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Modeling Multiple Myeloma by AID-Dependent Conditional Activation of MYC

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Efforts to create a mouse model that provides even a phenocopy of human multiple myeloma (MM) have been unsuccessful. In this issue of *Cancer Cell*, Bergsagel and colleagues describe an apparent solution to this problem by creating a model in which a MYC transgene containing a stop codon and flanking Igκ regulatory sequences is activated sporadically in germinal center B cells by AID-dependent somatic hypermutation that reverts the stop codon. Although much remains to be done to fully characterize this model, this approach is likely to impact the creation of sporadic models for other kinds of germinal center B cell tumors.

The term "multiple myeloma" was suggested by von Rustizky in 1873, when he found during autopsy *multiple* separate bone marrow (*myel*-) tumors (-*oma*) (Malpas et al., 2004). Multiple myeloma (MM) is a post-germinal center tumor of long-lived bone marrow (BM) plasmablasts/plasma cells (PC) that have undergone extensive somatic hypermutation of Ig heavy chain (IgH) and Ig light chain (IgL) genes, antigen selection, and productive IgH switch recombination. Most tumors produce IgG or IgA, with IgM produced by only 1% of tumors. MM usually is preceded by a similarly age-dependent premalignant tumor called monoclonal gammopathy of undetermined significance (MGUS), the most common lymphoid tumor in humans, occurring in nearly 3% of individuals over the age of 50. For MGUS, the serum monoclonal Ig (M-Ig) typically is 5–30 g/l (normal polyclonal Ig is 7–15 g/l), with the tumor cells comprising no more than 10% of BM mononuclear cells. Although MGUS usually remains stable for many years, there can be sporadic progression to frankly malignant MM expressing the same M-Ig at a rate of 1% per year. Smoldering MM, with a stable BM content of 10%–30% tumor cells, has a higher rate of sporadic progression. Frankly malignant MM usually is progressive and is associated with secondary pathologies, sometimes including osteoporosis, osteolytic lesions, anemia, immunodeficiency, and decreased kidney function. Similar to long-lived PC, MGUS and MM have a strong dependence on the BM microenvironment for survival and growth, although some individuals develop extramedullary MM, or PC leukemia, most often as a very late event. In contrast to long-lived PC, MGUS and MM have the ability to proliferate at a low rate, usually with only a few percent of cycling cells until advanced stages of MM.

Early oncogenic events that are shared by MGUS and MM include primary IgH translocations (40%) and hyperdiploidy (50%), with dysregulation of a CYCLIN D gene being a unifying event for all tumors (Chng et al., 2007). Based on these early events, it has been proposed that MGUS and MM can be classified into at least five distinct molecular groups (diseases) that are associated with different biological and clinical characteristics. No genetic abnormalities distinguish MM and MGUS, although activating RAS mutations occur in 30%–40% of MM tumors but only 5% of MGUS tumors. Among shared genetic events that occur during progression of MM, rearrangements of *MYC* (rarely *MYCN*) represent a very late progression event that appears to be associated with increased proliferation and decreased stromal cell dependence.

The development of animal models for MM, including both de novo and syngeneic (Vanderkerken et al., 2003) or xenogenic (human MM into SCID-Hu, SCID-Rab, or NOD-SCID mice) (Matsui et al., 2008; Yata and Yaccoby, 2004) mouse transplant models

Table 1. Some Properties of Ex Vivo Murine Multiple Myeloma Models and Human Multiple Myeloma

	Balb/c PCT	BCLXL × iMYC	EμXBP1	WT C57BL6	Vk*MYC	Human MM
Reference	Potter, 2003	Cheung et al., 2004	Carrasco et al., 2007	Radl, 1999	Chesi et al., 2008	Chng et al., 2007
Mouse strain	Balb/c	mixed #	C57BL6	C57BL6	C57BL6	—
Tumor location	EM	EM, BM	BM, EM	BM	BM	BM
Major M-Ig	IgA > IgG	IgG > IgM	IgM > IgG	IgG > IgM	IgG	IgG > IgA
Ig hypermutation	sometimes	sometimes	sometimes	always (?)	always	always
Proliferation	aggressive	aggressive	indolent	indolent	indolent	indolent
Transplantable	yes	yes	?	yes	yes	yes (to mice)
MYC dysregulation	yes	yes	?	?	yes	late only (?)

PCT, plasmacytoma; EM, extramedullary; BM, bone marrow; Vk*MYC, transgenic hu c-MYC with stop codon and Igκ regulatory elements; #, BCLXL on FVB/N background × iMYC on C57BL6 × 129SvJ background.

has been an ongoing effort. Although the present work (Chesi et al., 2008) clearly provides the most promising de novo model, some of the previously described de novo models are described below (Table 1).

Nearly 50 years ago, Potter developed the transplantable Balb/c mouse peritoneal plasmacytoma model (Potter, 2003). Important features of this model include susceptibility only in Balb/c and NZB mice, dependency on chronic inflammation, a long latency, and high penetrance. Significantly, Ig translocations that dysregulate *MYC* occur in all tumors, including preneoplastic foci of aberrant PC that are found in the peritoneal granuloma. The introduction of other genes (e.g., *v-abl*, *BCL2*, *BCLXL*, *IL6*) transgenically or by infection with virus changed some of the characteristics (above) of this model, but the outcome still was mostly extramedullary plasmacytoma, but sometimes with BM involvement. Despite the deficiencies of this model for MM, it has been seminal in many fundamental discoveries, including structure and rearrangements of Ig genes, biosynthesis of Ig, *MYC* translocations, the role of IL6 and the microenvironment in the survival and growth of plasma cell tumors, identification of tumor susceptibility genes, and hybridoma formation.

Van Ness, Janz, and colleagues have reported that PC tumors were generated in mixed inbred mice that coexpressed c-MYC and BCLXL driven by Ig enhancers (Cheung et al., 2004). These short-latency, aggressive tumors mostly were extramedullary plasmacytoma, but in some cases there also was BM involvement that sometimes was associated with lytic bone disease.

Radl and his colleagues reported a mouse model of MGUS in 1974, about 10 years after MGUS was described in humans (Radl, 1999; Vanderkerken et al., 2003). They found that C57BL6 mice had an age-dependent occurrence of serum M-Ig (IgG > IgM), with more than 50% of mice having at least one M-Ig at 2 years of age. The level of M-Ig was stable and was associated with BM PC that could be transplanted no more than twice. Subsequently, they identified seven continuously transplantable MM tumors in about 0.5% of 2-year-old C57BL6 mice. These tumors share most properties with human MM. Sometimes tumor cells were also found in spleen, perhaps reflecting the fact that the spleen is a significant site of hematopoiesis in mice but not in humans. Apart from the lack of *Ig/MYC* translocations by karyotype, very little is known about genetic abnormalities in these tumors, including identification of the genes that enable a high incidence of MGUS in these mice.

Recently, it was reported that an Eμ-XBP-1s transgenic C57BL6 model generated tumors that have the properties expected for MGUS and MM (Carrasco et al., 2007). However, there are major problems with this report: only a limited number of mice were analyzed for M-Ig and BM PC content, and more generally, the mice and tumors have been incompletely characterized: of six tumors, four produced IgM and only two produced IgG; only three of six tumors had somatically mutated Ig; evidence of clonality for many of these tumors was lacking or unconvincing; the tumors were not tested for transplantability; and analysis of the gene expression profiling data does not seem consistent with a PC phenotype. In addition, nontransgenic C57BL6 mice should have an incidence of M-Ig spikes comparable to what they found for their transgenic mice, and it is unclear why they did not find this in their control mice (Chesi et al., 2008; Radl, 1999). It is important that these problems are resolved before the Eμ-XBP-1s mouse can be accepted as a valid model for de novo MGUS and MM.

The present paper describes an elegant model in which a MYC transgene containing a stop codon and flanking Igκ regulatory sequences is activated sporadically in germinal center B cells by AID-dependent somatic hypermutation that reverts the stop codon (Chesi et al., 2008). Instead of the expected generation of Burkitt's lymphoma (which eventually occurred in 2 of 122 mice), all transgenic C57BL6 mice develop age-dependent mono- or pauciclonal PC tumors that mainly produce IgG, have a high frequency of Ig mutations, are minimally proliferative, and are localized in the bone marrow. The PC content of the BM is variable, consistent with a diagnosis of MGUS in some mice but MM in most mice. Anemia and osteoporosis occur in many mice, and some mice develop osteolytic lesions. In addition, chemotherapy sensitivity profiles for these tumors appear to be similar to clinical activity in human MM. Approximately one-third of these mice progress to extramedullary MM tumors that are localized in spleen or other lymphoid tissues, and sometimes in ascitic fluid. Immunization of the transgenic mice results in a similar age-dependent incidence of tumors, with about 25%

producing a M-Ig directed against the antigen, and about 75% of tumors progressing to extramedullary MM. One striking feature of human MM that is not present in most mice is the consistent suppression of polyclonal Ig (G, A, and M), but this may be due to low grade polyclonal PC transformation that does not generate detectable M-Ig.

Although the de novo model described here more fully recapitulates human MM than any of the other models (Table 1), many questions remain. First, is the role of MYC the same in this model as in human MM? Although difficult to prove, the authors propose that the dysregulation of *MYC* in these mice is a secondary event that drives one of the nascent MGUS tumors in the C57BL6 mouse towards MM. They provide evidence that MYC expression is increased in human MM compared to MGUS, but there is considerable overlap of MYC expression in human MGUS and MM. Moreover, full dysregulation of *MYC* by genomic rearrangements appears to be a late event in the progression of MM and is rarely, if ever, associated with the MGUS-to-MM transition (Chng et al., 2007). Second, is there truly an MGUS phase that progresses to MM in this model, or does the slow increase in tumor mass merely reflect the low rate of tumor proliferation? Third, apart from the dysregulation of *MYC*, what other genetic abnormalities occur in the MGUS, MM, and extramedullary tumors in this model, and do they mimic the oncogenic events that have been described in human MGUS and MM tumors? Fourth, how much tumor proliferation occurs during transplantation, and what is the phenotype of the cell that proliferates? The answers to these and other questions not only will reveal how well this model mimics the human disease, but may also provide new insights into the pathogenesis and biology of human MGUS and MM.

A final point of interest in this study is the observation that AID-deficient C57BL6 mice do not develop age-dependent MGUS (Chesi et al., 2008). Others have reported that AID is required for germinal center but not pregerminal center lymphomagenesis and have suggested that errors in AID-dependent somatic hypermutation and/or IgH switch recombination mechanisms are important contributors to pathogenesis of these tumors (Pasqualucci et al., 2008). There is no doubt that these AID-dependent processes sometimes cause oncogenic events (e.g., IgH translocations) in lymphoma and MM. Still, it seems likely that long-lived PC, and perhaps some kinds of germinal center or post-germinal center lymphocytes, cannot be generated in the absence of AID. Therefore, one cannot conclude that AID is required to mediate genetic changes in all germinal center and post-germinal center tumors.

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