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Updates on the hematologic tumor microenvironment and its therapeutic targeting

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ABSTRACT

In this review article, we present recent updates on the hematologic tumor microenvironment following the 3rd Scientific Workshop on the Haematological Tumour Microenvironment and its Therapeutic Targeting organized by the European School of Hematology, which took place at the Francis Crick Institute in London in February 2019. This review article is focused on recent scientific advances highlighted in the invited presentations at the meeting, which encompassed the normal and malignant niches supporting hematopoietic stem cells and their progeny. Given the precise focus, it does not discuss other relevant contributions in this field, which have been the scope of other recent reviews. The content covers basic research and possible clinical applications with the major therapeutic angle of utilizing basic knowledge to devise new strategies to target the tumor microenvironment in hematologic cancers. The review is structured in the following sections: (i) regulation of normal hematopoietic stem cell niches during development, adulthood and aging; (ii) metabolic adaptation and reprogramming in the tumor microenvironment; (iii) the key role of inflammation in reshaping the normal microenvironment and driving hematopoietic stem cell proliferation; (iv) current understanding of the tumor microenvironment in different malignancies, such as chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia and myelodysplastic syndromes; and (v) the effects of therapies on the microenvironment and some opportunities to target the niche directly in order to improve current treatments.

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The normal niches in development, adulthood and aging

A maladapted vascular niche induces the generation and expansion of tumor-initiating cells

Work from Dr. Rafii's laboratory, among others, has revealed the heterogeneity of endothelial cells, which comprise over 140 different types of endothelium in the human body. Each organ or tumor is vascularized by a specialized endothelium. It is believed that transcription factors belonging to the Ets family, such as Ets variant 2 (ETV2), Fli1 and the Ets-related gene (Erg), make endothelial cells organ-specific. Endothelial cells are important niche cells for hematopoietic stem cells (HSC) and their use as feeder cells in culture allows the expansion of HSC by ~150-fold.[1] As a refinement, a combination of reprogramming factors, including FBJ murine osteosarcoma viral oncogene homolog B (FOSB), growth factor independent 1 transcriptional repressor (GFI1), runt-related transcription factor 1 (RUNX1) and SPI1 (which encodes PU.1), can be combined with sustained vascular niche induction to generate HSC that are endowed with secondary repopulating activity.

However, a maladapted vascular niche can facilitate the expansion of tumor-initi-

ating cells in different organs. A paradigm-shifting concept over the past few years is that blood vessels not only deliver nutrients and oxygen to organs and tissues, but that they also sustain stem cells and cancer cells through an 'angiocrine' mechanism. Consequently, maladapted tumor-associated vascular endothelial cells may confer stem cell-like activity to indolent tumor cells. One example of this is the conversion of dormant lymphoma cells into aggressive lymphoma through the interaction with endothelial cells. This effect is dependent on Notch signaling, since Jagged1 abrogation in endothelial cells can slow down lymphoma progression.[2] Another example is the abnormal activation of the fibroblast growth factor receptor 1 (FGFR1)-ETS2 pathway in tumor-associated-vascular endothelial cells during chemotherapy. Specifically, tumor-derived FGF4 activates FGFR1 in endothelial cells and induces the expression of the transcription factor ETS2. Chemotherapy inhibits the tumor-suppressive checkpoint function of insulin growth factor binding protein 7 (IGFBP7)/angiomodulin and increases the expression of insulin growth factor 1 (IGF1) in endothelial cells, causing an FGFR1-ETS2 feedforward loop which renders naïve IGFR1$^+$ cancer cells resistant to chemotherapy.[3] This research helped to show that the FGF4-FGFR1-ETS2 pathway plays a crucial role in tumor-associated endothelium.

Angiocrine signals regulate quiescence and therapy resistance in bone

Kusumbe and colleagues characterized different vessel subtypes comprising endothelial and sub-endothelial/perivascular cells in murine bone marrow. Type H endothelium (named so because of its high expression of endomucin) nurtures bone-forming cells during development.[4] However, alterations of the vascular microenvironment can affect the fate of disseminated tumor cells.[5] Dormant tumor cells can be awakened through the production of factors such as periostin (POSTN) and transforming growth factor β-1 (TGFβ-1). Importantly, proximity to the sprouting vasculature supports cancer cell proliferation, whereas a stable vasculature keeps cancer cells dormant. In relation to this, vascular remodeling during aging might alter hematopoiesis. For instance, type H endothelium and its associated osteoprogenitor cells are reduced during aging, possibly affecting hematopoiesis. Consistent with these results, reactivation of endothelial Notch signaling can activate HSC in aged mice, although it cannot fully restore HSC self-renewal.[6] Age-associated vascular remodeling might facilitate the development of myeloid malignancies since it promotes myeloid cell expansion.[7]

The hematopoietic stem cell niche in aging

In this regard, Geiger *et al.* uncovered several microenvironmental contributions to HSC aging. It had been previously reported that aged stromal cells secrete more pro-inflammatory CC-chemokine ligand 5 (CCL5 or RANTES) but less osteopontin (OPN); these stromal changes imprint some aging-associated phenotypes in HSC.[8] Specifically, a decreased frequency of endosteal stromal cells and osteoblasts reduces OPN expression, which is associated with HSC aging (manifested as myeloid skewing). The bone marrow microenvironment of adult OPN knockout mice partly resembles an aged wildtype microenvironment in its increased number of HSC which exhibit reduced engraftment and polarity. However, treatment with OPN fraction D can attenuate the dysfunction of aged long-term HSC (LT-HSC) and ameliorates HSC by activating integrin $\alpha_9\beta_1$ in HSC.[9] Additionally, aged endothelial cells drive hematopoietic aging phenotypes in young HSC, whereas infusion of young endothelial cells enhances endogenous HSC activity in aged mice.[10]

Metabolism in the tumor microenvironment

Intense efforts are currently being expended to elucidate how cancer cells reshape their malignant microenvironment to increase their metabolic fitness and chemoresistance.

Subversion of systemic glucose metabolism as a mechanism to support the growth of leukemic cells

Work by Dr. Ye and colleagues in Dr. Jordan's laboratory has revealed how leukemic cells subvert the metabolism of systemic glucose for their proliferation. Insulin resistance, besides playing a key role in obesity and diabetes, may facilitate leukemogenesis: leukemic cells can actively reduce glucose utilization by normal tissues to increase their glucose bioavailability.[11] Collectively, the findings suggest that leukemic cells increase IGFBP1 production from adipose tissue, which can cause insulin resistance. An intricate communication with the gut causes loss of active glucagon-like peptide-1 (GLP1) and serotonin, which suppresses insulin secretion. Overall, these systemic perturbations are believed to cause desensitization of normal tissues to glucose, suggesting a novel therapeutic window based on the restoration of normal glucose regulation.

Mitochondrial trafficking in the tumor microenvironment

Mitochondria are emerging components in the molecular exchange between leukemic cells and their microenvironment. The ability of bone marrow mesenchymal stromal cells (BMSC) to donate mitochondria to different cell types[12] has emerged as a potentially important process in hematologic diseases. Mitochondrial transfer has recently been appreciated to be a previously unrecognized mechanism of intercellular communication associated with chemoresistance.[13,14] Tunneling nanotubules appear to be the primary mitochondrial exchange route used in acute myeloid leukemia (AML).[14] Work from Dr. Rushworth's laboratory indicates that NADPH oxidase 2 (NOX2)-derived reactive oxygen species, induced by H_2O_2 or daunorubicin, may enhance mitochondrial transfer from BMSC to AML blasts. The transferred mitochondria appear functionally active and capable of boosting metabolic activity in AML cells.[13] A similar process has been reported in multiple myeloma. Increased oxidative phosphorylation (OXPHOS) in multiple myeloma cells is associated with CD38-driven mitochondrial transfer.[15] It is worth noting that this process seems to affect malignant cells preferentially and is not frequently observed in their normal counterparts. Therefore, a potential therapeutic window might be available through blockade of mitochondrial transfer.

Fatty acid metabolism and bone marrow adipocytes in acute myeloid leukemia

Work from Dr. Tabe's and Dr. Andreeff's laboratories has revealed other metabolic changes in AML, particularly focused on the role of adipocytes and fatty acid metabolism. Fat cells are a predominant type of stromal cell in aged human bone marrow. BMSC can promote AML survival

through a metabolic shift from OXPHOS to fatty acid oxidation, which causes OXPHOS uncoupling.[16] In addition, leukemia stem cells express the fatty acid receptor CD36 and exhibit high levels of fatty acid oxidation, associated with cell quiescence and drug resistance.[17] However, a novel small molecule inhibitor of fatty acid oxidation, avocatin-B, selectively inhibits AML and leukemia stem cells without detectable toxicity in normal HSC. Avocatin-B increases fatty acid uptake and enhances the expression of fatty acid-binding protein-4 (FABP4) in adipocytes co-cultured with AML cells. However, concomitantly, avocatin-B increases glucose uptake and glycolysis in AML, thus contributing to AML survival.[18] Overall, these data highlight the limitations of targeting a single metabolic pathway, since leukemic cells may escape through metabolic adaptation. Accordingly, cytarabine-resistant AML cells exhibit increased fatty acid oxidation and OXPHOS. Fatty acid oxidation inhibition induces an energy shift from high to low OXPHOS that enhances anti-leukemia effects, but only in combination with cytarabine.[19] Inhibition of fatty acid oxidation additionally activates the endoplasmic reticulum stress activator transcription factor 4 (ATF4) and enhances cytarabine cytoxicity in AML cells co-cultured with bone marrow adipocytes.[20] These findings suggest that combined therapies containing inhibitors of fatty acid oxidation could be capable of targeting metabolic vulnerabilities in AML.

Inflammation and cell cycle

One hallmark of hematologic malignancies is a proinflammatory state whereby inflammatory cytokines affect the proliferation of normal and mutant cells. Inflammation is, therefore, one key trigger of the reshaped malignant microenvironment.

Impact of aged marrow macrophages on hematopoietic stem cells and their niche

Microenvironmental inflammation is another driver of hematopoietic aging. Previous studies have shown that aged CD41$^+$ LT-HSC accumulate during aging and their megakaryocyte bias results in increased circulating platelets in aged mice.[21-24] Calvi *et al.* have shown that aged bone marrow macrophages contribute to the expansion of platelet-based HSC through interleukin-1β.[25] Aged murine bone marrow macrophages exhibit an activated phenotype and defective phagocytic function, which causes reduced efferocytosis of senescent neutrophils. *In vitro* co-culture systems suggest that increased interleukin-1β and reduced Axl receptor tyrosine kinase and its associated protein growth arrest-specific 6 (Gas6) contribute to platelet skewing during aging.

Hematopoietic stem cells and their bone marrow niche under inflammatory stress

Inflammation can affect both HSC and their niches. Infection can cause stress and dysfunction in HSC responding to infection. Chemotherapy, transplantation or inflammatory cytokines, such as interferon (IFN)-α, can modify HSC quiescence and make HSC re-enter the cell cycle.[26-29] For example, acute or non-acute virus infections activate quiescent LT-HSC but also affect their function through IFN-I receptor signaling.[29] Non-acute murine cytomegalovirus infections alter the LT-HSC gene expression profile and impair HSC function upon transplantation. One mediator appears to be the extracellular matrix adaptor protein Matrilin-4 (Matn4), which is a candidate negative regulator of HSC proliferation under stress.[29] Under acute stress, Matn4 expression decreases, allowing for HSC expansion to replenish the blood system. Importantly, reduced expression of the Cxcl12/Sdf-1 receptor Cxcr4 in Matn4$^{-/-}$ HSC improves the reconstitution and expansion of HSC. On the non-hematopoietic side, endothelial cells proliferate after inflammatory stress or infection to maintain vessel integrity and permeability. The responses of endothelial cells to IFN-α *in vivo* are transient and dependent on the expression of IFN-α receptors. In this regard, vascular endothelial growth factor (VEGF) has emerged as one mediator of the activation of bone marrow endothelial cells by IFN-α-stimulated hematopoietic cells. In conclusion, as part of the dynamic crosstalk between HSC and their niches, inflammatory stress not only has an impact on HSC but also on their microenvironment and this altered bidirectional crosstalk affects the growth and function in each compartment.

The bone marrow microenvironment in myeloproliferative neoplasms

Associated with inflammation, bone marrow fibrosis is an extensive remodeling of the bone marrow extracellular matrix, which is typically observed in some myeloproliferative neoplasms. Previous studies found that damage to the bone marrow microenvironment contributes to the progression of myeloproliferative neoplasms.[30] However, the identification of fibrosis-driving cells and specific markers of a pre-fibrotic state are important therapeutic issues that remain only partially addressed. Schneider and colleagues described GLI family zinc finger 1 (Gli1)$^+$ mesenchymal stromal cells as fibrotic cells in different types of fibrosis. Gli1$^+$ cells appear to be myofibroblast precursors which contribute significantly to myelofibrosis. Accordingly, genetic ablation of Gli1$^+$ cells reduces fibrosis and improves hematopoiesis in experimental models.[31]

Regulation of dormant hematopoietic stem cells

Inflammation is only one of the mechanisms that can awaken dormant HSC, as shown in studies by Cabezas-Wallscheid and colleagues. Dormant HSC can now be identified with specific markers, such as Lineage$^-$Sca-1$^+$c-Kit$^+$ (LSK) CD150$^+$CD48$^-$CD135$^-$CD34$^-$ cells expressing the G protein-coupled receptor Gprc5c5.[32] Dormant HSC represent only a very small subset of bone marrow cells, but these cells harbor the highest long-term reconstituting potential. Dormant HSC are characterized by low levels of biosynthetic processes (transcription, mRNA processing and translation) which gradually increase as the HSC become activated. Retinoic acid/vitamin A-induced signaling is highly enriched in dormant HSC and contributes to maintain low levels of reactive oxygen species, protein translation and expression of the proto-oncogene c-myc in these cells. *In vivo*, pre-treatment with all-*trans* retinoic acid can preserve HSC quiescence upon stress induced by chemotherapy or lipopolysaccharide. These results suggest that retinoic acid might restrict HSC proliferation. In contrast, lack of vitamin A compromises HSC re-entry into dormancy after exposure to inflammatory stress.[32]

Molecular regulation and heterogeneity in the exit from quiescence by human hematopoietic stem cells

Not only the actual quiescence of HSC, but also the time that that these cells take to enter the cell cycle can be a

defining feature, as revealed by Laurenti and colleagues. In fact, LT-HSC take longer than short-term HSC to enter the cell cycle. Cyclin dependent kinase-6 (CDK6) expression controls the exit from quiescence in human HSC.[33] Consequently, enforced CDK6 expression can push LT-HSC to divide as quickly as short-term HSC. Human HSC heterogeneity and lineage commitment were dissected further in a subsequent study. The first lineage restriction appears to affect the $CD19^{-}CD34^{+}CD38^{-}CD45RA^{-}CD49f^{+}CD90^{+}$ HSC compartment's generation of myelo-lymphoid committed cells which are devoid of erythroid differentiation capacity. The expression of the C-type lectin domain family 9 member A (CLEC9A) and CD34 in these cells can be used to distinguish $CLEC9A^{hi}CD34^{lo}$ LT-HSC (with slow exit from quiescence) from $CLEC9A^{lo}$ $CD34^{hi}$ myelo-lymphoid-restricted HSC (with quicker entry into the cell cycle).[34] These results help identify human HSC subsets and will be very useful to study their interactions with bone marrow microenvironments.

Interaction of tumor cells with their microenvironment

Mapping the bone marrow microenvironment in sickness and in health

The development of single-cell technologies has made it possible to generate an atlas of different tissues at single-cell resolution. A recent study by Dr. Aifantis' group presented the transcriptional signatures of murine bone marrow vascular endothelial cells, perivascular cells and osteolineage/stromal cell populations under steady state or under stress (5-fluorocuracil), with a major emphasis on candidate cellular sources of key factors regulating hematopoiesis.[35] For example, the loss of the delta-like canonical Notch ligand 4 (DLL4) in endothelial cells caused profound transcriptional changes, which drove myeloid skewing of HSC/progenitors.

Targeting the microenvironment in smoldering myeloma

Like other hematologic malignancies, multiple myeloma involves a multistep transformation process[36] with concomitant remodeling of the BM microenvironment,[37] as shown by Ghobrial *et al.* However, studies on the human bone marrow microenvironment are frequently challenged by the scarcity and insufficient preservation of tissue biopsies for detailed studies. A potential way to replace bone marrow biopsies might be to combine whole-exome sequencing of circulating tumor cells and cell-free DNA, which might help our understanding of disease heterogeneity and evolution in multiple myeloma.[38] Cell-free DNA reveals a similar clonal structure as bone marrow biopsies,[39] potentially paving the path for less invasive mutational screening.

Investigating mechanisms regulating myeloma growth and dissemination using *in vivo* bone marrow imaging

Studies by Dr. Fooksman and others have showed the potential of intravital microscopy for studying the interactions of normal and mutant hematopoietic cells with their microenvironment. Antibody-secreting cells comprise mature plasma cells and more immature plasmablasts which can be identified by the expression of syndecan-1 (CD138), a marker with an unclear function until recently. CD138 has lately been found to promote the survival of antibody-secreting cells through IL-6 and A proliferation-inducing ligand (APRIL).[40] Therefore, ongoing studies are utilizing similar intravital imaging techniques to study the microenvironment in multiple myeloma and other hematologic malignancies.

The tumor microenvironment in chronic lymphocytic leukemia, plasma cell myeloma and myelodysplastic syndromes

Understanding and targeting tumor-microenvironment interactions in B-cell malignancies

Microenvironmental alterations can be putative therapeutic targets in B-cell malignancies, as revealed by Ringshausen *et al.* The expression of protein kinase C beta II (PKCβ2) and downstream activation of NF-kappa B (NFκB) in BMSC is required for the survival of malignant B cells.[41] Chronic lymphocytic leukemia (CLL) cells induce Notch2 signaling and complement C1q production by BMSC, which in turn inhibits glycogen synthase kinase 3 beta (GSK3β)-dependent degradation of β-catenin in CLL. Additionally, Notch2 activation in BMSC further stabilizes β-catenin in CLL through regulation of N-cadherin expression. Consequently, inhibition of Notch or Wnt pathways has therapeutic effects in experimental CLL models.[42]

The biological and clinical roles of the microenvironment in chronic lymphocytic leukemia

Work in Dr. Hallek's laboratory and others has illustrated how CLL becomes addicted to the microenvironment, and particularly to macrophages or nurse-like cells. A prominent example is the non-receptor tyrosine-protein kinase Lyn belonging to the SRC family, which is crucial both for B-cell receptor signaling and for microenvironmental support of the malignant cells.[43] Lyn-deficient mice present a reduced CLL burden. However, the loss of Lyn in B cells only reduces B-cell receptor signaling, but does not affect CLL progression. In fact, Lyn is required in microenvironmental cells (and particularly macrophages) for the expansion of CLL cells.

Pre-clinical modeling of myelodysplastic syndromes in murine xenograft models

The clinical heterogeneity and molecular complexity of myelodysplastic syndromes (MDS) make these diseases arduous to model and study. However, xenograft models have emerged as useful tools for studying MDS. Co-transplantation of $CD34^{+}$ cells with patient-derived BMSC has been reported in one study to increase long-term engraftment of human MDS in immunodeficient mice.[44] In that study, patient-derived hematopoietic cells prompted healthy BMSC to acquire MDS-BMSC-like features. Consequently, cytokines produced by MDS BMSC favored the propagation of MDS after orthotopic interfemoral transplantation into immunodeficient mice. However, this finding contrasts with that of another study which found similar engraftment of MDS regardless of the presence of human BMSC.[45] It is possible that technical differences and/or distinct diseases/stages underlie these divergent results. Moreover, due to recent advances in bioengineering and carrier materials, traditional xenotransplants are being progressively replaced by bioengineered humanized microenvironments. As one example, implantable scaffold

methods allow the study of multicellular interactions between human stromal cells and HSC.[46]

Targeting the tumor microenvironment

Targeting the tumor microenvironment in B-cell lymphomas

Other ways to target the tumor microenvironment in B-cell malignancies have been exemplified by research in Dr. Gribben's laboratory and take advantage of the fact that lymphoma cells live in an immune cell-enriched microenvironment. However, immune cells do not function normally because CLL or lymphoma cells reduce the F-actin immune synapse formation in tumor-infiltrating T cells. Nonetheless, impaired T-cell function can be therapeutically reverted by the immunomodulatory drug lenalidomide.[47] which has recently been approved for the treatment of lymphoma. In a subsequent study, the inhibitory B7-related molecules CD200, CD274 (PD-L1), CD276 (B7-H3) and VD270 (HVEM) were identified as key mediators of the T-cell synapse defect.[48] Consequently, the PD1-PDL1 axis has emerged as a highly promising target in CLL and lymphoma.[49,50] These results have been extrapolated to solid tumors, in which PD1 expression has become both a biomarker and a therapeutic target.

Engineering T cells to overcome the immunosuppressive tumor microenvironment

Engineered T cells can be used to overcome the immunosuppressive tumor microenvironment. Generating tumor-specific lymphocytes has proven challenging given that many tumors are not very immunogenic. A revolutionary approach in immunotherapy is to combine the variable regions of antibodies (which recognize epitopes shared by tumors) with the constant regions of the T-cell receptor to generate chimeric antigen receptor (CAR) T cells.[51] This approach has been improved recently by adding co-stimulatory domains. CD19-specific CAR-T cells have provided impressive results in acute lymphoblastic leukemia, with reported cure of chemorefractory disease. Some lessons learned from these studies are: (i) chemotherapy is essential; (ii) it is critical to include a co-stimulatory domain; (iii) targeting a single antigen may enable immune escape; and (iv) significant toxicities (neural, cytokine storm) should be avoided in the future by improving the specificity and efficacy of the approach (to reduce the number of CAR-T cells infused). However, despite the impressive positive results of CD19-specific CAR-T cells in acute lymphoblastic leukemia, AML has proven more difficult to treat. In this regard, integrated transcriptomics and proteomics have not identified single candidate targets in AML, although combinatorial strategies have been proposed.[52,53]

Targeting altered metabolism in the leukemia microenvironment

It is now clear that the bone marrow microenvironment rewires energy metabolism in AML; however, targeting metabolic vulnerabilities in AML has proven challenging given the high degree of metabolic adaptation in AML cells. One key driver of metabolic reprograming in the leukemic bone marrow microenvironment is hypoxia. Most tumors are typically hypoxic, as cancer cells avidly consume oxygen and blood vessels become progressively compressed or obstructed by the growing tumor mass. Cancerous tissue in both solid and liquid tumors develops chronic hypoxia, which is associated with resistance to therapy and immune suppression.[54] However, the role of (low) oxygen in the progression and chemoresistance of leukemia remains controversial. Recently, a hypoxia-activated prodrug (TH-302) was tested in models of AML *in vivo*.[55] TH-302 is able to eliminate cancer cells residing in hypoxic microenvironments. Hypoxic niches were increased in a syngeneic AML murine model, but AML cells surviving chemotherapy could be targeted by TH-302, which improved mouse survival. On the other hand, metabolic reprogramming was previously reported to become more dependent on glycolysis. However, recent findings have challenged this view by showing that many tumors rely primarily on OXPHOS. Although targeting OXPHOS clinically presents some obstacles, drugs such as IACS-010759, a highly effective and selective small-molecule inhibitor of complex I of the mitochondrial electron transport chain, can reduce tumor burden in experimental models of brain cancer and AML.[56]

Interferon in myeloproliferative neoplasms

Connected with the effects of IFN-α on HSC and their microenvironment described above, studies by Dr. Kiladjian and others have shown that IFN-α is one of very few drugs capable of reducing the mutant allele burden in myeloproliferative neoplasms. Ropeginterferon triggered a dose-dependent anti-proliferative effect in *JAK2*V617F-mutated cell lines, whereas it did not affect the differentiation of normal CD34$^+$ cells.[57] One possibility might be to combine IFN with JAK inhibition, since the latter does not seem to modify the allele burden, but does dampen inflammation. IFN has been shown to induce molecular histopathological responses in myelofibrosis but it also induces immune and inflammatory toxicity. Ruxolitinib may offset IFN toxicity and the combination of these two drugs might enhance the molecular and histopathological response rate. However, it is possible that the anti-JAK1 activity of ruxolitinib might antagonize IFN signaling. These issues remain to be investigated in future studies.

CXCR4 inhibitors

The CXCL12-CXCR4 axis regulates bone marrow homing, retention, proliferation and egress of HSC and also affects the traffic of leukocytes. In particular, CXCR4 expression in HSC is necessary to keep these cells in the CXCL12-enriched bone marrow microenvironment. Dr. Peled and others have shown that efficient blockade of CXCR4 mobilizes HSC from the bone marrow into the circulation. Plerixafor (AMD3100) is a first-generation CXCR4 antagonist which has low affinity for the receptor. It is approved for HSC mobilization (but only in combination with granulocyte colony-stimulating factor) for the treatment of multiple myeloma and non-Hodgkin lymphoma. The new-generation CXCR4 inhibitor BL8040 binds CXCR4 with higher affinity (1-2 nM) than AMD3100 (84 nM).[58] In addition, whereas AMD3100 rapidly dissociates from CXCR4, BL8040 behaves as an inverse agonist and has a slow off-rate, causing more sustained CXCR4 inhibition. CXCR4 directly or indirectly stimulates tumor growth and regulates stromal cell adhesion-mediated drug resistance to chemotherapy. BL8040 can induce the mobilization of AML cells into the circulation and promote AML differentiation and apoptosis. A synergistic effect can be observed in combination with FLT3 or BCL-2 inhibitors,[59] suggesting that combination therapies could be useful in AML.

New insights into early-stage bone colonization of disseminated cancer cells

Finally, solid cancers can metastasize into bone after hijacking the normal bone marrow microenvironment, as illustrated by the work of Dr Zhang and others. Upon colonization of the bone marrow, cancer cells dysregulate bone formation and degradation cycles and stimulate the release of factors that promote tumor growth in a vicious cycle. Ell & Kang stated that: "TGFβ, insulin-like growth factor (IGF), and calcium are released from the bone matrix during lysis, enhancing tumor proliferation and survival. TGFβ signaling in tumor cells enhances expression of bone metastasis proteins including parathormone-related protein (PTHrP), the Notch ligand Jagged1, connective tissue growth factor (CTGF), IL-11, and matrix metalloproteinases. Calcium signaling through the calcium-sensing receptor leads to increased proliferation and survival. Osteoblasts also secrete a number of proteins that positively regulate tumor growth, including IL-6, secreted protein acidic and cysteine rich (SPARC) and periostin. SPARC induces cancer migration and homing through the $\alpha_v\beta_5$ integrin, whereas periostin and IL-6 promote tumor survival".[60]

Whereas many molecules driving metastatic growth have been identified, there is an important lack of knowledge regarding mechanisms allowing cancer cell colonization and maintenance before expansion. The microenvironment of early-stage bone lesions appears to be primarily an osteogenic niche composed of alkaline phosphatase $(ALP)^+$ collagen-I $(ColI)^+$ cells which define a preosteolytic stage (osteoclasts are not yet predominant at this early stage). Cancer and osteogenic niche cells generate heterotypic adherent junctions formed by E-cadherin and N-cadherin. E-cadherin blockade can abolish spontaneous bone metastases in a manner dependent on mammalian target of rapamycin (mTOR) and p70.[61] Moreover, cancer cells and niche cells are connected by gap junctions formed by connexin (Cx)43, which is induced in cancer cells after bone marrow colonization. Cx43 allows for calcium transfer to cancer cells to drive mTOR-dependent metastatic growth. This pathway can be inhibited by danusertib or a combination of everolimus and arsenic trioxide.[62] This research illustrates how solid tumors may hijack normal bone marrow niches to drive metastatic growth.

Summary

Increasingly, the tumor microenvironment is the focus of studies addressing survival, growth and chemoresistance of solid tumors and hematologic malignancies since it plays critical roles in disease initiation, maintenance and relapse. A key challenge is the dual role of the microenvironment in regulating normal and malignant hematopoiesis, since inhibiting the development and maintenance of malignancies must be followed by the reestablishment of normal tissue function. Therapies targeting the tumor microenvironment (which not only comprises the immune system, but also the stromal and endothelial cells that interact with the malignant cells and the immune cells) must simultaneously eliminate chemoresistant cells and preserve/reestablish normal hematopoiesis. Multidisciplinary meetings uniting basic and clinical researchers concerned about the tumor microenvironment have proven a unique opportunity for cross-fertilization of scientific knowledge, ideas and approaches to identify key vulnerabilities of the malignant niches.

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