

REVIEW

Multiple myeloma may include microvessel endothelial cells of malignant origin

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Abstract

Multiple myeloma (MM) comprises B and plasma cell compartments that originate from the same parent B cell and share as a cancer signature the same clonotypic IgH VDJ gene rearrangement. Here, we hypothesize that functional interactions between MM plasma cells (MM-PC) and their sister population of MM monocytoid B cells lead to the generation of microvessel endothelium of malignant origin from the monocytoid B cell progenitors. Published reports confirm that endothelial cells can harbor a molecular cancer signature characteristic of a given malignancy. We predict that MM monocytoid B cells—in response to both paracrine and autocrine pathways—contribute to tumor neovascularization in the bone marrow of MM patients. Our hypothesis further predicts that in MM, endothelial cells of malignant origin coexist with those of normal origin. We speculate that malignant development of MM incorporates functionally distinct sister lineages arising from the same MM progenitor that—by working together—ensure survival of the MM clone. We hypothesize that these two arms of the malignant MM clone are functionally interlinked to promote growth of the MM-PC compartment; by providing its own microenvironment, MM clonal evolution may ensure neovascularization to support an expanding malignancy.

Keywords: *Myeloma, leukemic progenitor cells, molecular genetics*

Introduction

Multiple myeloma (MM) is an incurable cancer that accounts for 1% of all cancers, 10% of all hematological malignancies, and 19% of deaths from hematological malignancies. It kills over 15 000 North Americans each year, and the median survival is 3–4 years. MM is characterized by the presence of monoclonal immunoglobulin in the blood, lytic bone lesions, and monoclonal plasma cells in the bone marrow. MM may arise through progressive acquisition of diverse genetic abnormalities, and is characterized by complex chromosomal abnormalities. The MM clone is definitively identified by a unique IgH (immunoglobulin heavy) VDJ gene rearrangement, termed clonotypic [1]. As summarized below, a variety of work leads to the idea that the generative compartment in MM includes B lineage cells in the bone marrow, the blood, or both, at a stage of differentiation preceding that of plasma cells. Our

work shows that MM is a hierarchy of B lineage cells that includes circulating drug-resistant malignant B cells with stem cell-like properties [2–4]. Matsui and coworkers have also described a drug resistant CD20+ MM population with stem cell characteristics [5,6]. The B cell compartments of the MM clone, including MM monocytoid B cells and MM plasma cells, are clinically relevant, based on observations that xenografted MM B cells give rise to lytic bone disease and clonotypic progeny and are self-renewing in immunodeficient mice [7,8]. Clonotypic MM cells with the phenotype of hematopoietic progenitors xenograft human MM to immunodeficient mice [9], and aberrant splicing restricted to the MM B cell compartment correlated with reduced survival in a cohort of MM patients [10]. MM clonotypic B cells express the RHAMM (receptor for hyaluronan mediated motility) oncogene, a centrosomal protein [11] shown by us to mediate centrosomal and chromosomal abnormalities

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[12], and to correlate with poor outcome in MM patients [13].

Our work suggests that all compartments of MM B lineage cells arise from a CD20+ MM cancer stem cell that harbors the clonotypic IgH VDJ, defining it as a B cell [2]. MM cancer stem cells appear to localize in the endosteal niche of bone marrow, and have characteristics expected of cancer stem cells including self-renewal, harboring unique cancer signatures, proliferative quiescence, drug resistance, and the ability to regenerate the MM clone.

Hypothesis

- That the MM clone carries within itself the potential to generate its own microvessel endothelium to support neovascularization.

Corollaries

- That MM cancer stem cells give rise to at least two functionally distinct lineages, one leading to MM plasma cells (MM-PC) and another leading to MM monocytoïd B cells.
- That MM monocytoïd B cells, shown to harbor the MM clonotypic signature, give rise to endothelial cells harboring the clonotypic signature and tumor specific chromosomal abnormalities.
- That paracrine release of angiogenic factors by MM-PC, together with autocrine pathways, stimulates the differentiation of MM monocytoïd B cells to vascular endothelial cells (VEC).

Predictions

- That at least some endothelial cells in tumor neovascular endothelium harbor the clonotypic IgH VDJ gene rearrangement that is unique to each MM clone.
- That at least some endothelial cells in MM neovascular endothelium harbor chromosomal abnormalities characteristic of autologous MM-PC.
- That MM tumor neovascular endothelium is chimeric, being a mixture of normal endothelial cells with those of malignant origin.

Experimental support for the hypothesis that multiple myeloma cancer stem cells give rise to endothelial cell progeny

Paracrine and autocrine circuits in MM

Chen and colleagues [14] reported that MM plasma cells (MM-PC) produce pleiotrophin (PTN), an

angiogenic factor. They further showed that PTN derived from MM-PC, together with macrophage colony stimulating factor (M-CSF) and vascular endothelial growth factor (VEGF), stimulates the 'transdifferentiation' of vascular endothelial cells (VEC) from normal monocyte progenitors. Others have shown that in MM, presumptively normal hematopoietic stem and progenitor cells give rise to vascular endothelium [15], implying that this may in fact represent a normal differentiation pathway for hematopoietic progenitors, perhaps involving monocytoïc cells, rather than some type of aberrant 'transdifferentiation'. Integrating these observations with work of ourselves and others suggests a new paradigm for MM as a cancer able to generate a vascular network of malignant origin that contributes to tumor neovascularization. We have shown that MM monocytoïd cells have autocrine stimulatory networks [16], including an M-CSF/M-CSF receptor circuit. Here, we speculate that under the influence of angiogenic factors secreted by MM-PC, clonotypic MM cells with monocytoïd morphology undergo differentiation to cells of the endothelial lineage and contribute VEC of malignant origin to the microvessel endothelium in MM bone marrow (Figure 1). This suggests that in MM, VEC populations and microvessels may be chimeric; we speculate

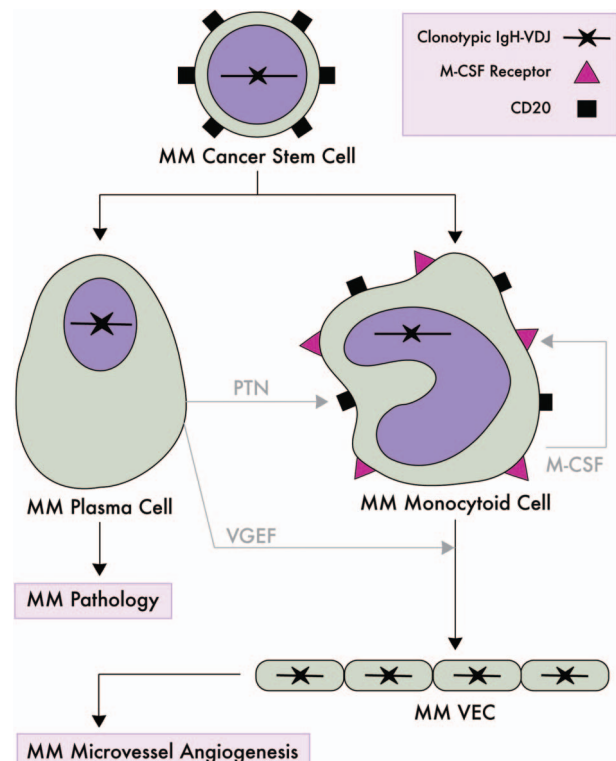


Figure 1. Generation of vascular endothelial cells (VEC) from multiple myeloma (MM) cancer stem cells.

that MM microvessel endothelium represents a mixture comprising VEC of malignant origin and VEC of non-malignant origin. VEC derived from normal monocytes will, by definition, lack MM-specific molecular signatures. VEC derived from MM monocytoïd cells will, by definition, harbor the MM-specific IgH VDJ molecular signature, and may also harbor MM-specific chromosomal abnormalities.

Malignant origin of endothelial cells in B lineage cancers

Recent findings show that endothelial cells derived from the bone marrow of patients with MM have an 'overangiogenic' phenotype and, in some respects, resemble transformed cells [17]. Several reports indicate that at least some endothelial cells from cancer patients harbor cancer signatures. As predicted above, in MM some VEC harbor MM-specific signatures, including the 13q14 deletion [18], and genomic clonotypic IgH VDJ gene rearrangements [19]. Clonotypic IgH VDJ, defined as the IgH VDJ gene rearrangement that characterized the malignant clone in each MM patient, was detected in endothelial progenitor cells from 5/7 patients tested [19]. Consistent with the idea that endothelial cell populations may be chimeric, 11–32% of circulating endothelial cells (CEC) harbored del 13 in MM [18]; the deletion was absent from CEC of MM patients whose malignancy lacked del 13, and CEC were found to be normal in patients with monoclonal gammopathy of undetermined significance (MGUS) even when the MGUS clone itself harbored del 13 [18]. Lymphoma-specific chromosomal aberrations are found in VEC of B cell lymphomas, harbored by 15–85% of microvascular endothelial cells [20], again indicative of chimerism. Taken together, these reports confirm that VEC can harbor a molecular cancer signature characteristic of a given malignancy. The aggregate population of microvessel endothelial cells in a given cancer patient may include a subset that originates from malignant progenitors, defined here as having a direct clonal relationship with the B lineage malignancy. Thus, based on this published evidence, at least some tumors appear to have the potential to generate microvessel endothelium of neoplastic origin, defined as sharing cancer-specific clonal markers that are otherwise thought to be restricted to malignant B lineage cells.

Monocytoïd B cells in the MM clone

Our work has documented that in MM, CD19+20+ B cells harbor clonotypic IgH VDJ gene rearrangements [1,3,21] and tumor-specific chromosomal abnormalities [22,23]. CD20+ clonotypic B cells

expand in three-dimensional cultures and have characteristics of cancer stem cells [2]. A large subset of these CD19+20+ cells have IgH VDJ rearrangements and express IgH molecules [21,24], defining them as B cells, but have the morphology of monocytes, as identified by histology and characteristic monocyte light scatter properties [3,21,25]. As is also the case for MM plasma cells, morphological characteristics remain the gold standard for identification of monocytes or monocytoïd cells. The relationship between MM monocytoïd cells and normal monocytes may be one of 'convergent evolution,' during which cells from different lineages acquire similar morphological characteristics, likely due to functionally driven imperatives. Here, the term 'monocytoïd' is used to designate an observed cell type that is clearly a B cell (based on its rearranged genomic IgH VDJ and expression of clonotypic IgH VDJ transcripts), but that has at least some of the physical/functional characteristics expected of monocytes. A similar naming convention has been applied to cells that have 'plasmacytoïd' characteristics but are not identifiable as *bona fide* plasma cells. MM monocytoïd B cells are adherent [26], as distinct from normal B cells but similar to normal monocytes. In contrast to normal monocytes, however, MM monocytoïd cells harbor signature molecular markers (clonotypic IgH VDJ rearrangements [1,3,24], DNA aneuploidy [21], and chromosomal abnormalities [22,23]) that identify them as malignant B cells. Like monocytes, MM monocytoïd B cells express CD11b [27,28], CD14 (unpublished), which is known to be expressed by B cells [29,30], and CD56 [25]. They also express CD34 [24] and CD151 [31]. Work by others has identified unusual cells in MM that express CD33 [32], including those with monocytoïd morphology [33], and CD117 [34]. However, given the many observations of phenotypic markers that are unexpectedly expressed on malignant cells, the presence or absence of a given marker or set of markers cannot be viewed as definitive evidence for or against particular lineage relationships.

Clonotypic MM monocytoïd cells express interleukin-6 (IL-6) and receptors IL-6R [16]. Further indicating a functional relationship with monocytes, and of particular relevance for this discussion, MM monocytoïd B cells express M-CSF receptors, and produce M-CSF (unpublished). They are drug resistant as defined by their persistence during and after chemotherapy [1,25,35], and constitutively express the p-glycoprotein drug transporter [36], a functional property shared with normal monocytes [37,38]. *Ex vivo* cell populations harboring MM monocytoïd B cells engraft human MM to the bone marrow of NOD SCID (non-obese diabetic/severe

combined immunodeficiency) mice [4,7], as defined by the presence of the IgH VDJ clonotypic signature. Despite the fact that multiple subsets of MM B lineage cells express CD20 [3], treatment with anti-CD20 has not proved to be a successful therapeutic strategy in MM [39], likely reflecting the observation that CD20+ B lineage cells, including MM monocytoid B cells, are not depleted during or after such therapy [3]. MM cancer stem cells, which have the morphology and scatter properties of lymphocytes [2], are a rare population of CD20+ cells that may be depleted by targeting CD20. In support of this idea, CD20+ clonogenic MM cells were depleted *in vitro* by anti-CD20 [5]. *Ex vivo* antibody targeting of lymphocytic and monocytoid B cells in G-CSF mobilized blood, followed by cytoreductive therapy and transplant of these 'purged' autografts, has been shown to improve event-free survival in MM [40], perhaps representing more effective depletion of B lineage subsets, and suggestive of their clinical importance.

Hypothesis: functional cooperation between two arms of the multiple myeloma clone generates microvessel endothelium of malignant origin

Chen *et al.* [14] show that MM-PC secrete PTN and VEGF, and that MM-PC-derived factors, together with M-CSF, stimulate the differentiation of healthy monocytes to VEC, and to microvessels. Our work shows that a large proportion of monocytoid B cells in MM are clonotypic [1] and DNA hyperdiploid [21], and harbor MM-specific chromosomal abnormalities [22,23]. Thus, in MM, a source of angiogenic factors (MM-PC) coexists with a set of putative endothelial cell progenitors of malignant origin (MM monocytoid B cells). Given the extensive similarities between MM monocytoid B cells and normal monocytes, it is plausible to suggest that both hold the potential for endothelial cell differentiation. Together, these two sets of observations indicate that the theoretical requirements for the generation of malignant microvessel endothelium have been experimentally met.

We propose that MM monocytoid B cells, like normal monocytes, respond to paracrine PTN and VEGF from MM-PC, under the influence of autocrine M-CSF, by generating VEC and tumor microvessels in MM-BM (Figure 1). Although it is possible that MM cells harbor the potential for cellular plasticity, it seems more likely that the generation of tumor microvessels occurs via a cytokine-directed program of differentiation inherent to at least some monocytoid compartments of the MM clone. Clearly this type of program also directs

normal monocytes toward formation of microvessels [14], perhaps as a rarely detected but important functional capability distinct from stem cell plasticity or cellular transdifferentiation, that bypass tissue restrictions. Microvessel endothelium in MM patients is predicted to be chimeric; unlike normal VEC, endothelial cells of malignant origin will have the clonotypic IgH VDJ signature in their genome. If correct, this means that at least some VEC are progeny of MM cancer stem cells, as predicted in Figure 1. The differentiation to VEC via MM monocytoid B cells is predicted to contribute to MM pathology by generating microvessel endothelium of malignant origin, thereby promoting clonal expansion of MM-PC and progression of MM.

Conclusions

We speculate that the malignant development of MM incorporates functionally distinct sister lineages arising from the same MM progenitor that—by working together—ensure survival of the MM clone (Figure 1). We have shown that MM includes multiple compartments of B lineage cells that share the same clonotypic signature [3]. As shown in Figure 1 and supported by a large body of evidence from our laboratory, clonotypic MM cancer stem cells, shown by us to be self-renewing B cells that express CD20 and genomic clonotypic IgH VDJ [2], generate both MM-PC and clonotypic MM monocytoid B cells [41]. We hypothesize that these two arms of the malignant MM clone are functionally interlinked by paracrine and autocrine pathways to promote growth of the MM-PC compartment. By developing the capability to provide its own microenvironment, MM clonal evolution ensures neovascularization to support an expanding malignancy. The existence of multiple compartments of the MM clone and the hypothetical involvement of VEC suggest the need for a combination of therapeutic approaches that together will target the entirety of the MM clone.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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