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Macrophages as mediators of tumor immunosurveillance

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Abstract

Tumor immunosurveillance is a well-established mechanism for regulation of tumor growth. In this regard, most studies have focused on the role of T- and NK-cells as the critical immune effector cells. However, macrophages play a major role in recognition and clearance of foreign, aged, and damaged cells. Macrophage phagocytosis is negatively regulated via the receptor SIRPα upon binding to CD47, a ubiquitously expressed protein. We recently showed that CD47 is up-regulated in myeloid leukemia and migrating hematopoietic progenitors, and that the level of protein expression correlates with the ability to evade phagocytosis. These results implicate macrophages in immunosurveillance of hematopoietic cells and leukemias. The ability of macrophages to phagocytose tumor cells might be exploited therapeutically by blocking the CD47-SIRPα interaction.

Introduction

Over the last 30 years, the dominant paradigm in cancer biology has held that tumor cells successively acquire multiple mutations and inherited changes in gene expression by epigenesis over a lifetime that allow cells to progress from normal tissue, to pre-malignant dysplastic growths, and eventually to fully malignant tumors. These mutations or epigenetic changes have been considered to be of two general types: some result in over-activity of genes that promote cancer growth (oncogenes), while others result in inactivation of genes that restrict tumor growth (tumor suppressors). Identification of mutations acquired in cancer, and determining their role in tumor progression, has been the fruitful result of the last few decades of research.

Perhaps the most informative cancer progression model has been the colorectal cancer model, described as a multistep set of events by Fearon and Vogelstein¹. Hanahan and Weinberg famously distilled the knowledge gained from many studies to enumerate six hallmarks that most cancers share: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, limitless replicative potential,

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sustained angiogenesis, and tissue invasion and metastasis². While this is a simplification of a complex process, these capabilities are almost universally found in varying degrees in tumors, indicating their profound importance in neoplastic progression. Nonetheless, recent work has shown that two more hallmarks may also be essential for development of cancer, namely the ability of cancer cells to acquire stem-cell like machinery to allow self-renewal (or for the inciting mutations to arise in a *bona fide* tissue stem cell)³, and the ability of cancer cells to evade elimination by the immune system. The cancer stem cell model has been reviewed extensively elsewhere⁴. Here we will instead focus on the role of macrophages as potential mediators of immunosurveillance, and its clinical implications.

Tumor immunosurveillance

The “tumor surveillance” hypothesis was first proposed by Macfarlane Burnet and Lewis Thomas in 1957⁵. The hypothesis suggests that tumor cells might have “altered-self” antigens that allow the immune system to recognize them leading to the tumor’s subsequent eradication. Recently, researchers have created mice with genetic deficiencies in various immune functions. For instance, mice that lack genes essential for immune development or effector responses, such as recombination activation gene 2 (RAG2^{-/-}), interferon gamma (IFN γ ^{-/-}), or perforin (Pfp^{-/-}), among others, have increased incidences of spontaneous and 3-methylcholanthrene induced cutaneous tumors and fibrosarcoma as well as increased incidence of spontaneous lymphomas and colon, breast, and lung carcinomas, indicating that a functional immune system is needed for tumor surveillance of at least some malignancies^{6,7}. Furthermore, in the last 10 years, hundreds of antigens expressed by human tumors have been identified that have limited or no expression by the vast majority of cells in normal tissues^{8,9}, allowing for the possibility of the adaptive immune system to recognize tumors as foreign.

While the adaptive immune response is now well recognized to be an important component of anti-tumor immunity, the role of the innate immune system has begun to be elucidated as well. Since the seminal discovery that NK cells can eliminate endogenous cells with decreased class I MHC expression¹⁰, the idea was born that tumors could be distinguished from normal tissues because of “missing self”. Since these landmark findings were first published, a wealth of information about activating and inhibitory receptor-ligand interactions on NK cells has been discovered. The most interesting of these, from a tumor immunology perspective, has been the natural killer group 2, member D (NKG2D) receptor, which can recognize MHC class I related proteins A and B (MICA and MICB)¹¹, and can also recognize antigens ectopically expressed on tumors, such as Rae1 or H60¹², leading to the destruction of tumor cells which over-express these proteins. Furthermore, it has recently been discovered that DNA damage can itself lead to upregulation of NKG2D ligands¹³, thereby linking pro-tumorigenic insults with the markings of “altered-self”.

Phagocytosis and tumor immunosurveillance

While there has been much data to support a role for T- and NK-cells in tumor surveillance, to date there has been a dearth of evidence to indicate that macrophages can directly kill tumor cells via phagocytosis. In the 1980s and 90s, there was interest in the idea that Kupffer cells, specialized macrophages that reside in the liver sinusoids, could prevent metastases from engrafting in the liver, but this avenue of research has been largely abandoned¹⁴⁻¹⁸. More recently, investigators have focused on the ability of macrophages to infiltrate solid tumors and modulate T-cell and stromal activity to either favor or inhibit tumor growth¹⁹. The general consensus is that macrophage activity in such situations is pro-tumorigenic via their ability to promote angiogenesis and metastases²⁰. These so-called tumor associated macrophages (TAMs) are thought to be recruited by tumor derived

chemokines and converted to a tumor permissive state²¹. These TAMs are called M2, or alternatively-activated macrophages, to distinguish them from M1, or classically-activated macrophages, and have been extensively studied²²⁻²⁴. Alternatively-activated macrophages produce less inflammatory cytokines, promote Th2 T-cell responses, favor wound healing, and increase local angiogenesis²⁵. Whether these TAMs can be converted from the pro-tumorigenic M2 type to the pro-inflammatory M1 type to attack tumors is still under investigation.

Perhaps one reason for the lack of evidence for the involvement of phagocytosis in tumor killing is that, until recently, many of the molecules involved in macrophage recognition of cells and particles had not been determined. The evolutionarily oldest of these recognition receptors are the C-type lectin receptors, which mediate binding to carbohydrate moieties on bacteria²⁶. In higher organisms, phagocytes can recognize foreign cells and particles that have been coated with antibody or complement via Fc receptors or complement receptors²⁷ (i.e. opsonization). Scavenger receptors were originally identified by their ability to recognize modified low density lipoprotein, but are now known to recognize bacterial cell wall components such as lipopolysaccharide (LPS) as well²⁸. In addition, the extracellular matrix proteins fibronectin and vitronectin can function as non-specific opsonins via integrin receptor mediated phagocytosis²⁷. Apoptotic cells express the ligand phosphatidylserine (PS) on their cell surface²⁹, and recognition by the PS receptor (most likely Tim4³⁰) on macrophages ensures prompt removal of apoptotic cells before they lyse and incite an inflammatory response. Even in the absence of apoptosis, neutrophils age over the day of their life, and when old, convert from being resistant to being sensitive to macrophage phagocytosis³¹; this process can regulate neutrophil numbers even when they have an artificially extended lifespan by enforced expression of Bcl2.

While these studies have illustrated the large variety of molecules involved in phagocytosis of foreign or apoptotic cells, it is the recent identification of ligands expressed on living self cells that can influence phagocytosis that might have more relevance for tumor surveillance. One such molecule is the pentaspanin CD47. Also known as integrin associated protein (IAP) because of its physical association with the integrins $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$, and $\alpha_4\beta_1$ ³², CD47 was first cloned as a marker expressed in ovarian tumors³³. Since then, it has been shown to be ubiquitously expressed on mammalian tissues in a variety of splice isoforms³⁴. The CD47^{-/-} mouse has a generally mild phenotype with the only dramatic deficit being rapid death following experimental induction of bacterial peritonitis, presumably due to defects in neutrophil migration³⁵. CD47 has also been implicated in cell migration^{36,37}, T-cell co-stimulation³⁸, neuronal development³⁹ and thrombospondin binding⁴⁰.

The first identification of a role for CD47 in phagocytosis was the observation that red blood cells (RBCs) from mice deficient in CD47 were rapidly cleared upon transfusion into wild-type hosts⁴¹. Indeed, as RBCs age, they lose expression of CD47 indicating that this may be a physiological mechanism by which they are cleared⁴². This observation was subsequently extended to leukocytes, where it was observed that CD47^{-/-} bone marrow was unable to rescue wild-type mice after lethal irradiation⁴³. The mechanism by which CD47 regulates phagocytosis is by interaction with signal regulatory protein alpha (SIRP α) on macrophages⁴⁴⁻⁴⁶. Upon ligation with CD47, the immunoreceptor tyrosine based inhibitory motif (ITIM) of SIRP α becomes phosphorylated and recruits SHP phosphatases to the membrane, resulting in inhibition of myosin accumulation at the cell surface, which thus precludes phagocytosis⁴⁷. Thus, CD47 appears to be a required signal to prevent macrophage phagocytosis of self. More recently, studies have described phagocytosis as the result of a balance between pro- and anti-phagocytic ligands. For example, when calreticulin, a pro-phagocytic cell surface marker, is blocked, CD47 expression is no longer required to prevent default phagocytosis⁴⁸. Seen in this light, any given macrophage

encounter will result in a series of stimulatory and inhibitory inputs, the sum of which determines whether phagocytosis is triggered (Figure 1a).

It is important to note that while many interactions can positively regulate phagocytosis, SIRP α -CD47 is the only known receptor-ligand pair that is a negative regulator of macrophage phagocytosis, highlighting the importance of CD47 for prevention of self phagocytosis. Some authors have proposed that plasminogen activator inhibitor-1 (PAI-1) has a “don’t eat me” function based on evidence that PAI-1 $^{-/-}$ or anti-PAI-1 antibody treated neutrophils are phagocytosed more readily than their wild-type counterparts⁴⁹. Thus far, a receptor for PAI-1 on macrophages has not been identified. Its effect may instead be due to a *cis* inhibitory interaction with calreticulin. In the absence of PAI-1, calreticulin appears to be exposed on viable neutrophils, allowing increased clearance by macrophages. Another molecule, CD31, can negatively regulate tethering of viable leukocytes to macrophages, and becomes disabled once cells undergo apoptosis. However, the function of this molecule appears to be limited to tethering, and does not appear to directly affect the phagocytic machinery in macrophages⁵⁰. A third molecule, CD200, has been shown to negatively regulate macrophage function in cancer. However, it functions primarily by altering inflammatory status and macrophage activation, and is not a phagocytic regulatory molecule per se (reviewed in 51). What these studies reveal is that phagocyte activation, binding, and engulfment is a complex, multi-step process with many players; however no ligand other than CD47 has been found that has a direct effect on phagocytosis via a macrophage inhibitory receptor. It remains to be seen if one of these signals is dominant over the others in the process of phagocytosis.

CD47 in hematopoiesis and leukemogenesis

CD47 is ubiquitously expressed at a low level on all hematopoietic cells, and its species-specificity may underlie some aspects of hematopoietic xenotransplant rejection^{52,54}. Indeed, immunodeficient non-obese diabetic, IL-2 receptor common gamma chain deficient (NSG) mice express a SIRP α variant that is better able to interact with human CD47, which may explain the tolerance for human hematopoietic grafts in these mice relative to other immunodeficient strains⁵⁴. Our recent evidence indicates that CD47 expression switches from low baseline levels to high levels rapidly on normal hematopoietic stem cells (HSCs) when they mobilize into the periphery after a strong inflammatory stimulus, such as granulocyte colony stimulating factor (G-CSF) or (LPS)⁵⁵. This upregulation is temporally precise, as CD47 levels quickly return to baseline within days. We hypothesize that this upregulation is necessary to prevent clearance of normal, healthy hematopoietic cells both during their constitutive recirculation from marrow to blood to marrow, and also during inflammation, which also mobilizes HSC and progenitors; a low-level of CD47 at steady state is sufficient for prevention of phagocytosis. In support of this model, 10 to 20% of the normal red blood cell density of CD47 is sufficient to prevent clearance of polystyrene beads by non-activated macrophages in the absence of other pro-phagocytic ligands⁴⁷. Furthermore, our evidence indicates that genetically reducing levels of CD47 on normal murine stem and progenitor cells in vivo, or antibody-mediated blocking of CD47 of normal human HSCs in vitro, is not sufficient to induce phagocytosis by macrophages at steady state. Our data also show that HSCs that are haplosufficient for CD47 (i.e. half the usual expression level) are more likely to be cleared after administration of LPS, suggesting that CD47 upregulation may occur to protect migrating HSCs from being phagocytosed as they transit past activated macrophages into hematopoietic tissues during the acute inflammatory response (reviewed in ⁵⁶). LPS has long been known to cause macrophage activation; one mechanism by which this may occur is through down-regulation of SIRP α expressed on some macrophage subsets after exposure to LPS⁵⁷, which may lead to a decreased threshold for phagocytosis. However, by increasing CD47 expression levels, sufficient engagement of

SIRP α may still occur (since this interaction may display high avidity characteristics like other immune receptor-ligand interactions⁵⁸), hence those cells would not be phagocytosed during this activated state (Figure 1c-d). In addition, pro-phagocytic markers may also be increased after mobilization/inflammation (see below), making CD47 up-regulation even more critical for cell survival.

Leukemic stem and progenitor cells from chronic myeloid leukemia (CML) during blast crisis and acute myeloid leukemia (AML) also constitutively upregulate CD47 on their cell surface^{55, 59}. To test whether CD47 upregulation affects tumorigenicity, we engineered a human AML cell line that does not engraft in RAG2^{-/-}, common gamma chain ^{-/-} mice (mice lacking T, B and NK cells, but that still have functional phagocytes), to express varying levels of murine CD47. The pathogenicity of the cell line *in vivo*, and the ability to evade phagocytosis *in vitro*, correlated with the level of CD47 expressed. Thus, the ability of leukemia cells to constitutively maintain high levels of CD47 is yet another example of a mechanism by which a tumor stem cell dysregulates a normal physiological process during pathogenesis.

Why would hematopoietic malignancies require additional protection against phagocytosis? One hypothesis is that the process of tumorigenesis itself creates a pro-inflammatory environment (Figure 2a). This could be due to the ability of normal stem cells to repopulate local or distant empty niches⁶⁰⁻⁶³, which is a migration begun by detaching from neighbor cells and could involve local dissolution of extracellular matrix to travel to adjacent or distant sites. Such an environment might lead to increased risk of phagocytosis due to increased macrophage activity and/or increased levels of opsonins, such as immunoglobulin in the serum or from dissolved components of the extracellular matrix such as fibronectin and vitronectin. In this case, there would be a strong selective pressure for those tumor clones that can escape phagocytosis by upregulating CD47.

A second hypothesis is that tumor cells themselves are more “phagogenic”, i.e. they are inherently more prone to eliciting phagocytosis, possibly because they express increased pro-phagocytic ligands, and escape variants eventually arise through selection that upregulate CD47 to compensate (Figure 1e-f, 2b). Similar to the way that NK cells patrol the epithelial environment for “stressed self”, macrophages may preemptively clear altered cells in the hematopoietic environment. Given that all major hematopoietic tissues are heavily infiltrated by macrophages, particularly at their sites of entry, phagocytosis of potentially dangerous variants could provide a potent tumor immunosurveillance mechanism. Moreover, identifying the pro-phagocytic ligands expressed on tumor cells could be useful in designing new therapies. Calreticulin is again implicated as one such molecule; it is upregulated in cells after irradiation⁶⁴ or anthracycline administration⁶⁵, providing a potential link between pro-tumorigenic insults and phagocytic clearance.

It remains possible that CD47 up-regulation has functions in addition to prevention of phagocytosis, as described above. In addition to its ability to bind to extracellular receptors, CD47 also has the ability to transduce signals into the cell after interaction with thrombospondin or integrins, leading to biological activities such as cell spreading, migration, or even apoptosis³². Our recent studies demonstrate that CD47 up-regulation does affect phagocytosis, though other functions may also be altered in these cells. However, the targeting of CD47 by therapeutic molecules in AML appears to primarily abrogate its function as a “don’t eat me signal”, without affecting survival or migration, leading us to speculate that its dominant function *in vivo*, in at least leukemic cells, is to prevent phagocytosis (Figure 1g).

Targeting CD47 to promote macrophage killing

We showed that upregulation of CD47 in human myeloid leukemias and their tumor-initiating leukemia stem cells contributes to pathogenesis through the engagement of CD47 with SIRP α leading to inhibition and evasion of phagocytosis by the host innate immune system^{55, 59}. This finding has therapeutic implications in that blockade of the CD47-SIRP α interaction by a blocking monoclonal antibody against CD47 could potentially enable phagocytosis of leukemia cells. Thus, we investigated whether antibody-mediated CD47-SIRP α blockade could enable phagocytosis of CD47-expressing leukemias⁵⁹. In our studies, monoclonal blocking antibodies targeting CD47 preferentially enabled phagocytosis of Lin-CD34+CD38-CD90- human acute myeloid leukemia stem cells (AML LSC, rare cells within the neoplasm capable of recapitulating disease in a xenotransplant model) by macrophages *in vitro*. When administered *in vivo* to human AML engrafted mice, anti-CD47 antibody eliminated leukemic disease and tumorigenic potential through targeting of AML LSC. Although this antibody is effective in eliminating AML in mouse models, there is accumulating evidence that anti-CD47 antibodies may be effective in other tumors. Blocking antibodies directed against CD47 have been shown to eliminate human chronic lymphocytic leukemia B cells *in vitro*⁶⁶, a multiple myeloma cell line⁶⁷ and lymphoid leukemia cell lines⁶⁸ *in vivo*. In solid tumors, anti-CD47 antibodies have been shown to eliminate human head and neck squamous cell carcinoma cell lines⁶⁹ as well as primary human bladder cancers *in vitro*⁷⁰. Such reports demonstrate that antibody-mediated blockade of CD47 signaling may be therapeutic in multiple hematopoietic and solid tumor malignancies.

What is the mechanism behind anti-CD47 antibody-mediated elimination of tumor cells? In our own studies, we have shown that the mechanism of antibody action is mediated by macrophages and is due to enabling of phagocytosis by blockade of the CD47-SIRP α that is independent from Fc-receptor (FcR)-mediated antibody killing. Several lines of evidence support this finding. First, this therapeutic effect was primarily mediated by phagocytes, as depletion of macrophages in AML-engrafted immunocompromised mice with liposomal clodronate abrogated the anti-CD47 therapeutic response. Second, two distinct anti-CD47 antibodies that block the CD47-SIRP α interaction enabled phagocytosis whereas a non-blocking anti-CD47 antibody did not, even though all three antibodies bound to target cells similarly. Third, an anti-SIRP α antibody also enabled phagocytosis of AML LSC. Fourth, an isotype-matched anti-CD45 antibody did not enable phagocytosis of LSC even though the antibody bound to the cells. Lastly, an F(ab)₂ fragment of the anti-CD47 antibody enabled phagocytosis of cancer cells even though the Fc receptor was absent (Chao et al., unpublished observations). Thus, these findings support a novel FcR-independent mechanism of antibody action for an anti-CD47 antibody.

Although we have demonstrated that an anti-CD47 antibody eliminates malignant cells by enabling of phagocytosis through inhibition of SIRP α , other mechanisms of anti-CD47 antibody killing have been proposed. Most notably, several groups have shown that anti-CD47 antibodies can eliminate tumor cells by direct apoptosis^{68, 71} that is caspase-independent⁶⁶. These reports raise the hypothesis that anti-CD47 antibodies induce apoptosis in tumor target cells that are then secondarily phagocytosed by macrophages and other phagocytes and that this process could be independent from the CD47-SIRP α interaction. However, these studies observed apoptosis only when anti-CD47 antibodies were immobilized or cross-linked, whereas apoptosis was not observed when antibodies were incubated in suspension. In our studies, anti-CD47 antibodies enabled phagocytosis of leukemia cells when incubated in suspension with no observed apoptosis⁵⁹. The functional outcomes in the manner in which CD47 is engaged (cross-linked or in suspension) may be a result of separate signaling mechanisms. In addition to SIRP α , CD47 binds,

thrombospondin-1, which forms a molecular bridge between phagocytes and apoptotic cells through its interaction with $\alpha v \beta 3 / CD36$ ⁷². It is possible that engagement of CD47 in some contexts induces apoptosis (via binding to thrombospondin-1⁷³, after irradiation⁷⁴, or with cross-linking antibodies⁶⁶), while in other contexts (anti-CD47 antibodies in suspension) blockade of CD47 enables phagocytosis by disrupting CD47-SIRP α . However, it is necessary to further elucidate the biochemical and functional downstream signaling consequences of cross-linked or soluble anti-CD47 antibody-mediated action.

Antibody targeting of CD47 on normal cells

CD47 is a widely expressed protein on many cells of the hematopoietic system as well as other tissues³⁴. Although much evidence supports the pre-clinical efficacy of anti-CD47 antibody-mediated elimination of tumor cells, given the widespread expression of CD47 in the hematopoietic system and other normal tissues, toxicity could be a barrier to translating the pre-clinical efficacy of an anti-CD47 antibody into a clinical therapy. To address this, the effect of an anti-CD47 antibody on normal cells was investigated on both human and mouse normal cell counterparts⁵⁹. Relatively minimal toxicity was observed. First, normal human CD34+ cells were not phagocytosed *in vitro* with an anti-CD47 antibody despite phagocytosis of human leukemia cells. Of note, CD34+ cells isolated from cord blood were shown to be resistant to anti-CD47 antibody-induced cell death⁷⁵. Second, when an anti-mouse CD47 antibody capable of enabling phagocytosis was administered to wild-type mice *in vivo*, minimal toxicity was observed with no reduction in the HSC pool.

If CD47 is expressed on multiple cell types in the hematopoietic system and in several organ tissues, why is significant toxicity with antibody-targeting of CD47 not observed? The therapeutic selectivity may be due to the presence of a yet unknown pro-phagocytic signal(s) on leukemia cells that is not present on normal cell counterparts, as discussed above. Thus, anti-CD47 antibody therapy may operate in a therapeutic window that is reliant on the presence or absence of pro-phagocytic signals in combination with the anti-phagocytic signal, CD47 (Figure 1e-g).

Macrophages as effector cells in an anti-CD47 antibody therapy

That macrophages play an important role in tumor immunosurveillance and clearance in myeloid leukemias is underappreciated. However, the notion that macrophages are major mediators of an anti-CD47 antibody therapeutic effect has important implications in translation of such a therapy to human leukemia patients. Standard therapy for human AML involves cytostatic drugs such as anthracycline-based combination chemotherapy which leads to long-term survival rates ranging from 10-75%⁷⁶, arguing for a need for improved therapy. Chemotherapy has been shown to induce an inflammatory response that attracts infiltrating macrophages into tumors, which in multiple tumors is associated with a better clinical prognosis⁷⁷⁻⁷⁹. Thus, an anti-CD47 antibody therapy administered in the post-chemotherapy setting may result in increased efficacy by utilizing the infiltrating macrophages as immune effector cells at the site of disease. However, if chemotherapy led to long-term expression of pro-phagocytic signals in normal hematopoietic stem and progenitor cells, the anti-CD47 therapy could have significant added hematopoietic toxicity.

In addition to modulation by chemotherapy, increasing macrophage cell numbers may also augment the efficacy of an anti-CD47 antibody therapy by expanding the available pool of effector phagocytic cells. Such macrophage manipulation could be achieved by increasing endogenous numbers of macrophages through administration of cytokines including macrophage-colony stimulating factor (M-CSF) or granulocyte-macrophage-colony stimulating factor (GM-CSF). The combination of such cytokines with tumor-specific antibodies has been shown to be therapeutically effective in tumor models^{80, 81}. In addition,

GM-CSF is clinically approved for use in AML patients and is generally well-tolerated (reviewed in 82). Therefore, combination therapy with GM-CSF and anti-CD47 antibody could be a viable therapy that could be translated clinically. In addition to increasing endogenous macrophage production, exogenous macrophages could be increased through *ex vivo* transplantation of macrophage progenitors including chronic myeloid progenitor (CMP) and granulocyte-macrophage progenitor (GMP) cells, which could be followed by anti-CD47 antibody administration to form an effective therapeutic strategy. We have previously shown that transplantation of either syngeneic⁸³ or fully allogeneic⁸⁴ mouse CMP/GMP into mice challenged with bacterial or fungal pathogens can increase myeloid effector cell numbers and prevent fulminant infection and death. Currently, we are investigating these therapeutic combinations in pre-clinical mouse models.

Conclusion

Evidence is emerging that for human myeloid leukemias, macrophage phagocytosis is a critical mediator of tumor immunosurveillance. These cancers can escape such immunosurveillance by up-regulating CD47, a potent “don’t eat me” signal. This appears to be a mechanism co-opted from normal HSCs, which also up-regulate CD47 as protection against phagocytosis in response to inflammatory or mobilizing agents. We are currently exploring the ability of blocking anti-CD47 antibody in combination with agents that increase macrophage activity to treat human myeloid leukemias in pre-clinical models.

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Figure 1.

Hypothetical model of pro- and anti-phagocytic signaling in hematopoiesis.

A) At steady state, CD47 signal strength is sufficient to overcome low pro-phagocytic ligand expression. B) When stem or progenitor cells undergo apoptosis, they increase expression of pro-phagocytic signals and phosphatidylserine, as well as decrease CD47, which allows for highly efficient phagocytosis. C) After administration of LPS, macrophages down-regulate SIRP α , which lowers the threshold for phagocytosis, allowing pro-phagocytic signals to dominate. D) The normal physiological response to inflammatory mediators is up-regulation of CD47 on hematopoietic cells, which allows lowered SIRP α on activated macrophages to be more efficiently engaged, thus preventing phagocytosis. E) Pre-leukemic clones may upregulate pro-phagocytic ligands, marking them for destruction via phagocytosis. F) Via natural selection, clones high for CD47 are selected over time, allowing these escape variants to survive. G) Blocking CD47 on leukemic clones masks an anti-phagocytic signal, allowing pro-phagocytic signals to dominate.

Figure 2.

Two possible models for selection of CD47 high-expressing leukemic clones

A) In the first model, tumor growth incites a localized inflammatory reaction that causes macrophage activation and SIRPα down-regulation. Only those clones that highly express CD47 survive in this toxic milieu. B) In the second model, pro-phagocytic ligands are up-regulated on early leukemic clones, marking them for phagocytosis. Over time, clones that are high expressers of CD47 are enriched because they are immune to clearance by macrophages.