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Cancer coopts differentiation of B-cell precursors into macrophages

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ABSTRACT

We recently reported that some cancers induce accumulation of bone marrow (BM) B-cell precursors in the spleen to convert them into metastasis-promoting, immunosuppressive B cells. Here, using various murine tumor models and samples from humans with breast and ovarian cancers, we provide evidence that cancer cells also coopt differentiation of the extra nodal B-cell precursors to generate macrophages (termed B-MF). We link the trans-differentiation to a small subset of CSF1R⁺ Pax5^{Low} cells within BM pre-B and immature B cells and cancer-secreted M-CSF that downregulates Pax5 via CSF1R signaling. Thus, cancer generates tumor-associated macrophages (TAM) from B-cell precursors in addition to their primary source, monocytes. Based on their differences from monocyte-derived TAM, such as a superior ability to induce FoxP3⁺ Tregs, suppress proliferation of T cells and more efficiently phagocytize apoptotic cells, we propose that cancer generates B-MF to mediate cancer escape.

The role of B cells in cancer remains poorly understood despite reports of both positive and negative associations of B cells with the disease outcome. Even in the same murine model, T2-MZ B cells and aging-associated B cells can either increase or mitigate B16-F10 melanoma growth in C57BL/6 mice, respectively (1, 2). B cells can also inhibit or enhance metastasis in BALB/c mice with orthotopic 4T1 breast cancer cells (3, 4). At least some cancer-promoting functions of B cells are attributed to their immunosuppressive, regulatory subsets termed Bregs, whose generation and function appear to be actively controlled by cancer itself. To enforce this regulation, cancer cells secrete various factors that affect survival and differentiation of B cells, including B lymphocyte stimulator (BLyS/BAFF), thymic stromal lymphopoietin (TSLP), colony stimulating factors (CSF such as M-CSF, GM-CSF and G-CSF) and lipid mediators such as 5-lipoxygenase (5-LO) metabolites (5-10). In BALB/c mice with orthotopic 4T1 breast cancer, a model for human triple negative breast cancer (11), our group linked 5-LO metabolite-induced activation of PPAR α to the conversion of B cells into TGF β ⁺CD25⁺ Bregs (tBregs) (7). These converted cells then promote metastasis acting as potent suppressors of antitumor T cell activity and inducers of FoxP3⁺ Tregs and MDSCs via the TGF β /TGF β RII axis (5, 12). Breast and ovarian cancer cells secrete TSLP both to prepare the metastasis “soil” in the lungs and to accumulate BM B-cell precursors in the spleen for conversion to tBregs. In the lungs, TSLP enables infiltration of CCR4⁺ cancer cells and their protector CCR4⁺FoxP3⁺ Tregs by upregulating expression of CCL17 (6, 13), while in the BM, TSLP downregulates expression of CXCR4 and α 4 β 1 in B-cell precursors, causing their premature emigration and accumulation in the spleen to be converted into TGF β ⁺CD25⁺ tBregs (10). M-CSF and GM-CSF from cancer cells promote differentiation

and survival of cancer-promoting myeloid suppressive cells and tumor-associated macrophages (TAM) from BM monocytes (8, 9). Although bifurcation of myeloid and lymphoid lineage from multipotent progenitors occurs before specialization of B-cell progenitors in BM, surprisingly some B-cell precursors appear to retain macrophage differentiation potential. For example, the differentiation of BM B-cell precursors can be diverted towards macrophages upon forced expression or deletion of a single transcription factor (14, 15). Anecdotal reports indicate that this transdifferentiation can also occur in BM of naïve mice, which is linked to a small subset of biphenotypic ($CD19^+B220^+CD16/32^{++}CD11b^+$) B-cell precursor cells (16). However, the biological consequence of this rare event and whether cancers affect this process remain unknown.

Here, we report that cancers frequently induce the transdifferentiation of the bona fide BM $Csf1R^+Pax5^{Low}$ pre-B and immature B (iB) cells into TAM (termed B-MF). To do this, cancers first drive accumulation of B-cell precursors/iB cells in the spleen and tumors. Unlike “classical” monocyte-derived TAM (9), cancer appears to use B-MF to mediate cancer escape via suppression of antitumor T cells. This does not appear to be a mouse specific phenomenon, as B-MF can be detected in patients with breast and ovarian cancers, and we present evidence of B-MF signatures in published scRNA sequence data of human cancers.

RESULTS

B-cell markers are expressed in TAM. We previously reported that some cancers mobilize BM B-cell precursors in the spleen and tumors (10) to subsequently convert them into $TGF\beta^+$ tBregs (5, 7, 12). Microarray transcription profiling of B-cell precursors and

tBregs from mice with BALB/c mice with orthotopic 4T1.2 breast cancer surprisingly revealed significant upregulation of macrophage-associated genes, such as *CD68*, *Csf1r* (encodes CSF1R), *Cebpb* (CCAAT Enhancer binding protein beta), *Cebpg* (CCAAT Enhancer binding protein gamma), *Ccl2* (CCL2), and *Csf1* (M-CSF) (Fig. S1A). Given that CEBPB and CSF1R play essential roles in defining macrophage fate (14, 17) and anecdotal reports on retention of macrophage differentiation potential in B-cell precursors (16, 18), we wondered whether the upregulation of macrophage genes indicates that cancer hijacks B-cell differentiation, pushing them to macrophages. To test this possibility, we FACS evaluated tumor-infiltrating B cells (TIB, gated on CD19⁺) and TAM (gated on F4/80⁺) in WT BALB/c mice and μ MT BALB/c mice (B-cell differentiation halted at the pro-B-cell stage (19)) bearing orthotopic 4T1.2 breast cancer. While both TIB and TAM in WT mice contained a small but significant proportion of cells co-expressing B-cell and macrophage markers, the double lineage expressing cells were not present in μ MT mice (Fig. 1A, gating strategy is in Fig. S1B). We also evaluated if these cells were present in other tumor models. TIB and TAM co-expressing B-cell and macrophage markers were found in WT BALB/c mice with orthotopic EMT6 breast cancer, and WT C57BL/6 mice with other orthotopic cancers (AT3 breast and MC38 colon cancer) or spontaneous ovarian cancer (Mogp), but were not detected in μ MT or J_HT mice with these cancers (Fig. 1B and Fig. S1C, D). Of note, in J_HT mice, B-cell differentiation is blocked at the pro-B-cell stage (20). A comprehensive FACS evaluation of TAM revealed they do indeed express additional B-cell-specific markers, including Mb1/CD79a, CD20, IgM, and IgD (Fig. S1D-G). To further confirm our finding, we used a B-cell lineage tracer Mb1-EYFP mouse model (21), which expresses enhanced yellow fluorescent protein (EYFP) as a readout for the Ig- α

receptor exclusively expressed in B cells (except plasma cells). Upon challenge with MC38 tumor cells, EYFP⁺ F4/80⁺CD11b^{inter/low} TAM were significantly increased in the tumor (Fig. S2). In sum, cancers cause a marked increase of macrophage-like cells derived from B cells (hereafter referred to as B-MF).

Cancer induces B-cell-to-macrophage differentiation. To explain the accumulation of B-MF, we tested whether cancer cells trans-differentiate them from B cells. Sort-purified Lin⁻CD19⁺B220⁺ B cells (>98% purity) from naïve mouse bone marrow (BM), spleen, lymph nodes (LN) and peritoneal cavity (PeC) were cultured for 7 days in conditioned medium (CM) of 4T1.2 cancer cells (4T1.2-CM). Of note, unless specified, from here on this incubation time was chosen because of a noticeable upregulation of macrophage markers (Fig. S3A). 4T1.2-CM, but not control medium, significantly upregulated F4/80 and CD11b in B cells from BM, but not from spleen, LN or PeC (Fig. 1C). The BM B cells acquired features associated with macrophages, such as adherence to plastic, enlarged vacuolar cytoplasm, and ability to phagocytize fluorochrome-labelled *E. coli* (Fig. 1D and Fig. S3B). Independent experiments with sort-purified B-cell subsets revealed that only BM B-cell precursors and iB cells, but not splenic transitional, follicular (FOB), or marginal zone (MZB) B cells, can become B-MF (Fig. S3C; gating strategy in Fig. S3D). Consistent with the accumulation of B-MF in mice with various tumors, CM from almost every type of cancer cell (except B16-F10 melanoma cells) induced the generation of B-MF from BM B-cell precursors/iB cells (BMBP) and pre-B-cell line 70z/3 (Fig. 1E-G and Fig. S3E). In single cell Imagestream FACS analysis, macrophage and B-cell markers were indeed co-expressed on single B-MF cells derived from BM Mb1-EYFP⁺ B cells (Fig. 1H). To rule out artifacts of *in vitro* experiments, we also performed a 7-day

in vivo conversion assay by i.p. implanting sort-purified BMBP from naïve Mb1-EYFP mice into PeC of C57BL/6 mice with or without ID8 ovarian cancer in PeC. Tumor-bearing mice contained significantly higher frequency of EYFP⁺ B-MF in PeC than naïve mice (Fig. 1I and Fig. S3F). These cells also markedly upregulated TGF β /LAP and PD-L1 (Fig. 1I), the two factors utilized in immunoregulation (22, 23). Taken together, we concluded that cancer uses BMBP to generate TAM in addition to their hitherto known source, monocytes (9).

B-MF and Mo-MF differ functionally. To understand why cancer generates TAM from B cells, we *ex vivo* generated B-MF and monocyte-derived macrophages (Mo-MF) from sort-purified BMBP and monocytes, respectively, by stimulating with 4T1.2-CM, as described above. While both populations adhered to plastic and comparably upregulated F4/80 and CD11b, only B-MF expressed the B-cell-specific markers (CD79a and IgM, Fig. 2A, B and Fig. S4A). mRNA microarray analysis of B-MF and Mo-MF revealed that regardless of origin, the two populations share expression of numerous macrophage-related genes and do not robustly express B-cell signature profiles (Fig. S4B-D and Table S1). Principal component analysis (PCA) and transcription profiling clearly separate B-MF from Mo-MF (Fig. 2C and Table S2). B-MF expressed higher levels of genes involved in fatty acid metabolism, cell cycle, steroid-cholesterol biosynthesis and downregulated expression of pro-inflammatory and IFN γ response genes (Fig. 2D, E). While Mo-MF had more robust M1-like transcription, M2-skewing was more pronounced in B-MF (Fig. 2E and Fig. S4E). The unique transcription profiles were also confirmed in single cell RNA sequencing (scRNA-seq) of *in vitro* generated B-MF (10,563) and Mo-MF (10,235) cells, with UMAP clustering identifying mostly separate cell-clusters of macrophages derived

from either B cells or monocytes (Fig. 2F and Table S3). We distinguished 12 cell clusters with the Leiden algorithm, using shared nearest neighbor (SNN) in PCA space and identified the key genes establishing the six clusters accounting for the majority of single cells (Fig. 2G, H and Fig. S4F). B-MF appeared to be more phagocytic than Mo-MF, as they markedly upregulated *Mrc1* (encodes CD206, Fig. 2I). Given the unique transcriptional signatures of the B-MF, we next examined scRNA profiles from four independent samples of TAM in mice with 4T1.2 cancer (Fig. 2J). Using signature genes identified from *in vitro* generated macrophages (Fig. 2H) we noted three clusters (0, 6, and 8) with robust expression of genes identified in B-MF (Fig. S4H). Cluster 8 also strongly overlapped with a mixed macrophage population (Cluster 4, Fig. 2G and Fig. S4F-H), suggesting that only clusters 0 and 6 were most similar to B-MF. We also examined expression of three genes robustly expressed in B-MF and they were also expressed in TAM clusters 0 and 6 (Fig. 2K). In contrast, expression of key Mo-MF genes was mostly found in clusters 1, 3, 4, and 5 (Fig. S4G), suggesting B-MF and Mo-MF retain traceable and different transcription profiles *in vivo*.

To confirm B-MF and Mo-MF differences at the functional level, first we examined their proliferation. Only B-MF expressed higher levels of Ki67 and readily incorporated BrdU (pulsed at day 6 and tested at day 7 of the culture) (Fig. 3A, B and Fig. S5A), indicating their proliferative and self-maintaining state. Given the changes seen in fatty acid metabolism (Fig. 2D), we stained the cells with filipin III, a fluorescent polyene antibiotic that binds to free cholesterol (24). Compared with Mo-MF, the cellular cholesterol content was significantly upregulated in both *in vitro* and *in vivo* generated B-MF (Fig. 3C, D and Fig. S1E-G). To evaluate phagocytosis, B-MF and Mo-MF were

cultured with fluorochrome-labeled apoptotic cancer cells. B-MF phagocytized apoptotic cells significantly better than Mo-MF (Fig. 3E, F). Given B-MF markedly upregulated TGF β /LAP and PD-L1 (Fig. 1I), we tested their function in a 4-day T-cell suppression assay (5). When B-MF or Mo-MF were cultured with naïve mouse splenic T cells stimulated with anti-CD3/CD28 antibodies, only B-MF significantly inhibited proliferation of CD4⁺ T cells and, at lesser extent, CD8⁺ T cells (Fig. 3G and Fig. S5B-E). Because TGF β is involved in induction of FoxP3⁺ Tregs (5), we also performed an *in vitro* Treg conversion assay by culturing the two macrophages with splenic CD25⁺CD4⁺ T cells in the presence of anti-CD3/CD28 antibodies and IL2 for 4 days. Compared with Mo-MF, B-MF induced higher levels of FoxP3⁺Tregs (Fig. S5F), further suggesting that cancer presumably uses B-MF to mediate immunoregulation and thereby tumor progression. We therefore tested whether B-MF can reverse the retarded growth of B16-F10 melanoma in μ MT mice (n=5/group, Fig. 3G), which we previously linked to the lack of B cells (12). Intravenous transfer of B-MF significantly decreased tumor-infiltrated CD4⁺ T cells (and at lesser extent CD8⁺ T cells) and their IFN-expressing subsets (Fig. 3H, I and Fig. S5H) and markedly increased growth of B16-F10 melanoma in μ MT mice (p<0.05, Fig. 3J and Fig. S5G). In contrast, T cells in the spleen and dLN were not affected by the B-MF transfer (not depicted), suggesting that B-MF primarily regulate tumor-infiltrating T cells.

Cancer mobilizes BMBP in the spleen to convert them to B-MF. Recently, we reported that breast cancer induces tBregs from BM pre-B cells. To do this, cancer first stimulates accumulation of BMBP in the spleen and tumor by causing their premature emigration from BM (10). We therefore reasoned that cancer targets the same pool of BMBP accumulated in the spleen to generate B-MF. In support of this hypothesis, the total

number of CD93⁺ BMBP were markedly increased in the circulation (but decreased in BM) of mice with 4T1.2 and Mogp cancers (Fig. 4A and Fig. S6A, B) as compared with naïve mice (Fig. S6A, B). Stimulation with 4T1.2-CM readily generated B-MF from CD93⁺ BMBP sort-purified from spleens of mice with 4T1.2 cancer (Fig. S6C) but failed to do so if B cells were isolated from spleens of naïve mice, regardless of CD93 expression (Fig. S6C and Fig. S3C), implying that cancer first drives accumulation of BMBP in the spleen to convert them into B-MF.

Cancer targets CSF1R⁺CD93⁺ BMBP by secreting M-CSF. To understand the mechanism of the B-MF generation, we analyzed CM of cancer cells for secreted factors that could affect differentiation of macrophages. M-CSF, a regulator of macrophage differentiation and survival (8, 17), was among the factors that was highly increased in the cancer cells that induce B-MF (Fig. 4B). Conversely, M-CSF was almost absent in CM from B16-F10 cells (Fig. 4B and Fig. S6D, E), which did not induce the generation of B-MF (Fig. 1E and Fig. S3E). Compared to naïve mice, serum M-CSF was also significantly upregulated in mice with 4T1.2 cancer (Fig. S6F). Importantly, BM CD93⁺CD19⁺ BMBP of naïve mice expressed its cognate receptor CSF1R (about 15% iB cells, 6% of pre-B cells and 1.5% pro-B cells, Fig. S6G). In mice with 4T1 cancer, CSF1R⁺CD93⁺CD19⁺ BMBP were markedly reduced in BM but increased in the spleen (Fig. 4A and Fig. S6G), consistent with their cancer-induced emigration from BM, as discussed above. To link these CSF1R⁺CD93⁺CD19⁺ BMBP to the generation of B-MF, we performed the *in vitro* macrophage conversion assay by stimulating sort-purified CSF1R⁺ and CSF1R⁻ B cells from BM and spleen of naïve mice with 4T1.2-CM. While CSF1R⁺ BM B cells readily generated B-MF, the BM CSF1R⁻ subset failed to do so (Fig. 4C). Consistent with an

inability of splenic CD93⁺ B cells of naïve mice to generate B-MF (Fig. S6C and Fig. S3C), we failed to convert naïve mouse splenic B cells into B-MF regardless of CSF1R expression (Fig. 4C). We also cultured primary BM BMBP or 70z/3 cells with 4T1.2-CM in the presence or absence of neutralizing M-CSF antibody (Ab) or Ki20227, a specific inhibitor of c-Fms/CSF1R (25). Both cells failed to generate B-MF upon M-CSF neutralization or CSF1R signaling inhibition (Fig. 4D, E and Fig. S6H, I). To rule out artifacts of *in vitro* assay, we created mice with conditional CSF1R deficiency in B cells (Mb1-CSF1R^{Flox/Flox} mice, gating strategy in Fig. S7). Unlike WT littermates or monocytes from Mb1-CSF1R^{Flox/Flox} mice, the loss of CSF1R in BMBP significantly impaired the cancer CM-induced B-MF differentiation (Fig. 4F, G). Of note, the residual macrophage differentiation seen in Fig. 4F is presumably due to CSF1R expression preceding Mb1 expression and thus only pro-B cells and onward will have *Csf1r* deletion.

Given that PAX5 is the key pro-B cell factor that represses *Csf1r* and other myeloid lineage-specific genes (15, 26), we reasoned that cancer decreases levels of this transcription factor using M-CSF. FACS staining confirmed that Pax5 was markedly decreased in BM CD93⁺ BMBP, particularly in CSF1R⁺ but not CSF1R⁻ subsets, from mice with 4T1.2 or Mogp cancer (Fig. 5A and Fig. S8A, C). Importantly, Pax5 was also significantly decreased in BM CSF1R⁺ BMBP from naïve mice and 70Z/3 cells upon treatment with 4T1-CM or M-CSF (Fig. 5B, C and Fig. S8B). As Pax5 deficiency alone is sufficient to render pro-B cells susceptible to myeloid differentiation (15), we concluded that cancer uses M-CSF to reduce expression of Pax5 in CSF1R⁺CD93⁺ BMBP and thereby promote macrophage differentiation.

To further understand the B-cell susceptibility towards macrophage conversion, we analyzed chromatin accessibility by performing ATAC-seq on CSF1R⁺ and CSF1R⁻ BMBP isolated from both BM and spleen. PCA clustering showed the most robust differences in chromatin profiles were driven by the location of the BMBP (BM vs spleen) regardless of CSF1R expression, driving the PC2 axis (blue & purple vs orange & green, Fig. 5D). The chromatin landscapes of the CSF1R⁺ and CSF1R⁻ BMBP isolated from BM (orange and green, Fig. 5D) also significantly differed from each other, driving the PC3 axis. We then examined the differentially accessible regions (DARs) between CSF1R⁺ and CSF1R⁻ cells isolated from spleen or BM. Whereas comparisons between CSF1R⁺ and CSF1R⁻ cells from spleen did not show any differences reaching our threshold for significance, confirming their close clustering on the PC3 axis; the BM CSF1R⁺ cells contained significantly more open chromatin than the BM CSF1R⁻ cells (Fig. 5D, E). These data suggest the BM CSF1R⁺ BMBP may have a more permissive chromatin environment, susceptible to macrophage differentiation signals. As the spleen-derived B cells and BM CSF1R⁻ cells were refractory to macrophage conversion (Fig. 4C), we looked at DARs with less accessibility in CSF1R⁻ compared to CSF1R⁺ BM cells (749 loci) in spleen cells to determine if these regions remain closed and potentially “lock in” the lymphoid lineage potential. Indeed, the overwhelming majority of regions with decreased accessibility in the BM CSF1R⁻ cells remained closed in the cells from spleen (679 of 749). Evaluation of these consensus open regions found in BM CSF1R⁺ cells for potential transcription factor binding sites permitting macrophage differentiation showed significantly increased accessibility of ERG and RUNX1 sites (Fig. 5F). ERG is known to be expressed both in myeloid and lymphoid progenitor cells (27) and has particular importance in early

hematopoietic progenitor cells as it binds to coregulators such as RUNX and GATA (28). RUNX1 regulates growth and survival of macrophages via binding to promoter and enhancer regions of *Csf1r* and upregulating its expression (29). Runx1 is also robustly expressed in early progenitor and myeloid-committed progenitor cells (30). Thus, the increased accessibility to binding sites of both ERG and RUNX1 suggests a potentially more primitive, permissive chromatin state allowing for myeloid lineage transformation of the BM CSF1R⁺ cells.

B-MF-generating CSF1R⁺ BMBP accumulate in humans with cancer. We recently reported that peripheral mobilization of BMBP also occurs in humans with breast cancer (BC) (10), suggesting the generation of B-MF. To test this possibility, we FACS evaluated PB of healthy donors (HD, n=7) and patients with BC (n=8). Compared with HD, PB of BC was markedly increased in CSF1R⁺ BMBP (Fig. S8D), as we described in mice with cancer. Moreover, microarray transcription profiling of sort-purified B cells from PB of BC patients revealed that they significantly upregulated macrophage-associated genes, such as *Cebpa*, *Marco*, and *Csf1r*, as compared with B cells from HD (Fig. 5G). We also FACS evaluated B-cells from PB of patients with ovarian cancer (OC, n=5). Compared with HD, OC patients significantly increased CSF1R⁺ BMBP (Fig. 5H and Fig. S8E) with upregulated expression of CD68 and LDLR (Fig. 5H), similar to mice with cancer. Using recently published scRNA-seq data of tumor-infiltrated immune cells from patients with breast cancer (31), we also found a macrophage cluster with overlapping signatures of B-MF-like cells (cluster 3, Fig. 5I) by examining genes with differential expression defined in murine *in vitro*-generated B-MF (cluster 0, Fig. 2H). In particular, cluster 3 was enriched for expression of *EGR1*, *IER2*, *IER3*, and *SLC40A1*, which were major drivers of identity

for murine *in vitro*-generated B-MF (Fig. 5I). Similarly, in the single-cell transcriptome data from human high-grade serous OC (32), we also detected the B-MF-like signature in macrophages (Cluster 0, Fig. 5J), although with a lesser overlap than in BC, further suggesting that human cancers can promote the B-cell transdifferentiation into macrophages.

DISCUSSION

BMBP undergo a series of subsequent and tightly regulated differentiation steps after their bifurcation from multipotent cells to committed myeloid/lymphoid lineage cells. Despite this, experiments with forced expression or inhibition of a single transcription factor or mutations that drive leukemogenesis (14, 15) reveal that BMBP retain plasticity and myeloid transdifferentiation potential. Unlike these artificial or rare events, here we report that the B-cell-to-macrophage transdifferentiation is commonly used by murine cancers to generate TAM/B-MF. In PB of humans with metastatic/recurrent triple-negative BC and high-grade serous OC, we also detect a significant increase of CSF1R⁺ CD68⁺LDLR⁺ BMBP which also express the macrophage-specific genes *Cebpa*, *Cebpb*, and *Marco*. Importantly, the B-MF signature is also identifiable within unique macrophage clusters using recently published scRNAseq profiles of tumor-infiltrating cells in patients with BC (31) and high-grade serous OC (32). Although the characterization of human B-MF is a topic of a separate study, our results suggest that human and murine cancers transdifferentiate BMBP into macrophages, adding one more feature to the heterogeneity and plasticity of TAM. The inflammatory and antitumor activities of TAM at the early stages of tumor can shift to proangiogenic and tumor-supporting M2-like phenotypes as

tumor progresses (33), presumably when B-MF would be induced. Interestingly, B-MF resemble both small (S)-TAM and large (L)-TAM (which associate with a poor disease outcome) recently identified in human colorectal liver metastasis (34). The lipid metabolism and phagocytosis genes of B-MF (*Fasn*, *Pltp*, *Acat1*, *C1qa*, and *C1qb*) are upregulated in L-TAM, while *LDLR*, *Hmgcr* as well *SI100a8*, *Vcan*, and *Thbs1* are increased in S-TAM.

Although TAM are primarily derived from monocytes (9), our results clearly show that at least some originate from bona fide B cells. The biological relevance of this redundancy in generation of TAM remains poorly understood; however, based on our comparisons of the side-by-side generated B-MF and Mo-MF, we think that the two macrophages may serve different purposes. Transcriptionally, B-MF preferentially upregulate expression of genes involved in cell cycle, fatty acid metabolism, and steroid-cholesterol biosynthesis, implying they utilize unique metabolic and inflammatory functions. Unlike Mo-MF, B-MF proliferate, i.e. self-maintain and thus may persist longer in the tumor. B-MF markedly upregulate surface expression of LDLR, which removes extracellular cholesterol/LDL (35), and this could explain the higher levels of intracellular cholesterol and lipids in B-MF compared to Mo-MF. Consistent with significant upregulation of genes associated with phagocytosis, M2-skewing and immunosuppressive functions (*PD-L2*, *B7-H3*, *Marco*, *TGF β*) and downregulation of pro-inflammatory and IFN γ response genes, B-MF express higher levels of surface MRC1 (CD206), PD-L1 (CD274), and TGF β /LAP and efficiently phagocytize apoptotic cells compared to Mo-MF. This efficient phagocytosis presumably occurs without overt inflammation, as LDLR-mediated cholesterol influx inhibits activation of the inflammasome (36). Our data show

that cancer generates phenotypically and functionally non-redundant TAM from BMBP and monocytes, where B-MF appear to promote cancer growth presumably by controlling antitumor T cell responses. First, unlike Mo-MF, B-MF efficiently suppress proliferation of T cells or induce the generation FoxP3⁺ Tregs *in vitro*. Second, B-MF enhance growth of B16-F10 melanoma in μ MT mice. To do this, they primarily decrease CD4⁺ T cells and inhibit IFN γ ⁺ CD4⁺ T cells in the tumor, presumably by utilizing the B-MF-expressed immunoregulatory factors, TGF β /LAP and PD-L1 (22, 23), and LDLR. For example, LDLR may enhance the TGF β responsiveness of target T cells by removing extracellular LDL/cholesterol that impairs TGF β binding and thus signaling via TGF β RII/TGF β R1 (37).

We propose that cancer primarily targets a small subset of CSF1R⁺Pax5^{Low} pre-B cells and iB cells recently emigrated from BM. First, B-MF are not found in tumor-bearing mice with the B-cell differentiation blockage at the pro-B cell stage. Second, splenic transitional B cells from naïve mice do not generate B-MF regardless of their CSF1R expression state, as cancer first needs to mobilize BMBP into the circulation as the source of B-MF. We and others have reported that cancers use TSLP and G-CSF to mobilize BM pre-B cells and HSPS in the circulation (10, 38). We also find the chromatin accessibility landscape of BM CSF1R⁺ BMBP to be significantly more open and permissive to macrophage differentiation signals. In contrast, CSF1R⁺ and CSF1R⁻ splenic B cells and BM CSF1R⁻ cells present a chromatin landscape that is refractory to macrophage conversion. The overwhelming majority of regions with decreased accessibility in the BM CSF1R⁻ cells remained closed in the spleen (679 of 749), presumably “locking in” the state of lymphoid lineage potential. It appears that BM CSF1R⁺ cells have a potentially more

primitive, permissive chromatin state allowing for myeloid lineage transformation. The BM CSF1R⁺ cells have more accessible ERG and RUNX1 binding sites, ~~the~~ two transcription factors expressed in myeloid and lymphoid progenitor cells (27) and early progenitor and myeloid-committed progenitor cells (30), respectively. Given that RUNX1 also upregulates expression of *Csf1r* by binding to its promoter and enhancer regions (29) and that the PAX5 deletion alone removes the repression of *Csf1r* and other myeloid lineage-specific genes and induces the BM B-cell precursor transdifferentiation (15, 26), we think that RUNX1 supports CSF1R expression in CSF1R⁺ BMBP to downregulate Pax5 in response to tonic and cancer-secreted M-CSF. Given that the CSF1R⁺ BMBP in our patients with metastatic/recurrent triple-negative BC and high-grade serous OC co-express macrophage-associated genes, human cancer may also target biphenotypic B-cell precursors reported to have a macrophage-differentiation potential (16). Overall, for the first-time we report the B-cell-to-macrophage transdifferentiation as a physiological and widely utilized phenomenon. Murine and possibly human cancers target the transdifferentiation to generate immunosuppressive TAM.

MATERIAL AND METHODS

Mice, cell lines: The animal protocol was approved by the ACUC committee of the National Institute on Aging (ASP 322-LMBI-2022) under the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23, 1985). The study used young (8-12 weeks old) female mice bred and housed in the same, specific pathogen-free environment at the National Institute on Aging (NIA). C57BL/6J, BALB/CJ, R26R-EYFP (B6.129X1-Gt (ROSA)26Sortm1(EYFP)Cos/J), *Csf1r*^{flox} mice (B6.Cg-Csf1rtm1.2Jwp/J) and μ MT mice (B6.129-*Ighm-tm1Cgn/J*) and J_HT mice (J_HT; B6.129P2-*Igh-Jtm1Cgn/J*) in C57BL/6 background mice were purchased from the Jackson Laboratory (Bar Harbor, ME); μ MT mice in BALB/c background were a gift from Dr. Thomas Blankenstein (Max-Delbrück-Center for Molecular Medicine, Berlin, Germany) (39). Mb1-Cre mice in C57BL/6 background (B6.C(Cg)-Cd79atm1(cre) Reth/EhobJ) were a gift from Dr. Richard Maraia (National Institute of Child Health and Human Development, Bethesda, MD) (40). Mogp-tag (Mogp) mice in C57BL/6 background were a gift from professor Dr. I. Miyoshi (Tohoku University Graduate School of Medicine, Miyagi, Japan) (12). To create mice with B-cell specific EYFP reporter (Mb1-EYFP) or CSF1R deletion (Mb1-CSF1R^{Flox/Flox}), Mb1-cre mice were bred with R26R-EYFP and *Csf1r*^{flox} mice, respectively.

4T1.2 cells-were a gift from Dr. Robin L. Anderson (Peter McCallum Cancer Center, Melbourne, Australia); MC38 colonic adenocarcinoma cells were a gift from Dr. Jeffrey Schlom (National Cancer Institute, Bethesda, MD) (41); mammary carcinoma AT3 cells were a gift from professor Scott I. Abrams (Roswell Park Comprehensive Cancer Center, Buffalo, NY); ID8-p53^{-/-}-RFP (ID8) cells were gift from professor Sharon Stack (University of Notre Dame, IN); and EMT6 cells and melanoma B16-F10 cells were

purchased from American Type Culture Collection (Manassas, VA). Cells were tested free of mycoplasma with Mycoplasma Detection Kits (Lonza Basel, Switzerland; and IDEXX BioAnalytics, Columbia, MO).

Tissues and blood processing: Human samples were collected with written informed consent at the Clinical Core Laboratory, NIA, under Human Subject Protocol # 2003054 and Tissue Procurement Protocol # 2003-071. Cancer patient PBMC were collected before the clinical trial (protocol # NCT02203513, NCI) from people with metastatic/recurrent triple-negative breast cancer and high-grade serous ovarian cancer (42, 43). The trial was approved by the Institutional Review Board of the Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA. All experiments were performed on PBMC which were cryopreserved after collection. Mouse BM cells were flushed out of femurs and tibias with cold cRPMI. Single cell suspension of BM, spleen, LN were prepared with 70µm strainer (Falcon, Bedford, MA). BM, spleen, and blood cells were treated with ACK buffer to remove red blood cells. Mouse tumor tissues were cut to 3-5 mm pieces and digested with mouse tumor dissociation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instruction.

Flow cytometry (FACS): For immune cell phenotyping, cells were pre-incubated with TruStain FcX™ solution before immunostaining with different combinations of anti-mouse or anti-human Abs (1µg per 10⁶ cells, Suppl. Table 4) and fixable viability dye, then fixed/permeabilized with eBioscience™ intracellular fixation & permeabilization buffer (Thermo Fisher, Waltham, MA). The samples were evaluated on FACSymphony™ (BD, Franklin Lakes, NJ), Amnis ImageStreamX MKII (Millipore, Burlington, MA) or

CytoFLEX (Beckman Coulter, Brea, CA). The results were analyzed with FlowJo v10(BD), IDEAS (Millipore) or Cytoexpert 2.3 (Beckman).

Cancer CM media preparation and cytokine quantification: Cells were cultured in RPMI1640 or DMEM (for ID8 cells) supplemented with 10% FBS, 1× HEPES, sodium pyruvate, nonessential amino acids solution, penicillin–streptomycin–glutamine (Gibco, Gaithersburg, MD), and 55 mmol/L β-mercaptoethanol in T75 flask to 70-80% confluency. CM was collected after 5 min centrifugation at 1500 rpm, filtered with 0.2μm filter, and stored at -80°C as single use aliquots. For cytokine tests, confluent cells were cultured with RPMI without FBS for 24h. Mouse serum was collected using BD Microtainer® Tubes following the manufacturer's instruction. Cytokines and M-CSF in filtered CM or sera were evaluated with Quantikine ELISA kit (R&D, Minneapolis, MN) or with Proteome Profiler Mouse XL Cytokine Array (R&D). Images were captured and analyzed with Fiji software.

In vitro B-MF conversion: BM Lin⁻ (TER119, CD11b, Gr-1, CD3ε, NK1.1 or CD49b, Ly6C, Ly6G, CD11c)⁻ CD19⁺ B cells were isolated from C57BL/CJ or BALB/CJ mice using FACSaria™ Fusion sorter and 10⁶/ml B cells were cultured in 50% cancer CM in cRPMI for 7 days in Nunc™ Multidishes with UpCell™ Surface (Thermo Fisher) without changing media for 7 days. 70z/3 pre-B cells (10⁵/ml) were cultured in 50% cancer CM for up to 30 days with replenishing culture medium every 3-4 days. Adherent cells (macrophages) were harvested by detaching them at 4°C for 15 mins in PBS. For Giemsa staining, B-MF were fixed with ethanol for 5 mins and Wright-Giemsa stained according to the manufacturer's instruction. CSF1R receptor signaling was blocked with Ki20227 (R&D).

In vitro assays: For bacterial uptake assay, *E. coli* (Thermo Fisher) labeled with pHrodo™ red (0.1 mg/ml) were cultured with B-MF generated from RAG-GFP for 2h. Cells were washed with PBS, fixed with 4% formaldehyde and stained with DAPI. For phagocytosis of apoptotic cancer cells, ID8-RFP cells (10^6 /ml) were pretreated with 300 nM gemcitabine hydrochloride (Sigma, St. Louis, MO) for 24h, then washed with PBS and cultured with macrophages for 2h. Macrophages were stained with anti-F4/80-FITC Ab and DAPI and phagocytosis was evaluated using Zeiss LSM 710 (Carl Zeiss AG, Jena, Germany) and analyzed with Fiji software. For macrophage proliferation test, BrdU (10 μ M, BD) was added to macrophage cultures at day 5 and the BrdU incorporation was quantified at day 7 using FACSymphony™ and analyzed by FlowJo.

T cell suppression assay was described elsewhere (13). Briefly, splenic T cells isolated with CD3⁺ T cell enrichment column (R&D) were labeled with eFluor™ 450 and cultured with macrophages at 1:10, 1:20 and 1:40 E: T ratios in 96-well flat bottom plates coated with 5 μ g/ml anti-mouse CD3e antibody (clone 145-2C11, BD) and free anti-mouse CD28 antibody (2 μ g/ml, clone 37.51, BD) for 4 days. The Treg conversion assay was described elsewhere (5). In brief, FACS-sorted splenic CD4⁺CD25⁻ T cells were cultured with macrophages at 1:5, 1:10 and 1:20 E:T ratios in plates coated with 5 μ g/ml anti-mouse CD3e antibody and free recombinant murine IL2 (5 ng/ml, PeproTech, Rocky Hill, NJ) in for 5 days. Control T cells were cultured with recombinant mouse TGF- β 1 (5 ng/ml, R&D) in cRPMI without macrophages.

In vivo experiments: For evaluation of macrophages in vivo, tumor cells were subcutaneously injected into congenic mice, such as 4T1.2 cells and EMT6 cells (1×10^6) in BALB/cJ and μ MT mice, and B16-F10, and AT3 and MC-38 cells (1×10^6) in

C57BL/6J, J_HT or Mb1-EYFP mice. ID8-p53^{-/-}-RFP cells (5×10^6) were intraperitoneally (i.p.) injected into C57BL/6J mice. For *in vivo* B-MF generation study, C57BL/6J mice with ID8-p53^{-/-}-RFP cells (5×10^6) in PeC were intraperitoneally injected with BM Lin⁻ CD19⁺EYFP⁺ B cells (5×10^6) sort-purified from Mb1-cre-EYFP mice and then 7 days later, the PeC lavage cells were FACS evaluated. To evaluate tumor-supporting role of B-MF *in vivo*, μ MT mice were intravenously injected with 3×10^5 *in vitro*-generated B-MF or PBS 3 and 7 days after subcutaneous challenge with B16-F10 melanoma cells (day 0, n=5). Tumor volume ($V=a \times b$, mm²) was measured at days 11, 14, 16, 18, and 21, and at day 21, mice were euthanized to evaluate tumor weight and T cells.

Cellular cholesterol content quantification: Macrophages were fixed with 4% formaldehyde solution in TBS for 5 mins, then after TBS washes, they were incubated with Filipin III at 1:100 dilution in TBS (5mg/ml stock in 100% ethanol, Cayman, Ann Arbor, MI) for 60 mins in the dark. Cells were washed with TBS and lipids were quantified with Zeiss LSM 710 and Fiji software, as described above.

mRNA microarray: For the collected biological samples, the standard RNA extraction protocol was performed by RNeasy Plus Micro kits (QIAGEN, Hilden, Germany), and genome-wide expression was measured using the Agilent platform (Mouse 8X60K v2 and Hs 8X60K v3, Agilent, Santa Clara, CA, USA) according to the manufacture's instruction. Principal Component Analysis (PCA) was performed using the Prcomp R function with expression values. Differential expressed genes (DEGs) was assessed using the moderated (empirical Bayesian) t-test implemented in the limma package (version 3.14.4)(44), and correction for multiple hypothesis testing was accomplished by calculating the Benjamini-Hochberg false discovery rate. Enriched

pathways were discovered by GSEA tool (45) with Molecular Signature Database v7.4. All microarray analyses were performed using the R environment for statistical computing (version 3.6.2).

scRNA-seq: Sort purified single cell suspensions were loaded into a 10× Chromium controller (10x Genomics, Pleasanton, CA, USA) and converted to barcoded single cell RNA expression library according to the standard protocol of the Chromium Next GEM Single cell 3' kit (v3.1 chemistry) in Laboratory of Immunology and Molecular Biology, National Institute on Aging, and the single cell 3' gene expression libraries were sequenced on NovaSeq 6000 (Illumina, San Diego, CA, USA) in the Genomics Core facility of the Johns Hopkins School of Medicine. Raw sequencing data was processed using the Cell Ranger version 5.0 (10x Genomics, Pleasanton, CA, USA) pipeline. The raw gene expression matrix was normalized and scaled using the SCTransform method (46) in the Seurat R package (version 4.0) (47). The minimum number of detected genes was set to 1,000, and genes were chosen when they were detected in more than 3 cells. Dimension reduction was performed using principal component analysis (PCA). For visualizing the generated clustering, we used the Uniform Manifold Approximation and Projections (UMAP) plot. We defined clusters with a leiden algorithm using shared nearest neighbor (SNN) in PCA space. From *in vitro* B-MF and Mo-MF, we generated a total of 12 clusters for *in vitro* samples (0-11). Integration of *in vivo* samples with canonical correlation analysis (CCA) was performed, and we generated 13 clusters for *in vivo* tumor macrophage samples (0-12). Finally, we performed a nonparametric Wilcoxon rank-sum test to search for highly expressed genes in the clusters. In addition, human tumor single cell transcriptomes were downloaded from GEO (GSE114725, and GSE146026) and also

processed with the same pipeline described. We used only macrophage clusters for downstream analysis. All single-cell analyses were performed using the R environment for statistical computing (version 4.0.5).

ATAC-seq analysis: We utilized a Hi-Seq 2000 machine to sequence the ATAC-seq libraries. We prepared 12 pair-end ATAC-seq libraries including BM CSF1R (+/-) and Spleen CSF1R (+/-) samples (n=3 per group). In total, 369M reads were sequenced and average 31M reads were sequenced per sample. We applied NIEHS TaRGETII ATAC-seq pipelines, which are available to the genomics community. All raw reads were trimmed using cutadapt package, and trimmed reads (>36bp minimum alignment length) were mapped against the mm10 reference genome using BWA aligner (48). We used de-duplicated and uniquely mapped reads for peak calling analysis after excluding black-list regions defined by ENCODE (49). The candidate peaks were predicted by MACS peak calling tool (50). In addition, we also applied the DESeq2 (51) to determine differentially accessible regions (DARs); cutoff: Fold change > 1.5, log2CPM > 1.2, FDR < 0.05. The differentially accessible regions were submitted for search of potential transcription factor binding sites using HOMER software (52). We used non DARs as background regions in de novo motif analysis.

Statistical analysis: The results are presented as the mean with each individual data point or in bar graph $\pm$ SEM. GraphPad Prism (Prism 6; GraphPad Software, Inc) was used to perform statistical analysis. Data were analyzed using Welch t-test or one-way ANOVA. A P-value less than 0.05 was considered significant (**** P<0.0001, ***P<0.001, **P<0.01, *P<0.05).

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AUTHOR CONTRIBUTIONS: C. C. performed the research, collected and analyzed data; E. Ra., M.B. and L.Z., performed experiments; B.P. worked on bioinformatics analyses, JMM provided clinical trial samples; A.B. and I.B. supervised the study and wrote the manuscript; and A.B. conceived and designed the study.

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DATA AND MATERIALS AVAILABILITY: The data associated with this study can be found in the paper or supplementary materials and ATAC-seq data are deposited at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE178716> and [GSE180285](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE180285)

SUPPLEMENTAL MATERIALS: Figures S1-S8 and Tables S1-4.

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Figure 1. Cancer induces differentiation of macrophages from BM B cells. **A** and **B**, FACS staining frequency $\pm$ SEM of co-expression for markers of B cells (CD19) and macrophages (F4/80⁺) in **A**, BALB/CJ and μ MT mice with orthotopic 4T1.2 breast cancer (n=5-6) and **B**, C57BL/6 and J_HT mice with spontaneous ovarian cancer (Mogp-tag and Mogp-J_HT, respectively, n=4) Frequency of F4/80⁺CD11b⁺ cells within CD19⁺ TIBs (left panels) and CD19⁺IgM⁺ cells within F4/80⁺ TAM (right panels). **C**, Representative flow plot for F4/80⁺CD11b⁺ macrophage of B cells isolated from BM, spleen, LN and PeC treated with CM from 4T1.2 breast cancer cells. **D**, Representative Giemsa staining in vitro-generated B-MF. Scale bar represented 20 μ m. **E**, Representative FACS plots of F4/80⁺CD11b⁺ macrophage of BM B-cells treated with CM from 4T1.2, EMT6, AT3, B16-F10, and MC-38 cells. **F** and **G** Representative FACS plots for CD68 and CD11b staining of pre-B cell line (70z/3) after 30-day stimulation with (RPMI) or without 4T1.2-CM and composite frequency analysis of F4/80⁺CD11b⁺ and CD68⁺CD11b⁺ cells from four independent experiments. **H**, Representative Imagestream imaging for surface markers F4/80, CD11b, CD19, and CD20 on *in vitro*-generated B-MF from naïve Mb1-cre-EYFP⁺ mice (bottom two panels, **H**) and WT mice (top two panels, **H**). **I**, Frequency $\pm$ SEM of CD274⁺ and LAP⁺ cells within EYFP⁺ or EYFP⁺F4/80⁺CD11b⁺ cells quantified 7-day after i.p. injection of BM B cells from naïve Mb1-cre-EYFP mice in C57BL/6 mice with ID8 ovarian cancer in PeC (n=3). ****P<0.0001, ***P<0.001, ** P<0.01, *P<0.05 between indicated comparisons.

Figure 2. Distinct gene expression profiles of B-MF and Mo-MF. **A**, Representative FACS plots of F4/80⁺CD11b⁺ macrophage of BM B cells or monocytes post 7-day treatment with 4T1.2-CM. Macrophage phenotype gated as F4/80⁺CD11b⁺ from B cell

(B-MF) and monocytes (Mo-MF) **B**, Representative expression of B-cell markers CD79a and IgM by FACs analysis on B-MF (Red) and Mo-MF (Orange). **C**, PCA plot of mRNA expression profiles generated from microarray data of sort purified B-MF (Blue), Mo-MF (Orange) and BM B cells (Green) (n=3). **D** and **E**, Bar plots of GSEA predicted pathways enriched in B-MF (D) or Mo-MF cells (E) from the Molecular Signature Database. **F** and **G**, UMAP plot of B-MFs (10,563 cells) and Mo-MFs (10,235 cells) analyzed using Seurat with colors depicting clusters by cell type (**F**) or by gene expression (**G**). **H**, Heatmap of top genes with differential expression characterizing the 12 major cell types of *in vitro* B-MF and Mo-MF single cell clusters. **I**, Overlay of Mrc1 expression for *in vitro* B-MF and Mo-MF single cell clusters. **J**, UMAP plot of sort purified TAMs (10,885 cells) generating 13 unique cell clusters from tumor tissues of 4 mice with 4T1.2 cancers. **K**, Violin plots of the expression of three *in vitro* derived B-MF up-regulated DEGs (Egr1, Ier3 and Slc40a1) in *in vivo* mouse 4T1.2 TAM single cell clusters.

Figure 3. B-MF and Mo-MF are functionally different. **A**, Ki67 staining (upper) and BrdU uptake (lower panel) frequency of B-MF and Mo-MF (frequency $\pm$ SEM, n=2). **B** and **C**, Representative Filipin III staining images and quantification (MFI). **D** and **E**, Representative images and quantifications of RFP⁺ apoptotic cells in B-MF and Mo-MF cultured with apoptotic ID8-RFP ovarian cancer cells for 2 h. Eight representative fields per sample were quantified and scale bars represent 20 μ m (C and E). **F**, Representative histograms of proliferated CD4⁺ T cells after co-culturing with macrophages at 1:10 (E:T) ratio in the presence of anti-CD3/CD28 Abs for 4 days. Control T cells were cultured alone with (activated) or without (non-activated) anti-CD3/CD28 Abs. Numbers represent

percentage of proliferated T cells. Macrophages were generated from BM B-cell precursors (B-MF) and monocytes (Mo-MF) with 4T1.2-CM treatment. The results in **B - F** were independently reproduced at least three times. **G – J, B-MF support tumor growth *in vivo***. μ MT mice were i.v. transferred with 3×10^5 *in vitro*-generated B-MF or PBS 3 and 7 days after s.c. challenge with B16-F10 melanoma cells (n=5). Each symbol is for a single mouse; Y-axis is for % $\pm$ SEM of tumor-infiltrated CD4⁺ T cells in CD45⁺ cells (**H**) and IFN γ ⁺ in CD4⁺ T cells (**I**) and tumor weight $\pm$ SEM (**J**). **** P<0.0001, ***P<0.001, ** P<0.01, *P<0.05 between indicated comparisons.

Figure 4. CSF1/CSF1R signaling axis is critical for B-MF differentiation. **A**, Numbers of CD19⁺ (left) and CD93⁺CD19⁺ (middle) B cells and frequency of CSF1R⁺ within CD93⁺CD19⁺ B cells (right) in the spleen of naïve and 4T1.2 tumor-bearing mice (n=5). **B**, ELISA measurements of secreted M-CSF in media conditioned by 4T1.2, EMT6, AT3, MC-38, B16-F10, and ID8 cells (n=6, pg/ml). **C**, Representative FACS plots of the F4/80⁺CD11b⁺ B-MF converted from sort-purified BM CSF1R⁺ and CSF1R⁻ B-cell precursors and splenic CSF1R⁺ and CSF1R⁻ B cells after 7-day treatment with 4T1.2-CM. **D** and **E**, Representative FACS plots of F4/80⁺CD11b⁺ (B-MF) from BM B-cell precursors after 7-day treatment with 4T1.2-CM (**D**) and pre-B cell line 70z/3 after 30-day treatment with 4T1.2-CM (**E**) with and without anti-MCSF Ab or Ki-20227 treatments (a specific MCSF-R inhibitor). **F** and **G**, Representative FACS plot (**F**) and quantification (**G**) of the generation of B-MF after 7-day 4T1.2-CM treatment on cells isolated from WT or mice with conditional CSF1R deficiency in B cells (Mb1-CSF1R^{Flox/Flox}) (n=6). Results for all panels were independently reproduced minimally three times. **** P<0.0001, ***P<0.001, ** P<0.01, *P<0.05 between indicated comparisons.

Figure 5. Cancer targets BM CSF1R⁺PAX5^{Low} B-cell precursors. **A**, FACS analysis of Pax5 expression of freshly isolated BM CSF1R⁻ or CSF1R⁺ B cell precursors (n=5,) **B**, FACS analysis of Pax5 expression of pre-B cell (70z/3 cells) treated with indicated cancer CM (n=3). **C**, FACS analysis of Pax5 expression of BM Lin-CD19⁺B220⁺CD93⁺IgM⁻IgD-CSF1R⁺ B cell precursors treated with M-CSF for 48h (n=3). **D**, 3D PCA plot of chromatin accessibility generated from ATAC-seq data of BM CSF1R⁺ and CSF1R⁻ B-cell precursors and spleen CSF1R⁺ and CSF1R⁻ follicular B cells **E**, Heatmap of differentially accessible regions (DARs) between BM CSF1R⁺ and CSF1R⁻ B-cell precursors (FDR<0.05). **F**, Top, highly significant de novo motifs are more open in BM CSF1R⁺ compared to BM CSF1R⁻ as well as Spleen CSF1R⁺ and CSF1R⁻ cells (678 sites). **G**, mRNA microarray heatmap of macrophage related-DEGs in B cells isolated from PB of patients with breast cancer (BC, n=8) compared to healthy donors (HD, n=7). Scale bar is for expression z-score. **H**, Cell frequency of CSF1R⁺ (left), CD68⁺ (middle) and LDLR⁺ (right) cells within CD19⁺CD10⁺ B cells of PB from healthy donors (HD) or patients with ovarian cancer (OC) (n=5-7). **I** and **J**, UMAP of scRNA sequencing data of macrophage cells (left) and expression levels of *in vitro* B-MF enriched genes (right) in published human BC (I) and OC (J) datasets. Error bars represent SEM, **** P<0.0001, ***P<0.001, ** P<0.01, *P<0.05 between indicated comparisons.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Supplementary Files

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