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Cytogenetics and molecular cytogenetics in multiple myeloma

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ABSTRACT

Multiple myeloma (MM) is characterized by frequent and complex genomic abnormalities that not only essentially contribute to the pathogenesis of this disease but also reflect its prognostic heterogeneity. There is evidence for two more or less mutually exclusive oncogenic pathways in the early development of clonal plasma cell disorders. Approximately half the tumours are non-hyperdiploid and carry translocations of the immunoglobulin heavy-chain *(IgH)* locus and various oncogenes, for example *Cyclin D1*, *Cyclin D3*, and *FGFR3*. The remaining hyperdiploid tumours exhibit recurrent trisomies – typically of chromosomes 5, 7, 9, 11, 15, 19, and 21 – but infrequently exhibit *IgH* translocations. While some chromosomal aberrations, such as deletion of chromosome arm 13q, deliver independent prognostic information that is already utilized for risk stratification within clinical trials, the prognostic significance of most other genetic aberrations in MM is undetermined.

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1. 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. 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), comparative genomic hybridization (CGH), and metaphase spectral karyotype imaging (SKY), multiple and complex chromosomal abnormalities are detectable in virtually all patients with MM and most, if not all, patients with MGUS. This article focuses on the pathogenetic and clinical implications of chromosomal abnormalities in plasma cell neoplasms and outlines the current methodology for the detection of genomic changes in these diseases.

2. Methodology

2.1. Cell sorting

The proportion of plasma cells in bone marrow aspirates from patients with MM is highly variable and can range from virtually 0% up to 100%. The number of tumour cells in a given specimen largely depends on the level of local bone marrow infiltration and the degree of sample dilution by bone marrow blood that mostly contains no or only few tumour cells. For the latter reason, the percentage of plasma cells on a bone marrow smear does not usually correlate with that in corresponding specimens provided for further analysis, using techniques such as cytogenetics. To yield an optimal plasma cell recovery, it is advisable to carry out several punctures within

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the anesthetized bone area and to draw no more than 3–5 ml of bone marrow per puncture. 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 (syndecan-1) expressed on both, normal as well as malignant plasma cells.[1] As CD138 is rapidly shed in apoptotic plasma cells, specimens should be processed as fast as possible.[2] To reduce the amount of microbeads necessary for purification, density gradient separation of the mononuclear cell fraction prior to plasma cell enrichment is advisable.

2.2. *Conventional cytogenetics*

The mitotic activity of tumour cells in MM as compared to other haematological cancers is generally very low as indicated by a median bone marrow plasma cell labelling index (PCLI, a measure of plasma cells in the S phase) of <1%[3]. Therefore, chromosome banding analysis in MM delivers informative (abnormal) karyotypes in only 30–40% of patients. In the remaining cases, no or only normal metaphases originating from non-tumourous (e.g. myeloid) cells can be procured.[3–10] Karyotypes are typically complex and exhibit more than 10 abnormalities in almost half of patients and even more than 20 aberrations in about 10% of cases.[11] While numerical and gross structural changes can be diagnosed without difficulty, small interstitial deletions or partial genomic gains as well as translocations with telomeric breakpoints, for example t(4;14)(p16.3;q32) and t(14;16)(q32;q23), can be easily overlooked due to the limited spatial resolution of the technique.[12–15]

2.3. *Molecular cytogenetics*

Modern molecular-based techniques, such as comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH), allow the detection of genetic abnormalities independently of proliferating cells. With these methods, chromosomal aberrations are found in virtually all patients with MM and most – if not all – patients with MGUS.

2.3.1. *Metaphase- and microarray-CGH*

CGH offers the advantage of examining the whole tumour cell genome for the presence of chromosomal imbalances – including amplifications – in a single hybridization experiment. Metaphase-CGH allowed a first approximate characterization of critically gained or lost regions in MM which was a prerequisite for the selection of appropriate DNA-probes applicable for FISH. However, metaphase-CGH has a number of limitations. It does not enable the detection of balanced aberrations, such as reciprocal translocations, as well as of genomic changes smaller than approximately 5–10 Mbp in size.[16–21] Microarray-based CGH facilitates the visualization of genome-wide aneuploidy at a very high resolution. This innovative approach is based on the hybridization of tumour DNA to glass slides with several thousands of immobilized reference DNA fragments.[22,23]

2.3.2. *FISH*

In contrast to CGH, FISH permits the reliable identification of both translocations as well as small deletions or gains.[24–30] Therefore, FISH became the most widely used technique for the analysis of chromosomal abnormalities in MM and MGUS. Interphase FISH should be performed in combination with immuno-fluorescent detection of light-chain restricted plasma cells (cIg-FISH).[31] Only in purified cell preparations, fluorescence antibody labelling is dispensable. In MGUS, where the proportion of clonal cells is generally low, plasma cell enrichment is mostly reasonable.

Most laboratories currently test for 13q and 17p deletions (13q-, 17p-) as well as the primary translocations t(4;14)(p16.3;q32) and t(11;14)(q13;q32). **However, we recommend to perform additional FISH experiments using DNA probes for the detection of the most frequent chromosomal abnormalities (e.g. +1q, +9q, +11q) in cases with a low plasma cell proportion before enrichment** (<1–2%) **and normal findings for all the aforementioned chromosomal loci. By this approach, a chromosomal aberration proving the clonality of the analysed plasma cells can be detected in virtually all patients.** For the detection of 13q loss, most laboratories apply probes mapping to chromosome band 13q14, although the critical region of 13q- (if present at all) is still poorly defined.[32–34] For the detection of 17p-, it is general practice to apply probes containing the *p*53 gene.[35–40] 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 (V_H) region of the *IgH* gene. This approach indicates t(14q32) by segregation of one C_H/V_H signal pair ("break-apart" strategy), which from our experience and that of other laboratories, can cause difficulties in the interpretation of hybridization signals and therefore deliver equivocal (false-positive) results.[15,28] Reciprocal translocations are reliably 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" strategy).[15,25,28,38,41–43]

From our experience and that of other laboratories, fluorescence signals can be less intense in myeloma cells as compared to other haematopoetic cells. Therefore, probes for the detection of deletions should always be combined with appropriate control probes indicating adequate hybridization efficiency. As disomy, for example of chromosome band 13q14, equals deletion of the respective chromosomal region in near-tetraploid karyotypes, ploidy should be determined in all tumours. Polyploidy can be reliably excluded by the use of control probes mapping to genomic regions that rarely display aneuploidy (e.g. chromosomes 2, 10, and 12).[16–21] Cut-off levels for positive results are based on data obtained from bone marrow specimens of healthy volunteers using the mean +3 SD percentage of cells with a given abnormality. For dual fusion or break-apart probes, a cut-off level of 10% is widely accepted. For imbalances or single fusion results with dual fusion probes, a cut-off level of 20% appears reasonable, although some laboratories apply a threshold of 10% for these probes.

2.3.3. *Metaphase Spectral Karyotype Imaging (SKY)*

SKY displays each of the 24 different human chromosomes in a different colour and enables the visualization of genomic rearrangements by a change in the colour at the point of the aberration. In contrast to conventional karyotyping, genomic

material of unknown origin, including insertions, complex translocations, and marker chromosomes which represent frequent findings in MM can be assigned to specific chromosomes by this technique.[14,44–46]

3. Pathogenetic relevance of chromosomal abnormalities

3.1. B-cell development and myelomagenesis

MM emerges from post-germinal-center B-cells that have been exposed to three specific DNA-modifying mechanisms: VDJ recombination, *IgH* switch recombination, and somatic hypermutation. VDJ recombination composes Ig V, D, and J gene segments to Ig heavy and light chain antigen receptors in precursor bone marrow B-cells.[47] Immature B-cells with functional surface IgM receptors leave the bone marrow and enter secondary lymphoid tissues as mature B-cells that can proliferate and differentiate to pre-germinal-center, short-lived plasma cells after antigen interaction. These short-lived plasma cells still mostly express IgM but can secrete other Ig isotypes after switch recombination. As part of the primary immune response, antigen-stimulated lymphoblasts undergo somatic hypermutation and antigen selection in germinal centers. Cells that do not express high-affinity antigen receptors undergo apoptosis while the remaining ones differentiate to memory B-cells or post-germinal-center plasma cells that migrate to the bone marrow and terminally differentiate into long-lived plasma cells that survive for > 30 days to years (Fig. 1) (reviewed in Refs. [48,49]). The high incidence of B-cell tumours arising from germinal-center or post-germinal center B-cells in general suggests that erroneous DNA remodeling is a crucial event in the development of lymphoproliferative diseases.[50,51] Clonal plasma cell disorders, like other lymphomas, are characterized by recurrent *IgH* translocations that are already detectable in about half of patients with MGUS.[25,52–55] This suggests that *IgH* rearrangements are seminal but not universal oncogenic events in early myelomagenesis.

3.2. Primary and secondary IgH translocations

While primary translocations occur early in pathogenesis (see above), secondary translocations – e.g. those involving the *c-myc* gene at chromosome band 8q24 – represent progression events that are structurally complex, do not employ the aforementioned B-cell specific recombination mechanisms, less often target the *IgH* locus, and rarely exhibit breakpoints in or near the J or switch region.[49] By the use of FISH and DNA probes embracing the *IgH* locus at chromosome band 14q32, *IgH* rearrangements are detectable in most myeloma tumours.[25,53] The incidence of the five most relevant primary translocations as well as the affected oncogenes is listed in Table 1. Of note, primary *IgH* translocations are more or less equally frequent in both, MGUS and MM (~40–60%), which strongly suggests that primary IgH rearrangements represent early pathogenetic events.[25,28,30,56,57] 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),[25,52,53] most likely reflecting a rising number of secondary *IgH* translocations which seem to be virtually absent in MGUS and smoldering MM. In comparison to heavy-chain translocations, light-chain translocations are rather infrequent in MM: the incidence of Igλ translocations in advanced tumours and HMCL is in the range of 20% while Igκ rearrangements seem to be very rare.[49]

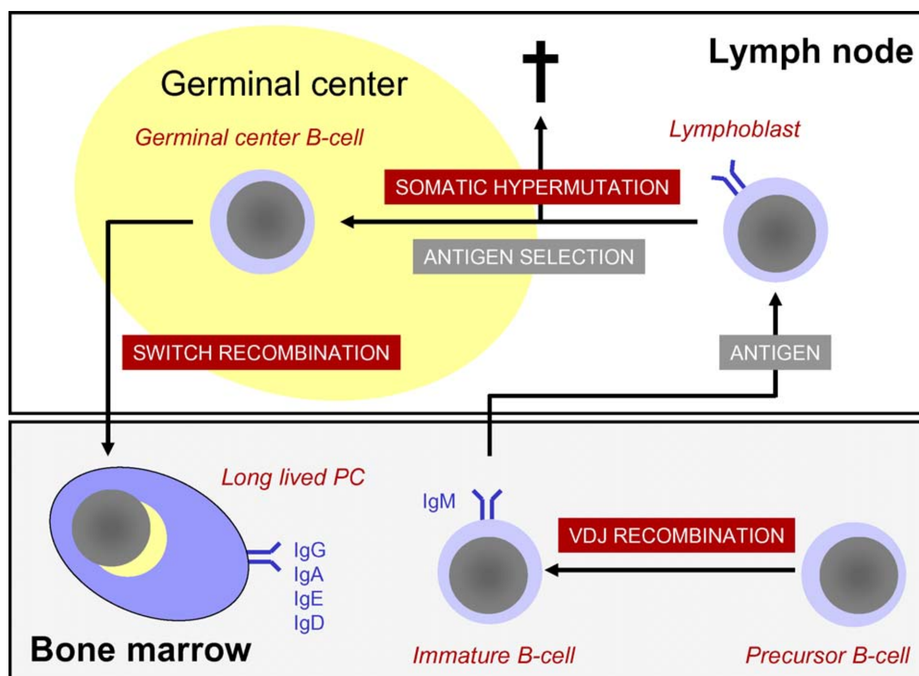

Fig. 1 – Plasma cell development and DNA-modifying mechanisms.[49]

Table 1 – Recurrent primary translocations in MM

Locus	Incidence	Involved oncogene(s)	Oncogene function
11q13	15–20%	*Cyclin D1*	cell cycle regulator
4p16.3	15–20%	*FGFR3 MMSET*	receptor tyrosine kinase nuclear set domain protein
16q23	2–10%	*c-maf*	bZIP transcription factor
6p21	3–5%	*Cyclin D3*	cell cycle regulator
20q11	2%	*mafB*	bZIP transcription factor

3.3. *Aneuploidy*

Almost all myeloma tumours and most cases of MGUS are aneuploid as demonstrated by DNA content measurements using flow cytometry,[58–60] conventional cytogenetics,[3–11] or molecular cytogenetics.[16–21,24–30] The most prevalent 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.[3] Four categories of aneuploidy can be defined by karyotyping: hypodiploidy (44–45 chromosomes), pseudodiploidy (44/45 to 46/47 chromosomes), hyperdiploidy (>46/47 chromosomes), and near-tetraploidy (>75 chromosomes).[3,61–63] Due to the frequent occurrence of chromosomal losses in tetraploid myeloma cells, near-tetraploidy has been classified together with hypo- and pseudodiploidy as "non-hyperdiploidy" which is observed in approximately half of tumours. In several studies comparing non-hyperdiploid and hyperdiploid MM, notable differences in the rate of specific structural abnormalities, e.g. a significantly higher incidence of chromosome 13 losses in non-hyperdiploid tumours, became apparent. Even more importantly, primary *IgH* translocations – such as t(11;14), t(4;14), and t(14;16) – were found to be much more prevalent in non-hyperdiploid as compared to hyperdiploid MM (>85% vs. <30%). [3,64–66]

3.4. *Genetic model of myelomagenesis*

Based on the seminal observations summarized above, a dichotomic model for the pathogenesis of monoclonal gammopathies has been developed (Fig. 2) (reviewed in Ref. [57]). The very high incidence of primary *IgH* translocations in non-hyperdiploid MM suggests that 14q32 rearrangements with consecutive oncogene dysregulation are mandatory for plasma cell immortalization in these tumours. In hyperdiploid MM, primary *IgH* rearrangements are comparably infrequent (<30%), suggesting that in these cases, gain of function of critical genes caused by duplications of chromosomal segments rather than by *IgH* enhancers by virtue of 14q32 translocations represent the pivotal transforming genetic event. For example, overexpression of *Cyclin D1* was demonstrated in almost 40% of tumours lacking a t(11;14) translocation but exhibiting polysomy of chromosome 11.[67–69] Consistent with this pathogenetic model, the hyperdiploid/non-hyperdiploid dichotomy – originally established in patients with MM – could recently be confirmed in a cohort of patients with MGUS analysed by FISH.[30] Disease progression from MGUS to MM and finally PCL is accompanied by an increasing rate of secondary genomic changes, such as activating mutations of *N-Ras*, *K-Ras* and *FGFR3*, *p53* inactivation by deletion or mutation, and *c-myc* translocations (reviewed in Refs. [48,49]. The role of chromosome 13 losses in this context remains a matter of debate (see below).

4. Clinical and prognostic implications of chromosomal abnormalities

4.1. *Aneuploidy*

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. Patients with a normal karyotype enjoy a significantly longer survival than those that are cytogenetically abnormal. Moreover, the classification of tumours with aberrant metaphases according

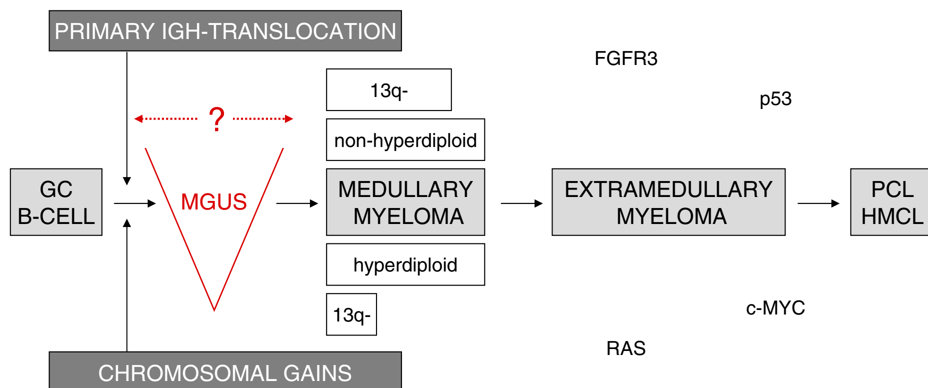

Fig. 2 – Genetic model of myelomagenesis.[57]

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.[3,10,61,70–75]

4.2. *Specific genomic imbalances*

4.2.1. *Monosomy 13/ 13q deletion (-13/13q-)*

- Incidence

  As determined by FISH in several studies, -13/13q- is present in about 40% to 50% of cases and thus is one of the most frequent abnormalities in MM.[39,76–79] 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 series.[3,6,7,73] The deletion is positively correlated with a number of other recurrent abnormalities. In patients with t(4;14)(p16.3;q32) or t(14;16)(q32;q23), the incidence of -13/13q- is approximately 90%.[25,29,80] The deletion is also significantly more prevalent in cases with chromosome 1q extra copies (65% vs. 30%)[66] and in non-hyperdiploid as compared to hyperdiploid tumours (65% vs. 25–35%).[3,61,64,65]There are controversial data about the proportion of patients with MGUS carrying the deletion. In one large FISH study (n = 147), the incidence of 13q- was 21%.[25] Accordingly, 13 of 59 (22%) patients with MGUS analysed by cIg-FISH on CD138-enriched bone marrow samples at our institution exhibited the deletion (personal data, September 2005). The comparably low incidence of 13q- in MGUS suggests that -13/13q- might be involved in the evolution of MM, but this hypothesis has not been proven yet by longitudinal genetic analyses. In two other studies using two or more 13q-probes to detect a genomic loss, a higher incidence of 13q-abnormalities (40–50%) was reported.[28,81]

- Clone size

  In a series of 760 patients with 13q- by FISH analysed at our institution, the median percentage of plasma cells carrying the deletion was 87% (range: 26% to 99%; personal data, September 2005). This data is in line with previously reported studies indicating that the fraction of cells with 13q- ranges between 75% and 90%.[32,33,77,82] However, there is variability in the size of the abnormal clone and in almost 10% of cases, the proportion of plasma cells carrying the abnormality is lower than 60% (Fig. 3). These data suggest that 13q- is a secondary event providing a growth advantage of the cell clone with the deletion. In 13 cases with MGUS and 13q-examined at our laboratory, the mean percentage of aberrant cells was 72% (range: 52% to 96%; personal data, September 2005). Considering a likely coexistence of clonal and normal plasma cells in these samples, one can assume that already on the MGUS level the majority of clonal plasma cells contain the deletion.

- Critical region

  As suggested by chromosome banding analyses, chromosome 13 deletion is consistent with monosomy 13 in the majority of cases. Although partial deletions are centered on the 13q14 region,[3–11] a minimally deleted genomic segment has not been defined yet. In line with these data, two FISH mapping studies showed that 80% to 90% of patients with known 13q- had monosomy 13 while an interstitial deletion was present in only 10% to 20% of cases.[32,82] Another small FISH analysis that applied a limited number of overlapping DNA probes mapping to chromosome bands 13q14-q21 proposed a 350 Kb critical region located distally

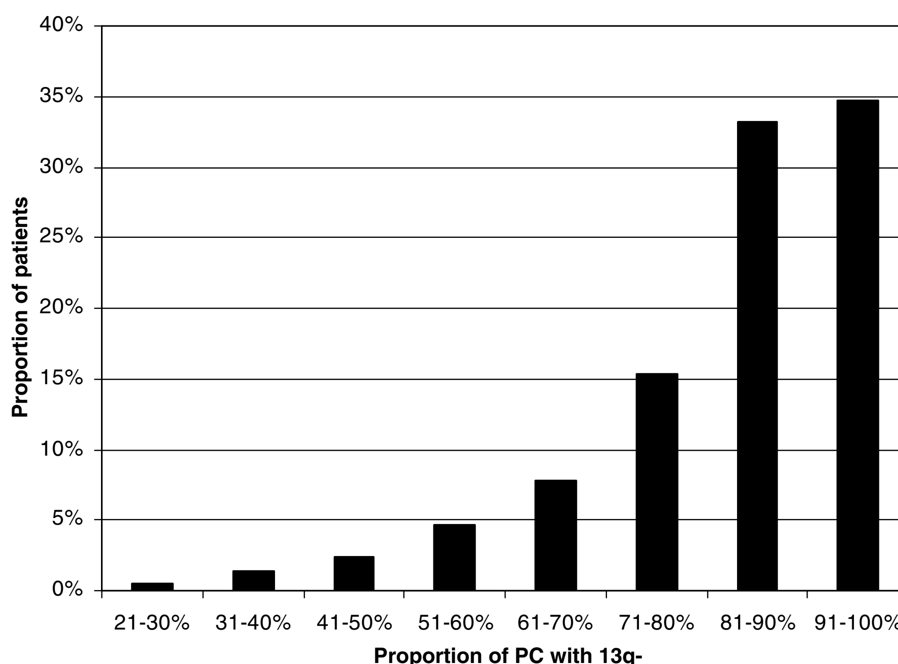

Fig. 3 – Clone size variability in 760 patients with deletion of chromosome band 13q14 as detected by cIg-FISH.

to the *RB1* gene.[34] We examined a series of 52 patients with MM using microarray-CGH. 267 of 6.400 DNA fragments represented on the chip were scattered along chromosome arm 13q. Our data were consistent with loss of the entire chromosome in 28 of 34 cases (82.3%). For the remaining six cases with a partial 13q loss, no commonly deleted region could be delineated.[23] As indicated by many FISH studies, 13q losses are almost exclusively monoallelic. Trisomy 13q14 is a rare finding.[32,33,39]

- Prognosis

Independent of the method of detection (conventional cytogenetics or FISH), -13/13q- is associated with significantly lower response rates, shorter event-free survival (EFS), and inferior overall survival (OS) in MM. This holds true for patients after conventional chemotherapy as well as for patients treated with high-dose chemotherapy (HD-CTX) and autologous stem cell transplantation (ASCT) (Table 2).[11,73,76–79,83] There is scarce data about the relevance of -13/13q- in the setting of allogeneic stem cell transplantation, but one study suggests that –13/13q- remains a negative prognostic marker in this context.[84] In contrast, the outcome of relapsed and refractory patients treated with the proteasome inhibitor bortezomib was not a significantly influenced by this aberration.[85] -13/13q- by karyotyping predicts 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 ("intrinsic" effect of chromosome 13 loss, higher rate of proliferating cells).[86] Conversely, significantly more 13q-deleted patients are identified by FISH as compared to conventional cytogenetics (40–50% vs. 15–20%).

4.2.2. *Chromosome 17p deletion*

- Incidence

Inactivation of the *p*53 tumour suppressor gene by monoallelic deletion or mutation is associated with disease progression in many human malignancies. Accordingly, *p*53 deletion is rather uncommon in intramedullary MM. In most FISH series, the incidence of *p*53 deletion among newly diagnosed patients was in the range of 5% to 10%.[36,38,39,87] 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.[88–91] In line with published data,[92] no *p*53 deletion was found in a cohort of 56 patients with MGUS analysed by FISH at our institution (personal data, September 2005).

- Clone size and critical region

In comparison to primary *IgH* translocations and 13q-, the median proportion of myeloma cells with 17p- was reported to be lower (~60%).[87] Among 43 newly diagnosed patients with monoallelic *p*53 deletion as diagnosed by FISH at our institution, we identified 6 patients with large 13q-deleted plasma cell clones and 17p-deleted subclones, again suggesting *p*53 inactivation as a secondary aberration (personal data, September 2005). The critical region of 17p losses has not been systematically evaluated.

- Prognosis

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.[35,38,40,87] In a recent study, the median progression-free survival (PFS) in patients with 17p- after one course of high-dose melphalan followed by ASCT was only 7.9 months as compared with 25.7 months in the remaining patients [87] In this particular study, 17p- was an independent prognostic marker and explicitly not correlated with 13q-. This contrasts with the highly significant positive correlation of both deletions in a series of 631 cases analysed at our institution ($P < 0.0001$; personal data, September 2005). Accordingly, 17p- was confirmed as an adverse prognostic marker but not as an independent variable in another study.[40]

4.3. *Other imbalances*

- Chromosome 1q

In cytogenetic studies, 1q abnormalities were associated with advanced disease and tumour progression, as well as with shorter event-free survival (EFS).[7,93] Data from a more recent study including gene expression data points to

Table 2 – Prognostic significance of chromosome 13 deletion in patients after conventional chemotherapy or high-dose chemotherapy with autologous stem cell transplantation

Authors	Treatment	n pts.	-13/13q-	Technique	mPFS (m)	p-value	mOS (m)	p-value	Independent variable?
Tricot et al. [71]	HD-CTX	155	14%	CC	22 vs. 43	0.001	29 vs. >50	0.001	yes
Desikan et al. [73]	HD-CTX	1000	16%	CC	n.a.	n.a.	16 vs. 44	<0.001	yes
Fonseca et al. [79]	C-CTX	325	54%	FISH	24.7 vs. 33.0	0.03	34.9 vs. 51.0	0.021	yes
Zojer et al. [77]	C-CTX	97	46%	FISH	n.a.	n.a.	24.2 vs. >60.0	<0.005	yes
Perez-Simon et al. [76]	C-CTX	48	33%	FISH	n.a.	n.a.	14.0 vs. 60.0	0.0012	yes
Facon et al. [78]	HD-CTX	110	38%	FISH	17.0 vs. 33.0	0.0005	27.9 vs. 65.0	<0.0001	yes
Liebisch et al. [83]	HD-CTX	111	48%	FISH	17.7 vs. 36.6[a]	0.002	43.2 vs. >74.9	0.004	yes

HD-CTX, high-dose chemotherapy; n pts., total number of patients; mPFS, mean progression-free survival; mOS, mean overall survival; CC, conventional cytogenetics; FISH, fluorescence in-situ hybridisation; n.a., not assessed.
a mEFS since first HD-CTX.

amplification and overexpression of the cell cycle regulator gene *CKS1B* at chromosome band 1q21 as a predictor of a particularly unfavourable prognosis.[94]

- Chromosome 9q

  A comprehensive multivariate risk factor analysis including clinical and genetic variables (imbalances of chromosome arms 1q, 9q, 11q, 13q, and 17p) in a series of 111 patients receiving upfront HD-CTX and ASCT revealed 13q- and trisomy of chromosome 9q (+9q) as the only independent markers of shorter EFS and OS. The median EFS in patients with +9q was 20.7 months as compared to 27.3 in patients without a chromosome 9q gain. Patients carrying +9q and 13q- had a very unfavourable outcome (median EFS: 12.2 months, median OS: 31.8 months) while in patients with disomy 13q and 9q, the estimated OS rate at 6 years was greater than 85%.[83]

4.4. Chromosomal translocations

4.4.1. 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.[95,96] Using FISH, the rearrangement can be identified in about 15–20% of patients with MM[25,40,42] and 15–30% of cases with MGUS.[25,28] 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.[42,97–100] As in mantle cell lymphoma (MCL), the translocation results in overexpression of *Cyclin D*1. Translocation breakpoints on chromosome 11 are dispersed over 330 kb centromeric to the *Cyclin D*1 gene locus and do not cluster in the *Major Translocation Cluster* (*MTC*) described for MCL, pointing to different mechanisms responsible for the translocations in these diseases (somatic hypermutation and switch recombination vs. VDJ recombination).[54]

The prognostic value of t(11;14) by FISH is not fully resolved. There was no impact of t(11;14) on survival of patients treated with conventional chemotherapy.[38] The same was true for a series of patients receiving HD-CTX and ASCT.[40,97] In a French trial, there was a trend towards a longer survival in t(11;14) positive patients as compared to t(11;14) negative patients, but the difference did not reach statistical significance (P = 0.055).[97] In line with these data, overexpression of *Cyclin D*1 mRNA as measured by RT-PCR in newly diagnosed patients was associated with a significantly longer duration of remission and a trend towards prolonged EFS (P = 0.055) after HD-CTX and ASCT.[101]

4.4.2. 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).[12,13,25,29,102] Of note, in about 20% of cases with t(4;14), *FGFR3* on der(14) is lost or not expressed.[103,104] The translocation was found to be more prevalent among tumours with an IgA isotype as well as in patients with aggressive clinical features.[38,40,97] Independently from the mode of treatment, t(4;14) is associated with an unfavourable clinical course. In one large HD-CTX trial, the median EFS of t(4;14) positive patients was 20.7 months as compared to 28.5 months in the t(4;14) negative cohort (P < 0.0001). The expected OS at 80 months was also significantly different (22.8% vs. 66%, P = 0.002).[97] In a multivariable analysis, t(4;14) was not an independent variable, most likely due to a strong relation between t(4;14) and 13q- observed in this and other studies.[29,97] A recent analysis confirmed the negative prognostic impact of t(4;14) in patients receiving HD-CTX: the median time to progression was only 8.2 months in patients with the t(4;14) as compared to 17.8 months in patients lacking the aberration (P = 0.001). Likewise, the median OS was significantly shorter in t(4;14) positive patients (18.8 months vs. 43.9 months, P = 0.001). In contrast to the aforementioned study, 13q- was present in only 26% of cases with t(4;14). Therefore, t(4;14) retained its independency in multivariable analysis.[40] In line with these data, the median PFS and OS was shorter in t(4;14) positive patients after treatment with conventional chemotherapy (17 months vs. 31 months, P < 0.001; 26 months vs. 45 months, P < 0.001). In this study, t(4;14) was associated with the highest hazard ratio (1.78, 1.23–2.50) in a multiple regression model including clinical and genetic variables.[38]

4.4.3. 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.[13,25,38] t(14;16) results in the upregulation of the basic leucine zipper (bZIP) transcription factor *c-maf*.[13] There is only scarce data about the prognostic impact of this *IgH* rearrangement. In one study, the abnormality was detected in 15 out of 323 patients (4.6%) by FISH. After treatment with conventional chemotherapy, t(14;16) positive patients had a significantly shorter median PFS (9 months vs. 30 months, P = 0.003) and OS (16 months vs. 41 months, P = 0.003) as compared to the t(14;16) negative cohort.[38]

5. Conclusions

The analysis of genomic rearrangements in MGUS and MM by the use of cytogenetics and molecular cytogenetics has significantly promoted our understanding of the development of clonal plasma cell disorders. However, the currently established pathogenetic model is still rather sketchy and many questions remain unresolved. Which genetic events are crucial for the transition of MGUS to MM? Which genes are critically dysregulated in hyperdiploid tumours? Which are the key genetic rearrangements associated with disease progression? To address these issues, further genetic, epigenetic, gene expression and protein analyses, including longitudinal studies, are warranted.

The prognostic relevance of most recurrent chromosomal abnormalities in MM is unknown. However, one can assume that the marked clinical heterogeneity of plasma cell malignancies is only in part mirrored by the currently know genetic markers. The evaluation of a comprehensive panel of chromosomal imbalances and translocations is under way within current clinical trials. To make treatment decisions based on genetic findings outside a clinical study is not recommended at present.

Conflict of interest statement

None declared.

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