has reached its propagatory stage) and probably have colonized in several other body parts.[73]

While such a diagnosis seldom leads to a good prognosis, microfluidic devices lately have shown some promise in isolating rare cell types from blood.[72] Using antibodies against the epithelial cell adhesion molecules (EpCAM) or other established molecular markers that are believed to be expressed on the plasma membrane of cancer cells but not on the membrane of regular blood cells, researchers were able to enrich the rare CTCs from blood samples.[74–78] Some groups have also used microfluidic versions of flow cytometry devices or microflow cytometers (μFCM) to sample rare cell types.[79] Microscale hydrodynamic, magnetic, or acoustic flow focusing strategies have aided μFCMs to sample atypical cells, which are present in low number.[80–82] Very recently, a method called ensemble-decision aliquot ranking (eDAR) has improved the efficiency of CTC isolation process further.[83] In this method, cell-surface markers are first labeled with fluorescent antibodies.[83] Then they are ranked by aliquots, looking at an ensemble of cells, and finally sorted. Here there are two major problems of antibody based CTC isolation approach. First, except for few cancer types, there is hardly any consensus molecular marker to pin-down a cancer cell. Second, to acquire necessary motile characteristic for invading the blood vessels, cancer cells often undergo a biochemical change called epithelial to mesenchymal transition (EMT). After this transition, cancer cells cease to express the EpCAMs, and therefore, according to the theory of EMT, CTCs should not have EpCAMs on their surface at the first place. In face of these problems, several research groups have contemplated on label-free detection of CTCs.[84–86] A list of various means that are applicable to segregate one specific cell type over others, includes dielectrophoresis, photoacoustic flowmetry, alternating current impedance cytometry, in-flow capacitance cytometry, coherent anti-Stokes Raman scattering cytometry, and time-of-flight optophoresis.[87–91] Any of these methods could hold the key for developing the next-generation CTC-chip.

Microfluidics application in cancer detection and prognosis is required specifically when a sample is available, or should be collected, in low quantity. For example, to characterize the activity of oncogenic kinases in the blood, bone marrow, and needle biopsy samples from suspected cancer patients, a group of researchers has manufactured a microfluidics platform that can reproducibly measure kinase activity from very few cells and applied it to measure ABL-kinase activity in leukemia patient samples.[92] Researchers have further developed another microfluidic system, called microfluidic image cytometry, which is capable of quantitative, single-cell proteomic analysis of multiple signaling molecules.[93] The large data from the microfluidic analysis are subsequently processed by bioinformatics, and in this way, the platform enables *in vitro* diagnostic technology for systems pathology analysis and personalized medicine. It is important to mention in this context that on the other side of the hallmarks-based conceptual generalization of cancer occurrences, cancer treatment in practice is probably gradually abandoning the hope of discovering a panacea and moving towards analyzing the nature of the disease, and advising the treatment course, on individual basis.[94] At this moment, however, any effort to improve personalized medicine in oncology has to meet several challenges including how to analyze small amount of tumor samples for wide-array of biomarkers.[95] We believe that to this end, employment of high-throughput microfluidic strategies, sooner or later, is inevitable.

In cancer treatment also, microfluidic systems can serve as useful tools, or provide some fundamental insights on the targeted process. For example, a promising route for chemotherapy is to use the embolic microspheres. They are made of biocompatible polymers, and when injected at a tumor site through a microcatheter, they could block the blood supply to the tumor, leading to tumor death. Alternatively, they can also be loaded with cytotoxic drugs or radioactive elements, and while being entrapped near tumor, they would release the therapeutics locally, thus conferring minimum side effect.[96–98] Preparation of microspheres with uniform size is a problem in this field, and microfluidic devices have already been proposed to solve that.[99] Also to this end, from the perspectives of fluid mechanics, it is interesting to study the dynamics of microspheres in a confined and complex fluid flow. A group of researchers has recently designed a microfluidic system with essential topological attributes of the circulatory network of the body and studied the spatial statistics of flow blocking by the embolic microspheres inside the system.[100] One can extend this study further by investigating how the

aforementioned process is affected by the pulsatile flow (as opposed to constant flow) and the surface characteristics of the microchannel. Microfluidic devices have also contributed to the field of treatment by pore formation or poration. It is an important way to make pores in the cell membrane towards introducing molecular therapeutic agent inside the targeted cells and can be achieved by either electro or acoustic (with cavitation microbubbles) means. Researchers have used microfluidic strategies to study the dynamics of both electroporation[101,102] and sonoporation.[103,104] Concerning the localized delivery of chemotherapeutic agents, we also believe that microneedle based drug delivery devices could be extremely useful for the next generation of cancer treatment.[105–107] In this relevance, one group of researchers has used a microfluidic chip for studying the bystander effects (non-targeted killing) of an anti-cancer drug called tamoxifen in breast cancer cells, by exploiting laminar flow patterning to ensure selective drug delivery.[108]

III. ONCOPHYSICS OR PHYSICAL ONCOLOGY

In 1917, the great British polymath, D'Arcy Wentworth Thompson wrote in his seminal work titled *On Growth and Form*: "*the form of an object is a 'diagram of forces.'*"[109] In this book, he argued that two important biological phenomena—growth and form—could not be explained by applying only the chemical rules; one had to consider the relevant physical forces after all.[109] His view, however, remained mostly unnoticed for a long time, amid the surge of molecular genetics. But now that we have come to know that genes do not act alone and that the intra- and extra-cellular physicochemical forces regulate their activity, biophysics is gaining a firm footing in cancer research.[110,111] So, what are these forces, and how do they act? As in microfluidic systems, forces that act upon a tumor tissue, have characteristic length scales of few millimeters or less. At this scale, we may safely ignore the gravitational force and expect that surface forces would dominate over the body forces. Not only that, fluid flow through a tissue, the interstitial flow, is a low Reynolds number event, marking a further resemblance between microfluidic channels and the tumor tissue matrix. The problem is, however, the complexity of a tissue and the entanglement of several of factors that lead to a specific change. One way to unscramble this mesh is to work on *in vitro* model systems with few variable parameters. In that kind of simulation of the dynamics of cancer cells, no alternative comes closer to show a dimensional parity with tumor tissue than the microfluidic systems do. Once such model system is available, and some interesting observation comes out it, one may then crosscheck its relevance in the tumor tissue. In this way, working back-and-forth between the model and the actual system, one could unravel the effects of physical forces in tumor development.

One of the first physical factors that turned out to be important in cancer biology was the stiffness of tumor tissue.[112,113] In body, every tissue has its typical "stiffness phenotype," and this parameter varies widely among tissues.[114] For example, lung, breast, and soft brain tissues have their characteristic elastic modulus between 500 and 1000 Pa, while for cartilage, it is in the order of 15 000 Pa, and for bone matrix, in the order of 10^9–10^{11} Pa. This variation in tissue stiffness is because of the variations in the protein density and composition of ECM, crosslinking of matrix fibrils, and their orientation. The tissue stiffness is an important physical factor, which enables the cells to perform their intended task in a proper manner, and if the ECM stiffness is found unusual, often cells stop showing their original characteristics.[115] Human mammary epithelial cells (HMECs), for example, do not express the milk protein, β-casein, *in vitro*, unless they are cultured on a compliant matrix having similar stiffness as the breast ECM.[116] Importantly for cancer research, breast ECM progressively stiffens with the development of breast cancer.[117] Clinicians detect this change with magnetic resonance imaging elastography and sono-elastography, or simply by feeling the tissue stiffness by touching it.[118,119] But, how far does this stiffening help the oncogenesis, and is it an essential part of cancer development? As the cells maintain a tensional reciprocity with ECM, altered tissue stiffness can induce changes in the rheological nature of the cytoplasm, by altering the architecture and composition of cytoskeleton, which could then act in favor of cancer cell migration during the invasion of blood vessels.[113,120–122] Also such changes in cytoplasmic rheology could well help the cancer

cells to penetrate the endothelial cell-cell junctions of blood vessel wall, a task that demands large elastic deformations of cell.[123,124] Very recently, a research group has used nanomechanical profiling by indentation-type atomic force microscope to provide quantitative indicators in the clinical diagnostics of breast cancer.[125] Remodeling of ECM orientation during stiffening could additionally provide the cancer cells with a guidance cue for invasive migration.[126,127] While stiffening of ECM initially occurs as a consequence of oncogenic genetic mutations, in later stage, ECM stiffening works as a positive regulator of a migrating, invasive "mesenchymal" phenotype during EMT.[128,129] With this revelation, initiatives of developing chemical agents that could restore the normal ECM stiffness, is underway. A burning question is how the cancer cells pass their acquired stiffness characteristics to the next generation of cells, which would require this trait for the successful colonization over other body parts.

Another important physical stimulus is the compressive stress in a tumor tissue.[130,131] Because of the overproliferation of cells within a very restricted space, flow passages within a developed tumor are often very narrow, and hence, there is a build-up of interstitial fluid pressure. Although the role of this pressure build up in transport of chemotherapeutic agents was known for years,[69] very recently, researchers have demonstrated that it can also activate cell migration, specifically affecting the cancer cells.[132] Normalization of interstitial fluid pressure, therefore, appears to be a possible future route for cancer therapy.[68]

Not only the fluid pressure, but the flow shear stress (FSS) also plays an important role in the process of metastasis. Cancer cells encounter significant FSS, while they are able to penetrate the blood vessel and are travelling to distal parts of the body, hitchhiking the circulatory system. To give an idea of how large FSS can be in vasculature, the time-averaged FSS in venous circulation varies between 0.1 and 0.4 Pa, while the same in arterial circulation can reach up to 3 Pa.[133] What is the effect of this variation in FSS on cancer cells? Exposure to very high shear stress, greater than 1.2 Pa, leads to cell cycle arrest of metastatic cancer cells, which are then attacked and removed by the surveillance cells of our immune system.[134] Low shear stress, on the other hand, could promote EMT and metastasis.[135] Furthermore, to colonize inside a new body tissue (e.g., breast cancer cells colonizing inside bone), the traveling cancer cells must attach to the endothelial cells of the blood vessels in the new tissue, and the magnitude of FSS affects this process of attachment.[136] Too low the FSS and cells might not have sufficient encounters with the endothelial cells expressing the proper surface molecules, which will enable the cancer cells to attach before they die in circulation. Too high the FSS and cells might not have enough residence time to initiate a strong bonding between them and the endothelial cells. Generally, it is the intermediate strength of FSS (0.5–1 Pa) that supports maximum tethering of cancer cells to the walls of blood vessel. At this range, FSS promotes a finite slipping velocity, which increases the binding rate. The optimum level of FSS for tethering is, however, a function of the association and dissociation rate constants between the ligand-receptor pair that mediates the interaction between the cancer and the endothelial cells. Since endothelial cells of different tissue express the surface molecules of different kinds and in different level, this might partly explain why cancer cells are able to colonize preferentially in certain tissues such as bone. Another mechanism by which cancer cell could bind to endothelial cells is by simple occlusion where vessel diameter is less than the diameter of cancer cells.[137] Such a case is often encountered in the lung. Occluded cells, however, show a surprisingly low efficiency of forming new colonies, probably because of the excessive fluid stress that inflicts cell death, or because of the lack of specific chemical adhesions to endothelial cells.[138] A definite reason behind this low efficiency is still missing.

Beyond the aforementioned extracellular mechanical forces, the distribution of intracellular forces can also be a rate-limiting factor for metastasizing cancer cells.[113] We know that during EMT, the intermediate filaments of cytoskeleton undergo a drastic change from cytokeratin to vimentin type. Its rheological and mechanical consequences, however, remain uninvestigated. Force distribution during cell migration is also a very important factor for metastasis, and it has a "problem of dimension" attached to it.[139] Efforts to prevent cancer cell migration in three dimensions, using the chemical agents that successfully stop cell migration in two dimensions, have failed. In three dimensions, cell migration seems to depend less on the active protruding

structures such as lammellipodia or pseudopodia and shows some unconventional varieties, including amoeboid movement, or by locomotion through membrane blebbing.[140–142]

In studying the effect of mechanical forces and physical factors on tumor growth, development, and metastasis, microfluidics could be a functional tool.[143] It can provide an *in vitro* model where physiological aspects of tumor microenvironment can be effective mimicked. Microfluidics also enables researchers to deliver physical cues such as flow shear stress, surface topography, matrix stiffness, and geometric confinements, in a controllable way. On top that, since using microscale flows, it is possible to provide chemical cues (e.g., concentration gradient of growth factor) independent of the mechanical cues, microfluidic systems could be used to study the physico-chemical equivalence of a stimulus. With respect to tumor metastasis, a group of researchers has earlier developed a microfluidic vasculature system to model interactions between circulating breast cancer cells with endothelial cells at prospective sites of metastasis.[144] Also, microfluidic systems are very appropriate for studying effects of haemodynamic stresses on cells. As we have indicated above, fluid shear stress has important consequences on the proliferation dynamics of cancer cells.[134,145,146] At cellular level, shear stress is first perceived at the membrane, by the deformation of the membrane structures and by the structural elements that are either embedded (e.g., ion channels) into or linked (e.g., cortical cytoskeleton) to the cell membrane.[147] Signal generated there then propagates downstream either directly by physical means through cytoskeletal elements, or indirectly by activating the signaling molecules that interact with the receptor. Importantly, whatever be the sensing and transduction mechanisms, mechanical signals almost always prompt the cells to undergo an active rearrangement, by changing their internal structure,[148] and altering the profile of gene expression.[149] It is also extraordinary that cells can differentiate between the types of mechanical signals, and even detect small fluctuations in intensity and nature (for example, steady versus pulsatile flow, or laminar versus turbulent flow). How do they do that is a question, which is currently being tackled by the microfluidic researchers using several microchannel designs[150,151] and novel cellular probes including stress sensitive dyes.[152] In future, it is possible to use such system to model how flow shear stress could influence the binding between cancer and endothelial cells for various types of ligand-receptor interactions. Though there exist microfluidic devices that enable analysis of intercellular and cell-substrate adhesion strength,[151,153] studying the deformability of cancer cells[124,154] and examining physical forces during metastatic migration,[25] with respect to the spectrum of forces that we described above, future opportunity is truly enormous. In this respect, it is also worth mentioning that many apparently non-physiological physical forces, such as electric field[155] or magnetic field could also have effects on the cell dynamics,[156] and microfluidic devices could be very appropriate system to investigate that in detail. There are also alternative and novel physical outlooks on cancer development, such as quantum metabolism,[157] density function theory of tumor growth,[158] and cancer propagation from the game theory perspective.[159] In future, it could be interesting for the researchers of microfluidics to investigate how far these ideas hold out in reality.

IV. EFFECT OF THE MICROCONFINEMENT

In the beginning of the review section, we indicated that microenvironment plays an important role in tumor development. Indeed, there is hardly any process in tumorigenesis that is not affected by the tumor microenvironment.[26] It consists of tumor cells, cells of other types, soluble growth and signaling factors, and extracellular matrix, which collaborate to promote cancerous transformation, encourage tumor growth, facilitate invasion, provide protection from the anti-tumor activity of host immune system, nurture resistance to therapeutics, and create metastatic niches.[160] On top of chemical signaling, as we have already narrated in the last section, mechanical cues from the microenvironment also play critical parts in tumor growth and progression. Mechanical stimuli such as increased matrix stiffness, proliferation-induced solid stresses, and high interstitial fluid pressure are integral parts of tumor development.[161] All these chemical and mechanical aspects of tumor microenvironment have lately received, or are

receiving, the research attention they deserve. What remains, however, relatively under-investigated is the effect of the geometric aspects of the microenvironment on tumor development (Fig. 1). Tumor cells, or rather most cells residing in body tissues, may grow within the geometric confinements that have their characteristic diameters in the range of 10-100 μm. The question naturally occurs: How does this microconfinement affect the behavioral dynamics of cancer cells and other tumor-associated cells? It is obvious that such geometric factors have enormous effects on the chemical and physical environment. For example, if we reduce the intercellular spaces, as it happens during the compression of bronchial epithelium, the tissue-concentration of growth factor effectively increases (Fig. 1(b)). This change in chemical condition, in turn, triggers further downstream signaling towards responding to the compressive stress.[162,163] Further, a small directional flow within a confinement space can create a concentration gradient of cell-secreted growth factors (Fig. 1(c)). This "autologous" gradient can then provide the directional cues for the migrating cells and is very instrumental in regulating the chemotaxis of cancer cells towards the draining lymphatics and in lymphangiogenesis (formation of new lymphatic vessels from existing ones).[164–166] In this relevance, very recently, a group of researchers has shown that in a microfluidic maze, both normal and cancer epithelial cells can migrate persistently and travel to the exit along the shortest path, even in the absence of any preexisting chemical gradient, and importantly, in absence of any flow.[167] They have noted that epithelial cells can create their own guidance-cue for migration exclusively under conditions of biochemical confinement. On the physical side, from the elementary knowledge of dimensional scaling, increasing confinement could mean increasing solid stress, interstitial fluid pressure, or shear stress. Given these facts, it is quite surprising that the role of geometric confinement in regulating the dynamics of cancer cells has not attracted much research attention.[22]

Nevertheless, there exist few reports in this subject, which have the potential to inspire the next level of research endeavors. For example, to explore how heterogeneous structures in tissues affect the dynamics of cancer cell migration, Irimia and Toner designed a microfluidic device, which mechanically constrain migrating cells.[168] They observed rapid and persistent movement of cancer within the confined space, even in the absence of any external guidance cue.[168] We have previously investigated how confinement alters the dynamics of cancer and normal cells in response to mechanical stimuli. We observed that when cultured inside microchannels having height less than 70 μm, cancer cells show increased speed of responding to flow shear stress (Fig. 2(d)).[169] One of the major problems in working with tissue is that an attempt to alter one parameter may alter some other parameter also, making it difficult to interpret how much of the observed change in cell behavior or dynamics could be attributed to the former. For instance, perturbations that alter matrix stiffness could simultaneously alter cellular confinement. To decouple the effects of these two factors in cancer cell migration, a group of researchers has very recently used a microfluidic system where matrix stiffness and cellular confinement can be varied independently of each other (Fig. 2(c)).[170] They found that confinement changes the relationship between cell migration speed and ECM stiffness. In this case, confinement increases the polarization of cell-ECM traction forces, which in turn perturbs the response of cells to ECM stiffness.[170] Though not quite using microfluidic system, another recent study has demonstrated that cell confinement controls the centrosome positioning and lumen initiation during epithelial morphogenesis.[171] We foresee that findings of this study could further be extrapolated to understand how breast cancer develops within mammary acini, and how cell confinement affects the process.

On the biophysical side, a relevant aspect could be probing the rheology of cancer cells, or of plasma membrane of the cancer cells, in such *in vitro* systems. There exist, however, several challenges in computational microfluidics, if one tries to model cancer-specific issues of cell-flow interaction in a confined microenvironment. The most profound among them is the deformability of biological entities.[172] At the simplest approximation, a living cell could be modeled as a deformable vesicle, and at this level, fluid-cell interaction is not trivial to simulate because of the two-way interactions between the flow and the biological features.[173] Deformation of cell, especially within a confined fluidic system, could lead to significant perturbation of the flow field, which in turn, could exert different level stress to cells than it would if the latter

were a non-deformable object (bluff body). Moreover, one has to also consider the intracellular rheology. While at the first level of modeling, one may assume that the rheology remains unchanged throughout the process, more realistic models should have provisions for incorporating stress induced hardening, or in some case softening, of the cytosol. Hence, in the end, one should consider the active rheology of the living system, as it could provide vital insight into how cancer cells adapt to fluid shear stress. Yet another important factor is the cell membrane (i.e., fluid-cell interface) that could also show active rearrangements in tension and rigidity, in response to applied flow stress. There exist specialized cholesterol-rich liquid-ordered nanodomains in plasma membrane called lipid rafts.[174] Researchers have shown that lipid rafts play important roles in oncogenesis.[175] Since, they also influence the stress adaptive response of cancer cells, a relevant challenge is to model their dynamics under a confined environment. Phase-field based models show some promises in solving such a complex scenario.[176] If one could incorporate exclusive cancer-specific information in the free energy expression in an order parameter based formalism, one could then couple flow dynamics with mechanotransduction within the cell as well as cancer-specific interaction with the micro-environment, and link intracellular signaling with extracellular events, specific to cancer progression in a confined microenvironment.

Cells-on-chip type devices originally came out of the increasing need to miniaturize the assays in cell biochemistry and biophysics. This motive was, and is still, very much important particularly for the processes that involve rare cell types or expensive reagents or both. Nevertheless, the presumption was that basic cell behavior would be unperturbed by such dimensional scaling-down. We now see that this assumption may not be universally true, which leads us to believe that the data obtained from cells-on-chip type studies should be carefully examined, taking the perturbation due to confinement effect into consideration. For example, within the confines of the microfluidic systems, and with the limitations in available media and oxygen concentration, temporal changes in pH and osmolarity could play vital roles in determining the cellular dynamics.[22] On the other hand, the ability to control the confinement parameter independently of other factors could be utilized to isolate the key parameters that affect the dynamics of cancer cells in three dimensional tissues. Confinement, especially a controlled one, is a unique feature of the microfluidic systems, as it could hardly be realized in conventional cell culture platforms. A pertinent challenge in this regard is to create a confinement that mimics the flexibility as well as the fluidic environment of a real microcapillary in a human body. Microfluidic channels are generally fabricated in materials like glass or polydimethoxysiloxane (PDMS), which have very large elastic modulus compared to soft-tissues. Since we have already seen that substrate elasticity has a very critical role in tumor development, microchannels with compliant wall surfaces are desired in many applications. Towards this goal, some progress had already been made, especially using hydrogels. For example, researchers have used laser induced focal photoablation to generate micron scale guidance structures in transparent hydrogels. They then used these photopatterned microchannels to guide the directional growth of neurites from dorsal root ganglia.[177] Such microchannels, embedded in a hyaluronan hydrogel, has also been by using hard x-ray irradiation.[178] In another approach, hydrogel mediated partitioning of microchannels has been used to create tunable 3-D cellular microenvironment.[179] To stretch the scope of applicability even further, a research group has recently reported a method to fabricate biofunctionalized polyethylene glycol hydrogel microchannels with adjustable circular cross-sections.[180] Remarkably, they were able to decorate the inner channel surfaces by gold nanoparticles, which enabled them to create nanopatterns for cell adhesion, against an inert yet biocompatible background. We believe that such system have immense relevance and prospective applications in cancer research. These, especially, can lead to a direct extrapolation of *in vitro* studies on isolated tumor cells towards understanding the biophysical aspects of tumor survival in narrow blood vessels. In the end, true potential of microfluidics will never be unleashed, if this fundamental aspect of the system is not used to illuminate hitherto uncovered phenomena happening inside three dimensional tissue. Revising the issue of tumor microenvironment, we envisage the answers to many related problems if the interactions among tumor-associated cell types are studied in a micro-confined environment.

V. THE PROBLEM OF A "RENEGADE CELL"

In preceding three sections, we have described how researchers have applied different microfluidic strategies to solve many problems in oncology, related to cancer model, detection, and treatment; we have delineated how physical forces act, alongside the genetic changes, in the process of tumor development, and what microfluidics has to do with it; and above all, we have exemplified how a microfluidic system could be useful in mimicking the tumor microenvironment and the physiological microconfinement. Having addressed these issues, we now tread into presumably the most important part of this review, where we endeavor to chart out some of the most challenging and unresolved problems in cancer biology. We believe that for understanding the future scope of microfluidics in cancer research, which indubitably looks immense, a good knowledge of the fundamental questions in this subject is necessary.

A. Primary tumor: Not-so-sweet home

Some say that cancer is a disease of epithelial tissue. This statement is mostly correct since 80% of cancer types are of epithelial origin. In our body, epithelial cells form a protecting monolayer structure encircling the body cavities or lumens (Fig. 1(a)). Lying over a special ECM layer called basement membrane, they form extremely impermeable intercellular junctions. Epithelial cells show distinct chemical polarities in their ECM-facing (basal) and lumen-facing (apical) membrane surfaces. Beyond the basement membrane, there exists an intermediate ECM-rich tissue layer called mesenchyme, in which many cell types, including fibroblasts, macrophages, and pericytes subsist (Fig. 1(a)). Mesechymal layer supports the epithelial tissue and bridges the gap between the later and the blood vessels. Upon one or several genetic mutations, cancer cells, originally of epithelial origin, first shed their monolayer forming properties and apico-basal polarity as they start growing over each other (Fig. 1(a)). In many cases, this unregulated growth fills up the entire lumen volume, and a compressive pressure develops therein. As the cell density increases enormously within a very restricted space, cancer cells begin to starve in nutrient and oxygen. This hypoxic condition then prompts them to secrete the growth factors that will attract endothelial cells towards the tumor to form new blood vessel around it. At the same time, cancer cells secrete enzymes that dissolve the basement membrane. Next, by chemical degradation of mesenchymal ECM and by physical means, they push towards the blood vessel (Fig. 1(a)). Taken together, this story essentially delineates "the standard model" of tumorigenesis. There are, however, a few unresolved questions in this model.

To begin with, we do not know yet whether and how genetic mutations that trigger unregulated cell division, also lead to cell migration. If we recall the hallmarks here, we realize that this problem is about building a connection between the sixth hallmark and the rest. Talking about the hallmarks, their universality is not unquestionable.[181,182] Except the sixth hallmark—the capability to metastasize—others are not unique features of cancer.[181] In fact, we need to note here that the terms, "cancer" and "neoplasia," are often restricted to the tumorous growth that has also acquired, or eventually acquire, the invasive ability. Other tumors are simply "benign" in nature. There are evidences that benign tumors could show some or all of the other hallmarks; yet they never become invasive. Why? That is the puzzle for the researchers who are attempting to generalize the process of oncogenesis.

Another relevant problem is about the intratumoral heterogeneity (ITH).[183–185] Do all cancer cells in a primary tumor bear identical genetic mutations? Are they equally capable of invading the neighboring tissue and migrating up to the blood vessel? With some recent revelations in this matter, answers to both questions look negative.[29] It is, therefore, likely that cancer initially sets in with few basic and essential mutations, which lead them to breach the growth regulation and at the same time, makes their genome vulnerable to further mutations. Then, as their descendants gather more mutations, some of them are selected, by a mechanism akin to Darwinian natural selection, for the traits that will enable them to invade the mesenchyme and thus, escape from the limiting environment of the primary tumor.[29] Though this clonal selection hypothesis explains the origin of ITH and why cancer takes some time to become invasive, there is no direct proof in its favor. Since one cannot monitor or visualize cells actually

evolving in a tumor tissue, one cannot study the dynamics of such clonal evolution of cancer cells. We wonder whether a microfluidic system, capable to maintaining a tumor mass for sufficiently long-time, could be used in this purpose. We presume that such microfluidic might be inspired from some of existing organ-on-chip type devices.[186,187]

In relation to the last issue, some researchers have argued that special types of cancer cells, the cancer stem cells (CSCs), constitutes the key players of oncogenesis.[188] They have associated CSCs with invasive properties, drug resistance, and disease relapse after initially successful chemotherapeutic treatment. There is, however, little consensus on how CSCs form in a tumor, how they could be identified, and to what extent they show stem cell like behavior. It is known that some chemicals, such as Salinomycin, are very good at specifically killing the putative CSCs.[189] Yet, at the same time, a fraction of non-CSCs was found to compensate for the killed CSCs. This seemingly confusing observation has undermined the prospect of CSC-killing agents of becoming effectual cancer drugs. The most prevailing view is that in primary tumor, CSC and non-CSC populations of cancer cells probably undergo a dynamic conversion between two phenotypes.[188] There is, however, little direct evidence in favor of such model. We note that though initially the CSC hypothesis was received with skepticism, very recently a body of evidences has accumulated over their existence.[190–192] We believe studying CSC formation and dynamics could be an interesting topic for the microfluidic research.

Some other problems related to the origin of primary tumor are around the question why a specific mutation leads to the tumor formation in a specific tissue while other tissues are left mostly unharmed.[193] For example, mutation in a gene called Retinoblastoma (Rb, named after the disease that its mutation causes) leads to lethal tumors in retina in children, while other body tissues remain normal. Similarly, mutation in Neurofibromin-II (NF-2, its mutation causes Neurofibomatosis Type II) causes the cancer of peripheral nervous system and specifically affects the Schwan cells, while the same mutation rarely causes cancer of other tissue. Probably this tissue-specificity has something to do with the microenvironment of respective tissue, as compared with that of others. Since microfluidic systems are very efficient in investigating the microenvironmental factors, they could be used to unravel the mechanism.

At last, some puzzles also remain on how cancer cells interact with the cells of the neighboring tissue (tumor-stroma interaction), and how they induce the migration of blood-vessel-forming endothelial cells towards the primary tumor. Traditionally, researchers believed that intercellular signaling at long distance was mediated by the fluid phase diffusion of soluble factors. Yet in recent times, another complementary mechanism of long distance communication has attracted some research attention.[194] Cancer cells were found to pack and secrete the pro-angiogenesis ingredients that are required to excite the endothelial cells, in small lipid-encircled capsules called microvesicles.[195] What is more interesting is that these microvesicles do not only contain the protein factors to induce the angiogenesis, but surprisingly, they also contain messenger ribonucleic acids (mRNAs) of the genes that will facilitate the migration of endothelial cells. As microvesicles get fused to the membrane of the endothelial cells, they release their content inside the cytoplasm, and as a result, cells start synthesizing the proteins that they would not do otherwise. The discovery of microvesicles has opened up new areas for chemically targeting the cancer cells. The characterization of microvesicles, however, remains a problem since they are not very abundant in blood or in any other body fluids. At least one microfluidics-based enrichment device has already come up with different solutions to this problem.[196] We anticipate that further improvements hereon will lead to a superior understanding of the formation and dynamics of microvesicles.

B. Dissemination and metastasis: Hitchhiking the circulatory system

Migrating up to the blood vessel, cancer cells can now access the connective system of body, and then, they become blood-borne. In a flowing environment, cancer cells are now exposed to several new challenges. We have already enlisted the physical forces that could in principle affect the success of a metastasizing cancer cell. There are, however, other physiological factors, which also have profound consequences on the success of metastasis. In this regard,

much of the challenges for travelling cancer cells come from the immune cells. Naturally evolved for eliminating the evading parasites, they also interact with the cancer cells both within the primary tumor and within the circulatory system. Some of them promote the process of tumorigenesis, while others exert inhibitory effect and are known to identify and kill the cancer cells. The established conception is that cell types belonging to innate immunity—especially tumor-associated macrophages (TAMs), mast cells, and granulocytes—contribute positively to tumor development.[39] They do so by regulating cell survival, tissue remodeling, and angiogenesis. Within the tumor microenvironment, their chronic activation also suppresses the anti-tumor responses of the cell types belonging to adaptive immunity. Interestingly, this view supports a very classical description of cancer as "a wound that never heals."[197] In last few years, researchers have attempted to portray the evolution of interaction between cancer cells and the immune system within a unifying framework called immunoediting. The basic concept is that the interaction evolves in three phases: elimination, equilibrium, and escape.[198] In the elimination phase, concomitant activation of innate and adaptive immunity ensures the destruction of the tumor, before it could grow out to become clinically apparent. If some cancer cells are able to survive the elimination phase, they then enter the equilibrium phase. In this phase, they live in a state of dormancy for a long time, but their outgrowth is prevented by the immune system. If some of these cells gather favorable mutations and become genetically unstable, they may then give rise to a new tumor variant that is no more recognized by the cells of adaptive immunity. Alternatively, they could also activate the immunosuppressive system within tumor microenvironment, possibly by chronically activating the cells of innate immune system. This variant of tumor cells is then able to escape the anti-tumor effects of the immune system. As in the case of clonal evolution hypothesis of cancer, very little is known of the dynamics of immunoediting. For example, we do not know yet what the effectors of this process are and what cell-cell interactions are important at each phase. Tumor microenvironment should also play an important role in the evolution between phases. The question here is whether we could rigidly segregate the immune cells in pro- and anti-cancer categories, or whether this segregation would eventually be marred by the contextual transitions between the categories. Reprogramming of tumor microenvironment is currently being used in developing innovating cancer immunotherapies.[199] We envisage that microfluidic systems mimicking the tumor microenvironment could contribute to such development to an enormous extent.

There are also some very interesting findings, including very recent ones, on how many different ways cancer cells may avoid recognition by the adaptive immunity. For example, one group has shown that expression of a protein called B7-H1 in skin cancer (melanoma) assists the cancer cells to resist the recognition and destruction by the adaptive immune system.[200] Another group of researchers has found that by remodeling the chromosome structure, cancer cells can reduce the level of the surface proteins (or "tags") that could lead them to be identified by the immune system.[201,202] Interestingly, a type of blood cells called platelets is known for providing a protective cover for the cancer cells during their dissemination and metastasis phase.[203,204] By secreting a protein called fibrinogen, platelets form an aggregate over the surface of the travelling cancer cells, and this cover, in turn, prevents recognition of cancer cells by the natural killer (NK) cells. It is here interesting to note that very recently a microfluidic device has come up to evaluate and modulate the tumor cell line's susceptibility to NK cell recognition.[205] Extrapolating over the aforementioned findings, it seems possible that the platelet-cloak, as it is called, could also shield the deleterious effects of fluid shear stress. In the absence of appropriate *in vitro* studies, however, this proposition remains untested. On the other hand, some reports suggest that by activating several surface receptors on either cancer cells or platelets, blood flow generated shear stress itself may facilitate their mutual interactions.[206–208]

VI. CONCLUDING REMARKS

In conclusion, we observe that though much advancements have already been made in using microfluidic system in cancer research, a vast part of the latter remains unexplored. As the importance of tumor microenvironment continues of amaze the cancer biologists, we

definitely have reasons to believe that microfluidics could be something more than a miniaturized assay system for oncology. One of the emerging dogmas in cancer biology is the system thinking.[209] It appeals to the researchers to think beyond the molecular details, and to contemplate on how the implications of an observed phenomenon could be weighed against the state of the system as a whole, in which it takes place. Since actual tissue could be too complex to isolate the principal components from the entangled network of several mutually interacting factors, *in vitro* systems are the best to begin with. In this matter, we argue that microfluidic systems are better than the others, not only because they enable performing assays with a very low volume of samples, superior sensitivity, and high-throughput analysis but also for the reason that they present a near perfect model of the confined tissue environment. With all these promising aspects, could microfluidics find its deserved importance in cancer research? The answer surely belongs to the future, but we can be sure that the future looks promising. Lastly, going back in a circle to where it all started—with the declaration of "the war on cancer" in the 1970s—some cancer researchers now believe that while we are winning some battles, we are probably losing the war in general.[181] We end this article with the hope that microfluidics could just be the right weapon to win it in the end.

ACKNOWLEDGMENTS

T.D. thanks the Max Planck Society, Germany for financial support. S.C. thanks Department of Biotechnology (Govt. of India) and British Council for financial support.

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