c-maf in multiple myeloma: An oncogene enhancing tumor-stroma interactions

Gene expression profiling studies reported by Hurt et al. in this issue of *Cancer Cell* reveal an unexpectedly frequent overexpression of *c-maf* in multiple myeloma and identify molecular targets of *c-maf* transactivation. The results define *c-maf* as a prototype of a class of oncogenes that not only stimulate cell cycle progression, but also promote pathological interactions between tumor and stroma cells.

Multiple myeloma (MM) is a currently incurable neoplasm of terminally differentiated B cells characterized by clonal expansion of malignant plasma cells in the bone marrow, development of lytic bone lesions, and the presence of monoclonal immunoglobulin (Ig) in serum and/or urine. MM is not an uncommon malignancy, as it accounts for 2% of all cancer deaths and nearly 20% of deaths caused by hematological malignancies. It is often preceded by a premalignant plasma cell disorder referred to as monoclonal gammopathy of undetermined significance (MGUS), which progresses to malignant MM or related disorders at an approximate rate of 1% per year. MM is usually confined to the bone marrow (intramedullary MM), as it is critically dependent on myeloma cell/bone marrow stroma interactions regulating tumor cell growth, survival, and migration. With terminal progression to a more aggressive disease phase, myeloma cells can become independent of the marrow microenvironment and acquire the ability to grow at extramedullary sites, such as blood ("plasma cell leukemia") or pleural fluid.

Insights into the molecular pathogenesis and biology of MM have been gained mainly from studies on the fundamental genetic abnormalities linked with the transformation and clonal evolution of malignant plasma cells and from studies delineating the interactions of myeloma cells with their bone marrow microenvironment. Relating the data from cytogenetics and Ig gene mutational analyses to clinical phases of the disease has generated a model of a multistep transformation process from normal plasma cells through MGUS to malignant MM (Kuehl and Bergsagel, 2002; Seidl et al., 2003). Apart from the advances in myeloma genetics, investigations elucidating the complex myeloma-host interactions have recently culminated in the identification of a wealth of targets for novel therapeutic approaches aiming at direct induction of myeloma cell apoptosis or at overcoming acquired drug resistance (Hideshima et al., 2003).

With the recent development of large-scale DNA microarray technology, global transcriptional characteristics of normal, premalignant, and malignant plasma cells can now be assessed in a far more comprehensive manner. Gene expression profiling has already been used to define the molecular portraits of the respective plasma/myeloma cells according to Ig types/light chain subtypes and in relation to the degree of transformation (Magrangeas et al., 2003; Zhan et al., 2002; Davies et al., 2003). Derived from such studies, novel candidate MM disease genes and a gene-based classification with prognostic relevance have been suggested (Zhan et al., 2002). In this issue of *Cancer Cell*, Hurt et al. (2004) provide an excellent example of how this novel technique can be employed to trace an oncogenic event and identify its molecular targets.

Profiling gene expression by use of DNA microarrays in a large panel of myeloma cell lines, the authors detected overexpression of *c-maf* in 50% of all cell lines studied, a finding confirmed in myeloma cells purified from a series of patient bone marrow samples (see Figure 1). *c-maf* is the cellular homolog of *v-maf*, the transforming gene of the avian retrovirus AS42, which was first isolated from a spontaneous fibrosarcoma in chicken. It is a member of the basic leucine zipper transcription factors belonging to the AP1 superfamily. In lymphoid cells, *c-maf* is a T-helper 2 cells

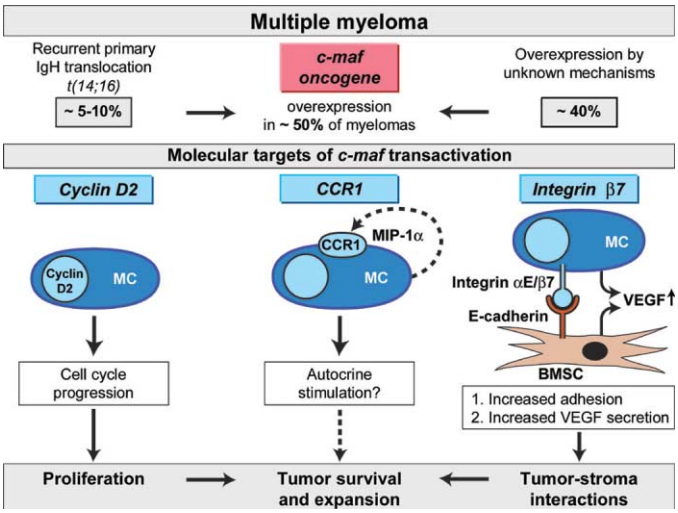

Figure 1. The oncoprotein *c-maf* and its transactivation targets in multiple myeloma

Overexpression of *c-maf* is a much more frequent oncogenic event in multiple myeloma than was expected from the low 5%–10% prevalence of the recurrent t(14;16) translocation in which the *c-maf* gene locus at 16q23 is dysregulated under the influence of enhancer regions of the IgH gene at 14q32. Hurt et al. detected significant levels of *c-maf* expression in 50% of myeloma cell lines and myeloma cells (MC) purified from patient bone marrow samples. Transactivation target genes were identified by differential expression profiling of *c-maf* positive and negative cell lines as well as MC transduced to overexpress *c-maf* or transduced with a dominant-negative form of the oncogene. *c-maf* increases the expression of *cyclin D2*, which drives the cells into cycling, and of *integrin β7*, which promotes adhesion of MC to bone marrow stroma cells (BMSC). The latter phenomenon is associated with increased secretion of vascular endothelial growth factor (VEGF) known to induce marrow neoangiogenesis and to stimulate MC in both an autocrine and a paracrine manner. It remains to be shown whether *c-maf*-induced upregulation of the *C-C chemokine receptor-1 (CCR1)* is part of an autocrine mechanism in which *CCR1* functions as receptor for myeloma-derived MIP-1α.

transcription factor controlling the expression of interleukin-4 (IL-4), while in macrophages it has been shown to potently activate the expression of IL-4 and IL-10. *c-maf* is expressed at high levels in myeloma cells carrying the translocation t(14;16) (q32;q23), which results in fusing Ig heavy chain and *c-maf* gene loci (Kuehl and Bergsagel, 2002; Seidl et al., 2003). However, this primary translocation is present in only 5%–10% of MMs. Thus, the first important finding from this paper relates to the molecular pathogenesis of MM as it identifies *c-maf* overexpression as an oncogenic event occurring in approximately half of all MM cases, mostly as a result of yet unknown mechanisms. Confirmation of this unexpected frequency will certainly be required in larger patient cohorts.

The second important aspect is the identification and functional characterization of the transactivation targets. Although unknown to date, it is perhaps not surprising that *c-maf* upregulates *cyclin D2*, a promoter of cell cycle progression. In fact, it has been shown that the vast majority of MM tumors dysregulate one or more of the cyclin D1, 2, or 3 genes. More of a surprise is the finding that the oncoprotein *c-maf* also increases the expression of *integrin* $\beta 7$, an adhesion molecule that heterodimerizes with integrin αE to bind to E-cadherin on the surface of bone marrow stroma cells. The functional studies performed by Hurt et al. confirm that *c-maf*-regulated expression of *integrin* $\beta 7$ is of pivotal importance for the adhesion of myeloma cells to E-cadherin and marrow stroma cells. Furthermore, the *integrin* $\beta 7$-mediated adherence of myeloma to stroma cells significantly increases the secretion of vascular endothelial growth factor (VEGF). VEGF is produced both by myeloma and marrow stroma cells and is one of the cytokines known to optimize the micromilieu for MM tumors via autocrine and paracrine stimulatory loops (Dankbar et al., 2000; Podar et al., 2001). VEGF also possesses potent angiogenic activity, thus establishing ties to marrow neoangiogenesis. Undoubtedly, the discovery of this *c-maf-integrin* $\beta 7$-mediated pathway of myeloma cell adhesion is an important new piece of information increasing our understanding of MM pathogenesis in general and of myeloma-stroma interactions in particular. In addition, these findings on *c-maf* and its targets are of broad interest, since they define a class of oncogenes that enhance mutual interactions between tumor and stroma rather than rendering the tumor self-sufficient in growth signals.

What are the clinical implications? Given the frequency of *c-maf* overexpression in MM, it will be interesting to explore its prognostic significance both in terms of survival and incidence of skeletal events. In addition, the elegant studies by Hurt et al. suggest *c-maf* as an intriguing new therapeutic target in MM. Indeed, recent developments indicate that oncogene inactivation or target redirection are promising approaches in cancer treatment (Felsher, 2003; Steffen et al., 2003). It should be kept in mind, however, that *c-maf* is widely expressed not only in embryonic but also in adult tissues. This fact may pose significant challenges to a *c-maf*-targeted therapy for MM.

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Selected reading

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