From Cancer Immunoediting to New Strategies in Cancer Immunotherapy: The Roles of Immune Cells and Mechanics in Oncology

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Abstract

For the last three decades, the concept of immunoediting has evolved to characterize our increasing understanding of the interactions between cells from the immune system and cancer development. Elucidating the role of immune cells in the progression of cancer has been very challenging due to their dual role; the immune system can either suppress tumor formation by killing cancer cells, or it can also promote tumor growth. Revealing how immune cells are hampered by the tumor microenvironment and how they aid tumor progression has signaled strategies to reverse these effects and control cancer cell growth; this has been the advent of immunotherapy design. More recently, the role of physical forces in the process of immunoediting has been highlighted by multiple studies focusing on understanding how force changes in the stiffness of the extracellular matrix and fluid flow shear stress contribute to tumor development. Using models in vitro that incorporate biomechanical components, it has been shown that these physical aspects are not only important during the formation and growth of primary tumors, but in the metastatic process as well. In this way, we have also gained insight into the interactions occurring within the vascular system, which are highly affected by the dynamics of physical collisions between cells and by shear forces. Here, we review the concept of cancer immunoediting with an emphasis on biomechanics and conclude with a summary on current immunotherapies and potential new strategies.

Keywords

Cancer immunoediting · Tumor microenvironment · Cell biomechanics · Cell signaling · Drug delivery · Cell-mediated drug delivery

7.1 Introduction

Cancer development is a complex process that requires the coordination of multiple cellular activities. In many instances, cancer cells take advantage of healthy cells, either suppressing their cytotoxic functions or feeding on their secreted cytokines to proliferate. For the last three decades, the role of the immune system in cancer develop-

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C. Dong et al. (eds.), *Biomechanics in Oncology*, Advances in Experimental Medicine and Biology 1092, https://doi.org/10.1007/978-3-319-95294-9_7

ment and progression has been a major focus of research in cancer immunology. It is clear now that the immune system can stop and completely eliminate cancerous cells from the body, but it can also selectively target and kill the cells that are more immunogenic, effectively enriching tumors with cells that are less immunogenic and more difficult to detect by the same immune system. In addition, it is clear now that cancer progression is stimulated by many factors including biomechanical properties of the tumor microenvironment. Recent evidence suggests that cancer cells are driven toward a more invasive phenotype through mechanical compression [1]. After cancer cells leave the primary tumor and enter the vascular system, they benefit from interactions with immune cells and biomechanical forces once again; by forming stable bonds with lymphocytes and neutrophils in circulation under low shear stress conditions, cancer cells can arrest on the vascular endothelium and extravasate toward secondary tissues. Understanding the underlying mechanisms of these interactions and the biomechanical conditions favoring them, is the basis for developing effective immunotherapies.

7.2 Immunoediting

The concept of immunoediting evolved in the last three decades to describe the dual role that the adaptive and innate immune systems play in the process of cancer development. Initially, the immune system was thought of as a control mechanism in the body to contain cancerous cells. The concept of immune surveillance was first described by Burnet [2]; and it described a defense mechanism used by long-lived animals to cope with somatic mutations and potential neoplasia. Any tumor formation was understood as a failure of this system. However, experiments by Stutman and colleagues showed that tumor occurrence in mice with major immune deficiencies (e.g., lacking lymphocytes) was similar to tumor formation rates in immunocompetent mice [3], partially disproving the immune surveillance theory. Later, with the use of mice lacking B and T lymphocytes and NK cells, the protective role of the immune system was revisited. These later studies showed an increased rate in tumor formation by carcinogen chemicals or viruses in immunodeficient versus immunocompetent mice [4]. Taken together, these results hinted at the complex role that the immune system plays in the context of cancer progression and gave rise to the term immunoediting. Depending on the stage of cancer progression, the immune system can suppress the growth of cancer cells, or it can shape tumors so that cancer cells develop specific traits to escape and grow uncontrollably.

Cancer immunoediting is separated in three phases: elimination, equilibrium, and escape. For a thorough review on this topic, we refer the reader to the work by Schreiber et al. [5]. The basis for the concept of immunoediting comes from the observation that tumor cells isolated from immunocompetent mice are less immunogenic than cells harvested from tumors grown in immunodeficient mice [4]. This suggests that the immune system not only can affect tumor growth, but it can shape the quality of the tumor cells. By killing more immunogenic cells in immunocompetent mice, the immune system effectively selects for those cells that do not carry antigens for their detection, cells that are less immunogenic (Fig. 7.1).

Elimination: In this phase cancer cells are recognized by the adaptive and innate immune systems and can be efficiently eliminated before the cells are clinically detected. Direct evidence for this phase is still lacking; however, its existence is inferred from mouse models [6] and clinical studies comparing tumor rate formation in patients with a deficient immune system and healthy adults [7, 8]. As early as 1943, it was observed that mice spontaneously recovering from chemically induced tumors using methylcholanthrene acquired immunity specific toward that tumor. Recurring inoculations after mice had recovered did not yield new tumors [9]. In addition, compared to wild-type mice, immunocompromised mouse models in which different cells of the immune system are genetically deleted have shown an earlier onset in tumor development in response to carcinogen chemicals, oncogenic viruses, and spontaneous tumor formation (reviewed in Ref. [6]).

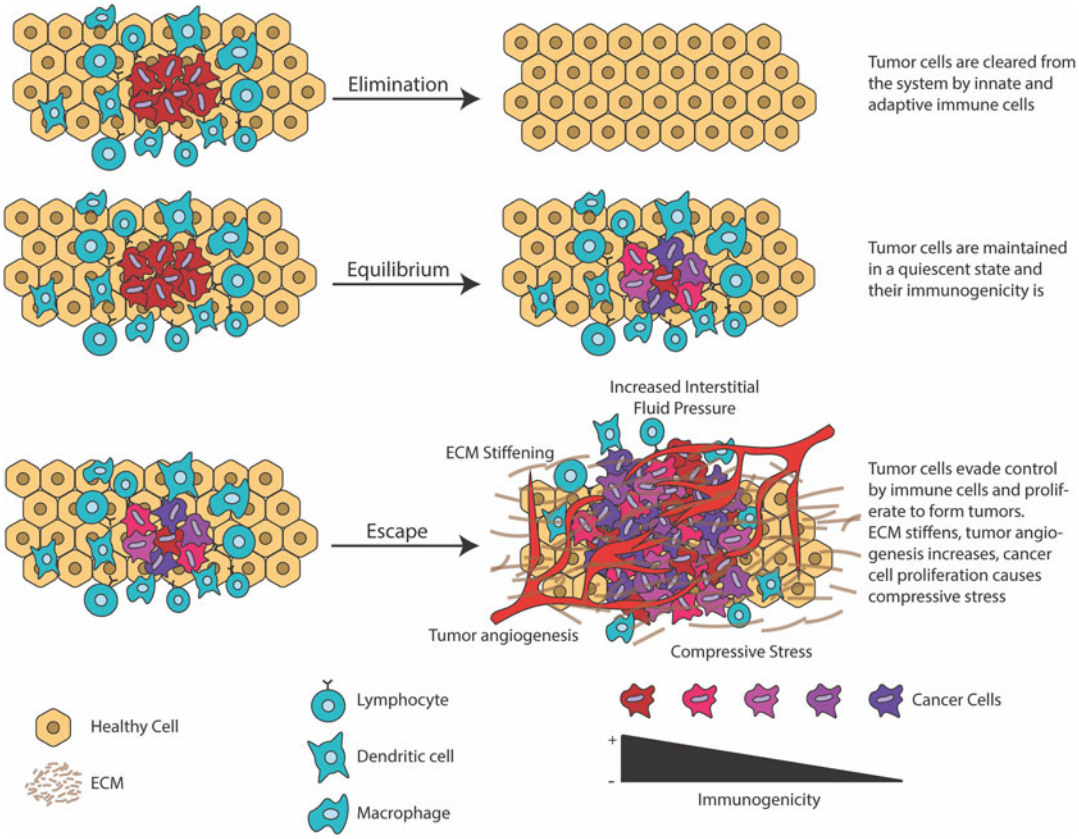

Fig. 7.1 Depiction of the three stages of immunoediting. Elimination: cancer cells are controlled and removed from the body by the innate (e.g., macrophages and dendritic cells) and adaptive (e.g., B- and T-lymphocytes) immune system. Equilibrium: cancer cells are kept at a dormancy state and tumor size is constant. Escape: cancer cells breach the immune system and grow uncontrollably. In addition, the ECM stiffens, tumor angiogenesis and interstitial fluid pressure increase, and cancer cell proliferation causes compressive stress in the interior of the tumor. In later stages, cancer cells collectively migrate and invade neighboring tissues and finally enter the vascular system to form metastasis in distant organs

In humans, clinical observations of patients with AIDS show a higher incidence for various types of cancer compared to the general population, with the exception of breast cancer [10–12]. Furthermore, multiple studies have found antibodies specific for tumor antigens in sera from healthy adults [8, 13], possibly suggesting that at some point, their system was exposed to cancerous cells, but these were controlled and eliminated by the immune system. If all cancer cells are successfully removed from the body during this phase, tumors do not grow, and this is the end of the process. In contrast, if some cancer cells breach the elimination phase, they progress toward the next phase, equilibrium.

Equilibrium: Cancer cells that progress into the equilibrium phase undergo what is called the editing process. By killing highly immunogenic cells, the adaptive immune system shapes the immunogenicity of the tumor. During the equilibrium phase, malignant cells stay in a dormancy state controlled by the immune system. This phase can last decades and it is thought to be the longest of the immunoediting process. Some of the most compelling evidence for the existence of the immune-mediated equilibrium phase was presented by Koebel et al. in an elegant experiment where mice were treated with low doses of methylcholanthrene and monitored for tumor occurrence for 200 days at which point the

immune system was challenged with a combination of monoclonal antibodies against CD4, CD8, and interferon-γ (IFN-γ). The results showed that after treatment with the carcinogen, most animals did not developed tumors during the first 200 days. However, after the immune system was challenged, half of the animals developed tumors in the site of the initial injection [14].

At the end of the equilibrium phase, there are two possible outcomes: tumor regression, if cancer cells are controlled and eventually eliminated, or tumor progression, if cancer cells become less immunogenic and eventually overcome the control by the immune system progressing into the last phase, escape.

Escape: This is the phase most widely studied and for which there is most evidence. During the escape phase, cancer cells grow uncontrollably developing sizable tumors that are clinically detectable. Cancer cells that progress from equilibrium to escape do so using three main routes; either cancer cells acquire the ability to circumvent recognition by the immune system, they become more resistant to cytotoxic effects by immune cells or they develop immunosuppression mechanisms that inhibit normal functioning of B and T lymphocytes and natural killer (NK) cells. Once in the escape phase, biomechanical changes in the tumor microenvironment take place and contribute to the malignant transformation of cancer cells and eventual tumor growth. Increased compression stress, stiffer extracellular matrix, higher interstitial flow, and fluid pressure contribute to change the normal behavior of stromal cells (e.g., immune cells, fibroblasts, and endothelial cells) surrounding cancer cells to support tumor growth [15].

Recently, genetically engineered mouse models of sarcomagenesis were used to address the importance of tumor-specific antigens (TSAs) in the recognition and editing process of cancer cells [16]. These models provide an advantage over carcinogen-induced tumor models because they allow researchers to analyze tumors with the same genetic and histopathological characteristics in different contexts, immune-competent vs. immunodeficient mice. Generally, carcinogen-induced tumor models are recognized as being more immunogenic than genetically induced tumors. However, to increase immunogenicity and test the role of T lymphocytes in the editing process, lentiviral vectors that express specific T-cell antigens combined with a luciferase reporter gene have been used. The results using this approach show that mice lacking reactive T cells and weak thymic expression are more susceptible to sarcoma formation than immune-competent littermates. Interestingly, tumors from immune-competent mice show a decrease in luciferase activity compared to tumors from immunodeficient counterparts, suggesting an editing process mediated by T cells. Moreover, when immune-competent mice are treated with anti-CD4/CD8 antibodies, they develop tumors at a similar rate compared to immunodeficient mice, and luciferase activity is restored. In addition, when tumors from immunodeficient mice are transplanted into wild-type mice, luciferase activity decreases, suggesting again a loss in antigen expression due to a T-cell-mediated editing process.

Experiments using murine tumor models of transplantable melanoma, sarcoma, and adenocarcinoma and a transgenic model of breast carcinoma show that T-cell function is not systematically reduced in the organism but that immunosuppression is a phenomenon triggered within the tumor microenvironment [17]. The tumor microenvironment and normal tissues are different in several aspects; malignant neoplastic tissues exhibit hypoxia, lower pH, and increased cytokine concentration reminiscent of chronic inflammation [18, 19]. Much of the research efforts have focused on the study and characterization of the tumor microenvironment in terms of the biological signals and chemical characteristics. However, in light of the latest results coming from the field of biomechanics, changes in the biophysical properties of the tumor microenvironment are getting more traction. It is clear now that mechanical forces affect cell behavior, cell-cell crosstalk, and how cells respond to stimuli.

Consequently, in cancer research, in addition to biological changes in the tumor microenvironment, mechanical changes have also been rec-

ognized as instrumental driving forces in cancer progression.

Increased interstitial pressure [20], stiffened extracellular matrix [21], and mechanical compression [1] are all characteristics of the tumor microenvironment. The fluid balance between the venous system, cytoplasm, and interstitial compartments is maintained by the difference in net forces between osmotic and hydrostatic pressures; this is described as the Starling forces [22]. As early as 1975, it was proposed that the increased interstitial pressure in solid tumors was due to the expansion of tumor angiogenesis combined with a deficient formation of lymphatic vessels for fluid drainage [23]. In contrast to normal angiogenesis, tumor angiogenesis is characterized by the aberrant growth of tortuous blood vessels which leads to vessel leakiness and accumulation of proteins from the plasma into the tumor tissue. Interstitial fluid pressure in normal tissues has been reported around 0 mmHg; in contrast, solid tumors can exhibit interstitial fluid pressure between 0 and 40 mmHg [24].

In healthy tissues and organs, the composition and mechanical properties of the extracellular matrix (ECM) are tightly regulated by synthesis, remodeling, and degradation processes. In cancer, these processes are deregulated leading to the disruption of the ECM dynamics. In most solid tumors, the ECM becomes rigid and disorganized [25]. Fibroblasts are the most common type of cell present in the tumor stroma, and one of their main functions is to maintain the ECM; by secreting collagen type I, III, IV, and V [26], fibronectin, laminin [27], and matrix metalloproteinases (MMPs) [28], they contribute to matrix turnover and sustain the basement membrane [29]. Initially, neoplastic lesions are contained within a basement membrane that is separated from the surrounding tissue; this is called carcinoma *in situ*. Together, the cells around the basement membrane, fibroblasts, capillaries, immune cells, and ECM are called the reactive stroma to differentiate them from the stroma in healthy organs. Fibroblasts present in the reactive stroma acquire an activated phenotype that resembles fibroblasts during the wound healing process and is different from their normal phenotype in healthy tissues [29]. Fibroblast activation is triggered by multiple growth factors including transforming growth factor-β (TGF-β) [30], fibroblast growth factor-2 (FGF-2) [31], epidermal growth factor (EGF) [32], and platelet-derived growth factor (PDGF) [33]. Once fibroblasts are activated, they promote degradation of the ECM and alter its composition by secreting higher levels of MMP2, MMP3, and MMP9 [26, 29]. While remodeling the EMC, activated fibroblasts also produce large amounts of insulin-like growth factor (IGF) [34], hepatocyte growth factor (HGF) [35], nerve growth factor (NGF) [36], EGF, and FGF-2 that increase proliferation of neighboring cells [29]. In conjunction, all these biological and mechanical changes have deleterious consequences leading to enhanced cancer cell growth and migration, epithelial to mesenchymal transition (EMT) and ultimately to cancer metastasis.

Mechanical compressive stress is generated by the uncontrolled growth of cancer cells in a confined space. Experiments on agarose gels show that compressive stress inhibits spheroid growth, but the effect is reversible; once the stress is reduced, spheroid growth is resumed [37]. Mechanical stress induces cell death in cancer cells via apoptosis; in spheroids under anisotropic mechanical stress, it was observed that cell death occurred predominantly in high compression regions, while cell proliferation resulted in areas under low compressive stress [38]. While initially mechanical compression might restrain cell growth, it is proposed that sustained compressive stress can effectively select for cancer cells with a more invasive phenotype and metastatic potential [1]. Nowadays, it is widely recognized that the biological function and phenotype of cells are not only responsive to biological or chemical cues but also to mechanical stimuli. Using microprinting techniques and a compressive device with a piston, Tse and colleagues maintained breast cancer cells under compressive stress for 16 h prior to performing a wound healing assay. The results show that compared to control cells, cells under compressive stress exhibit increased migration and cytoskeletal remodeling and form more stable focal adhesions, which leads to enhanced

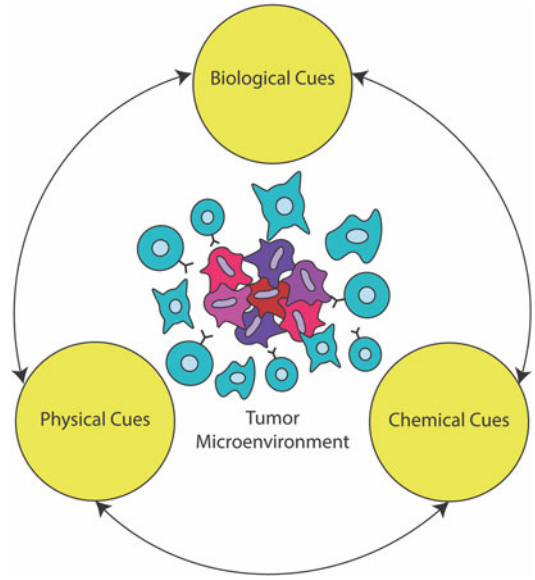

Fig. 7.2 Interdependence of biological, chemical, and physical cues affect the tumor microenvironment and how tumor cells interact with cells in the tumor stroma

collective migration and invasion [1]. A side effect of the increased mechanical compression in the tumor microenvironment is the collapse of blood vessels [39], which causes hypoxia. In turn, hypoxia stimulates production of growth factors like TGF-β and vascular endothelial growth factor (VEGF) that compromise the functionality of macrophages and cells in thetumor stroma [37].

In conclusion, biological, chemical, and mechanical changes in the tumor microenvironment contribute to cancer progression, and their effects are interdependent. Together these cues create feedback loops that feed on each other (Fig. 7.2). For example, changes in biological signals can lead to the release of enzymes that remodel the extracellular matrix changing its stiffness. In turn, stiffer matrices promote cell proliferation that changes the pH of surrounding tissue.

7.3 Interactions Between Cancer Cells and Leukocytes in the Vascular System

Blood circulation through the vascular system is essential for sustaining viable cells in the body. Circulating blood carries oxygen and nutrients to feed the cells and collects waste secreted by them. To sustain biological functions, each cell in the body must be at a distance of at least 100–200 μm from a capillary [40]. Thus, it is no surprise that cancer cells, which have a very high metabolic rate, co-opt blood vessels to increase angiogenesis and support tumor growth. Blood vessels near tumors differ from normal vessels in that flow is more irregular, the basement membrane is altered, and the endothelium is usually discontinuous [21]. This last characteristic of the tumor microenvironment is used by migratory tumor cells to escape from the primary tumor and enter the circulatory system, which often results in the formation of cancer metastasis.

One of the common traits signaling the promotion from equilibrium to the escape phase is the occurrence of secondary tumors distant from the initial location of cancer cells. To do this, cancer cells must travel through the circulatory or lymphatic systems. Even though these circulating tumor cells could in theory be more vulnerable to detection and attack by the immune system in the vascular circulation, evidence from our lab and others has shown that neutrophils mediate tumor cells transendothelial barrier crossing to form metastases (Fig. 7.3a) [41, 42]. Initially, experiments in vivo by video microscopy showed that tumor cell interaction with the endothelium was limited

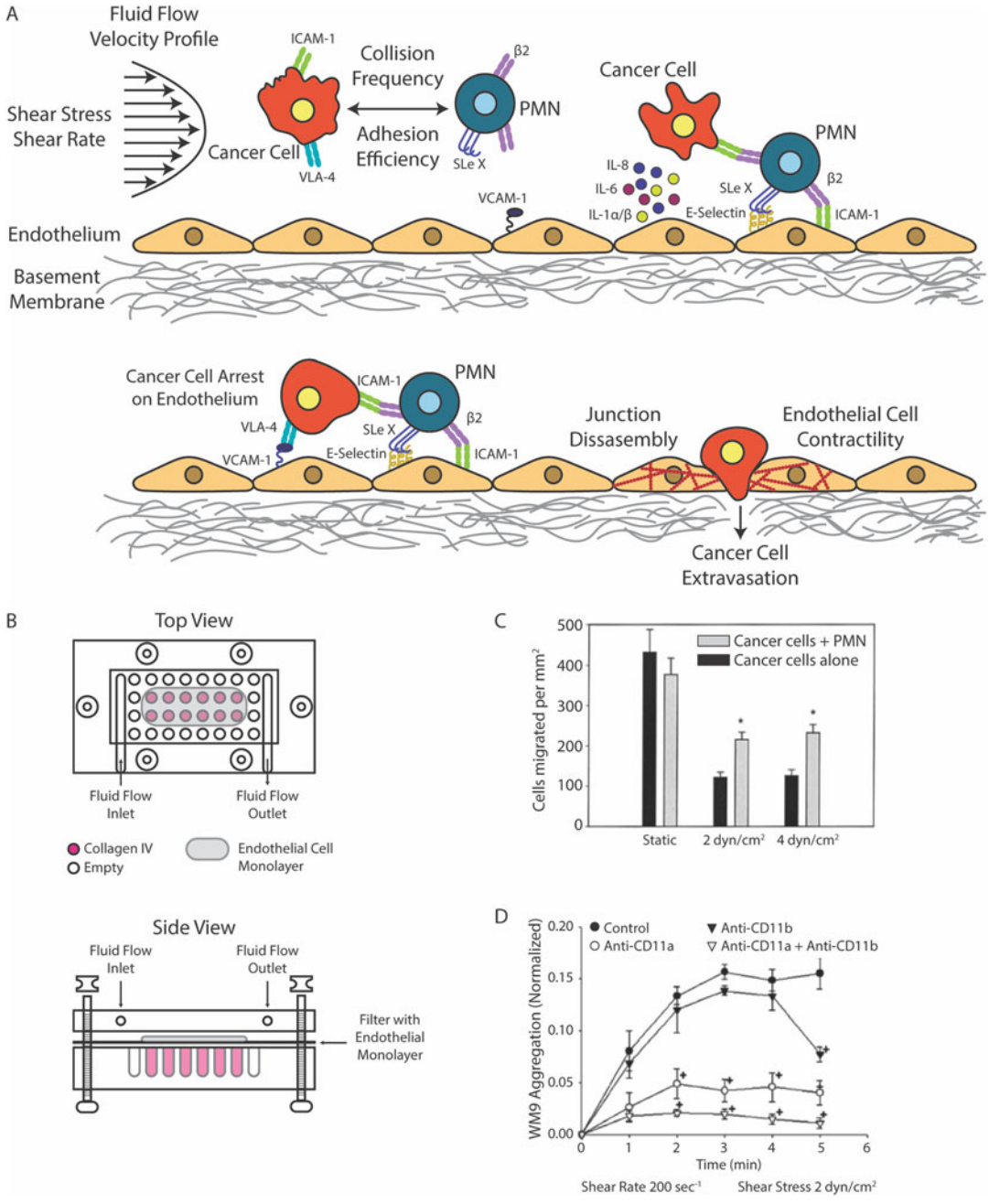

Fig. 7.3 (**a**) Interactions between cancer cells and PMNs in the vascular system depend on the frequency of collisions and the efficiency of adhesion. Under fluid flow conditions, cancer cells arrest on the endothelium assisted by neutrophils; they bind together through ICAM-1 and β2 integrin interactions (β2 integrin is essential to the formation of both receptors, Mac-1 and LFA-1) or through the very late antigen-4 (VLA-4) and vascular cell adhesion molecule-1 (VCAM-1) binding. Once cancer cells arrest on the endothelium, they initiate cell-cell junction disassembly and endothelial cell contractility to create gaps and extravasate. This final step is essential for the formation of secondary tumors. (**b**) Top and side views of the flow extravasation chamber that combines chemotaxis with dynamic flow conditions. (**c**) Migration of melanoma cells alone or assisted by neutrophils under static and dynamic flow conditions, the chemoattractant was collagen IV and migration was assessed after 4 h. Results represent mean ± SEM, $n = 3$ [41]. D. Relative contribution of Mac-1 (CD11b) and LFA-1 (CD11a) receptors on heterotypic cell-cell binding. Results show normalized WM9-PMN aggregation in a parallel plate flow assay. Results show mean ± SEM, $n = 3$ [60]

to the microcirculation [43]. This observation suggested that cancer cells were trapped in capillaries based on vessel-size restriction, and subsequent extravasation only occurred at these places. However, further evidence in vivo showed that melanoma cells can be arrested on the wall of presinusoidal vessels in mice pretreated with interleukin-1α (IL-1α) [44]. Later, in our lab, it was demonstrated, by comparing static and dynamic flow conditions, that the interaction between neutrophils and cancer cells, for cancer cell arrest on the endothelium, is particularly important under dynamic flow conditions (Fig. 7.3c). The endothelium has several adhesion molecules, upregulated in response to local environmental signals, that interact with multiple cells from the immune system, e.g., P- and E-selectins and intercellular adhesion molecule-1 (ICAM-1) mediate interactions with neutrophils and lymphocytes and vascular cell adhesion molecule-1 (VCAM-1) mediates interactions with eosinophils and basophils [45, 46]. Cancer cells, on the other hand, are heterogeneous in the expression of adhesion molecules. However, to metastasize successfully they must be able to either directly interact with the endothelium or elicit immune cells to mediate the adhesion or both.

Dynamic flow in the vascular system imposes mechanical restrictions that affect the interactions between cells in the circulation and the endothelial cells in the vessel wall. The binding of white blood cells (WBC) to the endothelium comprises a sequence of events mediated by a delicate balance of hemodynamic forces from the blood flow and adhesion forces between proteins in the plasma membrane. Microscopic analyses along with biomechanical models constructed to understand the effect of these forces in leukocytes revealed that the contact area between WBC and the endothelium increases with time as the WBC stretches and then decreases and as the trailing edge of the WBC retracts from the endothelium in the direction of the flow [47]. Model simulations based on experimental data reveal that changes in the ratio of the shear stress around the WBC and the drag force decreases with WBC deformation and increases with the diameter of the vessel. This implies that the net hemodynamic and adhesion forces are influenced by the deformability of the cell and the adhesion kinetics. A comparison of the model with data collected from in vivo experiments indicates that WBC deformability is an essential feature that aids in its adhesion to endothelial cells [47]; a flattened cell on the vessel wall causes fewer disturbances to the flow and experiences lower shear stress [48]. In contrast to WBC, cancer cells appear to be stiffer; using a suspended microchannel resonator to compare the deformability of cancer cells and blood cells, Shaw et al. concluded that blood cells are more deformable than cancer cells [49]. Thus, it is not surprising that cancer cells in circulation hijack white blood cells to adhere to the endothelium.

Cells in circulation experience shear stress around 1–6 dyn/cm^2 in the venous system and between 10 and 70 dyn/cm^2 in the arterial system on healthy adults [50]. Cancer cell extravasation is usually observed in the bifurcation of veins where the shear stress is lower, which suggests that cell adhesion is regulated by shear forces [51]. In addition, these sites in the circulatory system show hematocrit enrichment and high shear rates; this characteristics promote margination of leukocytes to the vessel wall and provide better chances for leukocyte rolling and adhesion to the endothelium [51]. Binding between cell adhesion molecules under high shear rate conditions requires a high on-rate for bond formation, and subsequent bond stability requires high tensile strength. To explain how cell rolling and adhesion can be enhanced under increased flow conditions, a new type of non-covalent bond was proposed, the “catch” bond. Intuitively, the lifetime of non-covalent bonds decreases as they undergo tensile forces; this is described as the “slip” bond, and multiple examples of this behavior have been widely observed in the interactions between cell adhesion molecules (CAMs) [52, 53]. In contrast, the lifetime of “catch” bonds increases as they

experience tensile forces. Binding molecules can experience changes in their 3D configuration under high shear forces that might strengthen the bond, suggesting a possible explanation for the mechanism of action of the "catch"-bond behavior. Single molecule experiments using atomic force microscopy (AFM) have demonstrated that selectins [54], integrins [55], and cadherins [56] exhibit "catch"-bond behavior up to a limit threshold and then transition into "slip"-bond behavior. This transition between "catch" and "slip" bonds provides a mechanism to mechanically regulate cell-cell adhesion under shear stress conditions. Indeed, studies have suggested that a minimum shear stress is needed for leukocyte rolling and adhesion to the luminal side of the endothelial wall. As shear stress grows, the number of adherent leukocytes increases to a point and then gradually decreases [54, 57].

As mentioned above, it has been previously shown that cancer cells can bind to the endothelium and extravasate in the absence of shear stress. However, when fluid flow is present, cancer cells rely on immune cells to arrest on the endothelium before escaping the vascular system. A study using melanoma cells found that the efficiency of extravasation increased 85% when melanoma cells were assisted by neutrophils (PMNs). Using a modified Boyden chamber that integrates shear flow and chemotactic migration (Fig. 7.3b), Slattery et al. showed that melanoma cells bind to PMNs through ICAM-1 and CD11b/CD18 (Mac-1) receptor interactions and that this binding is strong enough to arrest melanoma cells on the endothelium under 4 dyn/cm^2 shear stress and facilitate extravasation [41]. Furthermore, they show evidence that the interactions between neutrophils and cancer cells are not limited to bond formation for cancer cell arrest on the endothelium, but also cancer cells affect the normal functions of neutrophils. In agreement with multiple studies, Peng et al. [58] found that melanoma cells affect cytokine expression by PMNs; by increasing interleukin-8 (IL-8) secreted by PMNs, melanoma cells create a potential auto-stimulatory microenvironment [41]. IL-8 can increase Mac-1 expression on PMNs to strengthen melanoma cell adhesion and activate the endothelium for cell extravasation. A follow-up study by our group demonstrated that blocking CXCR1 and CXCR2 (receptors for IL-8) on PMNs decreased Mac-1 upregulation and reduced melanoma cell extravasation. Furthermore, we found that CD11a/CD18 (LFA-1) is also necessary for melanoma cells arrest in the endothelium. In fact, blocking monoclonal antibodies against CD11b showed that LFA-1 is necessary and sufficient for the initial arrest of melanoma cells, but Mac-1 is responsible for the stabilization of PMN-melanoma aggregates on endothelial cells. The initial rate formation of cell clusters in anti-CD11b-treated cells was the same as control; however, rapid disaggregation was observed after only 3 min (Fig. 7.3d). In contrast, PMN-melanoma control cells remained stable in clusters [59, 60]. All these studies suggest a more complex role between cancer cells in circulation and the endothelial wall that goes well beyond a simple entrapment due to vessel-size restriction.

One of the dominant forces in the circulation affecting tumor cells is the hemodynamic force created by blood flow. Interestingly, it has been shown that shear rate rather than shear stress plays a more significant role in the aggregation of melanoma and PMNs cells and its subsequent adhesion to the endothelium (Fig. 7.4a). By using high molecular weight dextran, Slattery et al. were able to modify the viscosity of the circulating medium, thus maintaining a constant shear rate while increasing the shear stress [61]. In a subsequent study, Liang et al. recognized that shear rate is inversely proportional to the cell-cell contact time (Fig. 7.4b). Using a similar experimental setup, they proposed a two-step adhesion mechanism in which PMNs first roll and arrest on the endothelium and then capture circulating melanoma cells. The study shows that endothelial E-selectin and ICAM-1 modulate the first step in response to shear rate and shear stress, and melanoma expressed ICAM-1 affects the second step in response only to shear rate [60, 62]. These results taken together suggest that once the bonds are formed, they are very stable, and larger hemodynamic forces do not increase dissociation rates. In contrast, hemodynamic forces regulate

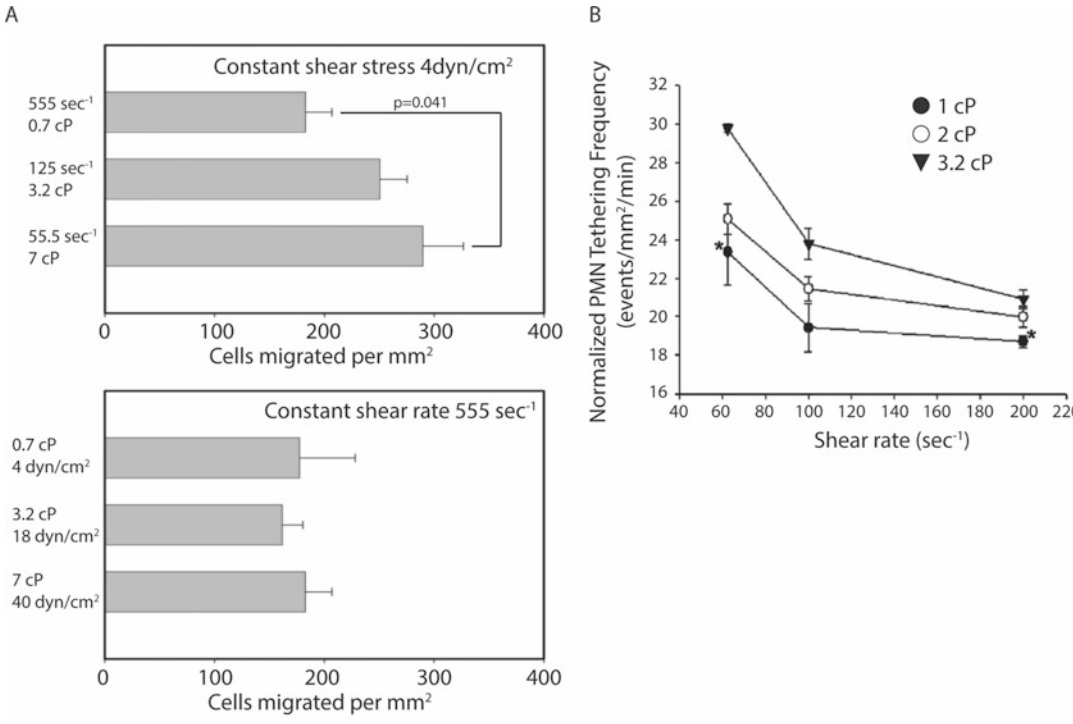

Fig. 7.4 (**a**) Effect of shear rate and shear stress on migration of melanoma cells assisted by neutrophils. Results show the mean ± SEM, $n = 3$ [61]. (**b**) Effects of shear rate and shear stress on tethering frequency of PMNs on a monolayer of endothelial cells. Results show the mean ± SEM, $n = 3$ [60]

cell-cell collision and larger shear rates decrease contact time between cells, effectively decreasing bond formation.

The binding of a receptor to a ligand can be considered like a chemical reaction. Thus, in the case of cellular adhesion, reaction kinetics can be used to study the rate of binding and dissociation. The rate of receptor-receptor binding depends on two parameters, the intrinsic kinetic constants of the molecules and the time of interactions that is governed by the hemodynamic flow. Multiple studies have determined the kinetic parameters for interactions between ICAM-1 on endothelial cells and β2-integrins on PMNs [63]. However, Hoskins et al. [64] estimated the kinetic parameters describing the interactions between ICAM-1 receptors in melanoma cells and β2-integrins expressed in PMNs to understand if the cell type or the molecular expression affects these parameters. Their results show that the dissociation rate (k_{off} ~0.3 s^{-1}) for melanoma cells and PMNs is higher compared with the dissociation rate for endothelial cells and PMNs (k_{off} ~0.1 s^{-1}); this suggests that the ICAM-1 receptors expressed in melanoma cells have lower affinity for β2-integrins in PMNs compared to endothelial cells [64]. It is worth noting that most of the experiments to calculate k_{off} rate using PMNs have been done using recombinant purified molecules immobilized on to a substrate. In contrast, the experimental setup implemented by Hoskins et al. used a monolayer of melanoma cells with circulating PMNs in a parallel plate flow chamber, which is a more complex system where other adhesion proteins are present; this can potentially confound the result.

Mechanistic studies in vivo using siRNA technology emphasize the importance of ICAM-1 expressed on melanoma cells binding to β2-integrins on PMNs for cell extravasation and progression of cancer metastasis. In the case of melanoma, B-Raf is the most commonly mutated gene; the single nucleotide polymorphism (SNP) at position 1799 changes a thymine (T) nucleotide for adenine (A), which in consequence changes amino acid 600 from valine (V) to glutamic acid (E) [65]. Initially, the location of the SNP was misidentified as amino acid 599; thus in the literature, it is sometimes referred to as V599E [66]. Knockdown of V600E B-Raf in melanoma cells shows a decreased in ICAM-1 expression resulting in reduced melanoma cell extravasation (Fig. 7.5a, b) [67]. In addition, lower ICAM-1 expression is a direct response to lower IL-8 production in the tumor microenvironment [67]. These experimental results in vitro were confirmed in vivo by performing tail vein injections of melanoma cells in nude mice and monitoring metastasis formations in the lungs [66]. Targeting V600E B-Raf using siRNA significantly reduced tumor formation in the lungs compared to buffer control or scrambled siRNA (Fig. 7.5c) [66]. In a follow-up study, PMNs were shown to be of great importance for melanoma cell extravasation in vivo, confirming previous results. Using nude mice, Huh et al. showed that melanoma cell retention followed by cell extravasation in the lungs was increased threefold when melanoma cell injection was followed by PMN injection, as opposed to injection of melanoma cells alone [68]. This study also identified IL-8 as a major modulator of the interactions between PMNs and melanoma cells; when melanoma cells were transfected with siRNA targeting IL-8, lung metastasis formation was significantly reduced. Similar results were found in multiple studies using animal models of liver metastasis. Neutrophils were found to increase cancer cell binding to sinusoids in the liver and promote metastasis. When neutrophils were depleted in mice before inoculation of cancer cells, the effect decreased. However, this effect was reversed when neutrophils were co-inoculated with cancer cells. Using intravital microscopy they showed that cancer cells generally arrest on top of neutrophils already adhered to the endothelial wall [69, 70].

Neutrophils are the most abundant type of myeloid cells present in the circulation [71]. Thus, it is not surprising that cancer cells interact with neutrophils while traversing the circulatory system. However, more recent evidence suggests that in addition to neutrophils, monocytes also aid circulating tumor cells to adhere to the endothelium and extravasate. In vitro experiments using breast cancer cells and THP-1 cells (a monocyte cell line) or primary monocytes show that binding interactions between monocytes and breast cancer cells are also strong enough to withstand disaggregating forces in circulation [72].

7.4 Direct Interactions Between Cancer Cells and Endothelial Cells

It is worth mentioning some evidence suggesting that under specific conditions, cancer cells can interact directly with endothelial cells on the vessel wall. Either through cytokine release or receptor-receptor interactions, cancer cells can affect the endothelial barrier dynamics. In the absence of PMNs, Liang et al. showed that binding between VLA-4 on melanoma cells and VCAM-1 receptor on inflamed endothelial cells mediates adhesion only under low shear conditions; under high shear rates, cancer cells cannot bind to the endothelium by themselves [73]. More recent evidence suggests that the direct interaction between cancer cells and the endothelium through VLA-4 and VCAM-1 interactions is preferentially used by highly metastatic melanoma cells compared to low metastatic cells [74]. Comparing WM35 low metastatic melanoma cells with A2058 high metastatic melanoma cells, we show that even though both cell lines are derived from melanoma lesions, the expression levels of cell adhesion molecules is different. Higher metastatic melanoma cells express more VLA-4 receptors. This difference is sufficient to disrupt the endothelial barrier and promote cancer cell

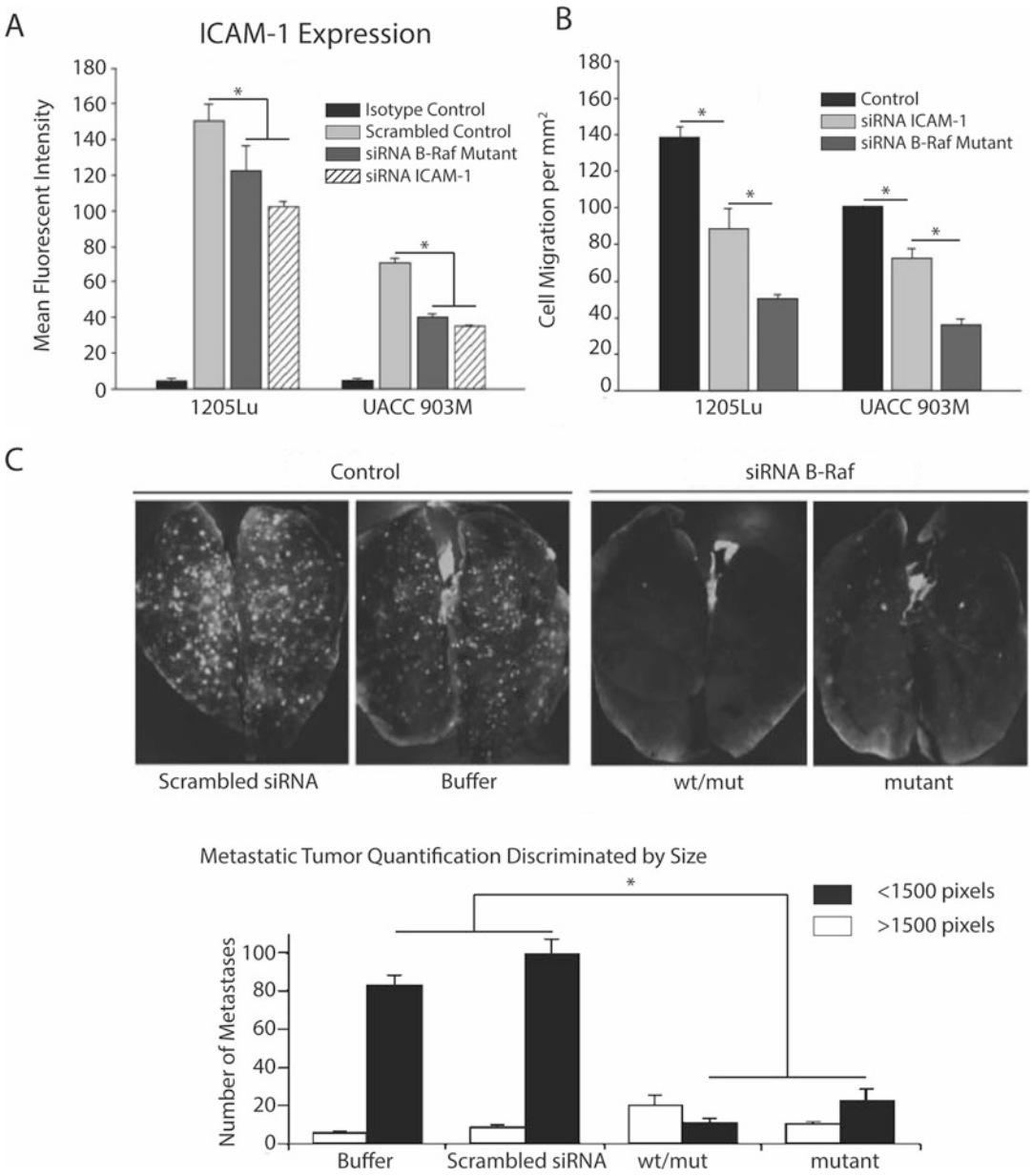

Fig. 7.5 (**a**) Knockdown of mutant V600 EB-Raf decreases ICAM-1 expression in melanoma cells. Results show mean ± SEM, $n = 3$ [67]. (**b**) Knockdown of mutant V600E B-Raf significantly decreased melanoma cell migration in vitro. Results show mean ± SEM, $n = 3$ [67]. (**c**) Knockdown of mutant V600E B-Raf significantly decreases metastasis formation in the lungs of nude mice [66]

extravasation (Fig. 7.6a). Interestingly, a previous study reported that cancer cells have the ability to change the biomechanical properties of endothelial cells when they come in direct contact. Using magnetic tweezer measurements, Mierke et al. show that the stiffness of endothelial cells decreases when they interact with breast cancer cells through the α5β1 integrin receptor [75]. This means that cytoskeletal remodeling dynamics increases in endothelial cells when they come in contact with breast cancer cells. In agreement with the previous study, new evidence from our

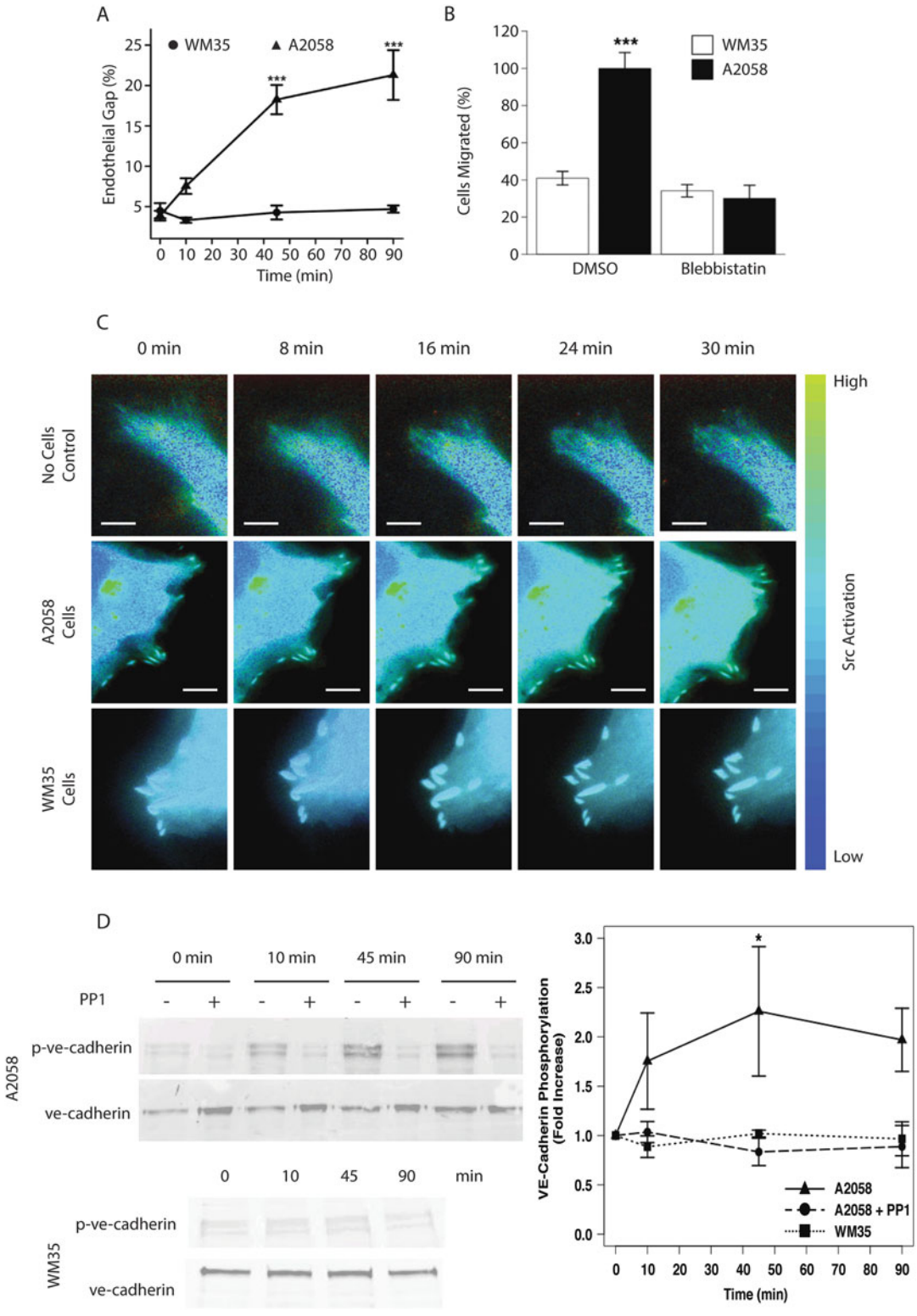

Fig. 7.6 (**a**) Effect of high metastatic melanoma cells vs. low metastatic melanoma cells on the disruption of the endothelial barrier measured as intercellular gap formation. (**b**) Cell migration across endothelial monolayers of high and low metastatic cells. In both cases the results show the mean ± SEM, $n = 3$. (**c**) Src activation monitored via FRET biosensor. Src activation was monitored for 30 min, and the results show time-lapse images of FRET signal [74]

lab suggests that cancer cell migration across the endothelium is decreased when contractility in endothelial cells is blocked (Fig. 7.6b) [74]. Using blebbistatin to block cell contractility, we show that migration of high metastatic melanoma cells (A2058) is significantly reduced when endothelial cells are not able to contract.

Melanoma cells also produce large amounts of IL-8 [76, 77]. This cytokine is considered a key mediator for endothelial barrier breakdown in the absence of PMNs. Initially, Khanna et al. [77] showed that low metastatic melanoma cells (WM35) express significantly lower levels of IL-8, compared to high metastatic melanoma cells (A2058 and 1205Lu). Using tumor-conditioned media collected from either cell line, the study shows that IL-8 produced by cancer cells promotes endothelial gap formation. The study also identified IL-6 and IL-1β as contributors to gap formation but to a lesser extent. Furthermore, they revealed that the p38 MAP kinase mediates this effect. By knocking down p38 Map kinase in HUVEC cells, extravasation of melanoma cells was decreased by 60% compared to control cells.

After some of the main modulators used by cancer cells to affect endothelial cells were identified, IL-8 and VLA-4, a follow-up study in our lab focused on finding possible mechanisms for endothelial barrier disruption induced by melanoma cells. The endothelial barrier is maintained by homodimer interactions of vascular endothelial (VE) cadherins located on the cell membrane of endothelial cells, which in turn are supported by the cytoskeleton in each cell. We proposed that gap formation in the endothelial barrier involves two main processes, cell-cell junction disassembly, meaning the disruption of ve-cadherin homodimers, and endothelial cell contractility. Phosphorylation of ve-cadherin is one of the main steps leading to homodimer disruption. Using a Src FRET biosensor in conjunction with western blot assays to monitor ve-cadherin phosphorylation, we show that high metastatic A2058 cells, but not low metastatic WM35 cells, activate Src in endothelial cells and that they do it through IL-8 secretion and engagement of the VCAM-1 receptor. Activation of Src by A2058 high metastatic melanoma cells results in phosphorylation of ve-cadherin and cell-cell junction disassembly (Fig. 7.6c, d). Multiple pharmacological inhibitors of cell contractility were used to show that endothelial cell contractility is necessary for melanoma cell extravasation. These results together show that metastatic cancer cells use cell-cell interactions and cytokines to disrupt the endothelial barrier and extravasate from the vascular system to reach distant organs.

7.5 Immunotherapies

Activating and harnessing the power and specificity of the immune system to fight against infectious diseases or cancers is a major goal of immunotherapy. The concept of treating cancer by active immunization was theorized in 1890s, when Paul Ehrlich and William Coley proposed the use of weakened tumor cells as a tumor-targeting vaccine [78–80]. Many immune-oncology approaches aim to unleash the potential of large numbers of functional, high T-cell avidity and cytotoxic T lymphocytes (CTLs) to penetrate tumors and kill cancerous cells [81, 82]. The first application of immunotherapies in the clinic was described in 1985 by Rosenberg et al. [83, 84] They described preliminary results after systemic administration of lymphocytes in combination with interleukin-2 (IL-2) in 25 patients with advanced cancer for whom standard treatment had failed. They reported that reduction of tumor volume of at least 50% was observed in 11 patients stressing the potential of immunotherapies. Their achievement ushered in a new era of adoptive immunotherapy (Table 7.1).

Cytokines: Cytokines are proteins produced in our body that play important roles in the body's normal immune responses and in the immune system's ability to respond to cancer. The two major cytokines used to treat cancer are interferons (IFNs) and interleukins (ILs). Tumor cells suppress major histocompatibility complex (MHC)-class I expression, which greatly reduces the antigenicity of tumor cells, thus preventing an immune response mediated by CTLs [85]. Im-

Table 7.1 Summary of currently available immunotherapies

Therapy	Mechanism and advantages	Disadvantages	References
Cytokines			
IL-2	· Stimulates the host's immune system · US FDA-approved	· Low response rates · Significant risk of serious systemic inflammation	[94, 95]
IFN- α	· Stimulates the host's immune system · Durable responses · Inhibits breast cancer progression	· Low response rates · Relative low toxicity	[94–96]
IFN- γ	· Generates mature dendritic cells for use in vaccines		[97]
Cell-based therapies			
Vaccines	· Stimulates the host's immune system · Minimal toxicity · Administered in the outpatient clinic	· Lack of universal antigens and ideal immunization protocols lead to poor efficacy and response	[95, 98]
Adoptive cellular therapy	· Omits the task of breaking tolerance to tumor antigens · Produces a high avidity in effector T cells · Lymphodepleting conditioning regimen prior to tumor-infiltrating lymphocyte (TIL) infusion enhances efficacy · Genetic T-cell engineering broadens TIL to malignancies other than melanoma	· Restricted to melanoma · Safety issues, serious adverse effects, and lack of long-lasting responses in many patients · Requires time to develop the desired cell populations · Expensive	[84, 95, 99–105]
Cell-mediated drug delivery systems			
Neutrophils	· Delivers liposomal antitumor drug to glioma		[106]
T cells	· Delivers chemotherapeutic agents in forms of nanoparticles/liposomes targeting lung cancer, lymphatic tumor · Delivers oncolytic virus targeting myeloma, colorectal cancer cells · Delivers immunomodulators to carcinoma		[107–110]
Natural killer cells (NK cells)	· Targets and kills tumor cells · Delivers TRAIL (tumor necrosis factor-α-related apoptosis inducing ligand)-coated liposomes to lymphatic tumor and circulating tumor cells (CTCs) · Delivers gold nanoparticles conjugated with antibodies that bind to neuroblastoma and melanoma and releases cytokines to kill cancer cells		[111–113]
Monocytes, macrophages	· Delivers therapeutics to lung cancer, melanoma		[82, 114]
Immune checkpoint blockade			
Anti-CTLA-4 monoclonal antibodies	· Unleashes pre-existing anticancer T cell responses · Exhibits strong antitumor properties · Extends overall survival	· Only a small fraction of patients obtain clinical benefit · Severe immune-related adverse events have been observed in up to 35% of patients	[95, 99, 115–117]

(continued)

Table 7.1 (continued)

Therapy	Mechanism and advantages	Disadvantages	References
Anti-PD1 and anti-PD-L1 antibodies	· Sufficient clinical responses which are often long-lasting · Therapeutic responses in patients within a broad range of human cancers · Reduced toxicity compared to anti-CTLA-4 antibodies	· Only a relatively small fraction of patients obtain clinical benefit	[95, 118–120]
Combination immunotherapy (immune checkpoint as the backbone)	· Improvement of antitumor responses/immunity	· May lead to increase in the magnitude, frequency, and onset of side effects	[95, 121, 122]

paired MHC expression is commonly observed in patients with melanoma and breast cancer [86, 87]. Natural killer cells (NK cells) become suppressed in their functional activity in MHC-deficient tumor cells in vivo. Pro-inflammatory cytokines were used to revert the functionality of NK cells within MHC-deficient tumors. Levin et al. treated MHC-deficient, tumor-bearing mice with a cocktail of recombinant IL-12 and IL-18 or a mutant form of IL-2, also called a "superkine," which strongly binds to the IL-2 receptor even when it lacks the receptor α-chain (CD25) [88]. Both treatments increased the survival of MHC class I-deficient tumor-bearing mice considerably by reverting the functionality of tumor infiltrating NK cells. Cytokine treatments were relatively nontoxic and also increased the life span of tumor-bearing animals.

The interferons (IFNs) are a family of pleiotropic cytokines that protect against diseases by directly affecting target cancer cells and by activating antitumor immune responses [89]. The production and action mechanisms of IFNs are closely controlled to achieve maximal protection and avoid the potential toxicity associated with excessive responses. As IFNs can be produced by, and act on, both tumor cells and immune cells (e.g., CD8+ T cells and dendritic cells), understanding this reciprocal interaction will facilitate the development of improved single-agent or combination therapies that exploit IFN pathways. The biological roles of IFNs offered the rationale for using exogenous IFN-α as an anticancer treatment, which proved efficient against several solid and hematological tumors [90]. Mature and differentiated CD8+ T cells and certain types of CD4+ T cells release IFN-γ that enhances the immune response by upregulating the expression of MHC class I and MHC class II molecules on both tumor cells and tumor-resident antigen-presenting cells (APCs) [89].

Vaccines: Cancer vaccines initiate the dynamic process of activating the immune system to successfully re-establish a state of equilibrium between tumor cells and the host [80]. Cancer vaccines introduce tumor-associated antigens to cause tumor regression by relying on a cascade of events that are coordinated by dendritic cells (DCs). Innate antigen recognition and processing are the responsibilities of DCs, which, upon activation, have a powerful ability to present tumor antigens processed onto MHC and to translate pathogenic danger signals into the expression of specific cytokines and stimulatory molecules that signal antigen-specific T-cell proliferation and differentiation. The administration of different combinations of cytokines that induce the production of DCs with various phenotypes and functions has been applied as vaccines to cancer patients. The Bacillus Calmette-Guerin (BCG) vaccine has also been used to infect DCs to augment their expression of MHC molecules, suggesting that the infected DCs have an increasing ability to increased the activation of T cells [91–93]. Those activated T cells induced cytotoxicity against BCG-infected bladder cancer cells. Patients with tumor-associated DCs prior to BCG treatment were more likely to experience bladder carcinoma recurrence after BCG therapy.

Adoptive cell transfer: More than a decade ago, it was evident that either directly stimulating T cells inside a patient or finding a good source of antitumor T cells for injection or releasing blocked checkpoints in lymphocytes in vivo could be viable approaches for new cancer therapies [123]. Lately, one of the therapy approaches drawing most attention is called adoptive cell transfer (ACT) that was first introduced by Rosenberg et al. in 1988 [124]. This is a highly personalized approach, and its goal is to supply the patient with large quantities of antitumor cells to cause an objective regression of the disease. As early as 1987, it was reported that tumor infiltrating lymphocytes (TIL) isolated from patients with metastatic melanoma exhibited cytotoxicity toward autologous tumor cells and could be a source of T cells for ACT therapy [125]. Lymphocytes for ACT therapy are isolated from the host and expanded in vitro. During the expansion process, cells are sorted to enrich a population already presenting tumor reactivity. One major advantage of ACT is that the host can be pretreated; the immunosuppressive microenvironment can be modulated before cells are injected. The key issue to make ACT therapy a success is the identification of target molecules differentially expressed in cancer cells and normal tissues; specific mutations on proteins expressed on the cell membrane of individual tumor cells are the ideal candidates [126]. Tumor-infiltrating lymphocytes (TILs) used in ACT therapy can be cultured from resected melanoma tumors. This approach has been shown to mediate durable, complete regressions of metastatic melanoma [127–129].

Chimeric antigen receptor T-cell (CAR-T) therapy: During the expansion process of TILs, cells are genetically modified to express specific antitumor cell receptors or chimeric antigen receptors (CARs), all of which will target tumor cells. The expanded population of CAR T cells is then infused into the patient. After the infusion, the T cells multiply in the patient's body and, with guidance from their engineered receptor, recognize and kill cancer cells that harbor the antigen on their surfaces. CARs are a type of antigen-targeted receptor composed of intracellular T-cell signaling domains fused to extracellular tumor-binding moieties, most commonly single-chain variable fragments (scFvs) from monoclonal antibodies. CARs directly recognize cell surface antigens, independent of MHC-mediated presentation, allowing the use of a single receptor construct specific for any given antigen in all patients. In the case of CAR T therapy, the host can also be pretreated to modulate the immunosuppressive microenvironment before cells are injected.

In spite of immune surveillance, tumors do develop and evade the presence of a functioning immune system [90]. Therefore, emerging technologies focus on overcoming the activation energy barrier presented by the immunosuppressive tumor microenvironment [130]. Recent preclinical and clinical results suggest that delivery of immunostimulatory molecules can rouse the immune system with greater rigor, leading to improved antitumor immunity and survival outcomes [130]. Different drug payloads are available to be incorporated in the immune cell-mediated delivery systems (DDSs) as introduced below.

Cell-mediated drug delivery: Cell-mediated DDSs have emerged as a promising strategy to deliver therapeutics to different cancers. This novel technology takes advantage of cell properties, such as long circulation time, abundant surface ligands, flexible morphology, cellular signaling, and metabolism, to offer a unique opportunity to maximize therapeutic outcomes as well as minimizing side effects [81].

Direct antitumor effect of immune cells: In addition to their role as carriers of viruses and drugs, there is a synergy effect between the viruses and the immune cells that improves the antitumor effects of both. For example, cytokine-induced killer (CIK) cells identify their targets via the NKG2D receptor and its ligands, including the stress response ligands, MICA and MICB. The ligands are upregulated in human tumors as a result of the various stresses imposed against tumor growth. Viral infection is one type of a stressor, and it can increase NKG2D ligand expression. When CIK cells enter cancer cells, CIK cells act like natural killer (NK) cells and try to kill the cancer cells.

Types of immune cells used in cell-mediated drug delivery: Leukocytes, or white blood cells (WBCs), play crucial roles in the immune system, by removing cellular debris and defending the body against infections and diseases [81]. Leukocytes are found in five major types: neutrophils (40–75%), lymphocytes (20–45%), monocytes (2–10%), eosinophils (1–6%), and basophils (less than 1%). Although the life span of leukocytes (up to 20 days) is typically shorter than that of red blood cells (RBCs), their specialized functions make them appealing drug delivery carriers because leukocytes are involved in various immune responses, cellular interactions, and cell-cell adhesion and are capable of penetrating through biological barriers into tissues.

Neutrophils are the first cells that arrive at the sites of infection or inflammation, produce cytokines to attract other cells, and are removed after a few days. Neutrophils can also engulf invading microorganisms or foreign substances and consequently eliminate the invaders using digestive enzymes or respiratory burst [131]. Scientists have utilized neutrophils to deliver therapeutic nanoparticles or liposomes across the blood vessel barrier or blood-brain barrier for the treatment of inflammation, infection, and cancers [82, 132]. Unfortunately, neutrophils have the average life span of 5.4 days in circulation and only a few hours after their isolation from blood. The short life span of neutrophils restricts their applications in DDSs.

Monocytes are mononuclear leukocytes with kidney-shaped nuclei and clear cytoplasm. They are produced from stem cell precursors in the bone marrow. Monocytes circulate in the bloodstream and migrate to tissues, particularly the liver, lymph nodes, and lungs. They also migrate to and accumulate at disease sites in association with infection or inflammation [133]. Once leaving the blood flow, monocytes differentiate into macrophages in response to various stimulations. Otherwise, they return to the bone marrow without activation. Macrophages play versatile roles in inflammation, cell recruitment, cytokine and growth factor secretion, and bacteria/cellular debris removal. Recent studies also indicate that macrophages are the major players in disease microenvironments and disease progression, such as in cancer invasion. Additionally, monocytes/macrophages present phagocytic capability that allows the spontaneous encapsulation of therapeutic vehicles [134].

Xie, Dong, and Yang et al. recently developed a smart, targeted, and living drug delivery system by using human monocytes/macrophages (THP-1 cells) to kill human melanoma cells (Fig. 7.7a) [82]. Once differentiated, macrophage-like THP-1 cells first took up and internalize biodegradable and photoluminescent poly (lactic acid) (BPLP-PLA) nanoparticles loaded with a melanoma-specific drug that inhibits B-Raf (PLX4032). The BPLP-PLA polymer is fully degradable with tunable fluorescent properties. Nanoparticle uptake efficiency by THP-1 cells was further enhanced by chemically conjugating muramyl tripeptides (MTPs) onto the surface of the nanoparticles. The internalization of nanoparticles did not alter the macrophage-like functionality of THP-1 cells as confirmed in the unaffected expression of CD11b (an alpha chain of the β2 integrin MAC-1) of the THP-1 cells after they take up the nanoparticles. In order to evaluate the therapeutic potential of macrophages in the environment similar to the bloodstream, the macrophages were allowed to bind to the melanoma cells on a cone-plate viscometer. THP-1 cells were pretreated with BPLP-PLA nanoparticles for 2 h and then co-cultured with GFP-tagged high metastatic melanoma cells (1205Lu) for 1 h under static conditions, and dynamic conditions with shear rates varied from 50 to 200 s^{-1}. The maintenance of CD11b on the surface of the macrophages even after NP uptake allowed the cells to bind to the melanoma cells through ICAM-1 under shear stress conditions (Fig. 7.7b). After the THP-1 cells successfully bound to the melanoma cells, the nanoparticle-drug complexes were released from the THP-1 cells by exocytosis and were able to release PLX4032 in a sustained manner to kill both high and low metastatic melanoma cells (1205Lu and WM35 cells, respectively).

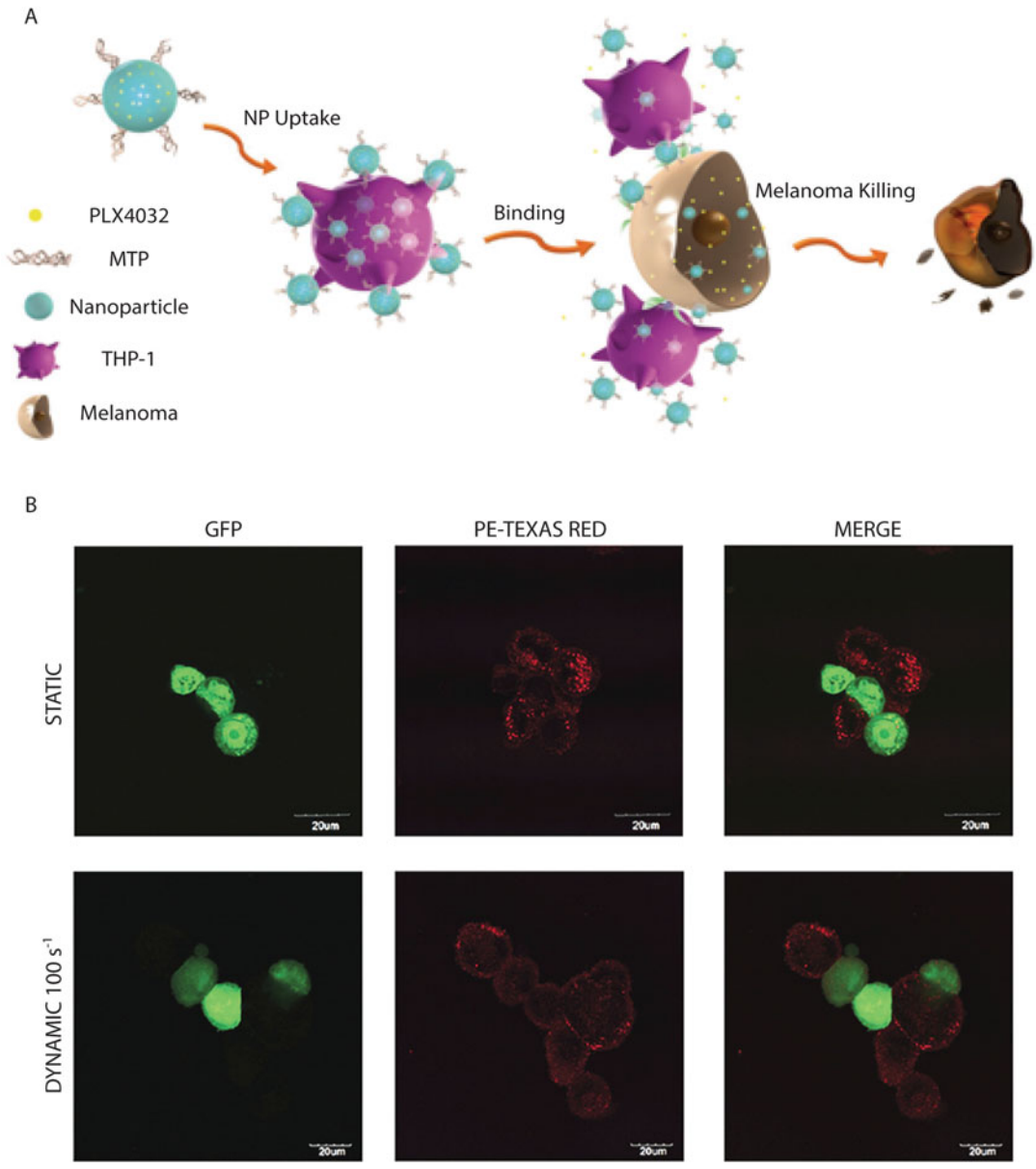

Fig. 7.7 (**a**) Schematics of the immune cell-mediated nanoparticle (NP) delivery system targeting melanoma cells developed by Xie et al. (**b**) Confocal images THP-1 (not stained)/GFP-1205Lu binding and nanoparticle (PE-Texas red) delivery, scale bar: 20μm [82]. Reprinted with permission from ref. 75

Lymphocytes are characterized by their large nucleus surrounded by a thin layer of cytoplasm with their average diameters between 7 and 15 μm. They are primarily located in the circulation and central lymphoid organs, including the spleen, tonsils, and lymph nodes [135]. T cells and B cells are the major types of lymphocytes and are responsible for the adaptive immune system. T cells mature in the thymus and play a critical role in cell-mediated immunity and can be broadly divided into helper T cells, cytotoxic T cells, and regulatory T cells [136]. When an antigen appears, antigen-presenting cells (APCs) recognize and present the antigen

to T cells. Then, helper T cells secrete various cytokines, which stimulate cytotoxic T cells to directly eliminate abnormal cells. Regulatory T cells are also activated to suppress immune response in order to maintain immunological tolerance. B cells are produced in the bone marrow and involved in humoral immunity. B cells make antibodies against antigens and can be characterized by the presence of immunoglobulin on their surface [137]. B cells can differentiate into memory B cells, which respond rapidly when exposed to the same antigen. Therefore, both lymphocytes present multiple functions in human immunity and are involved in numerous diseases: detecting antigens, infiltrating disease sites, and attacking abnormal cells. Clearly, lymphocytes could serve as a potential platform to deliver drugs specifically to cancer cells [107, 138, 139]. Overall, leukocytes have a rapid response and intrinsic homing properties with respect to infections, inflammations, and tumors. Such sensitive detections and biological barrier infiltration abilities give rise to opportunities for leukocytes-mediated drug delivery. However, vulnerable leukocytes are difficult to harvest and handle with relative short life spans, which hinder the manipulation processes for loading drugs.

Along with T cells, dendritic cells (DCs) play a critical role in the immune response by controlling both immune tolerance and immunity [130, 140]. DCs are bone marrow-derived cells that are found in all tissues. DCs sense their environment through both surface and intracellular receptors and promptly respond to environmental signals, differentiate into mature DCs, and transmit the information to both T cells and B cells. DCs initiate an immune response by presenting the captured antigen, which is in the form of peptide-major histocompatibility complex (MHC) molecule complexes, to naïve or antigen-inexperienced T cells in lymphoid tissues. As compared with antigen-presenting cells (APCs), such as macrophages, DCs are exceptionally efficient in stimulating very low numbers of T cells to respond.Dendritic cells also migrate to the tumor site and promote production of immunostimulatory cytokines such as IFN-γ, IFN-α, and IL-12. These properties render them the central candidates for antigen delivery and vaccination against cancer [122].

DCs can be produced ex vivo by culturing hematopoietic progenitor cells or monocytes with cytokine combinations and have been tested as therapeutic vaccines in cancer patients for more than a decade [141]. Sipuleucel-T (also known as APC 8015), which is a cellular product based on enriched blood APCs that are cultured with a fusion protein of prostatic acid phosphatase (PAP) and GM-CSF, was used in the treatment of metastatic prostate cancer [142, 143]. The treatment resulted in an approximately 4-month-prolonged median survival in phase III clinical trials, and sipuleucel-T has been approved by the US Food and Drug Administration (FDA) for the treatment of metastatic prostate cancer [122, 144].

Monoclonal antibodies and immune checkpoint blockade: Monoclonal antibodies bind to specific targets in the body. They can induce an immune response that can destroy cancer cells. Inhibitory receptors such as anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) protein expressed on tumor-specific T cells lead to compromised activation and suppressed effector functions such as proliferation, cytokine secretion, and tumor-killing effect. The immune checkpoint blockade refers to a new immunotherapy implemented to block negative regulatory receptors on T cells, in effect "taking the brakes off" the immune system and allowing endogenous natural immune responses against tumors to be unveiled [145]. This treatment was first introduced to treat advanced melanoma. Two different checkpoint blockade treatments targeting CTLA-4 and PD-1 have recently been approved by the FDA on the basis of striking clinical trial results in melanoma, renal cell carcinoma, and lung cancer. The anti-CTLA-4 antibody ipilimumab (Yervoy, Bristol-Myers Squibb) was the first immune checkpoint inhibitor drug ever to show

improved overall survival in phase III clinical trials and to be approved by the US FDA in March 2011 for the treatment of metastatic melanoma [146]. Although not yet compared in a randomized clinical trial, ipilimumab is generally considered more tolerable than high-dose IL-2. Both have promising durable response in melanoma. It is worthy of note that the response rate of ipilimumab may be less than that cited for IL-2. A recent follow-up study of 1861 melanoma patients treated with ipilimumab showed that about 20% survived 3 years, but most impressively, at this time the survival curve flattens, and most patients alive at 3 years are alive up to 10 years after therapy has been completed. Atypical patterns of tumor response to immunotherapies, including ipilimumab, make comparisons of response rates less informative; thus, milestone survival (e.g., at 3 years) may be a more appropriate measure of response to immunotherapy.

Monoclonal antibodies directed against PD-1 and its ligand, PD-L1 (programmed cell death ligand-1), have shown impressive antitumor responses with much potential in the treatment of melanoma, renal cell cancer, non-small cell lung cancer, and other tumors. Pembrolizumab (Keytruda®) and nivolumab are the first two anti-PD-1 checkpoint inhibitors that gained accelerated approval from the FDA for the treatment of ipilimumab-refractory melanoma [147]. The approval of pembrolizumab was based on the results from a phase II clinical trial of 123 patients with advanced or metastatic nonsquamous NSCLC (non-small lung cancer) without mutations in the EGFR gene or alterations in the ALK gene for which there exist targeted therapies. Patients in the trial had not been treated previously and were randomly assigned to receive either pembrolizumab along with chemotherapy or chemotherapy alone. In the trial, 55% of the patients who received pembrolizumab and chemotherapy had at least a partial response to the treatment, compared with 29% of patients who received chemotherapy alone. Medianprogression-free survival for the two groups was 13 months and 8.9 months, respectively.

7.6 Future Perspectives

In light of the evidence collected during the last three decades, the complementary role of biological signals and biophysical forces during cancer progression has been established. Nowadays, it is widely accepted that mechanical forces such as compressive stress in the tumor microenvironment contribute to shape the invasiveness and migratory ability of cancer cells. Further analysis of the interplay between biological, chemical, and biophysical cues in the tumor microenvironment will lead to better approaches for cancer diagnosis and therapies. Developing in vitro models that integrate all three components to study interactions between immune cells and cancer cells will result in approaches that better resemble the situation *in vivo*.

The idea of using immune cells to develop therapies for cancer patients is not a new one. However, this field is currently experiencing major advances, thanks to the development of better and more efficient technologies to genetically modify cells and new biocompatible materials to encapsulate drugs or molecules for diagnosis. This combination of improved techniques and materials will expand the breath of personalized medicine treatments available for cancer patients, and in the future, this field is only poised to expand.

Acknowledgments The authors sincerely thank the former members of the lab and their collaborators for their outstanding contribution to the work presented in this chapter, Dr. Margaret Slattery, Dr. Shile Liang, Dr. Hsin H. Peng, Dr. Meghan Hoskins, and Dr. Payal Khanna. This work was supported in part by NIH grants M01-RR-10732, CA-97306, C06-RR-016499, NIBIBEB012575, NCICA182670, and NHLBIHL118498; National Science Foundation (NSF) grants CBET-0729091, DMR1313553, CMMI1266116, and CBET-BME1330663; and the Pennsylvania Department of Health (PA-DOH)—Commonwealth Universal Research Enhancement (CURE) Program (Dong–Multi-P.I.), 2015–2017, "Development of Smart Drug Delivery Systems for Brain Tumors."

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