Molecular biology of myeloma

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**Abstract** Multiple myeloma (MM) is a B-cell malignancy characterised by the accumulation of clonal plasma cells (PC) in the bone marrow (BM). The molecular bases for this incurable disease have been widely investigated in the last years, and the development of modern genomic technologies has contributed to the understanding of the pathogenesis of MM. The molecular mechanisms that explain the cellular origin of myeloma cells, the cytogenetic abnormalities and their clinical implications, and the biological information provided by gene expression profiling analysis are reviewed in this paper. In addition, a molecular classification of MM in seven groups based on the relationship between gene expression profiling, chromosomal translocations and prognostic outcome is also presented. And finally, the recent hypothesis of a potential unifying event in the pathogenesis of MM, supported by cyclin D deregulation in virtually all MM tumours, will be summarised.

**Key words** Myeloma • Plasma cell • Cytogenetic abnormalities • Gene expression profiling


Multiple myeloma (MM) is a B-cell malignancy characterised by the accumulation of terminally differentiated clonal plasma cells (PC) in the bone marrow (BM), the production of a monoclonal immunoglobulin (Ig) detectable in serum and/or urine and the presence of lytic bone lesions. MM is often preceded by a premalignant condition called monoclonal gammopathy of undetermined significance (MGUS). Individuals with MGUS show significant risk of progression to MM, with a 0.8% annual risk of malignant transformation [1, 2]. Despite advances in the understanding of the pathogenesis of MM, it remains an incurable disease, the median survival being around 3–4 years (ranging from <1 to >10 years). In this review, we will focus on the novel insights into the molecular biology reported in the last years.

**Cellular origin of myeloma cells**

Normal differentiation from early B cells to PC is characterised by three B-cell-specific DNA remodelling mechanisms that modify Ig genes: VDJ rearrangement, Ig somatic mutation and Ig class switching recombination [3–6]. Rearrangements of the Ig genes of B-cell precursors to form a B-cell receptor (BCR) occur in the BM, while antigen recognition, selection, somatic hypermutations and class switch recombination take place in the lymph node at the germinal centre (GC). Within the lymphoid malignancies there is a marked predominance of GC and post-GC tumours (80%) over acute lymphoblastic leukaemias (ALL), and a possible explanation for this finding is that GC B cells can be exposed
to these three specific DNA-modification mechanisms that generate double-stranded breaks in DNA, whereas pre-germinal centre B cells and T cells are subjected only to VDJ recombination [7, 8]. After antigen encounter, naïve B cells may either differentiate outside GC into short-lived PC, in which Ig genes are not somatically hypermutated and generally secrete IgM, or they can enter GC of lymph nodes where they undergo repeated rounds of somatic hypermutations and antigen selection. The latter process results in the selection of B cells which produce antibodies with increased affinity for the antigen. These somatically mutated IgM-positive B cells may escape into the blood as memory cells, or undergo immunoglobulin heavy chain (IgH) switch recombination to IgG, IgA, IgD or IgE, which migrate to the BM where they interact with stromal cells and terminally differentiate to long-lived PC [9]. Sequence analysis of the Ig VH gene has shown that MM tumour cells are post-germinal, as they are heavily mutated cells, without intraclonal variation among them [10, 11].

Cytogenetic abnormalities

Molecular cytogenetic investigations of myeloma cells have demonstrated that almost all cases of MM are cytogenetically abnormal [12]. MM is characterised by a marked karyotypic instability. This genomic complexity, as well as the delay in identification of IgH translocation (Tx), due to the telomeric location of some translocation partners, has hindered the understanding of genetic bases of MM. The development of advanced genetic methodologies such as interphase fluorescent in situ hybridisation (FISH), multicolour FISH, comparative genomic hybridisation (CGH) and more recently microarray technologies has helped to unravel the meaning of some of the genetic abnormalities [13–17]. Nowadays, IgH Tx and ploidy status may explain two major genetic pathways in MM. Both aneuploidy and IgH Tx are seen in the very early stages of the PC disorders.

IGH translocations

A primary event in many kinds of B-cell tumours is dysregulation of an oncogene that, as a result of Tx to the IgH locus (14q32) or, somewhat less often, to the IgL locus (κ, 2p11 or λ, 22q11), is juxtaposed near one of the potent Ig enhancers. In MM, IgH Tx may be classified into primary or secondary [18]. Primary IgH Tx occur as initiating events during the pathogenesis of MM, whereas secondary Tx are involved in progression. Most primary IgH Tx result from errors in B-cell-specific DNA modification processes, mostly IgH switch recombination or less often somatic hypermutation. The breakpoints occur mainly within or immediately adjacent to IgH switch regions or JH regions. By contrast, secondary Tx would be mediated by other kinds of recombination mechanisms that do not specifically target B-cell-specific DNA modification processes.

Unlike other B-cell tumours, in MM there is a marked diversity of chromosomal loci involved in IgH Tx. About 40% of MM tumours have IgH Tx involving 5 recurrent chromosomal patterns (Fig. 1): 11q13 (CCND1), 4p16 (FGFR/MMSET), 16q23 (CMAF), 6p21 (CCND3) and 20q11 (MAFB) [13, 19].

\[t(11;14)\]

The prevalence of this Tx according to interphase FISH analysis is 15–20% of MM and is readily detectable by karyotyping. The breakpoints on 14q32 fall within either the JH region or the switch region. On 11q13, the breakpoints are dispersed over 330 Kb centromeric to cyclin D1 gene (CCND1), with no evidence of clustering in the Major Translocation Cluster (MTC) described for mantle cell lymphoma [20]. As a result of the translocation, CCND1 is juxtaposed to the powerful IgH 3’ enhancer(s) on der(14), and its expression is dysregulated, as indicated by gene expression profiling and RT-PCR in 100% of MM cases with t(11;14) [17].

\[t(4;14)\]

The t(4;14) is identified in approximately 15% of MM using FISH analysis, but this Tx cannot be detected by karyotyping techniques. In the IgH locus the breakpoints all occur in the switch region and dissociate the intronic enhancer from the 3’ enhancer. This Tx results in the simultaneous dysregulation of the fibroblast growth factor receptor 3 gene (FGFR3) on der(14) and the multiple myeloma SET domain gene (MMSET) on der(4) [21]. FGFR3 is one of the high-affinity tyrosine kinase receptors for the FGF family of ligands. Both FGFR3 and MMSET genes are not normally expressed in PC but are overexpressed as a result of the t(4;14). However, gene expression profiling and RT-PCR analysis have shown that only 75% of the MM with t(4;14) display a simultaneous overexpression of MMSET and FGFR3. In the remaining 25% of cases only MMSET is upregulated and the lack of FGFR3 expression is linked in most cases to loss of the FGFR3 gene on der(14) [22, 23]. These data suggest that MMSET may be the critical transforming event in MM harbouring the t(4;14) whereas FGFR3 could be dispensable. In some cases (10%) the translocated FGFR3 contains activating mutations that may be involved in MM progression [24].

The constitutive activation of FGFR3 results in the subsequent activation of the anti-apoptotic STAT-3 signalling pathway. Recent evidence, using different molecules such as the small molecule inhibitor PD173074 (Pfizer, Ann Arbor, MI) or the CHIR-258 (inhibitor of