Chronic inflammation leads to adapted immune cells forming local and distant tumors at sites of inflammation

Leili Shahriyari

Mathematical Biosciences Institute, Ohio State University, Columbus, Ohio OH 43210, USA

shahriyari.1@mbi.osu.edu

Hypothesis

It is hypothesized that chronic inflammation leads to a deficient immune system that forms tumors not only at the primary site of chronic inflammation, but in other inflammatory sites.

Abstract

The main drivers of some tumors are defective immune cells, not epithelial cells. In particular, epithelial cells are just the victims; mutations occur in epithelial cells due to high levels of inflammatory signals secreted from defective immune cells. Therefore, I hypothesize that some metastases are the result of inflammatory processes by defective immune cells at sites of inflammation rather than migration of tumor cells from one site to another site. Consequently, common cancer treatments such as chemotherapy are not effective for some tumors, because theses treatments cause inflammation in tumor and other organs. Furthermore, since chemotherapy increases the chance of inflammation in some organs such as lung and liver, it facilitates metastases to these organs.

Introduction

Many cancers arise from sites of chronic inflammation; for example, Imtiyaz et al. [1] found that after chronic ulcerative colitis had been induced in mice, 14 weeks later the mice developed colitis-associated cancer (CAC). Balkwill et al. [2] list inflammatory conditions that predispose an individual to cancer, and others have attempted to understand how chronic inflammation leads to carcinogenesis. For example, Hussain et al. [3] found that p53 had been mutated as a result of oxidative damage due to inflammation in the colon. p53 mutations have also been seen in rheumatoid arthritis (RA), a chronic inflammatory disease. Immune cells within a chronic inflammation site initiate tumor progression by releasing reactive oxygen or nitrogen species, which leads to DNA damage in epithelial cells [4]. Yamanishi et al. [5] found that microdissected RA synovial regions with an abundance of p53 mutations contained significant amounts of IL-6 mRNA [5], indicating an increased inflammatory response. From these findings, we may conclude that chronic inflammation leads to inactivation of tumor suppressor genes with the help of immune system.

In chronic inflammation, immune cells might become adapted to the wound healing process so that their phenotype and functionality could be permanently changed. Various adaptive immune cells have been seen in colitis-associated cancer [6]; for instance, CD4+ effector T cells create an environment for tumor initiation and progression by releasing tumor-promoting cytokines, e.g. IL-6 [4]. Moreover, under poorly regulated pro-inflammatory conditions, regulatory T-cells fail to inhibit, and may instead contribute, to a T helper (Th-17) pro-carcinogenic process [7]. Therefore, in chronic inflammation, T-cells might become adapted to send many proliferation signals, and regulatory T-cells might have been changed to prevent their inhibition. The constitutive activation of transcription factors, such as NF-κB and/or STAT3, can be important in mediating the immune response, angiogenesis and oncogenesis in almost all CACs. [6].

In sites of chronic inflammation, fibroblasts also differ from normal fibroblasts. Carcinoma-associated fibroblasts express pro-inflammatory genes, enhance macrophage recruitment, encourage vascularization, and can therefore assist tumor growth [8]. NF-κB can promote the activity of carcinoma-associated fibroblasts [8]. Genetic inactivation of Pten in stromal fibroblasts of mouse mammary glands accelerates the initiation and development of mammary epithelial tumors by remodeling the extracellular matrix, aiding the infiltration of innate immune cell, and increasing angiogenesis [9]. In a prostate cancer model, p53 mutation within the stroma occurs before p53 inactivation in the epithelium; loss of p53 facilitates fibroblast proliferation and tumor progression [10].

These findings suggest that cells at the site of chronic inflammation might be adapted to the wound healing process. For example immune cells might become permanently adapted to send signals of proliferation or angiogenesis, and epithelial cells might be adapted to proliferation (such as inactivation of tumor suppressor genes). These adaptations may lead to the initiation of a tumor (Figure 1). It is hypothesized that various types of adaptations occur, for example, only one type of immune cells (e.g. macrophages) at the site of chronic inflammation could become adapted to sending high levels of a specific inflammatory signal. Alternatively, only one type of immune cells is adapted to release high levels of several inflammatory proteins. It is possible that a set of immune cells becomes adapted, for instance macrophages and CD4+ T-cells. In some cases these adaptations might be local, i.e. only cells at the site of chronic inflammation become defective. However, in some other cases the bone marrow cells - the source of immune cells - might be adapted to produce one or several defective progenies with special

functionalities. Therefore, cancer patients could have different immune deficiencies, which lead to their having different types of tumors. For instance, patients with colitis-associated cancer might have different immune deficiencies leading to quite different types of tumors that respond differently to the same treatment.

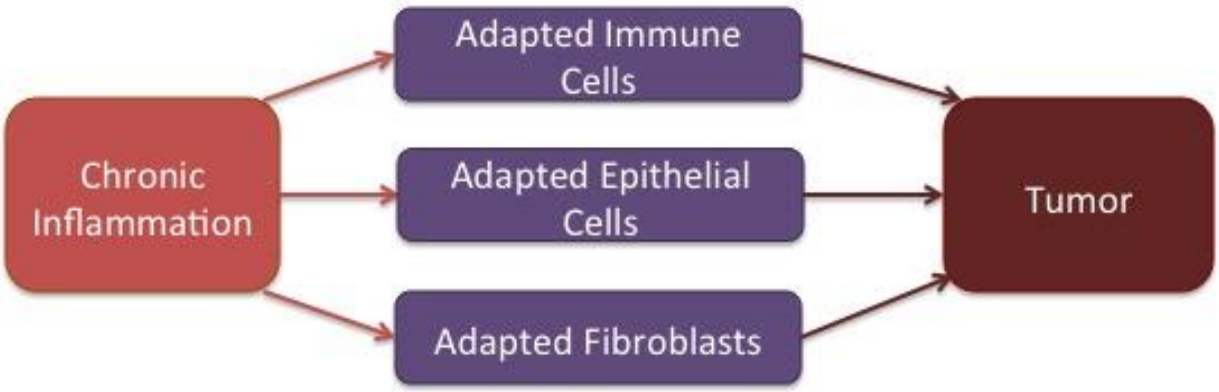

Figure 1: Tumor Initiation from Chronic Inflammation. Chronic inflammation leads to adapted tissue and/or adapted immune cells as the result of constantly going through the wound healing process. These cells become adapted to the wound healing process, including proliferation, angiogenesis and migration. These adaptations can cause tumor initiation and progression.

Metastasis

A strong correlation has been found between activated immune cells and metastasis in inflammatory breast cancer patients [11]. Importantly, the common metastatic sites (lymph nodes, bone, lung, and liver) are sites where immune cells are very active. Therefore, it can be hypothesized that some metastases are the result of the inflammatory processes by adapted immune cells at inflammation sites rather than migration of tumor cells from one site to other sites. If immune cells are adapted to send high levels of inflammatory signals, such as signals of proliferation and angiogenesis, then tumors would initiate at sites of inflammation as the result of the wound healing processes by these adapted immune cells. The following discussion offers some evidence to support this hypothesis.

Circulating tumor cells

There is a theory that metastasis is the result of cancer cells migration from the tumor extracellular matrix to the bloodstream or lymphatic vessels, becoming circulating tumor cells, and then infiltrating a new site. It seems that circulating tumor cells (CTCs) only use lymphatic routes to migrate to the nearby lymph nodes; they do not use lymphatic vessels in traveling long distances [12]. Cell migration could happen when tumor epithelial (E) cells lose their cell-cell adhesion and become motile mesenchymal (M) cells, i.e. the epithelial-mesenchymal transition (EMT). When these circulating tumor cells exit the bloodstream, they undergo a reverse process called mesenchymal-epithelial transition (MET) to continue their differentiation and develop a secondary tumor [13]. Another hypothesis suggests a relationship between EMT and cancer stem cells [14-16]. Although there are several studies on EMT and its role on metastasis, this hypothesis remains controversial [17-19].

Only a very small number of circulating tumor cells successfully form distant metastases [20]. B16F1 melanoma cells were injected intraportally to target mouse liver in one study, and only 36% of them survived more than 13 days. Furthermore, 2% of the injected cells gave rise to

micro-metastases by day 3, but only 1% of these micro-metastases continued to grow to form macro-metastases in the liver by day 13 [21]. In breast cancer patients, the presence of CTCs (CK+ cells) in the peripheral blood has not been statistically correlated with disease-free survival, whereas the presence of CTCs in bone marrow has been correlated with disease-free survival. Almost all patients with CTCs had positive bone marrows [22]. Circulating tumor cell clusters are rare compared with single CTCs; however CTC clusters, which usually include platelets and white blood cells, increase 23-50 fold in metastatic potential [23]. There is no correlation between the numbers of CTC-clusters and single CTCs [24]. However, in inflammatory breast cancer, there is a correlation between immune activation and CTCs with EMT characteristics [11]. Cohen et al. [11] hypothesized that EMT could be the result of inflammatory processes initiated by activated immune cells. Epithelial cells from the colonic epithelium of patients with benign colon diseases also circulate in the blood [25]. During wound healing, epithelial cells migrate and disperse as individual mesenchymal cells by downregulating cell-cell junctions [26]. Thus, circulating tumor cells in the blood could be the result of inflammation.

In examining published data sets, one can calculate the probability of finding no circulating tumor cells in the blood from patients with a range of metastatic cancers (Table 1). The probability of detection of CTCs in blood is based on the following formula.

$$P(CTCs\ in\ blood|Metastasis) = \frac{number\ of\ patients\ with\ metastatic\ tumor\ and\ CTCs\ in\ blood}{Number\ of\ patients\ with\ metastasis} \quad (1)$$

The probability of not detecting any CTCs in blood of patients with metastatic tumors is >0.3, which means there might be other phenomena besides circulating tumor cells that cause metastasis. Since no CTCs were detected in blood of 29% of metastatic breast cancer patients starting a first or new line of therapy, it is unlikely that treatments are responsible for the lack of detectable CTCs in blood [27]. Moreover, CTC counts do not change at different time-points during a 24-h period [28].

The probability of detection of circulating tumor cells clusters in various metastatic carcinomas (Table 2) uses the following formula:

$$P(CTCs\ clusters\ in\ blood|Metastasis) = \frac{number\ of\ \text{patients with metastatic tumor and CTC clusters in blood}}{Number\ of\ patients\ with\ metastasis} \quad (2)$$

Table 1: Probability of detecting and not detecting CTCs in blood from patients with metastatic cancer.

Cancer Type	P(CTCs | Metastasis)	P(No CTCs | Metastasis)	Ref.
Breast Cancer	0.4	0.6	[22, 27, 64–66]
Prostate Cancer	0.63	0.37	[27, 67]
Cancer of Unknown Primary Tumor	0.52	0.48	[27]
Ovarian Cancer	0.38	0.62	[27]
Gastric cancer	0.31	0.69	[27]
Colorectal Cancer	0.4	0.6	[27, 68]
Lung Cancer	0.2	0.8	[27]
Pancreatic Cancer	0.19	0.81	[27]
Bladder cancer	0.29	0.71	[27]
Renal Cancer	0.25	0.75	[27]

Table 2: Probability of detecting CTC clusters in blood, if there is metastasis.

Cancer	Probability	Ref.
Breast cancer	11⁄27 = 0.41	[24]
Prostate cancer	4⁄13 = 0.31	[24]
Melanoma	6⁄20 = 0.3	[24]

Pre-metastatic niche

Recent studies show that the microenvironments of metastatic sites change before any tumor cells exist there. It is suggested that recruited bone marrow progenitor cells change the host's environment to generate the "pre-metastatic niche", to which the tumor cells metastasize [29]. Bone marrow-derived hematopoietic progenitor cells (HPCs) that express vascular endothelial growth factor receptor 1 (VEGFR1) form cellular clusters at pre-metastatic sites before the appearance of a single tumor cell [30]. MMP9 induction in the pre-metastatic lung by endothelial cells and macrophages significantly increases lung metastasis. Matrix metalloproteinases (MMPs) play a key role in wound healing, inflammation, tumor invasion and metastasis. In a mouse model, suppression of MMP9 induction by deletion of either VEGFR-1TK or MMP9 markedly decreases lung metastasis [31]. The MMP9 levels in endothelial cells of healthy regions of the lungs from patients carrying tumors in other organs were significantly higher compared with those without tumors. Metastasis to a few lung lobes was actually found in 77% of patients with primary tumors [31].

Injury and metastasis

There is some evidence of metastases to the sites of injuries; for example, two patients with squamous cell carcinoma of the lung developed distant localized metastatic disease at sites of physical injuries. One patient developed a metastatic tumor in the knee injured in an accidental fall 6 weeks earlier, and the other patient developed metastatic tumors in the parts of the liver that were injured in a mechanical fall 2 months earlier [32]. In a mouse model of metastatic breast cancer, radiation-induced pulmonary injury leaded to chronic inflammatory responses and the development of pre-metastatic niches [33]. In another mouse model, hepatic ischemia-reperfusion injury increased the number of liver metastases of human pancreatic cancer (Capan-1) cells injected into the spleen [34]. Lung injury induced by the chemotherapeutic drug, bleomycin, increased the number of metastases, as also tumor cell adherence to extracellular matrix and fibrin at injured areas [35]. From these findings it can be concluded that the sites of injuries are potential metastatic sites.

Platelets

Platelets play a crucial role in metastasis; there is a significant correlation between platelet count and survival time in patients with solid tumors [36]. There is also experimental evidence showing that inhibition of platelet activation or platelet depletion decreases metastatic rates [37-39]. Furthermore, circulating tumor cells can be found as a multi-cellular cluster associated with platelets and white blood cells [40]. Metastasis seems to be reduced in one study when a host was platelet-depleted [41, 42]. Moreover, platelets in tumor cell clusters release

transforming growth factor-β (TGF-β) [43], and tumor cells that are bound to platelets highly express EMT-associated genes and MMP-9 [44]. Platelets are important in repairing injuries; the number of platelets will significantly increase, and they become activated in wound healing. Activated platelets release a number of mediators to promote blood clotting, cell division, cell migration and vasodilation [45]. Thus platelets could bind to cells, thereby increasing their migration and proliferation in helping to repair a wound.

The case of no primary tumor

Cancer of unknown primary origin (CUP) is defined by the presence of metastatic disease without an identified primary tumor. CUPs are 3.5% of all human malignancies, and one of the 10 most frequent cancers worldwide [46]. The most common subtypes of CUPs are adenocarcinomas that are well to moderately differentiated, poorly differentiated carcinomas and adenocarcinomas, squamous cell carcinomas, and undifferentiated neoplasms [47]. The major sites of these CUP subtypes are bones, liver, lung and lymph nodes [47]. In one study of ocular diagnosis in 420 patients, 278 reported a history of a primary tumor and 142 patients had no history of a cancer. No primary site was found in 73 of the 142 patients, and around half of these patients with no known primary tumor died of diffuse metastatic disease [48]. Chronic inflammation might cause bone marrow cells to become adapted to the wound healing process. Thus, the tumor would initiate at the sites where bone marrow derived cells are most active.

Wound healing and cancer treatment

Failure of traditional cancer therapies has been observed in almost all inflammatory cancers. Although the failures of cancer treatment has been widely linked to the existence of resistant cells or cancer stem cells [49], the high level of circulating inflammatory biomarkers is highly associated with such failures [50]. Unnaturally dying cells or necrotic cells, which have been caused by treatment, send signals to the immune system to replace them and repair the wound. Therefore, I suggest that the failure of cancer treatment can mainly be associated with the wound healing process in the tumor microenvironment after treatment. Extracellular high mobility group box 1 (HMGB1), one of these damage-associated molecular pattern (DAMP) molecules, is released from necrotic cells, thereby triggering inflammation and immunity [51]. HMGB1 is passively released from necrotic cells or actively secreted from stressed cancer cells and immune cells [52]. Release of HMGB1 in response to chemotherapy in leukemia increases resistance to the therapy [53]. Moreover, binding of HMGB1 to toll-like receptor 4 (TLR4) on dendritic cells (DCs) leads to early relapse after chemotherapy in breast cancer patients [54]. High levels of HMGB1 also occur in patients with non-small cell lung cancer (NSCLC) after tumors have been removed by surgery. Significantly high levels of both HMGB1 and transition factor p65 have also been seen in NSCLC tumors with node metastasis [55]. Nasopharyngeal carcinoma (NPC) patients with high levels of HMGB1 expression had poor overall and disease-free survival [56]. These insights imply that most common cancer therapies such as surgery, radiation, and chemotherapy cause necrotic cell death [57], which activates the immune system similar to the wound-healing process [58].

In one experiment, human ovarian cancer cells were added to bone marrow cells recovered from mice irradiated with 1000 cGy. These irradiated bone marrow cells significantly increased proliferation of human ovarian cancer cells compared to non-irradiated controls [59]. Furthermore, micro-metastases in bone marrow are frequently observed after chemotherapy; their existence significantly reduces survival rate [60]. Decrease in the CTC count has been seen during chemotherapy; however, after stopping the treatment the level of CTCs increased in

prostate cancer patients [28]. Since the increase in the number of CTCs could be the result of inflammatory processes initiated by activated immune cells [11], these observations suggest that chemotherapy reduces the survival rate because it causes inflammation in bone marrow and possibly other organs; inflammatory processes by these adapted immune cells lead to tumorigenesis.

A simple computational model has recently been developed to investigate cell dynamics during wound healing after cancer treatment that tend to kill epithelial cells, including surgery, chemotherapy and radiotherapy [61]. The model predicts that the involvement of high-fitness cancer cells in wound healing leads to faster relapse, and cancer cells outside of the wound can result in a slow recurrence of the tumor. The model also reveals that the absence of relapse after treatment implies a slow-developing tumor that might not reach an observable size in the patients' lifetime.

I argue that necrotic cells in the tumor microenvironment activate the immune system to initiate the wound healing process. If a tumor includes adapted tissue and/or adapted immune cells, these adapted cells start the wound healing process in the tumor microenvironment. Adapted activated immune cells send more signals of proliferation and/or angiogenesis than normal cells. If there were adapted cells, they would divide at a much higher rate in response to these signals than normal cells. Thus, a tumor might be expected to recur after the treatment and is likely to grow more aggressively (Figure 2).

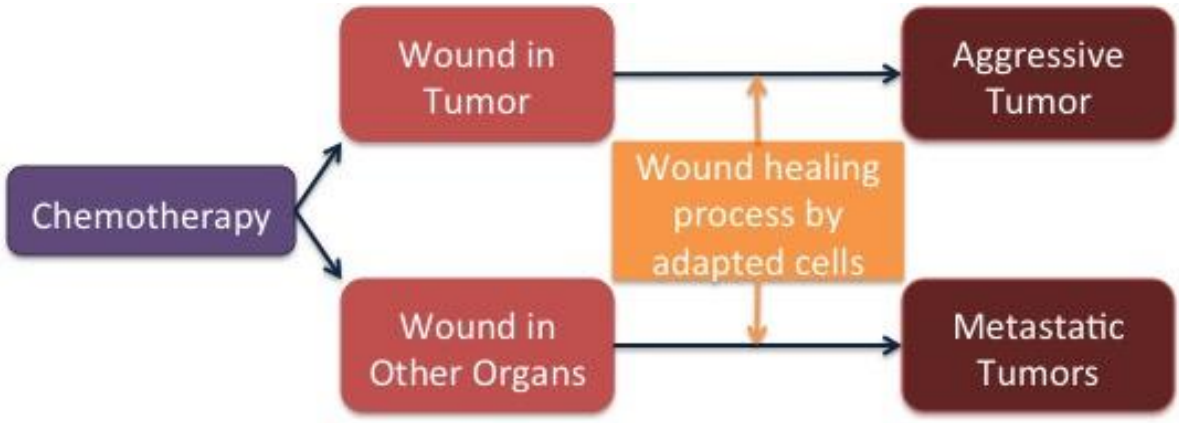

Figure 2: Chemotherapy might cause metastasis. Chemotherapy causes necrotic cell death in tumors and other organs. Necrotic cells activate immune cells, which initiate the wound healing process in sites of inflammation, including the tumor environment. If the tumor is an inflammatory one, then it might contain adapted tissue and/or adapted immune cells. Adapted cells respond to wound healing signals more strongly than normal cells. Thus, a more aggressive tumor would reappear at the primary site, and new tumors would form in other sites of inflammation. If chemotherapy is continued for a long period of time, chronic injury occurs in the tumor and some other organs. Chronic inflammation can lead to mutations, i.e. the formation of adapted cells, and adapted cells can initiate a tumor.

Conclusion

The hypothesis is that chronic inflammation causes adapted bone marrow derived cells (for

example, adapted macrophages or T-cells) and/or adapted tissue cells (for instance, adapted epithelial cells or stromal fibroblasts) to aid tumor initiation and progression. If adapted immune cells are present, the new site of inflammation might be vulnerable to these adapted immune cells and cause metastasis.

The new site of inflammation may recruit activated platelets that travel between sites of inflammation, including the site of inflammatory carcinoma. Tumor cells can link to adhesion receptors on platelets and travel along to these new sites of inflammation. Activated platelets start the wound healing process at the new site, which now includes some tumor cells. Since tumor cells respond to wound healing signals more strongly than normal cells, new tumors can be initiated at such sites of inflammation [62, 63].

Finally, it is posited that most regular cancer treatment schedules do not work well for inflammatory tumors, because these therapies cause inflammation in tumors and other organs, and the involvement of adapted cells in wound healing processes after treatment can lead to tumorigenesis.

References

1. H. Z. Imtiyaz, E. P. Williams, M. M. Hickey, S. a. Patel, A. C. Durham, L.-j. Yuan, R. Hammond, P. a. Gimotty, B. Keith, and M. C. Simon, "Hypoxia-inducible factor 2 α regulates macrophage function in mouse models of acute and tumor inflammation," *The journal of clinical investigation*, vol. 120, no. 8, pp. 2699–2714, 2010.
2. F. Balkwill, K. a. Charles, and A. Mantovani, "Smoldering and polarized inflammation in the initiation and promotion of malignant disease," *Cancer Cell*, vol. 7, no. 3, pp. 211–217, 2005.
3. S. P. Hussain, P. Amstad, K. Raja, S. Ambs, M. Nagashima, W. P. Bennett, P. G. Shields, A.-j. Ham, J. a. Swenberg, A. J. Marrogi, and C. C. Harris, "Increased p53 Mutation Load in Non- cancerous Colon Tissue from Ulcerative Colitis : A Cancer-prone Chronic Inflammatory Disease Increased p53 Mutation Load in Noncancerous Colon Tissue from Ulcerative Colitis : A Cancer- prone Chronic Inflammatory Disease," *Cancer Res*, pp. 3333–3337, 2000.
4. M. J. Waldner and M. F. Neurath, "Colitis-associated cancer: The role of T cells in tumor development," *Seminars in Immunopathology*, vol. 31, no. 2, pp. 249–256, 2009.
5. Y. Yamanishi, D. L. Boyle, S. Rosengren, D. R. Green, N. J. Zvaifler, and G. S. Firestein, "Regional analysis of p53 mutations in rheumatoid arthritis synovium.," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 15, pp. 10025–10030, 2002.
6. J. Terzi´c, S. Grivennikov, E. Karin, and M. Karin, "Inflammation and Colon Cancer," *Gastroenterology*, vol. 138, no. 6, pp. 2101–2114, 2010.
7. S. E. Erdman and T. Poutahidis, "Roles for inflammation and regulatory T cells in colon cancer.," *Toxicologic pathology*, vol. 38, no. 1, pp. 76–87, 2010.
8. N. Erez, M. Truitt, P. Olson, and D. Hanahan, "Cancer-Associated Fibroblasts Are Activated in Incipient Neoplasia to Orchestrate Tumor-Promoting Inflammation in an NF-kB-Dependent Manner," *Cancer Cell*, vol. 17, no. 2, pp. 135–147, 2010.
9. A. J. Trimboli, C. Z. Cantemir-Stone, F. Li, J. a. Wallace, A. Merchant, N. Creasap, J. C. Thompson, E. Caserta, H. Wang, J.-L. Chong, S. Naidu, G. Wei, S. M. Sharma, J. a. Stephens, S. a. Fernandez, M. N. Gurcan, M. B. Weinstein, S. H. Barsky, L. Yee, T. J. Rosol, P. C. Stromberg, M. L. Robinson, F. Pepin, M. Hallett, M. Park, M. C. Ostrowski, and G. Leone, "Pten in stromal fibroblasts suppresses mammary epithelial tumours.," *Nature*, vol. 461, no. 7267, pp. 1084–1091,

2009.

10. R. Hill, Y. Song, R. D. Cardiff, and T. Van Dyke, “Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis,” *Cell*, vol. 123, no. 6, pp. 1001–1011, 2005.
11. E. N. Cohen, H. Gao, S. Anfossi, M. Mego, N. G. Reddy, B. Debeb, A. Giordano, S. Tin, Q. Wu, R. J. Garza, M. Cristofanilli, S. a. Mani, D. a. Croix, N. T. Ueno, W. a. Woodward, R. Luthra, S. Krishnamurthy, and J. M. Reuben, “Inflammation Mediated Metastasis: Immune Induced Epithelial-To-Mesenchymal Transition in Inflammatory Breast Cancer Cells,” *Plos One*, vol. 10, no. 7, p. e0132710, 2015.
12. C. L. Chaffer and R. a. Weinberg, “A Perspective on Cancer Cell Metastasis,” *Science*, vol. 331, pp. 1559–1564, mar 2011.
13. J. H. Tsai and J. Yang, “Epithelial-mesenchymal plasticity in carcinoma metastasis,” *Genes & Development*, vol. 27, pp. 2192–2206, oct 2013.
14. M. K. Jolly, B. Huang, M. Lu, S. A. Mani, H. Levine, and E. Ben-Jacob, “Towards elucidating the connection between epithelial-mesenchymal transitions and stemness.,” *Journal of the Royal Society, Interface / the Royal Society*, vol. 11, no. 101, p. 20140962, 2014.
15. W. L. Tam and R. A. Weinberg, “The epigenetics of epithelial-mesenchymal plasticity in cancer,” *Nature Medicine*, vol. 19, pp. 1438–1449, nov 2013.
16. M. Lu, M. K. Jolly, J. . Onuchic, and E. Ben-Jacob, “Toward Decoding the Principles of Cancer Metastasis Circuits,” *Physics in Cancer Research Cancer Res*, vol. 74, no. 17, pp. 4574–87, 2014.
17. M. H. Chui, “Insights into cancer metastasis from a clinicopathologic perspective: Epithelial-Mesenchymal Transition is not a necessary step,” *International Journal of Cancer*, vol. 132, no. 7, pp. 1487–1495, 2013.
18. D. J. Ruiter, J. H. J. M. Van Krieken, G. N. P. Van Muijen, and R. M. W. De Waal, “Tumour metastasis: Is tissue an issue?,” *Lancet Oncology*, vol. 2, no. 2, pp. 109–112, 2001.
19. H. Ledford, “Cancer theory faces doubts,” *Nature*, vol. 472, pp. 273–273, apr 2011.
20. A. F. Chambers, A. C. Groom, and I. C. MacDonald, “Dissemination and growth of cancer cells in metastatic sites.,” *Nature reviews. Cancer*, vol. 2, no. 8, pp. 563–572, 2002.
21. K. J. Luzzi, I. C. MacDonald, E. E. Schmidt, N. Kerkvliet, V. L. Morris, a. F. Chambers, and a. C. Groom, “Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases.,” *The American journal of pathology*, vol. 153, no. 3, pp. 865–873, 1998.
22. J.-Y. Pierga, C. Bonneton, A. Vincent-Salomon, P. de Cremoux, C. Nos, N. Blin, P. Pouillart, J.-P. Thiery, and H. Magdel´enat, “Clinical significance of immunocytochemical detection of tumor cells using digital microscopy in peripheral blood and bone marrow of breast cancer patients.,” *Clinical cancer research : an official journal of the American Association for Cancer Research*, vol. 10, no. 4, pp. 1392–1400, 2004.
23. N. Aceto, A. Bardia, D. T. Miyamoto, M. C. Donaldson, B. S. Wittner, J. A. Spencer, M. Yu, A. Pely, A. Engstrom, H. Zhu, B. W. Brannigan, R. Kapur, S. L. Stott, T. Shioda, S. Ramaswamy, D. T. Ting, C. P. Lin, M. Toner, D. A. Haber, and S. Maheswaran, “Circulating Tumor Cell Clusters Are Oligoclonal Precursors of Breast Cancer Metastasis,” *Cell*, vol. 158, pp. 1110–1122, aug 2014.
24. a. F. Sarioglu, N. Aceto, N. Kojic, M. C. Donaldson, M. Zeinali, B. Hamza, A. Engstrom, H. Zhu, T. K. Sundaresan, D. T. Miyamoto, X. Luo, A. Bardia, B. S. Wittner, S. Ramaswamy, T. Shioda, D. T. Ting, S. L. Stott, R. Kapur, S. Maheswaran, D. a. Haber, and M. Toner, “A microfluidic device

for label-free, physical capture of circulating tumor cell clusters.," *Nature methods*, vol. 12, no. April, pp. 1–10, 2015.

25. K. Pantel, E. Deneve, D. Nocca, A. Coffy, J.-P. Vendrell, T. Maudelonde, S. Riethdorf, and C. Alix-Panabieres, "Circulating Epithelial Cells in Patients with Benign Colon Diseases," *Clinical Chemistry*, vol. 58, pp. 936–940, may 2012.
26. N. J. Zvaifler, "Relevance of the stroma and epithelial-mesenchymal transition (EMT) for the rheumatic diseases.," *Arthritis research & therapy*, vol. 8, no. 3, p. 210, 2006.
27. W. J. Allard, J. Matera, M. C. Miller, M. Repollet, M. C. Connelly, C. Rao, A. G. J. Tibbe, J. W. Uhr, and L. W. M. M. Terstappen, "Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases," *Clinical Cancer Research*, vol. 10, no. 20, pp. 6897–6904, 2004.
28. J. G. Moreno, S. OHara, S. Gross, G. Doyle, H. Fritsche, L. G. Gomella, and L. W. Terstappen, "Changes in circulating carcinoma cells in patients with metastatic prostate cancer correlate with disease status," *Urology*, vol. 58, pp. 386–392, sep 2001.
29. H. Peinado, S. Lavotshkin, and D. Lyden, "The secreted factors responsible for pre-metastatic niche formation: Old sayings and new thoughts," *Seminars in Cancer Biology*, vol. 21, no. 2, pp. 139–146, 2011.
30. R. N. Kaplan, R. D. Riba, S. Zacharoulis, A. H. Bramley, L. Vincent, C. Costa, D. D. MacDonald, D. K. Jin, K. Shido, S. a. Kerns, Z. Zhu, D. Hicklin, Y. Wu, J. L. Port, N. Altorki, E. R. Port, D. Ruggero, S. V. Shmelkov, K. K. Jensen, S. Rafii, and D. Lyden, "VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche.," *Nature*, vol. 438, no. 7069, pp. 820–827, 2005.
31. S. Hiratsuka, K. Nakamura, S. Iwai, M. Murakami, T. Itoh, H. Kijima, J. M. Shipley, R. M. Senior, and M. Shibuya, "MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis," *Cancer Cell*, vol. 2, no. 4, pp. 289–300, 2002.
32. N. D. Walter, P. L. Rice, E. F. Redente, E. F. Kauvar, L. Lemond, T. Aly, K. Wanebo, and E. D. Chan, "Wound Healing after Trauma may Predispose to Lung Cancer Metastasis: Review of Potential Mechanisms.," *American journal of respiratory cell and molecular biology*, 2010.
33. H. Gong, W. Hu, Q. Hu, X. Li, and Q. Song, "Radiationinduced pulmonary injury accelerated pulmonary metastasis in a mouse model of breast cancer," *Oncology Letters*, pp. 3613–3618, oct 2015.
34. H. Tajima, "Increased E-selectin in hepatic ischemia-reperfusion injury mediates liver metastasis of pancreatic cancer," *Oncology Reports*, pp. 791–796, 2012.
35. F. W. Orr, I. Y. Adamson, and L. Young, "Promotion of pulmonary metastasis in mice by bleomycin-induced endothelial injury.," *Cancer research*, vol. 46, no. 2, pp. 891–7, 1986.
36. D. Buergy, F. Wenz, C. Groden, and M. A. Brockmann, "Tumor-platelet interaction in solid tumors," 2012.
37. G. N. Tzanakakis, K. C. Agarwal, D. K. Veronikis, and M. P. Vezeridis, "Effects of antiplatelet agents alone or in combinations on platelet aggregation and on liver metastases from a human pancreatic adenocarcinoma in the nude mouse.," *Journal of surgical oncology*, vol. 48, no. 1, pp. 45–50, 1991.
38. A. Amirkhosravi, S. a. Mousa, M. Amaya, S. Blaydes, H. Desai, T. Meyer, and J. L. Francis, "Inhibition of tumor cell-induced platelet aggregation and lung metastasis by the oral GpIIb/IIIa antagonist XV454," *Thrombosis and Haemostasis*, vol. 90, no. 3, pp. 549–554, 2003.
39. J. S. Palumbo, "Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-

mediated elimination of tumor cells," *Blood*, vol. 105, pp. 178–185, jan 2005.

40. A. Mitrugno, G. W. Tormoen, P. Kuhn, and O. J. McCarty, "The prothrombotic activity of cancer cells in the circulation," *Blood Reviews*, jul 2015.

41. J. S. Palumbo, "Mechanisms linking tumor cell-associated procoagulant function to tumor dissemination," *Seminars in Thrombosis and Hemostasis*, vol. 34, no. 2, pp. 154–160, 2008.

42. B. Nieswandt, M. Hafner, B. Echtenacher, and D. N. M¨annel, "Lysis of tumor cells by natural killer cells in mice is impeded by platelets.," *Cancer research*, vol. 59, no. 6, pp. 1295–1300, 1999.

43. M. Yu, A. Bardia, B. S. Wittner, S. L. Stott, M. E. Smas, D. T. Ting, S. J. Isakoff, J. C. Ciciliano, M. N. Wells, A. M. Shah, K. F. Concannon, M. C. Donaldson, L. V. Sequist, E. Brachtel, D. Sgroi, J. Baselga, S. Ramaswamy, M. Toner, D. a. Haber, and S. Maheswaran, "Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition," *Science*, vol. 339, pp. 580–584, feb 2013.

44. M. Labelle, S. Begum, and R. O. O. Hynes, "Direct Signaling between Platelets and Cancer Cells Induces an Epithelial-Mesenchymal-Like Transition and Promotes Metastasis," *Cancer Cell*, vol. 20, pp. 576–590, nov 2011.

45. C. L. Jackson, E. W. Raines, R. Ross, and M. a. Reidy, "Role of endogenous platelet-derived growth factor in arterial smooth muscle cell migration after balloon catheter injury.," *Arteriosclerosis and thrombosis : a journal of vascular biology / American Heart Association*, vol. 13, no. 8, pp. 1218– 1226, 1993.

46. N. Pavlidis and K. Fizazi, "Carcinoma of unknown primary (CUP)," 2009.

47. J. D. Hainsworth and F. A. Greco, "Cancer of Unknown Primary Origin," *Goldman's Cecil Medicine: Twenty Fourth Edition*, vol. 1, pp. 1334–1337, 2011.

48. C. L. Shields, J. a. Shields, N. E. Gross, G. P. Schwartz, and S. E. Lally, "Survey of 520 eyes with uveal metastases.," *Ophthalmology*, vol. 104, no. 8, pp. 1265–1276, 1997.

49. A. Dhawan, M. Kohandel, R. Hill, and S. Sivaloganathan, "Tumour control probability in cancer stem cells hypothesis," *PLoS ONE*, vol. 9, no. 5, 2014.

50. B. L. Pierce, R. Ballard-Barbash, L. Bernstein, R. N. Baumgartner, M. L. Neuhouser, M. H. Wener, K. B. Baumgartner, F. D. Gilliland, B. E. Sorensen, A. McTiernan, and C. M. Ulrich, "El- evated Biomarkers of Inflammation Are Associated With Reduced Survival Among Breast Cancer Patients," *Journal of Clinical Oncology*, vol. 27, no. 21, pp. 3437–3444, 2009.

51. M. T. Lotze and K. J. Tracey, "High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal.," *Nature reviews. Immunology*, vol. 5, no. 4, pp. 331–342, 2005.

52. H. Wang, O. Bloom, M. Zhang, J. M. Vishnubhakat, M. Ombrellino, J. Che, A. Frazier, H. Yang, S. Ivanova, L. Borovikova, K. R. Manogue, E. Faist, E. Abraham, J. Andersson, U. Andersson, P. E. Molina, N. N. Abumrad, A. Sama, and K. J. Tracey, "HMG-1 as a late mediator of endotoxin lethality in mice.," *Science (New York, N.Y.)*, vol. 285, no. 5425, pp. 248–251, 1999.

53. L. Liu, M. Yang, R. Kang, Z. Wang, Y. Zhao, Y. Yu, M. Xie, X. Yin, K. M. Livesey, M. T. Lotze, D. Tang, and L. Cao, "HMGB1-induced autophagy promotes chemotherapy resistance in leukemia cells.," *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K*, vol. 25, no. 1, pp. 23–31, 2011.

54. L. Apetoh, F. Ghiringhelli, A. Tesniere, A. Criollo, C. Ortiz, R. Lidereau, C. Mariette, N. Chaput, J. P. Mira, S. Delaloge, F. Andr´e, T. Tursz, G. Kroemer, and L. Zitvogel, "The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy," 2007.

55. X. Zhang, H. Wang, and J. Wang, "Expression of HMGB1 and NF-?B p65 and its significance in non-small cell lung cancer," *Wspolczesna Onkologia*, vol. 17, no. 4, pp. 350–355, 2013.

56. D. Wu, Y. Ding, S. Wang, Q. Zhang, and L. Liu, "Increased expression of high mobility group box 1 (HMGB1) is associated with progression and poor prognosis in human nasopharyngeal carcinoma.," *The Journal of pathology*, vol. 216, no. 2, pp. 167–175, 2008.

57. W. X. Zong and C. B. Thompson, "Necrotic death as a cell fate," 2006.

58. W. W. Harless, "Cancer treatments transform residual cancer cell phenotype.," *Cancer cell international*, vol. 11, no. 1, p. 1, 2011.

59. P. M. Gunjal, G. Schneider, A. A. Ismail, S. S. Kakar, M. Kucia, and M. Z. Ratajczak, "Evidence for induction of a tumor metastasis-receptive microenvironment for ovarian cancer cells in bone marrow and other organs as an unwanted and underestimated side effect of chemotherapy/radiotherapy," *Journal of Ovarian Research*, vol. 8, no. 1, 2015.

60. S. Braun, C. Kentenich, W. Janni, F. Hepp, J. de Waal, F. Willgeroth, H. Sommer, and K. Pantel, "Lack of effect of adjuvant chemotherapy on the elimination of single dormant tumor cells in bone marrow of high-risk breast cancer patients.," *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, vol. 18, no. 1, pp. 80–86, 2000.

61. L. Shahriyari, "Cell dynamics in tumour environment after treatments," *Journal of The Royal Society Interface*, vol. 14, no. 127, p. 20160977, 2017.

62. L. Shahriyari, "Abstract A07: A new perspective on cancer: Tumor initiation, failure of treatments, and metastasis," *Cancer Research*, vol. 76, pp. A07–A07, 2016.

63. L. Shahriyari, "A new hypothesis: some metastases are the result of inflammatory processes by adapted cells, especially adapted immune cells at sites of inflammation," *F1000Research*, vol. 5, p. 175, feb 2016.

64. S. Riethdorf, H. Fritsche, V. Muller, T. Rau, C. Schindlbeck, B. Rack, W. Janni, C. Coith, K. Beck, F. Janicke, S. Jackson, T. Gornet, M. Cristofanilli, and K. Pantel, "Detection of Circulating Tumor Cells in Peripheral Blood of Patients with Metastatic Breast Cancer: A Validation Study of the CellSearch System," *Clinical Cancer Research*, vol. 13, pp. 920–928, feb 2007.

65. M. Cristofanilli, "Circulating tumor cells, disease progression, and survival in metastatic breast cancer," *N. Engl. J. Med.*, vol. 351, pp. 781–791, 2004.

66. E. Racila, D. Euhus, A. J. Weiss, C. Rao, J. McConnell, L. W. Terstappen, and J. W. Uhr, "Detection and characterization of carcinoma cells in the blood.," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 8, pp. 4589–94, 1998.

67. J. G. Moreno, M. C. Miller, S. Gross, W. J. Allard, L. G. Gomella, and L. W. Terstappen, "Circulating tumor cells predict survival in patients with metastatic prostate cancer," *Urology*, vol. 65, pp. 713–718, apr 2005.

68. S. J. Cohen, C. J. Punt, N. Iannotti, B. H. Saidman, K. D. Sabbath, N. Y. Gabrail, J. Picus, M. Morse, E. Mitchell, M. C. Miller, G. V. Doyle, H. Tissing, L. W. Terstappen, and N. J. Meropol, "Relationship of Circulating Tumor Cells to Tumor Response, Progression-Free Survival, and Overall Survival in Patients With Metastatic Colorectal Cancer," *Journal of Clinical Oncology*, vol. 26, pp. 3213–3221, may 2008.