Targeting myeloperoxidase (MPO) for multiple myeloma immunotherapy

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Summary

Among dysregulated functions within the tumour microenvironment, oxidative and inflammatory functions play a critical role in tumour development. One such protein that has both pro-oxidative and proinflammatory properties is MPO. It is primarily expressed in myeloid cells as an oxidative antimicrobial agent but has most recently been found within the bone marrow tumour microenvironment. Its properties make it a promising target for the regulation of the development of cancers such as MM. It was discovered that MM expression and tumour cell homing increased towards areas with elevated myeloid cell expression and MPO activity. Methodically MPO was able to express critical MM growth factors and suppress immune response through reduction in cytotoxic T-cell activity. Therefore finding an inhibitor for MPO is a potentially viable method for preventing the development of MM. The irreversible MPO inhibitor 4-ABAH (4-aminobenzoic Acid Hydrazide) administered in small concentrations was found to reduce the MM tumour burden on affected mice. In short, the collected data show that MPO contributed to the development of tumour growth and that MPO specific inhibitors can serve as potential pharmaceutical strategies to limit MM disease progression.

Multiple myeloma (MM) is an incurable haematological malignancy characterised by abnormal growth of plasma cells in the bone marrow, such as binucleated plasma cells and abnormal growth (figure 1). MM is the most common haematological disease with a global presence of approximately 230,000 patients in a 5 year period and a mean age of 70. [1] MM consists of the overproduction of immunoglobulins and immunoglobulin light chains with no function from abnormal monoclonal plasma cells. A MM diagnosis requires the presence of greater than 10% of clonal plasma cells within the BM. Those cells must also be accompanied with at least one feature of end stage organ damage most often referred to as “CRAB” features (hypercalcemia, renal failure, anaemia, lytic bone lesions). MM is preceded with a condition known as Monoclonal Gammopathy of Undetermined Significance (MGUS). MGUS has a less than 10% bone marrow plasma cell and absence of end stage organ damage seen in MM. To determine when a patient develops MM from MGUS, screening techniques are used to observe when symptoms characteristic of MM emerge. Less than 1% of MGUS cases annually develop into MM through uncertain pathophysiological mechanisms. Even with our latest technology, the rate of relapse is still 50% after 5 years. The survival rate is similar, with only 55% surviving for longer than 5 years after initial diagnosis. [1]

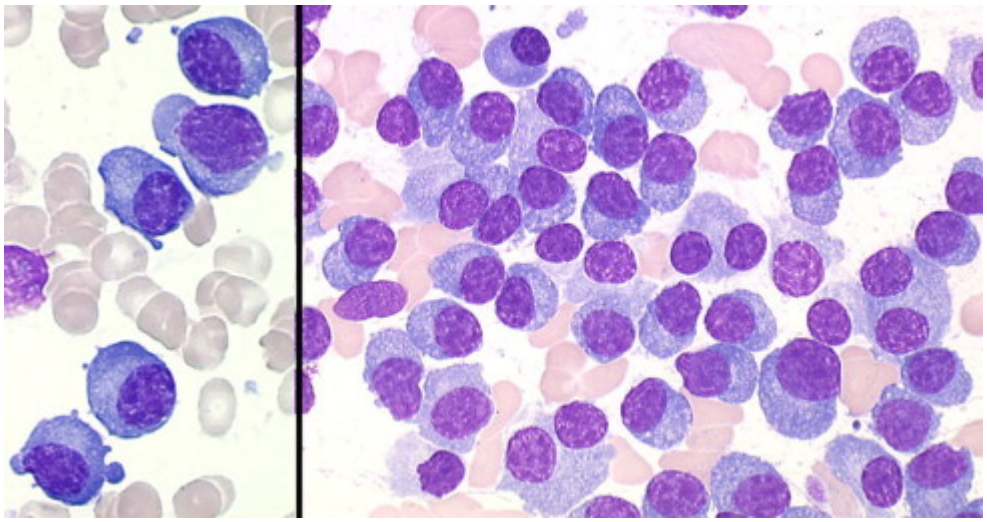

Figure 1: Plasma Cell Neoplasms in MM

Ribourtout, B., & Zandecki, M. (2015). Plasma cell morphology in multiple myeloma and related disorders. Morphologie, 99(325), 38–62. https://doi.org/10.1016/j.morpho.2015.02.001

MM plasma cells are characterised by their abnormal development. This includes cells with multiple nuclei, abnormally large nuclei, and high quantities of dysfunctional cells. These abnormalities can be seen in figure 1. The figure shows plasma cells from the bone marrow of a MM patient that have been stained purple. It can be clearly seen that some of the cells in the section on the right have 2 nuclei and a relatively large nucleus compared to the size of its cytoplasm. MM patients will have an average of 10% to 15% more plasma cells like the ones in figure 1 compared to healthy adults. Around 30% to 50% of patients will not have cases of plasma cells with multiple nuclei. Approximately 10% to 15% of patients will show abnormally small amounts of cytoplasm within plasma cells, independent of the size of the nucleus.

Myeloperoxidase (MPO) is a heme containing peroxidase expressed most abundantly in granular immune cells, such as polymorphonuclear leukocytes, lymphocytes, monocytes, and macrophages compared to most other cells. MPO is stored in azurophilic granules and released from them into the extracellular space by degranulation or exocytosis. The exact method for which MPO is excreted from the cell is not yet clear, however it is hypothesised that oxidative stress plays a key role in the release of MPO. [2] MPO plays a critical antimicrobial role in prominent immune cells such as neutrophils. Their antimicrobial effects are critical for an innate immune response facilitated by neutrophils. In addition to MPO, other compounds such as defensins, serine proteases, cathepsin G, alkaline phosphatase, lysozyme, NADPH oxidase, collagenase, lactoferrin, cathepsin, and gelatinase also have antimicrobial functions. However, of all those compounds within neutrophils, MPO constitutes 5% of neutrophil dry weight and 25% of azurophilic granule proteins [2]. The pathway of neutrophil responses to MPO can be seen in Fig 2.

MPO has its genetic coding location on the long arm of chromosome 17. The structure of MPO can be seen in figure 3. Its primary function is as an antibacterial agent by utilising H_2O_2 in addition to reactive oxygen and nitrogen species to oxidise several halides and pseudohalides to form hypohalous acids. The controlled release of MPO at the right location and time of infection is vital to its efficacy. Any unregulated release of MPO will exaggerate an inflammatory response and cause excessive damage even in an absence of inflammation. Diseases such as rheumatoid arthritis, diabetes, and certain forms of cancer have a reported correlation to MPO derived oxidants. As a result of this, MPO is one of the best biomarkers for diseases involving inflammatory and oxidative stress.

[2]

Novel studies have found a correlation between myeloid derived MPO and the bone marrow microenvironmental niche — where MM is localised. To test the correlation of MPO to the progression of MM, the 5TGM1 tumour bearing mouse model was used. Within the mouse model, it was shown that MM plasma cells can directly influence MPO expression in bone marrow derived myeloid cells. MPO itself can induce the expression of key MM growth factors and inhibit immunosuppressive antitumor T-cell capabilities. In the genetically identical syngeneic KaLwRij/5TGM1 mouse model of MM, the targeted inhibition of MPO using the irreversible inhibitor 4-ABAH demonstrated a significant reduction in overall MM tumour burden. These results show promise for the use of MPO therapeutically. [3]

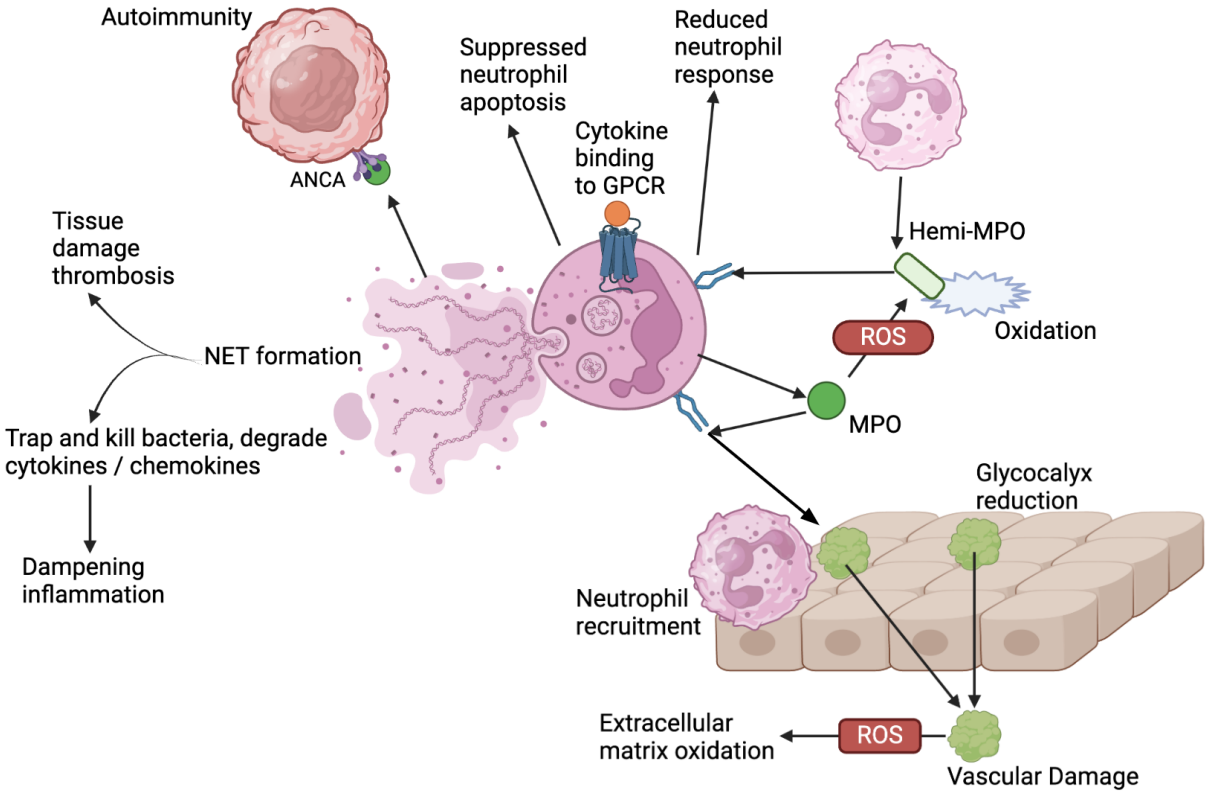

Figure 2: MPO trafficking within Neutrophils

Neutrophil activation through chemokine / cytokine receptors or activation through TLR9 leads to MPO release from primary granules. MPO function in causing tissue damage is formed through local formation of highly toxic oxidants. MPO positively regulates neutrophil movement to an inflamed area. It evokes neutrophil activity through degranulation and prevention of constitutive apoptosis from non-enzymatic processes as through CD11b. The MPO-CD11b complex creates a situational mechanism for controlling neutrophil mediated inflammation.

Dissociation of MPO into a smaller protein called hemi-MPO results in reduced activity and stunted neutrophil response.

MPO released intracellularly works in tangent with neutrophil elastase to promote chromatin decondensation, leading to the generation and exocytosis of Neutrophil Extracellular Traps (NETs).

NETs affiliated with MPO have more effective antimicrobial and chemokine/cytokine degrading properties. NETs have also been correlated to tissue damage and inception of thrombosis. Abnormal NET formation (or impaired NET degradation) leads to prolonged presentation of MPO antigens, triggering autoimmunity.

The glycocalyx regulates the movement of fluids between the endothelial cells and functions as barriers to macromolecules. In addition to the filter function, the glycocalyx is involved in cell-cell recognition, adhesion, membrane bending, tabulation, and moulding of the plasma membrane. Glycocalyx reduction can be achieved under hyperglycemic conditions (high blood sugar / glucose), where the glycocalyx is significantly reduced in thickness. This coincides with endothelial dysfunction and a general systemic increased vascular permeability in humans.

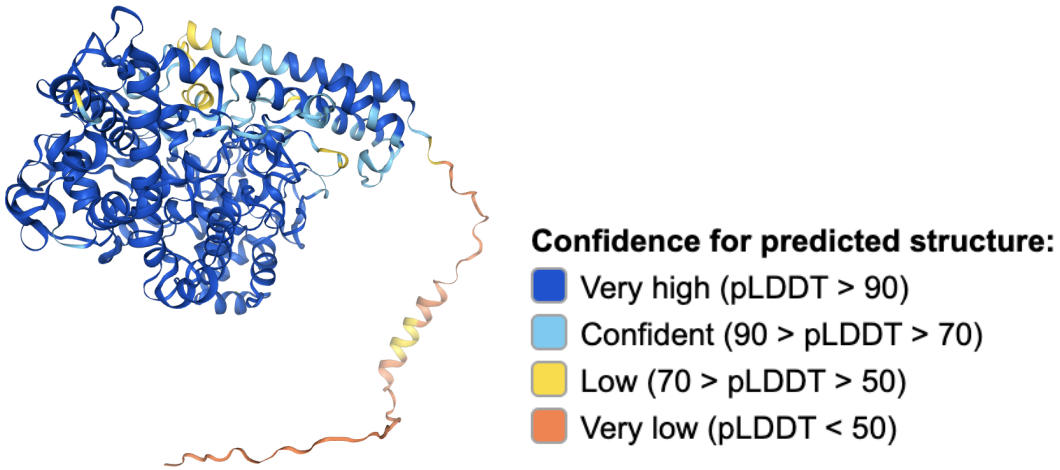

Figure 3: Structure of MPO
Image Credit: proteinatlas.org / ENSG00000005381-MPO

Abnormal MPO expression as a result of gene polymorphism can lead to DNA mutagenesis from MPO derived oxidants. In the promoter region of this gene, single nucleotide polymorphisms (SNPs) can potentially alter transcription and protein levels. Additionally, the specific substitution of thymidine in place of cytosine in codon 569 changes the amino acid expression from arginine to tryptophan, which can also cause certain genetic defects to MPO. [4]

In addition to gene polymorphism, MPO can also instigate the activation of specific genotoxic intermediates that can later go on to transform acrolein, a protein produced as a byproduct of saturated and unsaturated fatty acid metabolism. Acrolein can then be transformed into acrolein-protein adducts, which in humans causes benign colon cancer tumours to become malignant. There are many studies finding increased concentrations of MPO in patients with breast cancer compared to control groups. Breast cancer in particular is instigated from a variety of inflammatory enzymes, including those caused or derived from increased MPO concentrations. In this way MPO can serve as an effective marker for menopausal women suffering from breast cancer. [4]

MPO has also been found to be a hallmark enzyme of acute myeloid leukaemia (AML) from patients who showed high plasma MPO levels compared to control subject ranges. This demonstrates its presence in the bone marrow. [3], [5]

MPO is found in myeloid cells and is negatively associated with solid tumour development. Active myeloid cells are abundant in multiple myeloma (MM), and as such the function of MPO is brought into contention; however, the function of MPO and its downstream effects in the myeloma microenvironment are unknown. In this regard the function of MPO to impede MM progression and a potential role as a pharmaceutical target was investigated. Williams et al 2023 reported that in the 5TGM1-KaLwRij mouse model of myeloma, MM tumour development was associated with increased levels of CD11b+ myeloid cell populations along with an increase in MPO concentrations within the bone marrow. [4]

Taken together, this study from Connor Williams provides novel insights into the relationship between MM disease progression and myeloid-derived MPO. With limited available clinical therapies available to target the stromal environment; the identification of MPO as a potentially viable therapeutic target to reduce tumour progression in vivo offers potential for a new treatment strategy. Together with current procedures, this novel treatment can be incorporated to improve the quality of life of MM patients. [3]

Despite major improvements in treatments for patients with Multiple Myeloma (MM), an effective treatment that successfully eliminates long term clinical symptoms has yet to be discovered. In the last decade, innovations on the progression of MM have narrowed down the search to three main factors: immune evasion, disease progression, and persistence. Specifically, these factors were discovered to have an inverse relationship with MM plasma cells (PC) and the Bone Marrow (BM) microenvironmental niche. The inverse relationship is derived from MM PC and their reliance on the secretions of BM stromal cells for survival and growth. Therapeutic targeting of the BM microenvironment may prove to be an effective strategy for the elimination of root causes of MM and produce better therapies. The investigation of Myeloid-derived suppressor cells (MDSC), a heterogenous population of myeloid cells, is described to promote MM tumour progression through immunosuppression and induction of angiogenesis. Of the chemical messengers present in murine models of MDSCs, MPO experiences the greatest upregulation of gene expression. Being a key inflammatory enzyme for host defence, MPO accumulation in the tumour microenvironment has garnered interest in a multitude of studies aiming to describe the role of MPO in regulating cancer cell development due to its potent pro-oxidative and proinflammatory properties. [3], [6], [7]

William et al. reported the orally bioavailable and irreversible small molecule inhibitor of MPO (MPOi) was inoculated into 12-week old C57BL/6J mice intravenously using the preclinical Vk *MYC (Vk*14451-GFP) murine model.[7] Vk*MYC cell tumour progression was monitored through serum paraprotein electrophoresis (SPEP) while endpoint GFP+ cells in the bone marrow were quantified using flow cytometry. qPCR was used to measure MPO expression in myeloid derived cell populations from the Vk*MYC tumour landscape. The results showed the frequency of CD11b+ cells were significantly increased in the bone marrow of Vk*MYC tumour bearing mice compared to the vehicle control with MPO mRNA expression also notably greater. The researcher found that mice treated with MPOi had a significantly decreased tumour burden at the end of the 9 week treatment period compared to vehicle control mice. Specifically, hind limb GFP% and SPEP yielded a 15.2% and 31.4% decrease respectively of MM tumour development. MPOi was also found to potentially serve a role in regulating the bone marrow microenvironment and impede MM disease progression. A mRNA analysis of bone marrow cells from mice treated with MPOi demonstrated an

upregulation of critical cytokine IFN gamma and downregulation of the potent proangiogenic factor VEGF. However, there was no significant difference in end-point tumour burden when MPOi was administered after the first signs of MM (Identified through detection of monoclonal spike proteins from SPEP five weeks after tumour inoculation). These results suggest that the efficacy of MPOi to inhibit MPO is most effective during early stages of multiple myeloma tumour progression and loses effectiveness during progression. With limited therapies currently available to combat the stromal microenvironment in MM. The studies above indicate how MPOi can be a promising novel treatment when combined with current frontline therapeutic agents. [3]

Conclusion

The findings highlighted in this paper show some of the functional roles of myeloid derived MPO in the bone marrow microenvironment of MM. Specifically, it was shown that myeloid cell populations increased in the bone marrow of 5TGM1 tumour bearing mice inoculated with MM that have the capability to influence MPO gene expression. MPO was observed to induce expression of key MM growth factors and exerts immunosuppressive activity by inhibiting anti-tumour T-cell responses. The irreversible suppressor 4-ABAH and irreversible small molecule inhibitor MPOi both showed promise in reducing MPO activity and tumour burden of MM patients.

Myeloid Derived Suppressor Cells (MDSCs) are a heterogenous population of myeloid cells described to promote MM tumour progression through immunosuppression and induction of angiogenesis. One of the highest reported upregulated genes in murine models of MM is MPO. Its properties as a key inflammatory enzyme in host defence with pro-oxidative effects allows it to assist in the regulation of the tumour microenvironment. All of these factors of MPO make it attractive as a potential therapeutic target for the progression of MM.

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