CALR and CD47: An Insight into Their Roles in the Disease Progression of MDS and MPN

Kristian Boasman, Matthew J Simmonds and Ciro R Rinaldi

School of Life Sciences, College of Science, University of Lincoln, Lincoln, UK

Corresponding author: Ciro Rinaldi, School of Life Sciences, College of Science, University of Lincoln, Lincoln, UK Tel: +44 1205 446311; E-mail: crinaldi@lincoln.ac.uk

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Abstract

Myelodysplastic syndrome and myeloproliferative neoplasms are clonal myeloid disorders arising from haematopoietic stem cells that have the tendency to progress into acute myeloid leukaemia. Multiple prognostic scoring systems have been proposed and utilised in clinical practice to predict disease evolution, however none of them can predict treatment response. In solid tumours, the relationship between the pro-phagocytic calreticulin and the anti-phagocytic CD47 is repeatedly investigated. Overexpression of calreticulin has been documented to produce a pro-phagocytic signal in solid tumour and it is often counteracted by a concomitant expression of the anti-phagocytic CD47 as they act in response to one another, reflecting an apoptosis vs survival mechanism in response to chemotherapy. The role of both calreticulin and CD47 are currently poorly understood in myeloid malignancies including myelodysplastic syndrome and myeloproliferative neoplasms. The aim of this review is to elaborate on the current understanding round the roles and implications of calreticulin and CD47 signalling with in solid and haematological cancers, discuss potential roles for calreticulin and CD47 expression in transformation of myeloid cells in patients with MDS or MPN into AML and how these advances are starting to be used to design new therapeutic strategies to determine disease progression and treatment response in both solid cancer and myeloid malignancies.

Keywords: Haematological cancers; Solid cancers; Myelodysplastic syndrome; Myeloproliferative neoplasms; Acute myeloid leukaemia

Abbreviations: MDS: Myelodysplastic Syndrome; MPN: Myeloproliferative Neoplasms; CALR: Calreticulin; ET: Essential Thrombocythaemia; PV: Polycythaemia Vera; MF: Myelofibrosis

Introduction

Myelodysplastic syndromes (MDS), Myeloproliferative neoplasms (MPN) and acute myeloid leukaemia (AML) are myeloid malignancies which are a group of bone marrow diseases arising from clonal disorders of haematopoietic stem cells. MDS and MPN show tendencies to develop into AML as these diseases progress [1]. These diseases originate from a range of genetic events in the hematopoietic stem cells that lead to dysmyelopoiesis, abnormal differentiation, increased apoptosis with ineffective haematopoiesis, cytopenias or leucocytosis [2,3]. Scoring systems for prognosis of this disease are mainly based on bone marrow/peripheral blood features, cytogenetics and some clinical manifestations, with no specific genetic marker highlighting subtypes or stages of these conditions or predicting transformation and disease progression. In solid tumours, characterisation of the relationship between the pro-phagocytic calreticulin (CALR) and the anti-phagocytic CD47 markers is starting to show they act in response to one another, reflecting a possible apoptosis vs survival mechanism in response to chemotherapy. It has been suggested that calreticulin and CD47 could also play a similar role within myeloid malignancies, however their role within these diseases has been less well established. In this review using leading published data (searched for on Pubmed and Google scholar) we will discuss the current challenges in disease prognosis scoring and response to treatment within MDS and MPN, describe the current advances made in characterising the role of CALR and CD47 in solid tumours and the emerging evidence for a role of CALR and CD47 within MDS and MPN. We will also evaluate how these advances in our understanding of the role of CALR and CD47 are starting to aid in acting as diagnostic markers and in therapeutic treatments for these myeloid malignancies.

Challenges with Characterization and Prognostic Scoring within Myelodysplastic Syndrome and Myeloproliferative Neoplasms

Whilst both MDS and MPN are haematopoietic stem cell disorders that tend to develop on to AML, there are distinct differences between these disease subtypes. MDS develops from a group of clonal haematopoietic stem cell disorders, which are characterised by ineffective haematopoiesis which can develop into cytopenias [4]. Prognostic scoring of MDS is usually characterized on the basis of the extent of cytopenia, the percentage infiltration of the bone marrow blasts, and abnormal chromosomal structure upon karyotyping [5]. MPNs are chronic myeloid cancers that are characterized by over production of mature blood cells, which have the potential to evolve into AML [6]. The three most common forms of MPN are Essential thrombocythaemia (ET), polycythaemia vera (PV) and myelofibrosis (MF) [7], which are generally well defined from each other but quite often with overlapping presentations. Somatic mutations are common in MPNs; in the majority of patients JAK2 mutations, which affect JAK/STAT pathway signalling, have been described [8]. Although other mutations in MPN have been acknowledged, such as the CALR mutations or cMPL mutations [9], it is thought that all roads lead to
over activation of the JAK/STAT pathway, with these other mutations also affecting downstream parts of the JAK/STAT pathway.

Prognosis of MDS and MPN heavily depends on type and severity of disease. Over the past 25 years classification systems (French-American-British [FAB] and World Health Organization [WHO]) along with several prognostic-scoring systems, such as the International Prognostic Scoring System (IPSS), have been widely used [7,10,11]. Vast heterogeneity in these disorders results in a wide range of clinical indicators including: signs and symptoms of anaemia, infection and bleeding complications predominant, with systemic symptoms or features of autoimmunity being seen in some patients; perhaps indicative of disease pathogenesis [4].

In MDS, a standard measuring system ranking the risk factors of each disease into: very low, low, intermediate, high and very high risk is currently used (IPSS-R) [11] while in MPN a similar prognostic index (Dynamic International Prognostic Scoring System [DIPSS]) is only informative in the MF subtype, which is used for estimating survival from any point in the disease progression [12]. These scoring systems are mainly based on bone marrow/peripheral blood features, cytogenetics and some clinical manifestations such as systemic symptoms or splenomegaly but no specific genetic marker highlights specific subtypes or stages of those conditions or predicts transformation and disease progression.

Recent evidence has suggested that certain markers involved in controlling phagocytosis could be key in both disease progression and response to treatment within both solid cancer and myeloid malignancies, with the role of the pro-phagocytic CALR and anti-phagocytic CD47 recently coming to prominence. Overexpression of CALR has been documented to produce a pro-phagocytic signal in solid tumours [13] and it is often counteracted by a concomitant expression of the anti-phagocytic CD47[14], reflecting an apoptotic vs survival mechanism in response to chemotherapy. This expression pattern could be used as a potential target for therapeutics and prognostic scoring, as CALR and CD47 has a role in cancer progression and a potential emerging role in MDS and MPN, which will be discussed further.

The Roles of Calreticulin and CD47 in Ordaining/Controlling Phagocytosis

CALR is an endoplasmic reticulum (ER) protein, which has a crystal structure comprising of an extended proline-rich P-domain inserted between N- and C-terminal domains. CALR is involved in many different functions such as calcium homeostasis, protein folding and post translational modifications [15]. Although CALR is an ER protein, it is also found throughout the cell and outside the ER is predominantly located in the membrane and the cytosol [14] where it is involved in gene expression, cell death, programmed cell removal and wound healing [16].

The structure of CALR is split into 3 different domains each with a specific function. The acidic C-domain is responsible for calcium regulation and binding, predominantly within the ER, and the globular N-domain and extended P-domain are primarily responsible for the protein’s chaperone function [14,15]. The C-domain contains the KDEL amino acid sequence which prevents CALR from being secreted from the ER. Whilst the KDEL signal resides it to the ER it can however be translocated to other parts of the cell when needed [17]. KDEL-containing proteins bind to KDEL receptors on the ER affecting its translocation. If the protein is missing or has a mutated KDEL region cellular mis-localisation can occur. Localisation studies show that in myeloid cells CALR is upregulated, however when mutated, CALR stays located to the ER [17].

Expression of CD47 affects cellular proliferation, apoptosis, adhesion, migration and immune system homeostasis [18]. CD47 is comprised of an extracellular immunoglobulin (Ig) variable domain, a five times transmembrane-spanning domain, and a short alternately spliced cytoplasmic tail [18]. Different CD47 tissue expression patterns can be seen, which has been proposed to be due to different isoforms of CD47 determined by alterations in the range of length of the cytoplasmic tail from between 4-36 amino acid long [19].

The role of CD47 is in vacillating and increasing IgG mediated phagocytosis when responding to the presence of the arginine-glycine-aspartate (RGD)-containing extracellular matrix proteins [18]. As a cell surface glycoprotein of the Ig superfamily, CD47 is a transmembrane protein that interacts with several molecules including the protein signal regulatory protein alpha (SIRPa) [19,20]. CD7 blocks phagocytosis mediated by the SIRPα protein. Binding to SIRPa, CD47 stimulates the SIRPa to initiate a signalling cascade. Signalling causes recruitment of the src homology-2 domain containing protein tyrosine phosphatases SHP-1 and SHP-2, resulting in signalling to inhibit phagocytosis [21], giving CD47 the title of the “don’t eat me signal” [22].

CALR and CD47 in Cancer Prognosis

CALR expression on the cell surface acts as the pro-phagocytic signal activating the clearance of aged or damaged cells [23], but also, as described in different human cancers (ie AML, acute lymphocytic leukaemia, chronic myeloid leukemia, ovarian cancer, glioblastoma, and bladder cancer [13]), has roles in calcium signalling, macrophage-mediated immune evasion and activation of the unfolded protein response (UPR), which is responsible for upregulation of molecular chaperones and degrading misfolded proteins. In solid tumours this is often counterbalanced by the concomitant expression of the anti-phagocytic CD47 [14]. Overexpression of CALR mRNA and cell surface over-exposure of CALR correlates with poor prognosis in mantle cell lymphoma, superficial and invasive bladder cancer, and neuroblastoma [13]. Chao et al. [13], showed a correlation between CALR expression and survival with high expression of CALR associating with worse survival and high counteracting over expression of CD47.

In some MPN cases, mutation within exon 9 of CALR has been found, causing loss of the C-terminal acidic domain, multiple calcium-binding sites, and the KDEL sequence [24], resulting in a dysfunctional protein and contributing to over-activation of JAK/STAT pathway leading to myeloproliferation [17]. However, mutated CALR has also been shown to cause abnormal localising expressions in the cytosol and membrane [24,25] and activation of the UPR, which is often linked to disease progression via arresting protein translation through the ER compartment [26].

Several studies have shown that targeting CALR translocation to the cell membrane may represent a new strategy in cancer therapy [27,28]. Translocation from within the cell to the cell membrane is required for the initiation of the pro-apoptotic signalling of CALR [15]. Treatment with chemotherapy drugs anthracyclines and the reticulum Ca2+ ATPase Inhibitor thapsigargin, induces CALR translocation to the cell surface via Endoplasmic Reticulum Resident Protein 57 (ERP57) (a member of disulfide isomerase family), enhancing the immune
response and resulting in apoptosis of the cancer cell [27,29]. In contrast, knocking out 
Ebp57 causes a with decreased surface expression of CALR and prevents the cells from being attacked by T
cells resulting in a cell survival benefit [30].

Expression changes in CD47 are a marker of phagocytosis, with decreased expression in aged or damaged cells leading to homeostatic phagocytosis. Patients suffering with cancer show high expression rates of CD47 which correlate with poor clinical outcomes. This is shown in both solid tumours [21] and also in haematological cancers including AML [31], when CD47 expression increases on the surface of cancer cells it signals for survival by evading immune surveillance, acting as a marker of anti-phagocytosis [32,33].

Emerging Role for CALR and CD47 in Myeloid Disorders

The CALR/CD47 expression ratio seems to direct the immune response in MDS/MPN in different ways depending on which signal is predominant, with high CALR expression signalling for phagocytosis and high CD47 expression protecting the cancer cells from attack by the immune system [34]. Macrophage-mediated immunosurveillance is a form of programmed cell removal (PrCR) by the immune system, where target cells are recognized and phagocytosed [35], which is independent of apoptotic removal of cells by the immune system. Studies have shown CD47 to be the protector of cancer cells from the PrCR by overpowering the PrCR signals [34]. Blocking SIRPs and CD47 allows for PrCR of cancer cells and not normal cells to be targeted due to the higher expression of the eat-me signals, such as CD47 which correlate with poor clinical outcomes. This is shown in both solid tumours [21] and also in haematological cancers including AML [31], when CD47 expression increases on the surface of cancer cells it signals for survival by evading immune surveillance, acting as a marker of anti-phagocytosis [32,33].

CALR and CD47 Targeting for Anti-cancer and Myeloid Malignancy Therapy

CALR can be used as a potential target for anti-cancer therapy using molecules able to enhance CALR expression and stimulate phagocytosis of the affected cells [15]. In prostate and lung cancer cells, CALR targeting adenovirus (Ad-CALR/MAGE-A3) have produced an up regulation of CALR and consequently an increased rate of apoptosis in the cancer cells [42]. Administration of a chimeric construct of CALR along with a chemotherapy agent increased treatment response in mice with mammary tumours [43], indicating the potential for combined therapy in mammary tumours. In addition, after treatment with anthracycline, CALR expression on the cell surface correlated with the overall removal of cancer cells and survival of the patients [44], resulting in the activation of a tumour-targeting immune response in AML, neuroblastoma, non-small cell lung carcinoma, ovarian cancer, and colorectal carcinoma [44].

Anti-CD47 antibodies can block CD47-SIRPa signalling and induce cancer cells death. Dheilly et al. [45] showed how combining an anti-
CD49 with an anti-CD47 was effective in inducing cell death in leukemia, lymphoma, pancreatic, and ovarian carcinomas cell lines. Chemotherapy drugs are considered as the first line treatment for cancers, however, chemotherapy is not always effective. Combination therapies have been proposed, using specific chemotherapy drugs in conjunction with anti-CD47 [46]. Liu et al [47] generated pre-clinical assessment of a humanised anti-CD47 antibody (Hu5F9-G4) which in combination with rituximab chemotherapy drug treatment, resulted in an initial reduction of all blasts in vivo. After day 159 blood counts (white blood cells, hemoglobin, and platelets), were seen to return to normal range [48]. Concomitantly, Wang et al, showed that combining cytarabine in conjunction with anti-CD47 antibody, increases the antitumor effects in AML comparing with cytarabine alone [48].

Blocking CD47 using CD47 siRNA could also represent a therapeutic strategy. Wang et al [49] showed efficacy of CD47 siRNAs in melanoma and lung metastases regression. The CD47 siRNA were intravenously administered and resulted in reduction of tumour size. Ghohamin et al [50] demonstrated inhibition of CD47 using humanized anti-CD47 antibody Hu5F9-G4, which increases the presence of activated macrophages in brain tumours. SIRPa dependent phagocytosis induced this by blocking the CD47 signal [31]. Macrophages then become active due to the increase in the phagocytic signals, activating macrophages to phagocytose cancer cells [34]. Further studies are being carried out into both CALR and CD47 targeting which makes the future of the area very interesting.
Conclusion

The role of CALR and CD47 in myeloid neoplasms is currently poorly understood. In solid tumours overexpression of CALR produces a pro-phagocytic signal [13] and it is often counteracted by a concomitant expression of the anti-phagocytic CD47 [14]. Over expression of CALR has been seen to correlate with poor prognosis as it is counteracted by increased CD47 increased expression, which is critical for cancer cell survival [34]. Several studies demonstrated how CD47 is often overexpressed in AML [31], allowing the leukaemia cell to evade immune surveillance and survive [32,33]. During MDS progression onto AML, the levels of expression of CD47 increase but is immediately counteracted by the concomitant CD47 overexpression, resulting in a predominate anti-phagocytic message. The expression of CD47 in MPN is not well described yet. Preliminary data from our laboratory indicate in MPN samples no increase of CALR expression is seen on the cell surface, whilst CD47 expression is significantly elevated in ET, PV and MF, indicating a possible different mechanism of immunoresistance comparing to solid tumour and AML.

Emerging therapeutic strategies targeting CALR and CD47 expression have offered an interesting insight into the roles of CALR and CD47 in myeloid neoplasms and their implication into pathogenesis and disease progression. Targeting CALR with the aim of increasing its expression on the cell surface using an adenosine (Ad-CALR/MAGE-A3) [42] resulted in significant increase in cell death and apoptosis. Blocking the CD47-SIRPa signalling pathway [31] by using Hu5F9-G4 has resulted in reduction of CD47 on cancers cells and consequently increases in cancer cell removal and tumour burden reduction [47].

CALR and CD47 play vital roles in the pro/anti - phagocytic mechanisms of cancer cells. Better understanding is required for their involvement in myeloid malignancies. CALR and CD47 targeted therapies alone or in combination with standard chemotherapy could result in increased survival of patients suffering with both haematological and solid cancers. Further studies are required to better fully understand the implication of CALR and CD47 expression/ regulation and their prognostic role in disease progression and treatment response in cancer and the myeloid malignancies.

References


