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ABSTRACT

As patients with multiple myeloma (MM) have a variable clinical course, predictive markers would help determine the appropriate treatment strategy. Clinical staging is commonly used to predict outcome, but tumour marker expression and the underlying genetic changes are increasingly used to assess the biological aggressiveness of the disease. Recent studies have demonstrated the utility of immunohistochemistry in detecting prognostic markers, including fibroblast growth factor receptor 3, cyclin D1, c-maf and p53, which have been associated with various genetic aberrations, including t(4;14), t(11;14), t(14;16) and del(17p). While t(4;14), t(14;16) and del (17p) have been documented to confer a poor prognosis, t(11;14) appears to be a neutral or even favourable factor in some studies. CD56, CD33, CD20 and CXCR4 are promising surface markers due to their roles in MM progression, but further studies of larger cohorts are necessary to assess their prognostic relevance. In this review, the biological function and clinical relevance of the main prognostic markers in MM is discussed, and also the role of immunohistochemistry in the stratification of patients into appropriate risk categories.

Multiple myeloma (MM) is a clonal haematological neoplasm of plasma cells in the bone marrow.[1] A premalignant condition known as monoclonal gammopathy of uncertain significance (MGUS) results in the immortalisation of a plasma cell and subsequent accumulation of genetic abnormalities that facilitates progression to myeloma at a rate of approximately 1% per year.[2–4] Myeloma is differentiated from MGUS by the presence of significant amounts of monoclonal immunoglobulin in the serum and/or urine, bone marrow plasmacytosis with clinical manifestations, lytic bone lesions, resistance to apoptosis and drug resistance.[4 5] The clinical presentation of MM is well characterised, but the clinical course is variable, due in part to differences in the underlying genetic profile of the myeloma cells.[6]

Clinical and biochemical staging systems are used to assess patients with MM at presentation; but as the underlying disease differs in each individual,[7] molecular cytogenetic prognostic indicators are increasingly important for stratifying patients into risk categories for individualised treatment.[8 9] Karyotype and fluorescence in situ hybridization (FISH) analyses have been used to investigate these molecular cytogenetics prognostic factors, but more readily available, robust and inexpensive technologies would have clinical utility and facilitate exploration of the functional basis of a patient's disease.[10]

Recent studies indicate that immunohistochemical (IHC) detection of oncogenes, such as fibroblast growth factor receptor 3 (FGFR3) and the tumour suppressor gene p53, may be surrogates for the underlying genetic aberrations and valuable for the routine evaluation of MM.[10 11] We shall review the biology and clinical implications of the major prognostic genetic aberrations and surface markers in MM and discuss their detection by IHC.

GENETIC ABERRATIONS

t(4;14)-FGFR3

Translocations involving the immunoglobulin heavy-chain (IgH) switch region on chromosome 14q32 are frequent in MM.[12] A common IgH translocation is t(4;14), which occurs in approximately 15% of myeloma patients and results in the deregulation of the FGFR3 located on chromosome 4.[13–15]

FGFR3, a transmembrane receptor tyrosine kinase involved in regulating cell proliferation and differentiation, is overexpressed in many cancers.[16] On ligand binding, FGFR dimerises and autophosphorylates the tyrosine residues on its cytoplasmic domain, activating downstream signalling pathways.[11] In MM, FGFR3 translocation results in its ectopic expression in plasma cells due to regulation by strong 3′ IgH enhancers, suggesting an oncogeneic role of this protein in the pathogenesis of myeloma.[17 18] In addition, somatic mutations of FGFR3 following translocation have been found to produce a constitutively active receptor in the absence of ligand. The resulting activation of downstream signalling pathways, such as the mitogen activated protein kinase (MAPK) pathway, further contributes to MM progression.[19 20]

The t(4;14) translocation is a unique genetic event due to its ability to simultaneously activate another potential oncogene, MMSET, in addition to FGFR3.[21] MMSET nuclear protein is typically expressed in rapidly growing embryonic tissue, but may have a role in chromatin remodelling.[21] A fusion gene is produced by this genetic aberration; however, the role of this protein in the pathogenesis of MM remains controversial.[2]

The t(4;14) translocation is consistently associated with poor prognosis in patients with MM.[22–25] The importance of identifying these patients early in their clinical course has been highlighted by Jaksic *et al* who showed that patients with translocation t(4;14) were initially chemosensitive, but rapidly relapsed and became resistant to alkylating agents.[26] The overall and progression-free survival rates were lower even with high-dose chemotherapy and autologous stem cell transplantation.[25–27]

The t(4;14) translocation is reliably detected by FISH, but a more efficient diagnostic tool would be clinically useful.[25] Using reverse transcriptase-PCR (RT-PCR), Keats *et al* identified FGFR3 expression at the mRNA level in 74% of t(4;14) MM patients and found an association with reduced survival.[24 28] This adverse prognosis was maintained in t(4;14) patients independent of FGFR3 expression, suggesting additional mechanisms, such as the loss of der(14), mutations inhibiting the transcription of FGFR3, or alternative genes that may be regulated by t(4;14).[24] As the clinical implications of FGFR3 protein expression remain controversial, we investigated FGFR3 expression by IHC in bone marrow samples of a large MM series and determined a strong correlation with t(4;14) by FISH.[11] Of the 16 patients with t(4;14), 12 (75%) expressed FGFR3 by IHC. Patients with FGFR3 expression had a significantly shorter progression-free and overall survival. We found FGFR3 detection by IHC had a sensitivity of 75% and a specificity of 98% for predicting t(4;14).[11] These results suggest FGFR3 may be a surrogate IHC marker for the adverse prognostic factor t(4;14). In addition, it also identifies FGFR3 expression as an important contributor to the pathogenesis of MM, and studies have shown that inhibition of this tyrosine kinase results in apoptosis of MM cells.[14 29 30] It is clear that FGFR3 is of vital importance in novel drug therapy and quick identification of high-risk patients by IHC.

t(11;14)-cyclin D1

At a frequency of 15–20%, t(11;14) is the most common translocation in MM.[22 23 31] As a result of this translocation to the IgH locus, cyclin D1, which is not normally found in plasma cells or B cells, is aberrantly expressed.[32 33] Cyclin D1 is involved in cell cycle regulation and, in association with cyclin dependent kinase 4 (CDK4), phosphorylates and inactivates the retinoblastoma (Rb) protein. As Rb controls the transition from G1 to S phase, its inactivation by cyclin D1 facilitates cell cycle progression.[32]

The t(11;14) translocation is associated with a neutral or favourable outcome in MM.[22 23 25 27 31 34] Cyclin D1 is expressed in 17–50% of patients with MM, but its prognostic significance is controversial.[33] Early studies found cyclin D1 expression to be associated with a poor prognosis,[35–38] but recent studies have shown cyclin D1 expression to be associated with a more favourable clinical course, including a longer duration of remission, and median event-free and overall survival.[33 39]

Cyclin D1 expression is more frequent than t(11;14), suggesting that mechanisms such as gene amplification or trisomy 11 contribute to cyclin D1 overexpression.[38] However, studies correlating cyclin D1 expression with t(11;14), as detected by FISH, have found that cyclin D1 expression is a good indicator of the underlying genetic aberration.[39–41] Using RT-PCR, Specht *et al* were able to show that high levels of cyclin D1 mRNA were found exclusively in the presence of a t(11;14) translocation.[40] Similarly, combined IHC and FISH analyses in 39 patients showed cyclin D1 positivity in all of the cases (7/7) bearing the t(11;14) translocation.[41] Since the t(11;14) translocation confers a neutral or even a favourable clinical outcome, it may be useful to identify these patients by IHC.[9 27]

t(14;16)-c-maf

The t(14;16) translocation is a IgH translocation that fuses the IgH switch region to the c-maf gene loci on 16p23 and leads to c-maf overexpression.[42] As it also occurs in MGUS, t(14;16) may be involved in early stages of myeloma development.[43]

c-maf is the cellular homologue of the viral analogue v-maf from an avian transforming virus.[44] c-maf is a basic zipper transcription factor involved in DNA binding and dimeric protein interactions and it is involved in expression of the cytokine interleukin 4 in Th2 helper cells and macrophages.[4 45] In myeloma, c-maf may upregulate cyclin D2 and integrin β7.[46] As cyclin D2 is involved in regulation of the cell cycle at the G1/S phase, its upregulation by c-maf in MM cells would promote cell cycle progression.[4] Integrin β7 is an adhesion molecule that binds to E-cadherin expressed on bone marrow stroma cells.[46] Through this pathway, c-maf overexpression enhances interactions between myeloma cells and the stroma, including increased secretion of vascular endothelial growth factor (VEGF) and production of lytic bone lesions.[46 47] As VEGF is a potent stimulator of angiogenesis, increased levels would promote tumour progression.[46]

The frequency of translocation t(14;16) is low, identified in only 2–9% of patients with MM, and it is associated with a poor prognosis.[23 44 48] However, studies of c-maf mRNA levels in these patients are controversial. Rasumussen *et al* found six of 135 (4.4%) MM patients overexpressed c-maf.[45] This rate is comparable with the prevalence of t(4;16) and all six patients also possessed this genetic aberration.[45] A more recent study reported 50% of MM bone marrows had increased c-maf mRNA levels.[46] In our study, IHC detected that 30% of patients with MM expressed nuclear c-maf; but we did not find an association with an adverse prognosis.[44] Furthermore, unlike FGFR3 which correlated with an underlying t(4;14), only 5% of our MM cohort that overexpressed c-maf possessed a t(14;16) translocation, suggesting an unlikely role of c-maf expression as a surrogate for this genetic aberration.[44] Nonetheless, because of the functional role of c-maf in MM, it remains a possible therapeutic target for this disease.

del(17p)-p53

Somatic allelic deletions and point mutations of the p53 tumour suppressor gene are the most frequent genetic abnormalities in human malignancy.[49 50] Translocations of IgH are thought to be primary events in MM, while p53 alterations are present as secondary changes in MM progression.[51] Although the prevalence of p53 mutations is generally low (~3%) in MM, hemizygous p53 deletions are found in 10% of patients at diagnosis and are an established adverse prognostic factor that is correlated with a significantly poor survival.[52 53]

The p53 gene encodes a nuclear 53 kDa phosphoprotein transcription factor that is involved in cell cycle arrest upon detection of DNA injury and subsequent initiation of apoptosis if the damage is not repaired.[54] As a result, a loss or disrupted function of p53 is associated with advance disease and tumour progression.[52] In MM, we found that p53 deletions are common in patients with central nervous system involvement and in patients with plasma cell leukaemia who have a very poor clinical outcomes.[51 55]

Although it has been established that hemizygous p53 deletions are associated with poor prognosis, the role of the remaining p53 allele remains to be elucidated.[10] Detection of aberrant nuclear p53 protein has been seen in many cancers, including haematological malignancies, and it is associated with an adverse prognosis.[56–58] Since patients with a deleted p53 allele have an elevated degree of genetic instability, it is likely that the remaining allele has an increased chance of being mutated.[52 59] As a result of this mutation, the half-life of the mutated p53 protein is increased compared with that of the wild-type, enabling detection by IHC.[60–62] The incidence of p53 protein

expression in myeloma, as detected by IHC, has been reported to be between 8 and 39%, but its relationship with p53 gene deletion status has only recently been investigated.[41 52 63 64] In a study of 105 newly diagnosed MM patients, we demonstrated a strong correlation between nuclear p53 expression and the presence of a hemizygous p53 deletion as detected by FISH.[10] Furthermore, the overall survival of p53 immunoreactive patients was significantly less than the survival of p53 non-immunoreactive patients, suggesting that detection of p53 expression by IHC may serve as a prognostic marker of MM and a surrogate for the presence of del(p53).[10] Nonetheless, mechanisms other than mutation, such as the p14ARF upstream regulatory molecule, may increase p53 stability and also result in aberrant p53 expression.[65 66] Since the frequency of p53 mutations is low and does not always result in p53 protein expression, further investigation of alternative mechanisms of p53 dysregulation and functional consequences of its aberrant expression is required for a better understanding of this subset of patients with aggressive MM.[41]

SURFACE MARKERS

CD56

CD56, a 174–185 kDa neural cell adhesion molecule, is expressed on all natural killer cells and is frequently expressed on MM cells.[67 68] During embryogenesis, CD56 is expressed on neuroectoderm cells and facilitates the adhesion of neurons and muscle during neuronal growth.[69 70] In haematopoietic tissues, CD56 facilitates homing of cells to the bone marrow and increases osteolysis, while decreased expression in MM facilitates progression to a leukaemic phase of MM in the peripheral blood and cerebrospinal fluid.[70 71]

CD56 is expressed in 70–80% of patients with MM, as determined by both flow cytometry and IHC; however, it is the prognostic relevance of a lack of CD56 expression that has been the subject of debate.[70 72 73] Studies have associated patients with CD56-negative MM with extramedullary disease, elevated Bence Jones proteins, renal insufficiency and more aggressive disease.[67 74] Using flow cytometry, Sahara *et al* determined patients with CD56-negative myeloma had a shorter overall survival than patients with CD56-positive myeloma, suggesting that CD56 may be a prognostic marker for MM.[74] In contrast, studies employing IHC on paraffin-embedded bone marrow slides and cryopreserved samples found that CD56-negative myeloma had no prognostic significance despite a similar frequency of detection as studies using flow cytometry.[75 76]

We found no association between CD56 expression by IHC and genetic prognostic markers such as t(11;14) or t(14;14) despite an earlier study using flow cytometry that determined a correlation between CD56 negative myeloma and t(11;14).[72 75] CD56 expression may be useful for distinguishing between MM and MGUS, which infrequently displays strong CD56 expression.[67] In addition, we found that patients who develop central nervous system myeloma have CD56-negative myeloma.[71]

Although its prognostic value remains controversial, it is clear that CD56 is of biological importance in MM and can be detected by IHC. Further studies of larger cohorts are warranted to better understand the role of CD56 as a prognostic marker in MM.

CD20

The success of the chimeric anti-CD20 monoclonal antibody rituximab in treating non-Hodgkin lymphoma has prompted investigation of CD20 in other haematological malignancies, including MM.[77]

CD20 is expressed by mature B-cells and in 20% of MM cases.[77–79] Early studies found an association of CD20 with adverse prognosis,[80 81] but a recent study by Robillard *et al* found 10 of the 12 (83%) patients who expressed CD20 detected by flow cytometry had the t(11;14) translocation associated with a neutral or favourable prognosis.[79] IHC may therefore be a useful method for detecting CD20 as a surrogate for the underlying t(11;14) translocation in patients with MM.[77] CD20 expression has also been associated with a mature small cell MM morphology and pulmonary infiltrates, suggesting a possible biological role yet to be elucidated.[78 79]

Clinical trials of rituximab have had limited success in patients with MM.[82] Nonetheless, further investigation of the prognostic implications of CD20 expression is warranted to better understand this possible therapeutic target in MM.

CXCR4

CXCR4 belongs to the family of seven transmembrane domain, G-protein coupled, chemokine receptors.[84] In MM, CXCR4 is involved in the migration and bone marrow localisation of malignant plasma cells via the VLA-4 integrin in response to stromal cell secretion of its ligand, stromal cell-derived factor 1 (SD-1).[84 85]

Flow cytometry detects CXCR4 expression in 10–100% of myeloma cases.[86] CXCR4 has been detected by IHC in other cancers, but has yet to be systematically examined in MM.[87] Studies of CXCR4 expression in mobilised and bone marrow-confined MM cells show that SD-1, VLA-4 and chemokine expression are downregulated in mobilised cells, further supporting a role of CXCR4 in MM cell homing.[88]

Few studies have evaluated the prognostic significance of CXCR4 expression; however, Van de Broek *et al* found low CXCR4 expression was correlated with lower overall survival, lower albumin levels, and higher C-reactive protein and β2 microglobulin levels.[89] Although the reason for lower overall survival is unclear, it is speculated that lower levels of chemokine receptors are associated with higher disease activity since confinement to the bone marrow is minimised. Instead, low CXCR4 expression may promote mobilisation to extramedullary sites, thereby contributing to disease progression.[88 89]

CD33

On phosphorylation of tyrosines in its cytoplasmic tail, CD33, a 67 kDa sialic acid-dependent adhesion molecule, mediates intracellular inhibitory signals.[90 91] The role of CD33 in myeloma has only recently been considered, but it is normally expressed on myeloid cells and has been targeted by drugs in the treatment of acute myeloid leukaemia.[90 91] CD33 has been detected in 6–35% of MM patients in the few studies that included it in their flow cytometric immunophenotyping panel.[90–92] Sahara *et al* found that CD33-positive patients had a significantly shorter 3 year overall survival, a higher mortality in the first year after diagnosis, higher lactate dehydrogenase and β2 microglobulin levels, and more frequent anaemia and thrombocytopenia.[91] In another study, CD33-positive patients had a greater frequency of t(4;14), which, with the clinical characteristics discussed, would contribute to a poor prognosis.[90]

As CD33 is a potential prognostic marker, there is interest in using the anti-CD33 antibody Mylotarg to treat MM.[90] Nonetheless, the use of drugs in CD33-positive myeloma should be carefully monitored, as conventional therapeutic agents can increase CD33 expression and may be associated with drug

Take-home messages

- Multiple myeloma patients have a variable clinical course and benefit from the detection of prognostic markers for stratification into risk categories.
- Immunohistochemistry has been shown to detect surface molecules such as CD56, CD20 and CXCR4 as well as biomarkers including FGFR3, cyclin D1, c-maf and p53 that act as surrogates for underlying genetic aberrations in myeloma.
- While molecular cytogenetic methods and flow cytometry can also be used to detect prognostic markers, immunohistochemistry is more readily available enabling efficient identification of candidates for risk-adapted therapies.

resistance.[91] As our knowledge of this marker is limited, further studies using IHC to assess the role of this molecule in MM may have utility.

CONCLUSIONS

MM is incurable with current therapies and, as discussed in this review, the identification of new prognostic markers and potential therapeutic targets for new drug development is clinically important.[5] Identifying high-risk patients for more aggressive therapy is a critical step for improving their clinical course. Certain genetic aberrations and surface antigens are significant prognostic markers in MM; however, conventional cytogenetics is of little assistance to detect these changes because of the low proliferation of MM cells, while flow cytometry is not always accessible.[8 93] Since IHC is a widely available, robust and inexpensive technology and has been found to detect many clinically significant MM prognostic markers, it should be adopted in clinical practice to identify patients with myeloma who have an adverse prognosis and who may be candidates for risk-adapted therapies.

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