Austin Journal of Cancer and Clinical Research

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Research Article

Molecular Cytogenetics of Multiple Myeloma

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Received: July 28, 2014; **Accepted:** November 24, 2014; **Published:** December 01, 2014

Abstract

Multiple Myeloma (MM) is a plasma cell tumor characterized by multiple genetic lesions. Two main groups of abnormalities can be distinguished: Hyper diploid and hipodoploid, in which the chromosome number is mainly constituted by trisomies or monosomies, respectively. The advent of Fluorescence *In Situ* Hybridization (FISH) has permitted to identify genomic lesions that have been integrated into a prognostic score system that take in consideration also clinical parameters. This simple analysis can be part of the initial evaluation of the patient and can drive more accurately his treatment.

Keywords: Myeloma; Cytogenetics; Fish

Introduction

In contrast to other haematological neoplasms such as the acute leukaemias, the systematic investigation of chromosomal aberrations in Multiple Myeloma (MM) by the use of conventional cytogenetics has been hampered by the low mitotic activity of tumour cells in this disease [1,2]. With the introduction of molecular-based cytogenetic techniques into the analysis of MM, and its precursor condition Monoclonal Gammopathy of Undetermined Significance (MGUS), considerable advances in the understanding of the biology of plasma cell tumours has been achieved. Using Fluorescence *In Situ* Hybridization (FISH), multiple and complex chromosomal abnormalities are detectable in virtually all patients with MM and most patients with MGUS with great prognostic importance [3].

Conventional cytogenetics

Cytogenetic analysis in MM is informative in less than 30% of patients this is due to the mitotic activity of tumour cells that in MM as compared to other haematological diseases is generally very low. Karyotypes are typically complex and exhibit more than 10 abnormalities in almost half of patients [4].

Numerical changes

The numerical changes are monosomies of chromosomes 13, 14, 16, and 22 as well as trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 [5]. Independent of the detection of specific chromosomal aberrations, chromosome banding analysis provides valuable prognostic information that can firstly be extracted from the presence or absence of abnormal metaphases, and secondly from the tumour cell ploidy in informative cases [6]. Patients with a normal karyotype enjoy a significantly longer survival than those that are cytogenetically abnormal [7]. Moreover, the classification of tumours with aberrant metaphases according to their chromosome number is also of prognostic relevance. In several series, hypodiploidy was associated with a significantly inferior outcome. However, as other adverse genetic features – such as monosomy 13/13q deletion, t(4;14), and t(14;16) – are predominantly present in hypodiploid tumours, it remains controversial whether or not hypodiploidy is an independent prognostic marker [8-10]. While numerical changes can be diagnosed easily, small interstitial deletions are usually missed. Fluorescence *In Situ* Hybridization (FISH), allow the detection of genetic abnormalities independently of proliferating cells [3,11]. With these methods, chromosomal aberrations are found in virtually all patients with MM. It is very important that plasmacells are purified from the original sample [2]. This is due to variable infiltration of plasmacells in the bone marrow and the dilution in the bone marrow aspirate that can lead to false negative results. There are various techniques to enrich tumour cells in MM. The most widely used method applies magnetic microbeads that recognize the cell surface antigen CD138 expressed on both, normal as well as malignant plasma cells fish [12]. FISH permits the reliable identification of translocations as well as small deletions or gains. FISH became the most widely used technique for the analysis of chromosomal abnormalities in MM.

Fish

The indication in MM is to test for 13q14 and 17p deletions (13q-, 17p-), translocations of the IGH locus, in particular t(4;14) (p16.3;q32) , t(11;14)(q13;q32) , t(14;16) (q32; q23) and abnormalities of chromosome 1p and 1q [2]. Most of these probes are available. For the detection of 13q loss, most laboratories apply probes mapping to chromosome band 13q14, although the critical region of 13q- is still poorly defined. For the detection of 17p-, it is general practice to apply probes containing the *p*53 gene. Translocations involving the immunoglobulin heavy-chain *(IgH)* locus can be detected by the use of DNA probes mapping to the constant (C_H) and variable region of the *IgH* gene [13]. Reciprocal translocations are diagnosed by the colocalization of differentially labelled probes for *IgH* and the respective translocation partner (e.g. 11q13, 4p16, 16q23), ideally on both derivative chromosomes (double fusion). Cut-off levels for positive results are based on data obtained from bone marrow specimens of healthy volunteers. For dual fusion or break-apart probes, a cut-off level of 10% could be accepted. For single fusion results with dual fusion probes, a cut-off level of 20% can be good, although some laboratories apply a threshold of 10% for these probes, and it is important that each laboratory set proper cut off values [2].

13q deletion

By FISH in several studies, -13/13q- is present in about 50% of cases representing the most frequent abnormality in MM [14]. Using conventional cytogenetics, the incidence of chromosome 13 losses among patients with informative karyotypes is comparable, resulting in an overall incidence of 10% to 20% as shown in large cytogenetic

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Citation: Gozzetti A, Frasconi A and Crupi R. Molecular Cytogenetics of Multiple Myeloma. Austin J Cancer Clin Res 2014;1(4): 1020.

series. In patients with t(4;14)(p16.3;q32) or t(14;16)(q32;q23), the incidence of -13/13q- is approximately 90%. By conventional cytogenetics -13/13q- is associated with significantly lower response rates, shorter Event-Free Survival (EFS), and inferior Overall Survival (OS) in MM. This is true for patients after conventional chemotherapy as well as for patients treated with High-Dose Chemotherapy (HD-CTX) and Autologous Stem Cell Transplantation (ASCT) [15]. -13/13q- by karyotyping predict a more unfavourable prognosis than the detection of the same abnormality by FISH. This is most likely due to a combination of negative prognostic markers reflected by this finding (higher rate of proliferating cells) [16]. Moreover, significantly more 13q-deleted patients are identified by FISH as compared to conventional cytogenetics (40–50% vs. 15–20%).

17p deletion

Inactivation of the *p*53 tumour suppressor gene by monoallelic deletion or mutation is associated with disease progression in many human malignancies. In most FISH series, the incidence of *p*53 deletion among newly diagnosed patients was in the range of 5% to 10% [17,18]. However, functional loss of the gene is present in up to 40% of patients with advanced MM and in more than 60% of human myeloma cell lines, pointing to this abnormality as a marker of tumour progression [19]. Independent of the mode of treatment (conventional chemotherapy or HD-CTX), deletion of the *p*53 gene locus identified by FISH is a predictor of shorter survival [20].

Chromosome 1q

In cytogenetic studies, 1q abnormalities were associated with advanced disease and tumour progression, as well as with shorter event-free survival. The gene implicated seems amplificated and is the cell cycle regulator gene *CKS1B* at chromosome band 1q21 as a predictor of a particularly unfavourable prognosis [21-23].

IgH translocations

IgH translocations are equally frequent in, MGUS and MM (~40–60%), which strongly suggests that primary IgH rearrangements represent early pathogenetic events [24]. The overall rate of 14q32 translocations, however, significantly increases with disease progression and reaches up to 90% in advanced tumours and Human Myeloma Cell Lines (HMCL), most likely reflecting a rising number of secondary *IgH* translocations which seem to be virtually absent in MGUS and smoldering MM. Typically, there are five main translocations involving 11q13 (CCND1), 6p21 (CCND3), 16q23 (MAF), 20q12 (MAFB), and 4p16 (FGFR3 and MMSET) [25].

t(11;14)(q13;q32)

Tumours carrying the t(11;14)(q13;q32) can be identified either by chromosome banding analysis, FISH, or gene expression analysis. Using FISH, the rearrangement can be identified in about 15–20% of patients with MM and 15–30% of cases with MGUS [26,27]. The presence of t(11;14) has been correlated with a lymphoplasmacytic, mature morphology of plasma cells, CD20 expression, and the oligo-/asecretory MM subtype. There was no impact of t(11;14) on survival of patients treated with conventional chemotherapy. The t(11;14)(q13;q32) is identical to that observed in mantle-cell lymphomas. The breakpoints involve the IGH gene at 14q32, and the CCND1 gene at 11q13, encoding the cyclin D1 protein. Although the cyclin D1 has been involved in the activation of proliferation, the t(11;14) myelomas are characterized by a low proliferative index and a frequent morphology of small mature plasma cells. Clinically, this type of myeloma is not remarkable. Although preliminary reports did show a better survival, more recent and larger studies did not confirm this prognostic impact [28].

t(4;14)(p16.3;q32)

The karyotypically cryptic t(4;14)(p16.3;q32) is detectable in approximately 15–20% of primary tumour specimens by FISH and leads to the dysregulation of two oncogenes, *MMSET* on der(4) and *FGFR3* on der(14) [21,29,30]. Of note, in about 20% of cases with t(4;14), *FGFR3* on der(14) is lost or not expressed. The translocation was found to be more prevalent among tumours with an IgA isotype as well as in patients with aggressive clinical features. Independently from the mode of treatment, t(4;14) is associated with an unfavourable clinical course [31].

t(14;16)(q32;q23)

Like t(4;14), t(14;16)(q32;q23) is karyotypically silent but can reliably be identified by FISH. With an incidence of 2–10%, t(14;16) is comparatively rare. t(14;16) results in the upregulation of the basic leucine zipper (bZIP) transcription factor *c-maf*. There is only scarce data about the prognostic impact of this *IgH* rearrangement, but it seems associated with poor prognosis [32,33].

Conclusion

The analysis of genomic rearrangements in MGUS and MM by the use of cytogenetics and molecular cytogenetics has ameliorated our understanding of clonal plasma cell disorders. The evaluation of a comprehensive panel of chromosomal imbalances and translocations is currently important within clinical management.

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Austin J Cancer Clin Res - Volume 1 Issue 4 - 2014
ISSN : 2381-909X | www.austinpublishinggroup.com
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Citation: Gozzetti A, Frasconi A and Crupi R. Molecular Cytogenetics of Multiple Myeloma. Austin J Cancer Clin Res 2014;1(4): 1020.