Premetastasis

Yoshiro Maru

Department of Pharmacology, Tokyo Women's Medical University, Shinjuku-ku, Tokyo 162-8666, Japan

Correspondence: maru.yoshiro@twmu.ac.jp

Sterile inflammation within primary tumor tissues can spread to distant organs that are devoid of tumor cells. This happens in a manner dependent on tumor-led secretome, before the actual metastasis occurs. The premetastatic microenvironment is established in this way and is at least partly regulated by hijacking the host innate immune system. The biological manifestation of premetastasis include increased vascular permeability, cell mobilization via the blood stream, degradation of the extracellular matrix, immunosuppression, and host antineoplastic activities.

Die Gewesenheit entspringt der Zukunft, so zwar, daß die gewesene Zukunft die Gegenwart aus sich entläßt (Heidegger 1967). [The character of “having been” arises from the future, and in such a way that the future which “has been” releases from itself the present. (English translation, Macquarrie et al. 1992)].

Premetastasis is a concept in biology. I believe that it can be correlated to the philosophy of existentialism described by Martin Heidegger, in that the death of man occurs while tumor cells escape cell death (Hanahan and Weinberg 2011). A future which has death, makes man (Dasein) project (entwerfen) his way of living. Tumor cells (Dasein) try to survive in the future-metastatic (premetastatic) organ by projecting secretome elements into the circulation to establish the premetastatic microenvironment in the absence of tumor cells.

Here, I mean metastasis as the existence of tumor cells in organs distant from the primary site unless otherwise mentioned. Soil is a metaphor for the microenvironment and synonymously used.

Niche sounds less conceptual than soil and represents a small physical microenvironment in a given organ for tumor cells to spread and regrow and where both the processes are cultivated by the remote action of primary tumors.

Metastasis is a complicated biological process consisting of at least six steps (Fig. 1); (1) detachment of tumor cells from the primary tumor, (2) intravasation, which requires macrophage-mediated vascular microenvironment that causes vascular permeability (Robinson et al. 2009; Arwert et al. 2018), but may not necessarily require initial movement to the invasive edge of tumor as evidenced by a new live-animal model (Deryugina and Kiosses 2017), (3) circulation in the blood stream, (4) extravasation, (5) recruitment, and (6) regrowth in the presence of macrophages. Tumor cells in circulation are not always single. In an experimental circulating tumor cell (CTC), roughly 20% of cells were in clumps <200 µm in diameter, co-traveling from the primary site in association

Y. Maru

Figure 1. Metastatic progression of cancer. Metastasis consists of at least six steps. See text.

with at least carcinoma-associated fibroblasts (Duda et al. 2010).

According to a well-established model of colon cancer progression with the accumulation of genetic alterations (multistep carcinogenesis), the most malignant phenotype is distant metastasis in which the activated oncogenes continue to be required (Merlos-Suárez and Batlle 2008). However, clear evidence of metastatic signature of the transformed cells themselves is currently missing even in colon cancer. Nontransformed mammary epithelial cells have been shown to spread to lungs and stay for 120 d (this roughly corresponds to 12 yr under the assumption that life span of mouse and human is 800 d and 80 yr, respectively) before they transform to metastatic tumor cells in Myc-KRasD12 conditional transgenic mice (Podsypkina et al. 2008). Ectopic colonization, possibly through blood stream of noncancerous epithelial cells may be clinically exemplified in endometriosis (Assor 1972; Starzinski-Powitz et al. 1999). Thus, metastatic spread may take place at any stage of cancer progression.

The word “premetastatic” is not new. It has been described from the standpoint of both time and space. Growth responsiveness to intratumoral injection of cholestrin was more prominent in the premetastatic phase of the primary tumor than those in the metastatic phase, indi-

cating that the authors in 1913 recognized the metastatic progression of the primary tumor cells in a time-dependent manner (Robertson and Burnett 1913). A premetastatic organ was initially described in 1996 in local lymph nodes without “any evidence” of esophageal cancer cells. The authors showed that lymphocytes from the esophageal cancer-draining lymph nodes suppressed proliferative responsiveness to PHA and failed to acquire IL-2-activated cytotoxic activity (lymphokine-activated killer: LAK activity) against esophageal cancer cells (O’Sullivan et al. 1996). Currently, LAK therapy has developed into tumor-infiltrating lymphocyte (TIL) therapy against mutated proteins in a more personalized manner (Zacharakis et al. 2018). Given that lymph nodes are a host defense organ against invading microbes and tumor cells, I believe that premetastatic lymph nodes have both primary tumor-led activities favorable for metastasizing tumor cells, and host-led immunological responses unfavorable for tumor cells (see premetastatic niche elements section).

I wish to underline one practical condition in premetastasis experiments; levels of evidence that tumor cells are indeed absent in the future-metastatic organs. Our experimental conditions in premetastatic lungs established in 2 wk are (1) no histological detection of fluorescently labeled tumor cells in the lungs in 6 wk, (2) less than 100

tumor cells per lung lobe as calculated by the copy number of a marker gene integrated into the tumor cell genome (Fig. 2; Hiratsuka et al. 2006). In a different premetastatic lung experiment by other authors, premetastatic lungs were established in 2 wk but without tumor-expressing marker green fluorescent protein (GFP) being detected by either flow cytometry or polymerase chain reaction (PCR) even in 3 wk (Yan et al. 2010). Not all premetastasis papers have provided this essential information questioning their reliability.

An interesting question is “can we call the tumor-free left lung a premetastatic lung with the primary lung cancer in the right lung?” Tumor cells can make their own soil as shown by contralateral orthotopic transplantation experiments of breast cancer cells, where labeled tumor cells from the right-side tumor mass spread via the circulation into the tumor mass formed by the same but unlabeled tumor cells on the opposite side (Kim et al. 2009a). In tumor-bearing organs, tumor cell-free portions are found within the same organ in a different place from where the primary tumor develops. In 729 non-small-cell lung cancer patients with distant metastasis at the time of diagnosis, the lung was the second-highest organ for metastatic spread, ac-

counting for 32.1% of the total metastasis (Tamura et al. 2015). It has also been known for more than half a century ago that localized radiation therapy to the right-sided primary lung cancer could cause responses on the left-sided (out-of-target) lung metastatic cancer. This so-called abscopal effect is possibly mediated by immunological activities since the effect was recently shown to be enhanced by immunotherapy (Ng and Dai 2016). This indicates that tumor cells may cultivate a premetastatic soil for future-self within the same organ from which they originate, whether local or distant.

PAGET AND NON-PAGET MECHANISMS FOR PREMETASTASIS

Comparison of the results of disseminated organs in necropsy between 735 breast cancer patients and 340 septic patients with systemic abscess led Paget (1889) to speculate that tumor cells metastasize to predisposed organs. Importantly, however, he also recognized from 129 necropsies of melanoma that the organ propensity in metastasis was not so prominent for this disease. He used metaphors to express tumor cells as seed, and target organs of metastasis as soil or seats of election. Sepsis induces dissemi-

Figure 2. Experimental system for premetastasis. There are many experimental systems for metastasis (Maru 2015, 2016). One example here has two phases, premetastatic and metastatic. cDNA microarray analysis of lungs between tumor-bearing and nonbearing mice may reveal changes in expression levels of genes in premetastatic lungs.

Y. Maru

nated intravascular coagulopathy resulting in systemic fibrin deposition, but soil for living, tumor cells, and bacteria, should be more restricted than nonliving materials like thrombotic emboli. Frequent generation of thrombosis in left atrium in atrial fibrillation patients causes systemic embolic events. Large-scale analysis of 37,973 patients showed that extracranial embolism took place in extremities in 68% of cases (Bekwelem et al. 2015), indicating a clear difference between tumor cells and thrombotic emboli. It is an alleged notation that metastasis in muscle is rare. However, injection of B16F10 melanoma cells into left ventricle resulted in macroscopically recognizable metastasis in bones, ovaries, adrenal glands, and brain at days 12–17 (Jones et al. 2006). Our melanoma subline also exhibits a similar metastasis in systemic organs additionally including lungs, liver, and kidney but rarely in brain and muscle (Inada et al. 2015). Taken together, if metastatic process includes primary tumor-led preselection or precultivation of the target organ or soil in the total absence of tumor cells or seed in the tissue, then this should be called premetastatic organ. However, even if precultivated, seeding into an organ may not necessarily be successful. The abortive seeding to a fertile soil may take place stochastically depending on the arriving tumor cell number and traits that determine the matching between tumor cell and premetastatic niche. Precultivation of many organs followed by arrival of tumor cells biologically tolerant of any soil should manifest a more proportional organ distribution for metastasis. Thus, I believe that premetastasis needs time for primary tumors to cultivate the distant organs. The question is how to cultivate. The above-mentioned metastasis experiment by intracardiac injection of melanoma cells, which starts from the step 3, appears to skip the preparatory period of time.

Metastasizing tumor cells in the circulation (step 3) have been extensively analyzed as CTCs of liquid biopsy samples in clinical settings. Presence of CTCs without distant metastasis, namely localized cancer cannot be accurately investigated since CTCs are rare in nonmetastatic circumstances due to technical limitation in detection (Cabel et al. 2017) and the propen-

sity of CTCs to cell death (Méhes et al. 2001). However, growing tumor cell lines can be intravenously injected into animals and their fate traced. Two such experiments showed a rapid decline in cell survival within 3 h, and only 0.1% ~ 1.0% of injected tumor cells remained alive in the lungs 24 h after injection. This was followed by manifestation of pulmonary metastasis in 12–14 d later (Fidler 1970; Reid and Gibbins 1979). Thus, tumor cells are not necessarily dormant (see below) before metastatic colonization is accomplished. This implies that even experimental CTCs without transplanted tumors can hardly be drivers for premetastatic soil establishment in a strict sense. However, CTCs that are constantly supplied from the primary tumors may participate in the premetastatic soil formation in concert with the primary tumors. In the PyM—induced mammary tumor model from Pollard's laboratory, intravenous injection of tumor cells expressing CCL2, followed by that of monocytes in 7 h resulted in increased CCR2+ inflammatory monocyte in the lungs (Qian et al. 2011). Since inflammatory monocytes were not observed in premetastatic lungs in the tumor-bearing mice, CCL2+ CTCs might have induced CCL2 in the lungs shortly before the recruitment of CCR2+ monocytes that participate in premetastatic soil formation.

Tumor cells may reach the unprepared soil and overcome conditions unsuitable for their survival. In the strict sense of the word, I suppose that premetastasis is defined as a biological state of organs or tissues outside of the primary tumor without a single tumor cell. This raises a fundamental question of whether or not an organ can be called a premetastatic site when it lodges clinically or even experimentally indiscernible tumor cells, including dormant cells such as cancer stem cells (CSCs), in cancer patients or tumor transplantation models. Latent metastasis, after years, is often observed in breast, lung, and prostate cancers. If you consider metastasis as a process that is accompanied by regrowing metastatic tumor cells, excluding growth-arrested or quiescent cells, the microenvironment with a single or very small number of dormant tumor cells could be called premeta-

static niche. Lyden's group calls it a "silent" pre-metastatic niche (Peinado et al. 2017).

Ninety days after injection of human lung adenocarcinoma cell line capable of achieving latent metastasis in athymic mice, tumor cells were found as a single cluster of 20 cells frequently along vasculature, and formed metastasis in 120–210 d (12–21 yr in human, see the above-described assumption) (Malladi et al. 2016; Deguchi and Maru 2017). The quiescence of the tumor cells was accomplished by auto-crine expression of a WNT inhibitor DKK1. This experiment supports the idea that a metastatic niche is not necessarily preformed but could be postformed by the arrival of cells. An intravenous injection of CD90-CD24-defined CSCs derived from the MMTV-PyMT-derived breast cancer model showed efficient lung metastasis as compared to the non-CSC populations (Malanchi et al. 2012). The induction of periostin and its subsequent WNT signaling in the CSCs facilitated survival and regrowth of the cells. Switching between quiescence and regrowth makes a difference.

An interaction that confers antiapoptotic and quiescence signals on tumor cells was also reported between metastasis-associated macrophage in the lungs and breast cancer cells expressing VCAM-1 (Chen et al. 2011), or thrombospondin-1+ endothelial cells and breast cancer cells (Ghajar et al. 2013). Since the niche is formed after tumor cell arrival in those experiments, I propose to define the post-formed premetastatic niche. If you define tumor-led predisposition of premetastatic organs as Paget-type, stochastic settlement of CTC with acquisition of the switching properties is of non-Paget-type. Hematopoietic stem cells (HSC) in bone marrow can be competitively evicted from the osteoblastic niche by metastasizing prostate cancer cells (Shiozawa et al. 2011), and those cancer cells can stay dormant there by WNT5a repressing canonical WNT3a activity in a reversible manner (Ren et al. 2019). Here the "lodge" is physiologically preestablished originally for HSC even before prostate cancer development. Therefore, in this case the premetastatic niche is preestablished fully for HSC but maybe partially for tumor cells, and

completed as a metastatic niche upon tumor cell arrival (Maru 2015).

TUMOR-LED SECRETOME

Tumor-secreted elements (I define secretome as a mass of anything released from primary tumors) affect not only premetastatic soil establishment but also metastatic behaviors such as extravasation of tumor cells by themselves. The discoveries of CCL5 secreted from colocalizing mesenchymal stem cells and an integrin-binding protein Nidogen1 by a well-designed secretome analysis belongs to the latter case (Karnoub et al. 2007; Alečkovič et al. 2017). The secretome includes many growth factors and their related proteins including VEGF, TNF α , TGF β (Hiratsuka et al. 2006; Huang et al. 2009), CCL2 (Qian et al. 2011; Hiratsuka et al. 2013), LOX (lysyl oxidase) (Erler et al. 2009; Cox et al. 2015), RANKL (Jones et al. 2006), versican (Kim et al. 2009b), soluble forms of ephrin-A1 (Ieguchi et al. 2014), HMGB1 (Dejean et al. 2012; Bald et al. 2014), and so forth (Fig. 3).

In many experiments, conditioned medium from tissue culture of tumor cell lines or organs (tumor-conditioned medium or TCM) is often used to establish premetastatic soil in a tumor cell-free condition. Secretory exosomes or extracellular vesicles containing active molecules such as particular miRs or integrins should also be included in the secretome term. For ex-

Tumor-derived secretome

- 1) VEGF
- 2) TNF α
- 3) TGF β
- 4) CCL2
- 5) LOX (Lysyl oxidase)
- 6) RANKL
- 7) Versican
- 8) Soluble form of ephrin-A1
- 9) HMGB1
- 10) Nodogen 1
- 11) Secretory exosome (integrin, miR)
- 12) Dormant tumor cell
- 13) Companion cell

Figure 3. Tumor-led secretome. Well-known examples of factors released from primary tumors. See text.

Y. Maru

ample, intravenous injection of tumor-derived exosomes containing miR-105 resulted in reduced expression of the tight junction protein ZO-1 with subsequent enhancement of vascular permeability in the lungs and brain, which facilitates metastasis in these organs (Zhou et al. 2014). I propose to include both exosomes and single or very few tumor cells (see above section) in the secretome irrespective of the time before or after they acquire quiescence.

In addition, CTCs in the form of oligo-clonal homotypic clusters showed 23- to 50-fold greater efficiency than single CTC for lung metastasis in an orthotopic xenograft model (Aceto et al. 2014). Since this cell-cell adhesion depends on plakoglobin, their metastatic potency is abrogated through the ablation of plakoglobin expression. While the biological significance of carcinoma-associated fibroblasts in primary tumors has been well documented (Orimo et al. 2005; Karagiannis et al. 2012; Paauwe et al. 2018), fibroblasts were also shown to facilitate lung metastasis by participating in heterotypic tumor cell-fibroblast clusters in CTCs (Duda et al. 2010). Peritoneal dissemination by ovarian cancer cells also utilizes such a companion strategy with fibroblasts in spheroids to enhance dissemination (Gao et al. 2019; Schreiber 2019). EGF produced by fibroblasts stimulates expression of $\alpha 5$ integrin subunit in cancer cells that facilitates their adhesion to the peritoneum.

In addition to the time-dependent migration of metastasizing tumor cells by themselves (Fig. 1), the development of the premetastatic soil also follows a stepwise process in which the primary tumors take the initiative (Fig. 4). The premetastatic process comprises of the following sequential steps: (1) release of growth factors and secretory exosomes from primary tumors, (2) activation of sterile inflammation in future-metastatic (premetastatic) organs, (3) mobilization of leukocytes from bone marrow and other organs to the premetastatic organs, (4) mobilizing leukocytes that participate in the formation of premetastatic niche and soil. However, even with the premetastatic step 1, steps 2 through 4 cannot necessarily be accomplished diffusely throughout the organ. Therefore, the premetastatic organ may have preme-

tastatic soil in a mottled fashion. For example, in our experiment examining premetastatic lungs, the premetastatic soil has increased vaso-permeability that takes place in a segmental manner (Hiratsuka et al. 2013) (see premetastatic niche elements section).

Needless to mention, modification of the primary tumors largely affects premetastatic soil. This is especially important in clinical settings since surgery, chemotherapy, and irradiation are applied to the primary tumors with no detection of metastasis by the current imaging techniques. If the premetastatic soil is established at the time of surgery, perfect removal of the tumor can abrogate the soil. In our premetastatic lung experiments, TNF α and VEGF released from the primary tumor induced increased expression of S100A8 and SAA3 in the premetastatic lungs, which recruited CD11b+ TLR4/MD-2+ myeloid cells from the bone marrow. Both S100A8 and SAA3 can serve as endogenous ligands for TLR4/MD-2 (see below) (Fig. 4B; Hiratsuka et al. 2008, 2013). S100A8 induces SAA3 expression in premetastatic lungs. S100A8 up-regulation was not observed in liver nor kidney in the premetastatic phase (Fig. 2). Anti-S100A8 neutralizing antibody abrogated the injected tumor cell recruitment in the lungs, but not in liver or kidney indicating that S100A8 is necessary to establish the premetastatic niche in the lungs but not liver or kidney. Both S100A8 and SAA3 levels are increased in the serum during the premetastatic period, 4–14 d after tumor transplantation (Maru 2009, 2010). Removal of the primary tumor decreased both CD11b+ cell number and those chemokine expressions (Maru 2016). This result suggests that premetastatic soil formation could be reversible but not plastic.

Another example of reversibility in premetastasis is found in the osteolytic bone niche induced by tumor-secreted lysyl oxidase (LOX) independently of RANKL (see also Hijacking homeostasis section) (Fig. 4C; Cox et al. 2015). Of note, it is established in a focal manner. Both anti-LOX neutralizing antibody and shRNA-mediated knockdown of LOX in tumor cells reversed the formation of the osteolytic lesions that serve as premetastatic niche. The authors

Figure 4. Basic premetastatic process consists of at least five steps. See text. (A–G) Examples of mechanisms of premetastatic soil formation. See text for each step.

Y. Maru

used intraperitoneal injection of TCM containing the secretome, to induce premetastatic soil in bone in a perfectly tumor-free manner. Recombinant LOX protein-induced osteolytic lesions, indicating that LOX is necessary and sufficient.

Although, apart from Th2 cytokines such as IL-4, 5, and 13, precise factors that can work in place of the tumor-secretome are unclear, steroids can reverse the lung metastasis in the background of the OVA-asthma model that is CD4+ T cell-dependent (Taranova et al. 2008). Inhibition of the cultivation of premetastatic soil by secretome elements that are specific and dominant for its establishment should be effective in blocking it.

Skin metastasis is frequently observed in anaplastic large-cell lymphoma carrying chromosomal translocation t(2;5). In a mouse model, HMGB1, also shown to be an endogenous ligand for TLR4/MD-2, was shown to be released from lymphoma cells where it activates expression of IL-8 in keratinocytes. This IL-8 in turn induces invasive mobilization of CXCR1/CXCR2-expressing lymphoma cells (Fig. 4D; Dejean et al. 2012). The idea was supported by the demonstration of expression of both HMGB1 and CXCR1/CXCR2 in clinical skin biopsy samples.

Damage of tumor tissues either chemically or physically provokes sterile inflammation that further intensifies the premetastatic soil. Paclitaxel can do so by making the primary tumor release extracellular vesicles of 100–150 nm in size containing annexin A6, capable of NF- κ B-dependent activation of the endothelial cell. This results in CCL2 up-regulation in the lungs, with recruitment of Ly6C+ CCR2+ monocytes that are essential also for extravasation of tumor cells (Qian et al. 2011; Keklikoglou et al. 2019). Importantly, the authors demonstrated that the vesicle release happened in an apoptosis-independent manner and the purified extracellular vesicle could recapitulate premetastatic soil without any tumor burden. Although the lungs were not analyzed, UV-induced HMGB1 released from melanoma cells increased its serum levels and led to subsequent lung metastasis (Bald et al. 2014).

PREMETASTATIC NICHE ELEMENTS

Premetastatic niche elements are divided into two categories—physical and functional. Physical elements include chemokines, cells (resident or migratory), and extracellular matrix (ECM). The ECM matrisome is nicely reviewed by Høye and Erler (2016). Core matrisome includes fibronectin, collagen IV, periostin, fibrinogen and fibrin, tenascin C. Matrisome-associated proteins are Sema, VEGF, MMP, and LOX (lysyl oxidase), and so forth. Functional elements include increased vaso-permeability, osteolysis, degradation of ECM, chemokine gradients, survival, immunosuppression and switching between quiescence and regrowth (Fig. 5).

A leading paper from Lyden's laboratory showed a close linkage among premetastatic niche elements in a given context (Kaplan et al. 2005). An undefined tumor-secretome, described as tumor-derived conditioned media in their experiments, up-regulates the expression of fibronectin and SDF-1 in the lungs. SDF-1 in turn stimulates the mobilization of both CXCR4+ VEGFR1+ fibronectin receptor $\alpha 4 \beta 1$ + myeloid cells from bone marrow (bone marrow-derived cells, BMDC) and CXCR4-expressing tumor cells from the primary tumor (Fig. 4E). Interactions here include SDF-1 and CXCR4 by chemokine gradient, fibronectin, and its receptor. Experimental cell labeling revealed that 97% of tumor cells were colocalized with BMDC clusters in the lungs 18 d after subcutaneous transplantation of tumor cells. BMDC clusters with increased expression of fibronectin serve as niche for metastasizing tumor cells. In a similar experiment in our laboratory, the efficiency of colocalization was only 5% (Hiratsuka et al. 2006), suggesting that niche settlement of tumor cells can be largely abortive. Tumor cells were detected near terminal bronchioles lined by club cells. Club cells are preexisting resident epithelial cells in the lungs and participate in the premetastatic niche formation by expressing SAA3 (Tomita et al. 2011). The extra domain A of cellular fibronectin also binds TLR4 in models of fibrosis (Malara et al. 2019).

An earlier study by Gabrilovich et al. (1998) showed that VEGF induced splenic expansion of

Figure 5. Metastatic niche elements. Our premetastatic model shows one example of how prometastatic versus antimetastatic elements are engaged in the lungs in the absence and presence of metastasizing tumor cells. See text for details.

immature Gr-1 (antibody detecting Ly6G and Ly6C) positive myeloid cells. This type of cells, currently called myeloid-derived suppressor cells (MDSCs), have been extensively investigated and their suppressive nature against T cells and NK cells is reviewed (Tcyganov et al. 2018). In mice, MDSCs are Gr-1 + CD11b+. They are grouped into two classes: polymorphonuclear (PMN)-MDSC (CD11b + Ly6G + Ly6Clow) and monocytic (M)-MDSC (CD11b + Ly6G-Ly6Chigh). Both cell populations are found in the tumor microenvironment and can suppress both antigen-specific and nonspecific T cell activities in a manner mediated by arginase I, iNOS, and ROS (Corzo et al. 2010). PMN-MDSCs dominate over M-MDSCs in number in primary tumors, and M-MDSC can differentiate into macrophages. Premetastatic lungs also have increased numbers of both populations with up-regulated arginase expression levels, which are either augmented or suppressed in

number by VEGF or a TLR4/MD-2 inhibitor eritoran, respectively (Hiratsuka et al. 2006; Deguchi et al. 2016).

As described in the previous section, S100A8-dependent recruitment of MDSCs is required for premetastatic niche formation in the lungs. In our work, an increased number of MDSCs were not observed in liver or bone during the premetastatic phase.

PMN-MDSCs impair NK cells in premetastatic lungs (Sceneay et al. 2012). In human, high expression of LOX-1 (lectin-type oxidized LDL receptor-1) can distinguish PMN-MDSC from morphologically similar physiological PMN cells that lack it (Condamine et al. 2016). However, in mice, no particular trait in MDSC was observed in our premetastatic lung models (Takita and Maru, unpublished results). In our experiments, premetastatic lung-derived MDSCs displayed low but appreciable suppressive activities against T cells as compared with those from

Y. Maru

primary tumors (Deguchi and Maru, unpublished results). The underlying mechanisms might be S100A8-TLR4/MD-2-NF κ B signaling cascade (Hiratsuka et al. 2006) (see Hijacking homeostasis section) that could potentially interact with HIF-1 α under normoxic conditions (Görlach and Bonello 2008).

In a latent metastasis model, down-regulation of ligands for NK activating receptor NKG2D and proapoptotic receptor FAS in dormant tumor cells was observed. NK cell-mediated restrictive effect on regrowth is lifted in postformed premetastatic niche by anti-NK1.1 antibody. Dormant CSCs obtain permissive regrowth resulting in overt metastasis (Malladi et al. 2016).

S100A8 expressed in both macrophages and endothelial cells forms paracrine networks with CCL2 and TNF α in premetastatic lungs, resulting in increased vascular permeability (Fig. 6, see below). Therefore, endothelial cells constitute an important element in the premetastatic niche. Endothelial cell-derived thrombospondin-1 could induce quiescence of injected breast cancer cells in the lungs (Ghajar et al. 2013). Premetastatic vasculature of lungs (but not liver) is also accompanied by perivascular cell activation with fibronectin production in a tumor WISP-1-stimulated KLF-4-dependent fashion (Murgai et al. 2017). Production of CXCL10 in pericytes was shown to regulate monocyte transmigration in blood-brain barrier (Niu et al. 2019).

Degradation of ECM Matrisome

Synergic effects of angiopoietin-2, MMP3 and MMP10, all of which were up-regulated in the

Figure 6. Paracrine networks may form vicious cycles. Among epithelial cells, endothelial cells, macrophages (and tumor cells if present), premetastatic soil is cultivated by paracrine cascades of growth factors/chemokines (Maru 2011b; Ieguchi et al. 2013). See two vicious cycles with red arrows.

premetastatic lungs, caused increased vascular permeability with fibrinogen/fibrin deposition by disrupting basement membrane as observed by transmission electron microscopy (Fig. 4F; Huang et al. 2009). The deposition started to be observed 8 d after tumor cell implantation. Antiangiopoietin-2 blocking antibody restored vascular stability and decreased lung metastasis (Keskin et al. 2015). We also used antifibrinogen antibody but could hardly discriminate between fibrinogen and fibrin (Hiratsuka et al. 2013, 2018). Although we do not know clearly whether fibrin deposition is a tumor-led active process (since fibrinogen is produced in lung endothelial cells) or a host response to prevent vascular leakage from the destabilized endothelial sites (proteolytic elimination of fibrinogen by coagulation factor X that is expressed by B220 + CD11c + NK1.1 + NK cells), inhibited tumor cell homing in the premetastatic lungs (Hiratsuka et al. 2018), suggesting that fibrinogen/fibrin deposition is a prometastatic activity.

Coagulation is not only a passive response to hemorrhage in tissue invasion by tumor and immune cells, but also actively stimulated by tumor-derived factors as well known in tumor-induced disseminated intravascular coagulopathy. While the essence of inflammation is increased vascular permeability and leukocyte mobilization, both of which actually take place in premetastatic lungs, coagulation involves platelets. Currently, precise roles of platelets in premetastasis still remain to be revealed, it is no wonder that it includes immune-thrombotic events. A recent paper suggests that platelets, and not leukocytes, are necessary to protect against infection-induced lung injury (Bain et al. 2019). I have proposed a triangle structure formed by angiogenesis, inflammation, and coagulation with tumor in the center (Fig. 7; Maru 2011a, 2016). This idea can be applied to premetastatic soil whose triangle is connected with that of primary tumor by inflammation (Gil-Bernabe et al. 2012).

The lung endothelium is surrounded by a basement membrane that mainly consists of collagen type IV and laminin. Primary tumor-secreted LOX cross-links collagen IV in the lungs, to which BMDCs adhere and produce MMP2 that is known to cleave collagen IV, thereby am-

Figure 7. Coagulation participates in premetastasis. Conceptual extension of the field in the primary tumor, in which angiogenesis, inflammation, and coagulation are interdependent, to premetastatic tissues as evidenced in our premetastatic lung system. See text for details.

plifying the recruitment of BMDCs by generating chemoattractive collagen IV proteolytic peptides (Erler et al. 2009). Colocalization of LOX and fibronectin precedes entry of BMDCs. Cleaved peptides of fibronectin by MMP2 were also shown to mediate attachment of ovarian cancer cells to the peritoneum (Kenny et al. 2008). Those mechanisms facilitate tumor cell entry to the metastatic sites. LOX mediated the expression of MMP2 but not MMP9.

Our cDNA microarray analysis of lungs from tumor-bearing vs. nonbearing mice (Fig. 2) revealed up-regulation of MMP9 depending on the tumor cell-types but not MMP2 or MMP14 (Hiratsuka et al. 2006). However, tumor cell homing was abrogated in premetastatic lungs of MMP9 knockout mice (Hiratsuka et al. 2002).

Focal Vascular Permeability and Osteolysis

VEGF, CCL2, MMP, S100A8, and SAA3 all can induce increased vascular permeability in the

lungs (Hiratsuka et al. 2013). VE-cadherin disruption was observed in premetastatic lungs in which MMP9-expressing BMDCs are increased in number (Yan et al. 2010). We found that tyrosine kinases EphA1 and its most homologous neighbor EphA2, and their membrane-anchored ligand ephrin-A1 are expressed in lung endothelial cells, and their binding serves as an adhesion machinery (Ieguchi et al. 2014). ADAM12-cleaved soluble forms of ephrin-A1 stimulated lung endothelial cells and caused increased vascular permeability by cell contraction in the lungs, which may accompany endocytosis of VE-cadherin, facilitating tumor cell entry (Yamazaki et al. 2009). Ephrin-A1 was originally discovered as an early-response gene to TNF α in endothelial cells (Holzman et al. 1990). It is intriguing that both LOX and EphA1 bind the carboxy-terminal portion of fibronectin (Masuda et al. 2008). One of the features of those potent factors in vaso-permeability and cell mobilization is that they constitute vicious cycles for interdependent up-regulation of expression (Fig. 6).

The increased vascular permeability in premetastatic lungs occurs in a segmental fashion (Hiratsuka et al. 2011, 2013). The osteolysis in premetastatic bone takes place in a focal manner (Cox et al. 2015) (see above section). The mechanisms of the mottled occurrence of premetastatic niches are unknown but maybe stochastic. It raises a question of whether or not portions of the same lungs or bone without increased vascular permeability or osteolysis, respectively, can be called premetastatic soil. I think the answer to this question is no.

ORGANOTROPISM

This is a concept speculated on by Stephen Paget with the suggestion that the mode of spreading of metastatic tumor cells to certain distant organs is a nonrandom process. However, it is often confusing since interpretations of experimental results differ depending on whether or not the word “spreading” or “distribution” of metastasizing tumor cells includes metastatic step 6, that is regrowth. For example, in a Perspective by Schild et al. in *Cancer Cell* (Rahmann

Y. Maru

and Gilbertson 2018), the authors intriguingly describe the hijacking of adipocyte energy metabolism by ovarian cancer cells that make a metastatic progression to the omentum, but they mentioned that “omental tropism was impaired” in gene ablated mice for the fatty acid-binding protein 4 (Nieman et al. 2011). First, this is postmetastasis and second, the knockout mice simply exhibited a decrease in metastatic tumor mass by a loss of energy transfer but not the degree to which ovarian cancer cells are oriented to omentum as expressed by factors such as, the number of tumor cells that reached the omentum from the primary site. This is a similar way of thinking of the extension of the conceptual field to the above-mentioned inclusion of dormant cells in premetastasis. Organotropism could be used as manifestation of regrowth of dormant tumor cells; an organ with dormant cells, to which the tumor cells are apparently nonorganotropic, changes itself to a metastatic organ to which the tumor cells are organotropic after regrowth.

Lyden's group showed that a combination of integrin subunits contained in tumor-released exosomes could determine the organotropism of the parent tumor by being up-taken by the organ-specific resident cells. According to this idea, one organotropism cannot exclude another organotropism, thereby enabling a given tumor to metastasize to more than one organ. Exosomes are no more an avatar than a growth factor is. Just as the mode of differential packaging of different integrin subunits in released exosomes is unknown (Costa-Silva et al. 2015; Hoshino et al. 2015), the choice in the combination of secreted growth factors from primary tumor is poorly understood (Hiratsuka et al. 2006). For example, matching between $\alpha\beta4$ with S100A4+ lung fibroblasts in lungs in laminin-rich soil, $\alpha\beta5$ with liver Kupffer cells in fibronectin-rich soil, eventually in each case provokes sterile inflammation.

HOST-LED ACTIVITY IN PREMETASTATIC SOIL

The tumor-led activities that are necessary to accomplish a premetastatic niche have been ex-

tensively studied. However, as in the case of reciprocal innervation in the autonomic nervous system, premetastatic soil includes host-led responses against prometastatic activities in premetastatic niche formation and arrival of metastasizing tumor cells.

The orthotopic transplantation of 4T1 tumor model shows spontaneous metastasis to the lungs in 2 wk. Ly6G+ neutrophils start to accumulate in the lungs on day 7 post tumor engraftment before metastasis, and neutrophil depletion augmented lung metastasis as assessed on day 25 without influencing CTC numbers (Granot et al. 2011). Given that the authors showed their killing activities against tumor cells, those cells should serve as antimetastatic elements in the premetastatic soil. While the lack of both S100A8 up-regulation and increased vaso-permeability in their experiments is inconsistent with our experimental results (Hiratsuka et al. 2013), increased CCR2 expression in the premetastatic lungs is in agreement with the results of our premetastatic lung system. Recruitment of tumor cell-killing neutrophils may be a host-led response concomitantly occurring with tumor-led premetastasis-supporting activities.

We have identified B220 + CD11c + NK1.1+ NK cells with antimetastatic activities. They originate from the bone marrow and are “educated” in the liver before moving to the premetastatic lungs, where they not only eliminate fibrinogen deposition but also attack future-arriving tumor cells by IFN γ (Hiratsuka et al. 2018). This finding implies a complicated nature of metastasis with a multiorgan linkage.

Sema3A expression is down-regulated in premetastatic lungs while its receptor, NP-1, expression changes are not so obvious (Hiratsuka et al. 2006). Sema3A is a potential angiogenesis inhibitor, and expression of both Sema3A and NP-1 is down-regulated in tumor progression (Maione et al. 2009). Sema3A was shown to stimulate antitumor macrophages, which can activate NK cells in an NP-1-dependent manner (Wallerius et al. 2016). Thus, Sema3A can be called antimetastatic, and its down-regulation in tumors favors tumor progression. However, Sema3A can also exert antimigration effect on macrophages in an NP-1-independent fashion

(Casazza et al. 2013). Therefore, in premetastatic lungs, down-regulation of Sema3A could also be a host-led response against BMDC recruitment to the lungs.

HIJACKING OF HOMEOSTASIS

Host defense programs, especially innate immune programs executed in inflammation, are often utilized by primary tumors to hijack in metastasis since the antimicrobial activities are usually accompanied by movement of leukocytes via circulation.

Endogenous Ligands for TLR4

In our experimental lung metastasis model, the transplanted tumors secrete VEGF, TNF α , and CCL2, stimulating the lungs in the absence of metastasizing tumor cells. The activated lungs in turn express S100A8 and SAA3 both of which are endogenous ligands of TLR4/MD-2 complex, a molecular sensor for microbial endotoxin or lipopolysaccharide (LPS), and released into the circulation (Maru 2009, 2010; Deguchi et al. 2013, 2016). TLR4/MD-2 is expressed in a variety of cell types including blood cells of myeloid origin, endothelial cells, and tumor cells. Production of both S100A8 and SAA3 in the lungs may make a chemokine gradient between the circulation and the lungs, resulting in mobilization and recruitment of cells that express TLR4/MD-2. Injection of reporter cells of TLR4 signaling into tumor-bearing mice with premetastatic lungs showed that the up-regulated endogenous ligands could activate TLR4 in vivo (Deguchi, Tsukahara, Maru, unpublished results) (Fig. 8). TLR4/MD-2-expressing bone marrow-derived Gr-1 + CD11b+ myeloid cells (BMDCs) may misinterpret the overproduction of S100A8 as the presence of microbes producing LPS, and thus be destined to reach the lungs as a host defense machinery against extrinsic pathogens. This recruitment is blocked by MD-2 antagonist eritoran (Deguchi et al. 2016). S100A8 and the concomitantly induced CCL2 (Hiratsuka et al. 2013) increased vascular permeability in the premetastatic lungs, which is possibly intensified by MMP9

expressed by BMDCs. BMDCs are also responsible for inhibiting IFN γ production by F4/80 + CD11b+ lung macrophages (Yan et al. 2010). The significance of pulmonary inflammation in metastasis was supported by two separate experiments in which, intra-tracheal exposure of bacteria or LPS and ovalbumin-induced CD4+ T cell-dependent asthma, both enhanced lung metastasis after intravenous injection of tumor cells (Taranova et al. 2008; Yan et al. 2013). This may give an alarm to clinicians since both pneumonia and asthma are rather common. Mechanistically, lung inflammation seems to contribute to premetastatic niche formation.

This mimesis between exogenous LPS and endogenous ligands appears to be a hijacking strategy of tumors against the host defense program in a manner in which S100A8 mimics LPS. We believe that LPS comes first in evolution, and then S100A8. However, it can be assumed that LPS mimics S100A8 but the cellular responses happen to be much larger in the imitator LPS than S100A8. Mimicry of host elements by exogenous ones has been reported in many circumstances including histone mimicry by H3N2 influenza A virus for transcriptional alteration (Tarakhovsky and Prinjha 2018).

NETs and Omental Metastasis

Ovarian cancer cells have an organotropic metastasis to the omentum that consists of adipocytes and connective tissues but not to other intraperitoneal adipose tissues. Primary tumor-led production of a subset of chemokines, including IL-8 and G-CSF but not CCL2, is required to induce mobilization of neutrophils from the bone marrow to the omental reticulo-endothelial system called “milky spots” embedded in the adipose tissue, a first-defense machinery of ~250 μ m in diameter and two spots per cm² in density in human adults (Shimotsuma et al. 1993; Lee et al. 2018). They consist of macrophages, lymphocytes, fenestrated endothelial cells and mesothelial cells, and their size and number is increased by inflammation. The mobilized neutrophils are abundantly recruited to high endothelial venules in milky spots. Although the mechanisms of how those neutro-

Y. Maru

Figure 8. Endogenous ligands activate TLR4/MD-2 in premetastatic lungs. An IL-3-dependent cell line Ba/F3 with an NFκB-dependent transcription cassette of the luciferase gene was transformed by BCR-ABL and injected into tumor-bearing mice with premetastatic lungs in which both S100A8 and SAA3 were up-regulated. Significant reporter activities were recognized. See text.

phils are attracted to the particular fat tissue omentum and of why NET takes place in the absence of microbes is unknown. However, released neutrophil DsDNAs and antimicrobial peptide such as LL37, capable of influencing availability of TLR4 ligands (Suzuki et al. 2016) and of stimulating cell migration of neutrophils through formyl peptide receptor-like 1 (FPR1) (De et al. 2000), may attract metastasizing tumor cells that ectopically express their functional receptors. One clue may come from an involvement of coagulation by neutrophil serine protease in which tissue factor and coagulation factor XII are required (Massberg et al. 2010).

Why can tumor cells settle down and even regrow under the trapping and killing activity of NET against invading microbes? Tumor cells attach to neutrophils in a NET-dependent manner since the attachment was not observed with a neutrophil-specific knockout of peptidylarginine deaminase 4 (PAD4) essential for NET formation by converting methyl-arginine to citrulline in histones for chromatin condensation. Citrullinated histones by themselves are not toxic to microbes and released histones are reported to activate neutrophil and endothelial cells in ARDS (acute respiratory distress syndrome) models in a manner dependent on TLR4 and P-selectin (Zhang et al. 2016). Since ARDS is an extreme

of LPS-induced TLR4 activation whose sterile version is the premetastatic lungs with low-level inflammation as I mentioned above. Once settled down tumor cells by overcoming the anticancer activity of neutrophils in the omentum in post-metastatic phase may initiate a parasitic metabolic program on adipocytes for fatty acids as an energy source (Nieman et al. 2011).

Bone Homeostasis by the RANKL-RANK System

RANKL is a highly abundant cytokine in bone marrow capable of inducing differentiation of osteoclasts that express its receptor RANK in physiological conditions. RANK is also expressed in breast cancer cells and RANKL can induce their directional migration (Jones et al. 2006). In this case, the preformed premetastatic niche in bone marrow can attract the RANK-expressing primary tumor cells but the premetastatic niche may be immature on the tumor cell arrival. Therefore, the preformed premetastatic niche may be completed by intraniche tumor cells by themselves (Fig. 4G).

As described above, tumor-secreted RANKL induces osteolytic lesions in bone, which serve as premetastatic bone niche before tumor cell colonization. It was shown that the osteolytic lesions were also created by CD4⁺ T cells found in the bone cavity and draining lymph nodes in a manner in which tumor antigen-stimulated T cells express RANKL (Monteiro et al. 2013).

CONCLUSION

The molecular basis that underlies the biology of premetastasis is sterile inflammation that is provoked by tumor-led secretome and spread from the primary tumor tissues to metastatic organs. The essential features are increased vascular permeability and active cell migration. The physiological property of a metastatic organ and the traits associated with metastasizing tumor cells may determine organotropism.

ACKNOWLEDGMENTS

I thank my colleagues in my laboratory, A Deguchi and K Ieguchi, for discussions and pre-

liminary data. This work was partly supported by grant-in-aid from The Vehicle Racing Commemorative Foundation.

REFERENCES

- Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H, et al. 2014. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* **158**: 1110–1122. doi:10.1016/j.cell.2014.07.013
- Alečković M, Wei Y, LeRoy G, Sidoli S, Liu DD, Garcia BA, Kang Y. 2017. Identification of Nidogen 1 as a lung metastasis protein through secretome analysis. *Genes Dev* **31**: 1439–1455. doi:10.1101/gad.301937.117
- Arwert EN, Harney AS, Entenberg D, Wang Y, Sahai E, Pollard JW, Condeelis JS. 2018. A unidirectional transition from migratory to perivascular macrophage is required for tumor cell intravasation. *Cell Rep* **23**: 1239–1248. doi:10.1016/j.celrep.2018.04.007
- Assor D. 1972. Endometriosis of the lung: report of a case. *Am J Clin Pathol* **57**: 311–315. doi:10.1093/ajcp/57.3.311
- Bain W, Olonisakin T, Yu M, Qu Y, Hulver M, Xiong Z, Li H, Pilewski J, Mallampalli RK, Nouraei M, et al. 2019. Platelets inhibit apoptotic lung epithelial cell death and protect mice against infection-induced lung injury. *Blood Adv* **3**: 432–445. doi:10.1182/bloodadvances.2018026286
- Bald T, Quast T, Landsberg J, Rogava M, Glodde N, Lopez-Ramos D, Kohlmeyer J, Riesenberger S, van den Boorn-Konijnenberg D, Hömig-Hölzel C, et al. 2014. Ultraviolet-radiation-induced inflammation promotes angiogenesis and metastasis in melanoma. *Nature* **507**: 109–113. doi:10.1038/nature13111
- Bekwelem W, Connolly SJ, Halperin JL, Adabag S, Duval S, Chrolavicius S, Pogue J, Ezekowitz MD, Eikelboom JW, Wallentin LG, et al. 2015. Extracranial systemic embolic events in patients with nonvalvular atrial fibrillation: incidence, risk factors, and outcomes. *Circulation* **132**: 796–803. doi:10.1161/CIRCULATIONAHA.114.013243
- Cabel L, Proudhon C, Gortais H, Loirat D, Coussy F, Pierga JY, Bidard FC. 2017. Circulating tumor cells: clinical validity and utility. *Int J Clin Oncol* **22**: 421–430. doi:10.1007/s10147-017-1105-2
- Casazza A, Laoui D, Wenes M, Rizzolio S, Bassani N, Mambretti M, Deschoemaeker S, Van Ginderachter JA, Tamagnone L, Mazzone M. 2013. Impeding macrophage entry into hypoxic tumor areas by Sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. *Cancer Cell* **24**: 695–709. doi:10.1016/j.ccr.2013.11.007
- Chen Q, Zhang XH, Massagué J. 2011. Macrophage binding to receptor VCAM-1 transmits survival signals in breast cancer cells that invade the lungs. *Cancer Cell* **20**: 538–549. doi:10.1016/j.ccr.2011.08.025
- Condamine T, Dominguez GA, Youn JI, Kossenkova AV, Mony S, Alicea-Torres K, Tcyganov E, Hashimoto A, Nefedova Y, Lin C, et al. 2016. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol* **1**: aaf8943. doi:10.1126/sciimmunol.aaf8943

Y. Maru

- Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, Cho HI, Celis E, Quiceno DG, Padhya T, et al. 2010. HIF-1 α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med* **207**: 2439–2453. doi:10.1084/jem.20100587
- Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, Becker A, Hoshino A, Mark MT, Molina H, et al. 2015. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol* **17**: 816–826. doi:10.1038/ncb3169
- Cox TR, Rumney RMH, Schoof EM, Perryman L, Hoye AM, Agrawal A, Bird D, Latif NA, Forrest H, Evans HR, et al. 2015. The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. *Nature* **522**: 106–110. doi:10.1038/nature14492
- Deguchi A, Maru Y. 2017. Dickkopf-1 helps metastasis by immune evasion. *Transl Cancer Res* **6**: S1422–S1424. doi:10.21037/tcr.2017.10.43
- Deguchi A, Tomita T, Omori T, Komatsu A, Ohto U, Takahashi S, Tanimura N, Akashi-Takamura S, Miyake K, Maru Y. 2013. Serum amyloid A3 binds MD-2 to activate p38 and NF- κ B pathways in a MyD88-dependent manner. *J Immunol* **191**: 1856–1864. doi:10.4049/jimmunol.1201996
- Deguchi A, Tomita T, Ohto U, Takemura K, Kitao A, Akashi-Takamura S, Miyake K, Maru Y. 2016. Eritoran inhibits S100A8-mediated TLR4/MD-2 activation and tumor growth by changing the immune microenvironment. *Oncogene* **35**: 1445–1456. doi:10.1038/onc.2015.211
- Dejean E, Foisseau M, Lagarrigue F, Lamant L, Prade N, Marfak A, Delsol G, Giuriato S, Gaits-Iacovoni F, Meggetto F. 2012. ALK⁺ALCLs induce cutaneous, HMGB-1-dependent IL-8/CXCL8 production by keratinocytes through NF- κ B activation. *Blood* **119**: 4698–4707. doi:10.1182/blood-2011-10-386011
- Deryugina EI, Kiosses WB. 2017. Intratumoral cancer cell intravasation can occur independent of invasion into the adjacent stroma. *Cell Rep* **19**: 601–616. doi:10.1016/j.celrep.2017.03.064
- Duda DG, Duyverman AM, Kohno M, Snuderl M, Steller EJ, Fukumura D, Jain RK. 2010. Malignant cells facilitate lung metastasis by bringing their own soil. *Proc Natl Acad Sci* **107**: 21677–21682. doi:10.1073/pnas.1016234107
- Erlor JT, Bennewith KL, Cox TR, Lang G, Bird D, Koong A, Le QT, Giaccia AJ. 2009. Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. *Cancer Cell* **15**: 35–44. doi:10.1016/j.ccr.2008.11.012
- Fidler IJ. 1970. Metastasis: quantitative analysis of distribution and fate of tumor emboli labeled with 125 I-5-iodo-2'-deoxyuridine. *J Natl Cancer Inst* **45**: 773–782.
- Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, Carbone DP. 1998. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood* **92**: 4150–4166.
- Gao Q, Yang Z, Xu S, Li X, Yang X, Jin P, Liu Y, Zhou X, Zhang T, Gong C, et al. 2019. Heterotypic CAF-tumor spheroids promote early peritoneal metastasis of ovarian cancer. *J Exp Med* **216**: 688–703. doi:10.1084/jem.20180765
- Ghajar CM, Peinado H, Mori H, Matei IR, Evason KJ, Brazier H, Almeida D, Koller A, Hajjar KA, Stainier DY, et al. 2013. The perivascular niche regulates breast tumour dormancy. *Nat Cell Biol* **15**: 807–817. doi:10.1038/ncb2767
- Gil-Bernabé AM, Ferjancic S, Tlalka M, Zhao L, Allen PD, Im JH, Watson K, Hill SA, Amirkhosravi A, Francis JL, et al. 2012. Recruitment of monocytes/macrophages by tissue factor-mediated coagulation is essential for metastatic cell survival and premetastatic niche establishment in mice. *Blood* **119**: 3164–3175. doi:10.1182/blood-2011-08-376426
- Görlach A, Bonello S. 2008. The cross-talk between NF- κ B and HIF-1: further evidence for a significant liaison. *Biochem J* **412**: e17–e19. doi:10.1042/BJ20080920
- Granot Z, Henke E, Comen EA, King TA, Norton L, Benezra R. 2011. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. *Cancer Cell* **20**: 300–314. doi:10.1016/j.ccr.2011.08.012
- Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: the next generation. *Cell* **144**: 646–674. doi:10.1016/j.cell.2011.02.013
- Heidegger M. 1967. *Sein und Zeit*. Max Niemeyer Verlag, Tübingen, Z326.
- Hiratsuka S, Nakamura K, Iwai S, Murakami M, Itoh T, Kijima H, Shipley JM, Senior RM, Shibuya M. 2002. MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. *Cancer Cell* **2**: 289–300. doi:10.1016/S1535-6108(02)00153-8
- Hiratsuka S, Watanabe A, Aburatani H, Maru Y. 2006. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. *Nat Cell Biol* **8**: 1369–1375. doi:10.1038/ncb1507
- Hiratsuka S, Watanabe A, Sakurai Y, Akashi-Takamura S, Ishibashi S, Miyake K, Shibuya M, Akira S, Aburatani H, Maru Y. 2008. The S100A8-serum amyloid A3-TLR4 paracrine cascade establishes a pre-metastatic phase. *Nat Cell Biol* **10**: 1349–1355. doi:10.1038/ncb1794
- Hiratsuka S, Goel S, Kamoun WS, Maru Y, Fukumura D, Duda DG, Jain RK. 2011. Endothelial focal adhesion kinase mediates cancer cell homing to discrete regions of the lungs via E-selectin up-regulation. *Proc Natl Acad Sci* **108**: 3725–3730. doi:10.1073/pnas.1100446108
- Hiratsuka S, Ishibashi S, Tomita T, Watanabe A, Akashi-Takamura S, Murakami M, Kijima H, Miyake K, Aburatani H, Maru Y. 2013. Primary tumours modulate innate immune signalling to create pre-metastatic vascular hyperpermeability foci. *Nat Commun* **4**: 1853. doi:10.1038/ncomms2856
- Hiratsuka S, Tomita T, Mishima T, Matsunaga Y, Omori T, Ishibashi S, Yamaguchi S, Hosogane T, Watarai H, Omori-Miyake M, et al. 2018. Hepato-entrained B220⁺CD11c⁺NK1.1⁺ cells regulate pre-metastatic niche formation in the lung. *EMBO Mol Med* **10**. doi:10.15252/emmm.201708643
- Holzman L, Marks R, Dixit V. 1990. A novel immediate-early response gene of endothelium is induced by cytokines and encodes a secreted protein. *Mol Cell Biol* **10**: 5830–5838. doi:10.1128/MCB.10.11.5830
- Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, Di Giannatale A, Ceder S, et al. 2015. Tumour exosome integrins

- determine organotropic metastasis. *Nature* **527**: 329–335. doi:10.1038/nature15756
- Høye AM, Erler JT. 2016. Structural ECM components in the premetastatic and metastatic niche. *Am J Physiol Cell Physiol* **310**: C955–C967. doi:10.1152/ajpcell.00326.2015
- Huang Y, Song N, Ding Y, Yuan S, Li X, Cai H, Shi H, Luo Y. 2009. Pulmonary vascular destabilization in the premetastatic phase facilitates lung metastasis. *Cancer Res* **69**: 7529–7537. doi:10.1158/0008-5472.CAN-08-4382
- Ieguchi K, Omori T, Komatsu A, Tomita T, Deguchi A, Maru Y. 2013. Ephrin-A1 expression induced by S100A8 is mediated by the toll-like receptor 4. *Biochem Biophys Res Commun* **440**: 623–629. doi:10.1016/j.bbrc.2013.09.119
- Ieguchi K, Tomita T, Omori T, Komatsu A, Deguchi A, Masuda J, Duffy SL, Coulthard MG, Boyd A, Maru Y. 2014. ADAM12-cleaved ephrin-A1 contributes to lung metastasis. *Oncogene* **33**: 2179–2190. doi:10.1038/onc.2013.180
- Inada M, Takita M, Yokoyama S, Watanabe K, Tominari T, Matsumoto C, Hirata M, Maru Y, Maruyama T, Sugimoto Y, et al. 2015. Direct melanoma cell contact induces stromal cell autocrine prostaglandin E2-EP4 receptor signaling that drives tumor growth, angiogenesis, and metastasis. *J Biol Chem* **290**: 29781–29793. doi:10.1074/jbc.M115.669481
- Jones DH, Nakashima T, Sanchez OH, Kozieradzki I, Komarova SV, Sarosi I, Morony S, Rubin E, Sarao R, Hojilla CV, et al. 2006. Regulation of cancer cell migration and bone metastasis by RANKL. *Nature* **440**: 692–696. doi:10.1038/nature04524
- Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, et al. 2005. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* **438**: 820–827. doi:10.1038/nature04186
- Karagiannis GS, Poutahidis T, Erdman SE, Kirsch R, Riddell RH, Diamandis EP. 2012. Cancer-associated fibroblasts drive the progression of metastasis through both paracrine and mechanical pressure on cancer tissue. *Mol Cancer Res* **10**: 1403–1418. doi:10.1158/1541-7786.MCR-12-0307
- Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. 2007. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* **449**: 557–563. doi:10.1038/nature06188
- Keklikoglou I, Cianciaruso C, Guc E, Squadrito ML, Spring LM, Tazzyman S, Lambein L, Poissonnier A, Ferraro GB, Baer C, et al. 2019. Chemotherapy elicits pro-metastatic extracellular vesicles in breast cancer models. *Nat Cell Biol* **21**: 190–202. doi:10.1038/s41556-018-0256-3
- Kenny HA, Kaur S, Coussens LM, Lengyel E. 2008. The initial steps of ovarian cancer cell metastasis are mediated by MMP-2 cleavage of vitronectin and fibronectin. *J Clin Invest* **118**: 1367–1379.
- Keskin D, Kim J, Cooke VG, Wu CC, Sugimoto H, Gu C, De Palma M, Kalluri R, LeBleu VS. 2015. Targeting vascular pericytes in hypoxic tumors increases lung metastasis via angiopoietin-2. *Cell Rep* **10**: 1066–1081. doi:10.1016/j.celrep.2015.01.035
- Kim MY, Oskarsson T, Acharyya S, Nguyen DX, Zhang XH, Norton L, Massagué J. 2009a. Tumor self-seeding by circulating cancer cells. *Cell* **139**: 1315–1326. doi:10.1016/j.cell.2009.11.025
- Kim S, Takahashi H, Lin WW, Descargues P, Grivennikov S, Kim Y, Luo JL, Karin M. 2009b. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature* **457**: 102–106. doi:10.1038/nature07623
- Lee W, Ko SY, Mohamed MS, Kenny HA, Lengyel E, Naora H. 2018. Neutrophils facilitate ovarian cancer premetastatic niche formation in the omentum. *J Exp Med* **216**: 176–194. doi:10.1084/jem.20181170
- Macquarrie J, Robinson E. 1992. *Being and Time*, translated from the German Sein und Zeit seventh edition, Blackwell, Oxford.
- Maione F, Molla F, Meda C, Latini R, Zentilin L, Giacca M, Seano G, Serini G, Bussolino F, Giraudo E. 2009. Semaphorin 3A is an endogenous angiogenesis inhibitor that blocks tumor growth and normalizes tumor vasculature in transgenic mouse models. *J Clin Invest* **119**: 3356–3372.
- Malanchi I, Santamaria-Martínez A, Susanto E, Peng H, Lehr HA, Delaloye JF, Huelsken J. 2011. Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* **481**: 85–89. doi:10.1038/nature10694
- Malara A, Gruppi C, Abbonante V, Cattaneo D, De Marco L, Massa M, Iurlo A, Gianelli U, Balduini CL, Tira ME, et al. 2019. EDA fibronectin-TLR4 axis sustains megakaryocyte expansion and inflammation in bone marrow fibrosis. *J Exp Med* **216**: 587–604. doi:10.1084/jem.20181074
- Malladi S, Macalinalo DG, Jin X, He L, Basnet H, Zou Y, de Stanchina E, Massagué J. 2016. Metastatic latency and immune evasion through autocrine inhibition of WNT. *Cell* **165**: 45–60. doi:10.1016/j.cell.2016.02.025
- Maru Y. 2009. A concept of homeostatic inflammation provided by endogenous TLR4 agonists that function before and after danger signal for metastasis. *Anti-Inflamm Anti-Allergy Agents Med Chem* **8**: 337–347. doi:10.2174/187152309789839064
- Maru Y. 2010. Premetastatic milieu explained by TLR4 agonist-mediated homeostatic inflammation. *Cell Mol Immunol* **7**: 94–99. doi:10.1038/cmi.2009.113
- Maru Y. 2011a. Inflammation in tumor progression. *Folia Pharmacol Jpn* **138**: 155–160. doi:10.1254/fpj.138.155
- Maru Y. 2011b. Endogenous ligand-induced activation of TLR4 in pre-metastatic phase is both downstream and upstream of TNF signaling. *Adv Exp Med Biol* **691**: 261–268. doi:10.1007/978-1-4419-6612-4_26
- Maru Y. 2015. The lung metastatic niche. *J Mol Med (Berl)* **93**: 1185–1192. doi:10.1007/s00109-015-1355-2
- Maru Y. 2016. *Inflammation and metastasis*. Springer, Japan.
- Massberg S, Grah L, von Brühl ML, Manukyan D, Pfeiler S, Goosmann C, Brinkmann V, Lorenz M, Bidzhekov K, Khandagale AB, et al. 2010. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med* **16**: 887–896. doi:10.1038/nm.2184
- Masuda J, Usui R, Maru Y. 2008. Fibronectin type I repeat is a nonactivating ligand for EphA1 and inhibits ATF3-dependent angiogenesis. *J Biol Chem* **283**: 13148–13155. doi:10.1074/jbc.M702164200

Y. Maru

- Méhes G, Witt A, Kubista E, Ambros PF. 2001. Circulating breast cancer cells are frequently apoptotic. *Am J Pathol* **159**: 17–20. doi:10.1016/S0002-9440(10)61667-7
- Merlos-Suárez A, Batlle E. 2008. Eph-ephrin signalling in adult tissues and cancer. *Curr Opin Cell Biol* **20**: 194–200. doi:10.1016/j.ceb.2008.01.011
- Monteiro AC, Leal AC, Goncalves-Silva T, Mercadante AC, Kestelman F, Chaves SB, Azevedo RB, Monteiro JP, Bonomo A. 2013. T cells induce pre-metastatic osteolytic disease and help bone metastases establishment in a mouse model of metastatic breast cancer. *PLoS One* **8**: e68171. doi:10.1371/journal.pone.0068171
- Murgai M, Ju W, Eason M, Kline J, Beury DW, Kaczanowska S, Miettinen MM, Kruhlak M, Lei H, Shern JF, et al. 2017. KLF4-dependent perivascular cell plasticity mediates pre-metastatic niche formation and metastasis. *Nat Med* **23**: 1176–1190. doi:10.1038/nm.4400
- Ng J, Dai T. 2016. Radiation therapy and the abscopal effect: a concept comes of age. *Ann Transl Med* **4**: 118. doi:10.21037/atm.2016.01.32
- Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R, Zillhardt MR, Romero IL, Carey MS, Mills GB, Hotamisligil GS, et al. 2011. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat Med* **17**: 1498–1503. doi:10.1038/nm.2492
- Niu F, Liao K, Hu G, Sil S, Callen S, Guo ML, Yang L, Buch S. 2019. Cocaine-induced release of CXCL10 from pericytes regulates monocyte transmigration into the CNS. *J Cell Biol* **218**: 700–721. doi:10.1083/jcb.201712011
- Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA. 2005. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **121**: 335–348. doi:10.1016/j.cell.2005.02.034
- O'Sullivan GC, Corbett AR, Shanahan F, Collins JK. 1996. Regional immunosuppression in esophageal squamous cancer: evidence from functional studies with matched lymph nodes. *J Immunol* **157**: 4717–4720.
- Pauw M, Schoonderwoerd MJA, Helderma R, Harryvan TJ, Groenewoud A, van Pelt GW, Bor R, Hemmer DM, Versteeg HH, Snaar-Jagalska BE, et al. 2018. Endoglin expression on cancer-associated fibroblasts regulates invasion and stimulates colorectal cancer metastasis. *Clin Cancer Res* **24**: 6331–6344. doi:10.1158/1078-0432.CCR-18-0329
- Paget S. 1889. The distribution of secondary growths in cancer of the breast. *Lancet* **133**: 571–573. doi:10.1016/S0140-6736(00)49915-0
- Peinado H, Zhang H, Matei IR, Costa-Silva B, Hoshino A, Rodrigues G, Psaila B, Kaplan RN, Bromberg JF, Kang Y, et al. 2017. Pre-metastatic niches: organ-specific homes for metastases. *Nat Rev Cancer* **17**: 302–317. doi:10.1038/nrc.2017.6
- Podsypanina K, Du YC, Jechlinger M, Beverly LJ, Hambardzumyan D, Varmus H. 2008. Seeding and propagation of untransformed mouse mammary cells in the lung. *Science* **321**: 1841–1844. doi:10.1126/science.1161621
- Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, Kaiser EA, Snyder LA, Pollard JW. 2011. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature* **475**: 222–225. doi:10.1038/nature10138
- Rahmann EP, Gilbertson RJ. 2018. Multiomic medulloblastomas. *Cancer Cell* **34**: 351–353. doi:10.1016/j.ccell.2018.08.010
- Reid GH, Gibbins JR. 1979. Fate of cultured cells after injection into the circulation of syngeneic animals. *Cancer Res* **39**: 4724–4731.
- Ren D, Dai Y, Yang Q, Zhang X, Guo W, Ye L, Huang S, Chen X, Lai Y, Du H, et al. 2019. Wnt5a induces and maintains prostate cancer cells dormancy in bone. *J Exp Med* **216**: 428–449.
- Robertson TB, Burnett TC. 1913. The influence of lecithin and cholesterol upon the growth of tumors. *J Exp Med* **17**: 344–352. doi:10.1084/jem.17.3.344
- Robinson BD, Sica GL, Liu YF, Rohan TE, Gertler FB, Condeelis JS, Jones JG. 2009. Tumor microenvironment of metastasis in human breast carcinoma: a potential prognostic marker linked to hematogenous dissemination. *Clin Cancer Res* **15**: 2433–2441. doi:10.1158/1078-0432.CCR-08-2179
- Sceneay J, Chow MT, Chen A, Halse HM, Wong CS, Andrews DM, Sloan EK, Parker BS, Bowtell DD, Smyth MJ, et al. 2012. Primary tumor hypoxia recruits CD11b⁺/Ly6C^{med}/Ly6G⁺ immune suppressor cells and compromises NK cell cytotoxicity in the premetastatic niche. *Cancer Res* **72**: 3906–3911. doi:10.1158/0008-5472.CAN-11-3873
- Schreiber H. 2019. Fibroblasts: dangerous travel companions. *J Exp Med* **216**: 479–481. doi:10.1084/jem.20181850
- Shimotsuma M, Shields JW, Simpson-Morgan MW, Sakuyama A, Shirasu M, Hagiwara A, Takahashi T. 1993. Morpho-physiological function and role of omental milky spots as omentum-associated lymphoid tissue (OALT) in the peritoneal cavity. *Lymphology* **26**: 90–101.
- Shiozawa Y, Pedersen EA, Havens AM, Jung Y, Mishra A, Joseph J, Kim JK, Patel LR, Ying C, Ziegler AM, et al. 2011. Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. *J Clin Invest* **121**: 1298–1312. doi:10.1172/JCI43414
- Starzinski-Powitz A, Handrow-Metzmaier H, Kotzian S. 1999. The putative role of cell adhesion molecules in endometriosis: can we learn from tumour metastasis? *Mol Med Today* **5**: 304–309. doi:10.1016/S1357-4310(99)01497-5
- Suzuki K, Murakami T, Hu Z, Tamura H, Kuwahara-Arai K, Iba T, Nagaoka I. 2016. Human host defense cathelicidin peptide LL-37 enhances the lipopolysaccharide uptake by liver sinusoidal endothelial cells without cell activation. *J Immunol* **196**: 1338–1347. doi:10.4049/jimmunol.1403203
- Tamura T, Kurishima K, Nakazawa K, Kagohashi K, Ishikawa H, Satoh H, Hizawa N. 2015. Specific organ metastases and survival in metastatic non-small-cell lung cancer. *Mol Clin Oncol* **3**: 217–221. doi:10.3892/mco.2014.410
- Tarakhovsky A, Prinjha RK. 2018. Drawing on disorder: how viruses use histone mimicry to their advantage. *J Exp Med* **215**: 1777–1787.
- Taranova AG, Maldonado D, Vachon CM, Jacobsen EA, Abdala-Valencia H, McGarry MP, Ochkur SI, Protheroe

- CA, Doyle A, Grant CS, et al. 2008. Allergic pulmonary inflammation promotes the recruitment of circulating tumor cells to the lung. *Cancer Res* **68**: 8582–8589. doi:10.1158/0008-5472.CAN-08-1673
- Tcyganov E, Mastio J, Chen E, Gabrilovich DI. 2018. Plasticity of myeloid-derived suppressor cells in cancer. *Curr Opin Immunol* **51**: 76–82. doi:10.1016/j.coi.2018.03.009
- Tomita T, Sakurai Y, Ishibashi S, Maru Y. 2011. Imbalance of Clara cell-mediated homeostatic inflammation is involved in lung metastasis. *Oncogene* **30**: 3429–3439. doi:10.1038/onc.2011.53
- Wallerius M, Wallmann T, Bartish M, Ostling J, Mezheyski A, Tobin NP, Nygren E, Pangigadde P, Pellegrini P, Squadrito ML, et al. 2016. Guidance molecule SEMA3A restricts tumor growth by differentially regulating the proliferation of tumor-associated macrophages. *Cancer Res* **76**: 3166–3178. doi:10.1158/0008-5472.CAN-15-2596
- Yamazaki T, Masuda J, Omori T, Usui R, Akiyama H, Maru Y. 2009. EphA1 interacts with integrin-linked kinase and regulates cell morphology and motility. *J Cell Sci* **122**: 243–255. doi:10.1242/jcs.036467
- Yan HH, Pickup M, Pang Y, Gorska AE, Li Z, Chytil A, Geng Y, Gray JW, Moses HL, Yang L. 2010. Gr-1+CD11b+ myeloid cells tip the balance of immune protection to tumor promotion in the premetastatic lung. *Cancer Res* **70**: 6139–6149. doi:10.1158/0008-5472.CAN-10-0706
- Yan L, Cai Q, Xu Y. 2013. The ubiquitin-CXCR4 axis plays an important role in acute lung infection-enhanced lung tumor metastasis. *Clin Cancer Res* **19**: 4706–4716. doi:10.1158/1078-0432.CCR-13-0011
- Yang D, Chen Q, Schmidt AP, Anderson GM, Wang JM, Wooters J, Oppenheim JJ, Chertov O. 2000. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPR1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *J Exp Med* **192**: 1069–1074. doi:10.1084/jem.192.7.1069
- Zacharakis N, Chinnasamy H, Black M, Xu H, Lu YC, Zheng Z, Pasetto A, Langhan M, Shelton T, Prickett T, et al. 2018. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. *Nat Med* **24**: 724–730. doi:10.1038/s41591-018-0040-8
- Zhang Y, Guan L, Yu J, Zhao Z, Mao L, Li S, Zhao J. 2016. Pulmonary endothelial activation caused by extracellular histones contributes to neutrophil activation in acute respiratory distress syndrome. *Respir Res* **17**: 155. doi:10.1186/s12931-016-0472-y
- Zhou W, Fong MY, Min Y, Somlo G, Liu L, Palomares MR, Yu Y, Chow A, O'Connor ST, Chin AR, et al. 2014. Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. *Cancer Cell* **25**: 501–515. doi:10.1016/j.ccr.2014.03.007

Cold Spring Harbor Perspectives in Medicine

Premetastasis

Yoshiro Maru

Cold Spring Harb Perspect Med 2020; doi: 10.1101/cshperspect.a036897 originally published online October 15, 2019

Subject Collection [Metastasis: Mechanism to Therapy](#)

Cellular Plasticity during Metastasis: New Insights Provided by Intravital Microscopy

Andreia S. Margarido, Laura Bornes, Claire Vennin, et al.

The Etiology and Impact of Muscle Wasting in Metastatic Cancer

Anup K. Biswas and Swarnali Acharyya

Normal Aging and Its Role in Cancer Metastasis

Mitchell Fane and Ashani T. Weeraratna

Zebrafish In Vivo Models of Cancer and Metastasis

Katy R. Astell and Dirk Sieger

Extracellular Vesicles and Metastasis

Shizhen Emily Wang

The Different Facets of Liquid Biopsy: A Kaleidoscopic View

Zahra Eslami-S, Luis Enrique Cortés-Hernández, Laure Cayrefourcq, et al.

Myeloid Cells in Metastasis

Agnieszka Swierczak and Jeffrey W. Pollard

Cancer Cell Dormancy in Metastasis

Matthew A. Summers, Michelle M. McDonald and Peter I. Croucher

Premetastasis

Yoshiro Maru

Bone Tropism in Cancer Metastases

Hai Wang, Weijie Zhang, Igor Bado, et al.

Impact of Immunometabolism on Cancer Metastasis: A Focus on T Cells and Macrophages

Nina C. Flerin, Sotiria Pinioti, Alessio Menga, et al.

New Approaches on Cancer Immunotherapy

Jong-Ho Cha, Li-Chuan Chan, Min Sup Song, et al.

Cancer Stem Cells and Epithelial-to-Mesenchymal Transition in Cancer Metastasis

Toni Celià-Terrassa and Mohit Kumar Jolly

Clinical Perspectives in Brain Metastasis

Krutika Deshpande, Ian Buchanan, Vahan Martirosian, et al.

Brain Metastasis Organotropism

Arseniy E. Yuzhalin and Dihua Yu

The Immune Microenvironment and Cancer Metastasis

Asmaa El-Kenawi, Kay Hänggi and Brian Ruffell

For additional articles in this collection, see <http://perspectivesinmedicine.cshlp.org/cgi/collection/>