Myeloma and marrow adiposity; unanswered questions and future directions

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Abstract

Multiple myeloma (MM) is a haematological malignancy characterised by the proliferation and accumulation of terminally differentiated abnormal plasma cells in the bone marrow. Patients suffer from bone pain, factures, anaemia, osteolytic lesions and renal failure. Despite recent advancement in therapy MM remains an incurable disease due to the emergences of drug resistance and frequent relapse. For many decades, research has been heavily focused on understanding the relationship between bone cells such as osteoblast, osteocytes and osteoclasts and the infiltrating tumour cells. However, it is now clear that the tumour-supportive bone microenvironment including cellular and non-cellular components play an important role in driving MM progression and bone disease. One of the most abundant cell types in the bone microenvironment is the bone marrow adipocyte (BMAd). Once thought of as inert space filling cells, they have now been recognised as having specialised functions, signalling in an autocrine, paracrine and endocrine manner to support normal systemic homeostasis. BMAds are both an energy store and a source of secreted adipokines and bioactive substances, MM cells are able to hijack this metabolic machinery to fuel migration, growth and survival. With global obesity on the rise, it has never been more important to further understand the contribution these cells have in both normal and disease settings. The aim of this review is to summarise the large body of emerging evidence supporting the interplay between BMAds and MM cells and to delineate how they fit into the vicious cycle of disease.

Introduction

Multiple myeloma (MM) is a debilitating haematological malignancy characterised by the clonal expansion of abnormal plasma cells in the bone marrow. Normal, functional polyclonal Ig populations are replaced by massive amounts of non-functional monoclonal Ig that are produced by the myeloma cells. The accumulation of these cells within the marrow leads to an overall reduction in blood cell production as the haematopoietic tissue is progressively replaced, resulting in systemic complications such as anaemia, immune deficiencies and renal failure. Moreover, MM cells do not just change the cellular landscape and compromise the immune system they also cause alterations in the cellular behaviour of resident cells leading to significant changes in marrow architecture, one of the most prominent changes is the uncoupling of bone remodelling resulting in the development of osteolytic bone disease [1]. Bone homeostasis is normally a balanced and dynamic process of bone resorption followed by new bone formation. MM cells disrupt this process, promoting increased bone resorption and suppressing new bone formation resulting in the formation of lytic bone lesions leading to bone pain, pathological fractures, cord compression, hypercalcemia and generalised osteoporosis. Even though MM management has come a long way since the introduction of the chemotherapy agent melphalan in the 1960s [2], with the development of new therapeutic agents such as immune-modulators (e.g. thalidomide, lenalidomide and pomalidomide), proteasome inhibitors (e.g. bortezomib, carfilzomib and ixazomib) and monoclonal antibodies (e.g. elotuzumab and daratumumab) MM remains a cripplingly incurable disease with the vast majority of cases relapsing [3]. So why is myeloma such a successful disease? MM is a genetically complex and heterogenous disease resulting from multiple genetic events, over time these changes lead to drug resistance due to clonal evolution, alterations in glucose metabolism, upregulation or mutations of pro-survival pathways [4]. MM cells manipulate their neighbouring cells to create a protective and permissive environment, driving disease progression by promoting changes in the bone microenvironment. So, what makes the bone an attractive environment? One of the main reasons is the constant active process of bone

remodelling, osteoclasts secrete factors that break down bone matrices, generating a bone-derived growth factor rich environment with the release of matrix proteins such as transforming growth factor β (TGFβ) and insulin-like growth factor (IGF), as well as calcium into the bone microenvironment. These factors bind to receptors on the tumour cell surface and activate SMAD and MAPK signalling, extracellular calcium binds and activates calcium pumps leading to tumour cell proliferation [5] thereby driving MM progression within the endosteal niche. The bone is a hub of activity both on the inner surface and within the central marrow, providing structural integrity to the skeleton as well as a home to an abundance of cell types such as nerve cells, endothelial cells, hematopoietic stem cells, macrophages, and lymphocytes, osteocytes, chondrocytes and adipocytes. In order to maintain healthy bone and normal blood cell production these cells provide a supportive niche via cellular cross talk. All these cell types communicate with each other via mechanisms such as cell-cell contacts, exosome release, or by soluble factors. MM cells are incredibly adept in manipulating this supportive communication network, disrupting or changing the levels of communication between these cells both directly and indirectly to provide a permissive environment in which to evade treatment and promote aggressive clonal expansion. To start to fully understand how MM cells shape their surrounding environment a broader more holistic view needs to be taken. Bone cells may be central to bone destruction, but it is now clear that there are other cells within the marrow that have an influential effect. One of the most abundant of these is the bone marrow adipocyte (BMAds). In the past this population of cells have been overlooked, regarded as inert space “fillers”, however, in the last few decades that has changed quite considerably as the research community has learnt more about their function and the soluble factors (adipokines) they secrete. It is becoming clearer that these cells play an important role in regulating both the local bone microenvironment as well as contributing to overall body homeostasis.

The importance of fat

Adipose tissue is the largest endocrine organ in the body responsible for energy storage as well as local and systemic signalling via the secretion of numerous different adipokines, cytokines and chemokines that play important roles in normal metabolic homeostasis [6]. With the growing global pandemic of obesity, it is becoming even more vital that the role of adipose tissue is fully understood in both normal and disease settings. When we think of adipose tissue this usually conjures up an image of white subcutaneous fat (fat just underneath the skin) that contributes to a person's body shape, this is not surprising as subcutaneous adipose tissues store >80% of total body fat in the body [7]. However, there are four distinct populations of adipocytes in the body: white, brown, beige and marrow. They all have their own distinct morphology and adipokine profile [8]. Out of the four populations, bone marrow adipose tissue (BMAT) is the least studied, primarily due to its accessibility within the bone cavity. Over the last few decades this difficulty and lack of data and understanding of the functional roles of these cells has started to be addressed with the development of new tools such as advances in non-invasive MRI- and CT-based imaging technologies [9], and advances in our understanding of how to provoke changes in BMAT levels such as high fat diet, PPARγ agonist treatment and irradiation [10]. These new innovations coupled with new transgenic reporter mouse strains and the ability to quantify BMAT *in vivo* [11] has allowed the field to grow and start to appreciate the role that BMAT plays in the maintenance of healthy bone, as well as its role in bone loss and disease establishment and progression. Osteoblasts and adipocytes arise from a common mesenchymal stem cell (MSC) progenitor within the bone marrow [12] and many osteoporotic states, such as aging, medication use, over and undernutrition are associated with an increase in marrow adiposity. The bone marrow is unique in the fact that it is spatially restricted, therefore BMAT expansion can come with a cost. As with many processes in the body there is a fine but competitive balance between osteoblastogenesis and adipogenesis, with MSCs shifting towards either lineage in response to a complex interplay of systemic and local mediators, such as elevated glucocorticoid levels, estrogen withdrawal, oxidative stress and sedentary behaviour promoting adipogenesis and pro-ostogenic factors such as growth hormone, IGF-1, Wnt proteins, estrogen and mechanic output promoting

osteoblastogenesis [13, 14]. Thus, when environmental ques tip in favour of adipogenesis this can have a negative impact on bone integrity and fragility. A patterned increase of marrow fat within the long bones is a normal process of aging with red hematopoietic tissue, mainly in the bones of the extremities, gradually being replaced in a symmetrical centripetal fashion from the periphery to the central skeleton by non-hematopoietic MSCs that form adipocytes. The exact reason for this huge conversion from red to yellow marrow is not clear. Although it is thought that the amount of red marrow may correlate with the number of healthy proliferating MSCs. As we age our MSC pool capable of supporting haematopoiesis decreases promoting the accumulation of adipose tissue [15] and thus, negatively regulating hematopoiesis [16]. Many diseases such as cancer preferentially effect the elderly. As we age our bodies become more susceptible to mutations, DNA damage and dysregulated gene function. BMAT expansion changes the cellular landscape altering the ratio of resident cells promoting new direct and indirect cellular interactions and possibly promoting a more permissive environment for tumour cells to establish and thrive.

Adiposity and MM progression

Aging and obesity are well-known risk factors for MM, obesity has been shown to increase risk by 20% compared to a lean individual [17]. Furthermore, diet-induced obesity has been shown to increase IGF1 levels in mice creating a permissive microenvironment for the progression of MM from a preceding condition known as monoclonal gammopathy of undetermined significance (MGUS) [18]. These are important associations, understanding the factors that contribute to the transformation of MGUS to MM could aid MM management and inform new prevention guidelines [19]. Studies often use body mass index (BMI) as a measure of obesity. However, the contribution to disease progression may vary depending on which fat depots are significantly enlarged and or involved at the disease site. Central adiposity, as measured by waist circumference, was shown to be associated with increased MM mortality [20]. The transition from MGUS to full-blown MM was shown to be related to total abdominal

adipose tissue surface and metabolic activity [21] and, excess visceral adipose tissue (VAT) was associated with poor treatment response [22]. Excess BMAT is no exception being implicated in both poor prognosis and drug resistance. Patients with higher BMAT prior to treatment were found to have less favourable outcome with BMAT affording a level of protection against chemotherapy induced cell death [23]. Moreover, studies have shown that as well as external pressures such as diet, irradiation and medication BMAT volume can be directly influenced by MM cells themselves. Patients suffering from MM exhibit an expansion of BMAT and have an increase in the number of preadipocyte as well as significantly larger mature adipocytes than in normal bone marrow [24]. Changes in adipocyte number was also observed early in disease onset in a preclinical murine model of myeloma [25]. Whether this is a consequence of osteoblast dysfunction skewing lineage cell fate or whether it is a direct result from the infiltrating tumour cells has not yet been elucidated. However, what is clear is that these findings support the growing link between obesity and MM and highlight some of the changes that occur in the bone microenvironment to aid MM establishment and progression. Moreover, the pressures that MM cells exert on BMAds not only alter cell number and size, but they also alter the factors these cells secrete.

Adipokine changes associated with MM progression

The serum levels of the adipokine leptin are related to energy stores such that leptin levels correlate with fat mass and increased adipocyte size, leptin has been shown to be upregulated in MM patients [26] [27] and also found to correlate with clinical stage [28]. Leptin is one of the most well-known and highly studied adipokine. It is a peptide hormone that acts centrally via the hypothalamus to regulate food intake and energy expenditure. Its main function is to mediate satiety, stimulate lipolysis and suppress lipogenesis. However, it also elicits pro-tumour effects by enhancing the expression of autophagic proteins, reducing apoptosis, stimulating MM cell proliferation and reducing the anti-tumour effect of chemotherapy via activation of AKT and STAT3 pathways as well as the upregulation of Bcl-2 expression and

the inhibition of caspase-3 activation. [28]. Increased leptin secretion also indirectly supports MM progression suppressing anti-tumour immunity by promoting invariant natural killer (iNKT) cell dysregulation [29]. Thus, targeting leptin-leptin receptor signalling maybe a promising therapeutic approach.

Another adipokine that is dysregulated in MM is adiponectin, this is the most abundantly expressed adipokine in the bone marrow and is often referred to as a 'guardian' adipokine as it has anti-inflammatory, anti-atherogenic and insulin sensitizing properties [30]. High levels of adiponectin have been linked with leanness and longevity with overexpression increasing adipocytes number rather than size, allowing for an expansion of triglyceride storage capacity whilst avoiding stress from excessive lipid content. Adiponectin also exhibits anti-tumour effects activating cell cycle arrest and apoptosis through the activation of p21 and p53 [31]. As well as decreasing cellular proliferation and increasing apoptosis of MM cells via the activation of AMPK and MAPK [32]. Low levels of adiponectin have been associated with several obesity-related complications such as type 2 diabetes, hypertension, insulin resistance and cardiovascular disease as well as several types of cancers such as breast, liver, colorectal cancer and myeloma [32, 33]. Serum levels of adiponectin were found to be significantly lower among patients with smoldering (an intermediate disease stage) and fully developed myeloma compared to MGUS patients suggesting that adiponectin could potentially enhance prediction models for progression from MGUS to MM [34]. Moreover, obesity induces a hypoxic microenvironment, which is known to inhibit adiponectin transcription via hypoxia inducible factor 1 alpha (HIF1α) [35]. Furthermore, obesity-induced chronic inflammation also leads to overexpression of TNFα, IL-6 and IL-18 which also inhibit adiponectin [36]. Therefore, obese MGUS patients may potentially be at risk of exacerbating their condition and accelerating transformation to full blown disease. The full mechanisms associated with the reduction of adiponectin are still being debated with numerous contributing factors such as transcription factors, soluble factors and post-translational modifications [33]. In the context of MM, adiponectin was also found to be downregulated by MM cells themselves via the secretion of

208 TNF-α [25], suggesting that it is an important step in MM cell survival. Furthermore,
209 adiponectin was found to inhibit osteoclast differentiation and maturation via the mTOR
210 pathway [37], thereby contributing to MM associated bone disease. In addition, decreased
211 adiponectin expression increased bone pain sensitivity through the regulation of nerve growth
212 factor (NGF), suggesting that adiponectin could also have analgesic properties [38]. Targeting
213 adiponectin is complex as it works in concert with other important hormones including insulin,
214 leptin, and various cytokines making its pharmacological exploitation more challenging.
215 Various strategies have been developed to modulate adiponectin levels in order to harness its
216 beneficial effects [32]. However, unfortunately these drugs have shown minimal effect in the
217 clinic. While pharmacological intervention has not shown promise, simple weight loss
218 strategies may be effective in MGUS patients or in conjunction with other conventional
219 therapies. Even though adiponectin intervention alone may not be sufficient to confront MM it
220 deserves some appreciation in the protective role it plays in the pathology of the disease.

221 IL-6 is a major morbidity factor for MM [39] and is a key mediator of tumour cell proliferation
222 and survival with elevated levels found in the serum of MM patients. It is secreted by immune
223 and stromal cells as well as adipocytes and tumour cells themselves and creates a pro-
224 oncogenic environment stimulating anti-apoptotic effects, tumour expansion, drug resistance
225 and angiogenesis as well as increased osteoclastic differentiation and activity leading to bone
226 destruction [40]. Due to the integral role of IL-6 in MM progression it was thought that clinically
227 targeting it alongside conventional therapies could be a promising approach. However clinical
228 trial outcomes have been disappointing, showing no additional benefit to chemotherapy in
229 patients with high-risk smouldering MM [41]. These results could be attributed to MM cell
230 plasticity, with the evolution and expansion of clones that are less dependent on IL-6 or that
231 conventional therapies already dampen IL-6 expression. Although IL-6 blockade may not be
232 a therapy that can be widely administered, it could have some promise in personalised
233 medicine, only being administered to patients that have persistently high IL-6 expression even
234 after cessation of conventional treatments.

Other less known adipokines/cytokines have also been identified as mediators of disease. Both MCP-1 and (SDF)-1α promote MM cell migration [24]. Apelin [42] and chemerin [43] are shown to be elevated in the serum of MM patients and have been identified as potential diagnostic markers. Whilst others such as adipsin [44] and resistin [45] been implicated in the protection of MM cells from chemotherapy induced apoptosis. This seems to be a common theme linking BMAds and MM cells, with a combination of factors contributing to support BMAds protective behaviour. A recent study by Fairfield and colleagues further demonstrated this MM promoting relationship, whereby MM cells alter BMAds gene expression and cytokine secretory profile, and in return BMAds provide resistance to dexamethasone-induced-cell-cycle arrest [23]. These are interesting findings and pose the question as to whether MM cells manipulate BMAds for the soluble factors they secrete, or whether the link between these two cell types is also a physical one.

MM and BMAd metabolism

In late-stage MM the bone marrow is packed with infiltrating tumour cells coupled with a significant loss in BMAT. It is not clear what happens to these BMAds, whether they are out crowded and destroyed, they shrink, or they dedifferentiate in response to the tumour cells. However, a recent study showed that loss of BMAT did not correlate with tumour burden, dismissing the notion that this effect was due to out crowding alone. Furthermore, *in vitro* co-culture experiments have demonstrated that when in direct contact with MM cells BMAds numbers and size decrease [23, 25]. This was found to be associated with a marked reduction in adipogenic transcripts coupled with the expression of a senescence-associated secretory phenotype (SASP), increasing basal oxygen consumption rates and nonmitochondrial respiration [23]. Furthermore, these changes were also observed in preadipocytes that had been exposed to MM cells early in the differentiation process suggesting that MM cells may influence cells at all stages, from mesenchymal stem cells (MSCs) to mature adipocytes [46]. These metabolic changes induced cellular dysfunction resulting in the up-regulation of

inflammatory pro-survival factors such as IL-6 and IL-18 [23]. Emerging studies have also shown that intracellular lipolysis is critical for fuelling cancer cells, and that the overexpression of lipid trafficking proteins such as fatty acid binding proteins have been associated with lipid transfer from adipocytes to cancer cells promoting tumour growth and survival [47, 48] Furthermore lipolysis has also been shown to promote drug resistance, prostate cancer cells co-cultured with BMAds increased expression of IL1-β stimulating BMAd lipolysis in a positive feedback loop that decreased prostate cancer cell sensitivity to docetaxel and up-regulated pro-survival signalling via prostaglandin E2 (PGE2) synthesis in BMAds [49]. Lipid transfer has been demonstrated between BMAds and MM cells [50], although its impact on MM progression and survival is still not well understood, adipocyte-supplied lipid may provide essential fatty acids for new membrane formation, play a role in the biosynthesis of lipid signalling molecules as well as provide vital energy [51]. Targeting fatty acid transport or uptake proteins has been shown to impede MM cell growth [52], more studies are needed to further understand the implications of targeting fatty acid metabolism in MM and whether modulating dietary fatty acid intake could also be a new therapeutic approach [53].

Exosomes

Recently, exosomes have received a great deal of attention as important mediators of inter-cellular signalling between tumour cells and cells of the microenvironment. Exosomes are membrane-bound vesicles that are shed by a wide variety of cell types inclusive of bone marrow stromal cells (BMSCs) (progenitors of BMAds) and tumour cells. They mediate intercellular communication both locally and systemically by transporting cell-specific bioactive substances such as lipids, nucleic acids and proteins. They exert their functions by transferring their cargo to recipient cells either by endocytosis or direct fusion with the cell membrane [54]. In the context of MM, this horizonal transfer of molecular or/and genetic information ensures constant crosstalk between MM cells and both neighbouring and distant cells within the microenvironment, supporting MM pathogenesis by promoting immunosuppressive effects,

angiogenesis, osteolysis and drug resistance [55]. miRNAs are among the most important signalling molecules which are packaged into exosomes, and the miRNA profile found in MM patients can differ significantly compared to healthy individuals. MM patients exhibit elevated levels of miRNAs such as miR-21 [56], miR-181a/b [57] miR-146a [58], let-7b and miR-18a [59] which promote MM proliferation, survival and migration, while others such as the tumour suppressor miR-15a and miR-16-1 are markedly reduced [60, 61]. The exosome release from BMAds is relatively unexplored, however several studies have demonstrated the role of exosomes in the crosstalk between white adipose tissue (WAT) and tumour cells [62-64], this coupled with the known contribution of BMSCs suggests that BMAds may also actively shed biologically relevant material. Moreover, the proteome of exosomes released for WAT showed an enrichment in proteins associated with lipid metabolism (e.g., FAO-catalysing enzymes) which significantly enhanced FAO in melanoma cells [64]. It may be that BMAd-originating exosomes might exacerbate tumour progression by remodelling cancer cell metabolism.

Spatial imaging

In order to study the direct interactions between BMAds and tumour cells more understanding of their spatial distribution and cellular relationships within the marrow is needed. Many valuable conclusions are drawn using murine tissue as to the distribution of both proliferating and non-proliferating MM cells in the bone niches. The use of flow cytometry and mass cytometry on human liquid biopsies have also contributed a wealth of knowledge as to lineage cell numbers as well as the specific markers they express [65, 66]. However, by using this technique any spatial information is lost making it impossible to address any geographical questions. Spatial data can shed light as to which cells sit next to which, and whether there are any common cellular constellations. These sorts of neighbourhood studies allow further investigation into the topography of the disease, studying it in a more holistic fashion which may reveal new cellular behaviours and interactions. New imaging techniques using next generation imaging such as the fluorescence-based microscopy systems CODEX® (CO-

316 Detection by indEXing) and Vectra® Polaris® Automated Quantitative Pathology Imaging
317 System or the mass cytometry system, Hyperion, are starting to bridge this gap by using single
318 cell technology combined with high-multiplex imaging on intact tissue [67]. These new
319 technologies allow us to address important questions. One evolving area of research that
320 could benefit from this is tumour cell dormancy, addressing the question as to whether the
321 exact spatial location of a MM cell in relation to its surrounding cells is important in inducing
322 this latent state. Human MM differs in its pattern of distribution in the marrow, which is
323 sometimes overlooked when using an animal model, as the cells that are used to inoculate
324 the animals are often from the same clone thus behave in a similar manner in the marrow. In
325 human MM, different cases can show differing morphology patterns. By studying a large
326 portion of the cellular landscape on the same tissue section more information can be drawn
327 as to why these cells behave differently. These new imaging techniques allow for around 40
328 different markers to be visualisation in parallel while preserving cellular morphology and tissue
329 architecture. Thus, using a well-designed panel of reliable markers the vast majority of cell
330 populations can be simultaneously tagged and analysed making it possible to map and decode
331 the precise cellular and spatial relationship between MM cells and the cells of the bone
332 microenvironment. These imaging techniques are not the only advances in studying spatial
333 interactions. The development of more sophisticated *in vitro* 3D co-culture models using
334 bioengineered materials to create a more robust physiological environment is paving the way
335 for more intimate studies of direct cellular interactions and signalling between neighbouring
336 cells. Fairfield and colleagues developed the first tissue engineered bone marrow adipose
337 model using silk fibroin scaffolds. They found that adipocytes took on a more physiological
338 morphology with sustained growth for up to 3 months, which is far greater than the average
339 2D culture. The addition of MM cells caused delipidation and shrinkage of the adipocyte
340 populations mimicking the changes seen in 2D cultures and some mouse and human tissue
341 samples [68]. These new engineered approaches along with new and advanced imaging
342 techniques will undoubtedly push research forward.

Conclusions

MM is a highly complex disease with the contribution of many cell types and signalling pathways supporting its pathogenesis. MM cells thrive by hijacking the host's metabolic machinery, promoting an environment that is protective and nutrient rich (figure 1). BMAds still hold many unlocked secrets that are slowly but surely being discovered. As with most multifactorial diseases the outcome is a sum of its parts and BMAds are an integral part of the puzzle. Targeting one cell type or signalling pathway is unlikely to overwhelm the disease, and as its history has dictated MM is capable of adapting and evolving to utilise its surrounding environment to promote continually survival. With the onset of obesity comes an expansion of cells that are pushed to their limits, secreting an increased level of pro-inflammatory factors in response to stress. MM cells are able to use these factors for their own survival advantage thereby promoting disease progression. New advancements in technologies will continue to aid the identification of novel secreted factors that drive MM progression and its associated bone disease, further picking apart the symbiotic relationship between MM cells, BMAds and the environment that surrounds them.

References

1. Kumar, S.K., et al., *Multiple myeloma.* Nat Rev Dis Primers, 2017. **3**: p. 17046.
2. Alexanian, R., et al., *Melphalan therapy for plasma cell myeloma.* Blood, 1968. **31**(1): p. 1-10.
3. Bazarbachi, A.H., et al., *Relapsed refractory multiple myeloma: a comprehensive overview.* Leukemia, 2019. **33**(10): p. 2343-2357.
4. Lohr, J.G., et al., *Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy.* Cancer Cell, 2014. **25**(1): p. 91-101.
5. Mundy, G.R., *Mechanisms of bone metastasis.* Cancer, 1997. **80**(8 Suppl): p. 1546-56.
6. Deng, T., et al., *Obesity, Inflammation, and Cancer.* (1553-4014 (Electronic)).
7. Lee, M.J., Y. Wu, and S.K. Fried, *Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications.* Mol Aspects Med, 2013. **34**(1): p. 1-11.
8. Craft, C.S., et al., *Bone marrow adipose tissue does not express UCP1 during development or adrenergic-induced remodeling.* Sci Rep, 2019. **9**(1): p. 17427.
9. Singhal, V. and M.A. Bredella, *Marrow adipose tissue imaging in humans.* Bone, 2019. **118**: p. 69-76.

10. Sebo, Z.L., et al., *Bone Marrow Adiposity: Basic and Clinical Implications.* Endocrine reviews, 2019. **40**(5): p. 1187-1206.
11. Cawthorn, W.P., et al., *Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction.* Cell Metab, 2014. **20**(2): p. 368-75.
12. Hasegawa, T., et al., *The PPARgamma-selective ligand BRL-49653 differentially regulates the fate choices of rat calvaria versus rat bone marrow stromal cell populations.* BMC Dev Biol, 2008. **8**: p. 71.
13. Sadie-Van Gijsen, H., et al., *The interrelationship between bone and fat: from cellular see-saw to endocrine reciprocity.* Cell Mol Life Sci, 2013. **70**(13): p. 2331-49.
14. Hardouin, P., T. Rharass, and S. Lucas, *Bone Marrow Adipose Tissue: To Be or Not To Be a Typical Adipose Tissue?* Front Endocrinol (Lausanne), 2016. **7**: p. 85.
15. Gurevitch, O., S. Slavin, and A.G. Feldman, *Conversion of red bone marrow into yellow - Cause and mechanisms.* Med Hypotheses, 2007. **69**(3): p. 531-6.
16. Naveiras, O., et al., *Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment.* Nature, 2009. **460**(7252): p. 259-63.
17. Lauby-Secretan, B., et al., *Body Fatness and Cancer — Viewpoint of the IARC Working Group.* New England Journal of Medicine, 2016. **375**(8): p. 794-798.
18. Lwin, S.T., et al., *Diet-induced obesity promotes a myeloma-like condition in vivo.* Leukemia, 2015. **29**(2): p. 507-10.
19. Chang, S.H., et al., *Obesity and the Transformation of Monoclonal Gammopathy of Undetermined Significance to Multiple Myeloma: A Population-Based Cohort Study.* J Natl Cancer Inst, 2017. **109**(5).
20. Teras, L.R., et al., *Body size and multiple myeloma mortality: a pooled analysis of 20 prospective studies.* Br J Haematol, 2014. **166**(5): p. 667-76.
21. Veld, J., et al., *Abdominal adipose tissue in MGUS and multiple myeloma.* Skeletal Radiology, 2016. **45**(9): p. 1277-1283.
22. GroΔ, J.P., et al., *Body fat composition as predictive factor for treatment response in patients with newly diagnosed multiple myeloma - subgroup analysis of the prospective GMMG MM5 trial.* Oncotarget, 2017. **8**(40): p. 68460-68471.
23. Fairfield, H., et al., *Myeloma-Modified Adipocytes Exhibit Metabolic Dysfunction and a Senescence-Associated Secretory Phenotype.* Cancer Res, 2021. **81**(3): p. 634-647.
24. Trotter, T.N., et al., *Adipocyte-Lineage Cells Support Growth and Dissemination of Multiple Myeloma in Bone.* (1525-2191 (Electronic)).
25. Morris, E.V., et al., *Myeloma Cells Down-Regulate Adiponectin in Bone Marrow Adipocytes Via TNF-Alpha.* J Bone Miner Res, 2020. **35**(5): p. 942-955.
26. Pamuk, G.E., et al., *Leptin and resistin levels in serum of patients with hematologic malignancies: correlation with clinical characteristics.* (1812-9269 (Print)).
27. Reseland, J.E., et al., *Abnormal adipokine levels and leptin-induced changes in gene expression profiles in multiple myeloma.* (1600-0609 (Electronic)).
28. Yu, W., et al., *Adipocytes secreted leptin is a pro-tumor factor for survival of multiple myeloma under chemotherapy.* (1949-2553 (Electronic)).
29. Favreau, M., et al., *Leptin receptor antagonism of iNKT cell function: a novel strategy to combat multiple myeloma.* Leukemia, 2017. **31**(12): p. 2678-2685.
30. Ouchi, N. and K. Walsh, *Adiponectin as an anti-inflammatory factor.* Clin Chim Acta, 2007. **380**(1-2): p. 24-30.
31. Nigro, E., et al., *New insight into adiponectin role in obesity and obesity-related diseases.* (2314-6141 (Electronic)).
32. Fowler, J.A., et al., *Host-derived adiponectin is tumor-suppressive and a novel therapeutic target for multiple myeloma and the associated bone disease.* Blood, 2011. **118**(22): p. 5872-82.

33. Parida, S., S. Siddharth, and D. Sharma, *Adiponectin, Obesity, and Cancer: Clash of the Bigwigs in Health and Disease.* Int J Mol Sci, 2019. **20**(10).
34. Hofmann, J.N., et al., *Circulating Adiponectin Levels Differ Between Patients with Multiple Myeloma and its Precursor Disease.* Obesity (Silver Spring), 2017. **25**(8): p. 1317-1320.
35. Chen, B., et al., *Hypoxia dysregulates the production of adiponectin and plasminogen activator inhibitor-1 independent of reactive oxygen species in adipocytes.* Biochem Biophys Res Commun, 2006. **341**(2): p. 549-56.
36. Ellulu, M.S., et al., *Obesity and inflammation: the linking mechanism and the complications.* Arch Med Sci, 2017. **13**(4): p. 851-863.
37. Liu, Z., et al., *Adiponectin inhibits the differentiation and maturation of osteoclasts via the mTOR pathway in multiple myeloma.* Int J Mol Med, 2020. **45**(4): p. 1112-1120.
38. Olechnowicz, S.W.Z., et al., *Multiple myeloma increases nerve growth factor and other pain-related markers through interactions with the bone microenvironment.* Scientific reports, 2019. **9**(1): p. 14189-14189.
39. Gu, J., et al., *Cytokine profiles in patients with newly diagnosed multiple myeloma: Survival is associated with IL-6 and IL-17A levels.* Cytokine, 2021. **138**: p. 155358.
40. Harmer, D., C. Falank, and M.R. Reagan, *Interleukin-6 Interweaves the Bone Marrow Microenvironment, Bone Loss, and Multiple Myeloma.* Front Endocrinol (Lausanne), 2018. **9**: p. 788.
41. Brighton, T.A., et al., *Randomized, Double-Blind, Placebo-Controlled, Multicenter Study of Siltuximab in High-Risk Smoldering Multiple Myeloma.* Clin Cancer Res, 2019. **25**(13): p. 3772-3775.
42. Maden, M., O.N. Pamuk, and G.E. Pamuk, *High apelin levels could be used as a diagnostic marker in multiple myeloma: A comparative study.* Cancer Biomark, 2016. **17**(4): p. 391-396.
43. Westhrin, M., et al., *Chemerin is elevated in multiple myeloma patients and is expressed by stromal cells and pre-adipocytes.* Biomark Res, 2018. **6**: p. 21.
44. Liu, Z., et al., *Mature adipocytes in bone marrow protect myeloma cells against chemotherapy through autophagy activation.* (1949-2553 (Electronic)).
45. Pang, J., et al., *Resistin induces multidrug resistance in myeloma by inhibiting cell death and upregulating ABC transporter expression.* (1592-8721 (Electronic)).
46. Fairfield, H., et al., *Multiple Myeloma Cells Alter Adipogenesis, Increase Senescence-Related and Inflammatory Gene Transcript Expression, and Alter Metabolism in Preadipocytes.* Front Oncol, 2020. **10**: p. 584683.
47. Yang, D., et al., *Utilization of adipocyte-derived lipids and enhanced intracellular trafficking of fatty acids contribute to breast cancer progression.* Cell Commun Signal, 2018. **16**(1): p. 32.
48. Nieman, K.M., et al., *Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth.* Nat Med, 2011. **17**(11): p. 1498-503.
49. Herroon, M.K., et al., *Prostate Tumor Cell-Derived IL1β Induces an Inflammatory Phenotype in Bone Marrow Adipocytes and Reduces Sensitivity to Docetaxel via Lipolysis-Dependent Mechanisms.* Mol Cancer Res, 2019. **17**(12): p. 2508-2521.
50. Falank, C., H. Fairfield, and M.R. Reagan, *Signaling Interplay between Bone Marrow Adipose Tissue and Multiple Myeloma cells.* Front Endocrinol (Lausanne), 2016. **7**: p. 67.
51. Diedrich, J.D., et al., *The Lipid Side of Bone Marrow Adipocytes: How Tumor Cells Adapt and Survive in Bone.* Curr Osteoporos Rep, 2018. **16**(4): p. 443-457.
52. Farrell, M., et al., *Targeting bone marrow adipose tissue and the FABP family increases efficacy of dexamethasone in MM.* Clinical Lymphoma Myeloma and Leukemia, 2019. **19**(10, Supplement): p. e89-e90.
53. Jurczyszyn, A., et al., *Plasma fatty acid profile in multiple myeloma patients.* Leuk Res, 2015. **39**(4): p. 400-5.
54. Zijlstra, A. and D. Di Vizio, *Size matters in nanoscale communication.* Nature cell biology, 2018. **20**(3): p. 228-230.

55. Faict, S., et al., *Exosomes play a role in multiple myeloma bone disease and tumor development by targeting osteoclasts and osteoblasts.* Blood cancer journal, 2018. **8**(11): p. 105-105.
56. Leone, E., et al., *Targeting miR-21 inhibits in vitro and in vivo multiple myeloma cell growth.* Clin Cancer Res, 2013. **19**(8): p. 2096-106.
57. Rossi, M., P. Tagliaferri, and P. Tassone, *MicroRNAs in multiple myeloma and related bone disease.* Ann Transl Med, 2015. **3**(21): p. 334.
58. De Veirman, K., et al., *Induction of miR-146a by multiple myeloma cells in mesenchymal stromal cells stimulates their pro-tumoral activity.* Cancer Letters, 2016. **377**(1): p. 17-24.
59. Manier, S., et al., *Prognostic role of circulating exosomal miRNAs in multiple myeloma.* Blood, 2017. **129**(17): p. 2429-2436.
60. Roccaro, A.M., et al., *BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression.* J Clin Invest, 2013. **123**(4): p. 1542-55.
61. Li, F., et al., *MicroRNA-15a/16-1 cluster located at chromosome 13q14 is down-regulated but displays different expression pattern and prognostic significance in multiple myeloma.* Oncotarget, 2015. **6**(35): p. 38270-82.
62. Rios-Colon, L., et al., *The Role of Exosomes in the Crosstalk between Adipocytes and Liver Cancer Cells.* Cells, 2020. **9**(9).
63. Wang, S., et al., *Exosomes secreted by mesenchymal stromal/stem cell-derived adipocytes promote breast cancer cell growth via activation of Hippo signaling pathway.* Stem Cell Res Ther, 2019. **10**(1): p. 117.
64. Lazar, I., et al., *Adipocyte Exosomes Promote Melanoma Aggressiveness through Fatty Acid Oxidation: A Novel Mechanism Linking Obesity and Cancer.* Cancer Res, 2016. **76**(14): p. 4051-7.
65. Fakhari, S., et al., *CD93 is Selectively Expressed on Human Myeloma Cells but Not on B Lymphocytes.* Iran J Immunol, 2019. **16**(2): p. 142-150.
66. Smets, T., et al., *Deep Profiling of the Immune System of Multiple Myeloma Patients Using Cytometry by Time-of-Flight (CyTOF).* Methods Mol Biol, 2018. **1792**: p. 47-54.
67. Tan, W.C.C., et al., *Overview of multiplex immunohistochemistry/immunofluorescence techniques in the era of cancer immunotherapy.* Cancer Communications, 2020. **40**(4): p. 135-153.
68. Fairfield, H., et al., *Development of a 3D bone marrow adipose tissue model.* Bone, 2019. **118**: p. 77-88.