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Genetics and molecular profiling of multiple myeloma: Novel tools for clinical management?

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ARTICLE INFO

Article history:
Received 3 April 2006
Accepted 3 April 2006
Available online 3 July 2006

Keywords:
Multiple myeloma
Cytogenetics
Gene expression profiling
Microarray
Proteomics
Molecular markers

ABSTRACT

The understanding of molecular events involved in multiple myeloma (MM) development as well as of mechanisms underlying sensitivity/resistance to anticancer drugs has been dramatically increased by the wide-spread use of modern technologies for genetic analysis, global gene expression and proteomic profiling. Such analytical approaches, which are presently supported by reliable bioinformatic tools, have depicted a new scenario for the development of molecular-based anti-MM agents and for predicting clinical outcome. IgH translocations or a hyperdiploid state are emerging as early genetic signatures of MM which lead to deregulated expression of cyclin D. At present however, the major challenge remains the definition of the potential role of cytogenetic techniques and molecular profiling technologies in individual patient management. Here we will describe the prospective potential and current achievements of such technologies which might produce major advancements in the treatment of this still incurable disease.

1. Introduction

Multiple myeloma (MM) is presently an incurable plasma cell (PC) malignancy which involves 15 000 new patients per year in US with a median expectation of life of 3–5 years and a prevalence accounting for approximately 50 000 patients alive annually. MM is characterized by accumulation of tumour cells within the bone marrow (BM) and a heterogenous clinical course, which can be often preceded by a premalignant condition, defined monoclonal gammopathy of undetermined significance (MGUS), and may progress to extramedullary and/or disseminated disease. The pathophysiology of PC disorders and specifically of MM appears strictly dependent on the BM microenvironment as well as on the molecular events which underline B-cell and PC differentiation.[1,2] Cytogenetic techniques and molecular profiling technologies offer valuable tools for the study of such processes and have yielded new insights into basic molecular events underlying the development of MM as well as into mechanisms of anticancer drug sensitivity/resistance. Presently, a major challenge is how to translate the knowledge derived from these studies into new diagnostic, prognostic and therapeutic applications.

Here, we will describe the most recent findings which have been achieved by the use of cytogenetic analysis and molecular profiling approaches and we will discuss their potential, but still undefined role in the management of individual MM patients.

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2006.04.005

Table 1 – The most recurrent IgH translocations in MM are reported with overall prevalence, main molecular target and prognostic impact

Translocation	Prevalence	Signature	Prognostic relevance
(11;14)(q13;q32)	15–30%	cyclin D1 upregulation	Favourable
(4;14)	15–20%	MMSET/FGFR3/cyclin D2 upregulation	Unfavourable
(14;16)	2–10%	c-maf/cyclin D2 upregulation	Unfavourable
(6;14)	3–4%	cyclin D3 upregulation	Unknown

2. Cytogenetic features of plasma cell discrasias

Malignant PCs may carry multiple and complex chromosomal abnormalities which are likely to be involved in the pathogenesis of PC diseases such as MGUS, smoldering MM (SMM), MM and PC leukaemia. The onset of PC neoplasms may be characterized by a low tumour burden (*i.e.* PCs ranging between 1% to 30% of BM cellularity in most of the newly diagnosed MGUS, SMM and MM) and low PC labelling index, indicating a reduced mitotic rate.[3] These characteristics have hampered the identification and classification of chromosome abnormalities by conventional cytogenetics. Introduction of fluorescence *in situ* hybridization (FISH) allowed the analysis of abnormal karyotypes both in metaphases and interphases providing a new tool for outcome prediction.[4] Chromosomal aberrations can be classified as numerical and structural.

2.1. *Numerical anomalies*

Monosomies, which usually involve chromosomes 13, 14, 16, and 22, and trisomies (chromosomes 3, 5, 7, 9, 11, 15, 19, 21) are commonly observed in PC discrasias.[5,6] Accordingly, investigators distinguish two main aneuploidy categories: hyperdiploid, if the number of chromosomes is between 48 and 74, and non-hyperdiploid when the chromosomes number is less than or equal to 46/47 (hypodiploid and pseudohypodiploid features, respectively) or more than 74 (near tetraploid feature).[7,8] These categories are also indicated by the corresponding DNA index value as marker of ploidy. In this case, the cut-off values are the following: hyperdiploid: $>1.05 < 1.75$; non-hyperdiploid: <1.05 (hypodiploid) or >1.75 (near tetraploid); non-hyperdiploid abnormalities are associated with shorter survival.[9–12]

2.2. *Structural anomalies*

Translocations involving the immunoglobulin heavy-chain (IgH) and light chain (IgL) genes are frequently present in PC neoplasms. IgH translocations have been detected by FISH analysis in nearly 50% of MGUS and SMM and up to 70% of MM samples.[13,14] IgL translocations have been less studied, but the prevalence of IgL translocations in MGUS and MM is 10% and 20%, respectively.[15]

IgH translocations are thought to participate to disease pathogenesis mainly through deregulation of transcription factors and/or growth factor receptors, leading to downstream activation of cyclins D1, D2 and D3. The most recurrent IgH translocations are strongly associated with non-hyperdiploid karyotypes and poor prognosis (Table 1).[16]

According to the partner chromosome, we can distinguish:

1) the translocation t(11;14)(q13;q32), which is an anomaly characterized by the overexpression of the cyclin D1[17] and is associated with a good prognosis in MM patients undergoing high-dose therapy (HDT) and stem cell support.[18] The prevalence of this anomaly is estimated in 15–30% of MGUS and MM;[19,20]
2) the t(4;14), which implies upregulation of Multiple Myeloma SET domain (*MMSET*) and *FGFR3* genes.[21] MMSET is likely to be involved in the progression of the disease, although more data are needed to clarify its role. In some cases, activating mutations of *FGFR3* are present. These data suggested that the inhibition of FGFR3 may be a potential therapeutic strategy at least for a specific subset of patients.[22] Finally, down-stream activation of cyclin D2 has been observed in the presence of t(4;14).[23] This translocation is associated with poor prognosis after HDT.[24] The prevalence is 0–10% and 15–20% of MGUS and MM, respectively;[21,25]
3) the t(14;16), which is a translocation associated with upregulation of the transcription factor c-maf and cyclin D2. This anomaly is variably detected in 2–10% of MM patients;[26]
4) the t(6;14), which is a relatively uncommon translocation (3–4% of MM),[27] and it is associated with upregulation of cyclin D3.[28]

Overall, IgH translocations may be considered as an early immortalizing event, which confers survival and proliferative advantage and probably stromal independence for the tumour clone, as they are highly represented in PC leukaemias.[29] However, this event would not be sufficient for progression of disease, as MGUS patients may carry the translocation without further evolution. Several data suggest that a secondary set of translocations and/or other chromosomal anomalies (e.g.involving c-myc, RAS and p53) provide additional hits for transformation and expansion of the PC clone. Such a scenario may clarify the pathways of transformation in MM tumours with non-hyperdiploid karyotypes. Hyperdiploid MM does not usually show the main IgH translocations. Hyperdiploid karyotype is likely to confer advantage to PC clone through other mechanisms, such as chromosomal duplication. Indeed, cyclin D1 expression has been observed in absence of t(11;14) in MM patients carrying hyperdiploid karyotypes and chromosome 11 polysomy.[30]

2.3. *Abnormalities involving chromosome 13*

Monosomy and structural anomalies of chromosome 13 have been initially described by conventional cytogenetics and associated with shorter survival.[31] A correct estimate of prevalence has been hampered because of the difficulty to obtain metaphases. A prevalence of 30–55% in MM has been reported by interphase FISH.[32] The most recurrent abnormalities are monosomy and interstitial deletions.[33] A non-hyperdiploid karyotype together with t(4;14) and t(14;16) or del(17)(p13.1) has been associated to chromosome 13 anomalies.[13] Conventional cytogenetic techniques were not able to identify t(4;14) and t(14;16) or del(17)(p13.1) in the presence of chromosome 13 anomalies. These abnormalities are often cryptic and can be detected only by metaphase and/or interphase FISH.[4] Recent studies have shown that the presence of these chromosomal aberrations, particularly t(4;14) and t(14;16), affects prognosis rather than the concomitant detection of chromosome 13 anomalies.[4] Prognosis is different whether anomalies of chromosome 13 were detected in metaphase or interphase. Metaphase FISH have shown that chromosome 13 anomalies are identified together with t(4;14), t(14;16) or del (17)(p13.1) in almost 60% of cases.[4] In the same study, interphase FISH revealed this association in 39% of cases. These data clarify previous reports indicating that chromosome 13 anomalies have prognostic significance mostly in abnormal metaphases, whereas their detection in interphase only reveals a less stringent association with t(4;14) and t(14;16) or del (17)(p13.1), which have a real prognostic impact.

2.4. *Other anomalies*

Deletions in the *p*53 locus (17p13) and activating RAS mutations have been described in 10% and 35–50% of MM patients, respectively. The presence of these alterations is associated with advanced stage and poor prognosis.[13,34] Therefore, it can be speculated that such anomalies play a critical role in the progression of the disease.

2.5. *Diagnostic work up for chromosomal abnormalities*

Introduction of molecular techniques has changed the diagnostic work up in PC discrasias.[4] Conventional cytogenetics must be integrated by metaphase and interphase FISH. Indeed, abnormal metaphases assessed by conventional cytogenetics or FISH often underline an active disease, with a high mitotic ratio and a poor outcome. In the context of a low mitotic ratio and tumour burden, interphase FISH can help to discriminate patients carrying poor prognostic abnormalities and more likely to develop progressive disease. Therefore, interphase FISH and ultimately PC FISH provide additional tools to assess the outcome of these patients.

3. Gene expression profiling

The relative expression of tens of thousands of genes can be analysed by microarray technologies, which have provided novel powerful and reliable tools for the study of tumour cell biology in the preclinical as well as in the clinical setting. New tumour classifiers have been produced and prognostic models based on microarray analysis have been developed in a variety of human tumours. This approach has led to important advances in the understanding of biology of human lymphoid malignancies.[35]

There are at least two major reasons which make MM an interesting malignancy for gene expression studies. First, MM is a clinically heterogenous disease, which individually is associated with significant differences in survival and second, genetic abnormalities underlying MM are very complex and variable, making it difficult to correlate between these molecular lesions and the clinical outcome. Pioneering studies using microarray by Zhan and coworkers identified novel MM associated genes suggesting a gene-based classification system for MM.[36] Complementary DNA arrays led De Vos and coworkers[37] to important findings on the role of intercellular signalling genes in the biology of malignant PCs. cDNA microarray analysis has been used for the study of the genetic changes underlying doxorubicin resistance in MM cells *in vitro* and provided evidence that selection of doxorubicin-resistant MM cells leads to changes in gene expression that reduce the apoptotic response to death-inducing stimuli.[38] Oligonucleotide arrays provided important insights to clarify the antitumour activity of dexamethasone (Dex) in MM. Particularly, transient activation of genes involved in DNA repair is followed by induction of apoptotic pathways and downregulation of genes promoting growth and survival. Moreover, mechanism of Dex-resistance have been elucidated in the same study and data originally derived from cell lines have been confirmed in primary cells, demonstrating the clinical relevance of such findings.[39] A further study from the Anderson's group demonstrated that 2-methoxyestradiol (2ME2), an estrogenic derivative with anti-MM activity, is able to modulate genes involved in Dex-resistance as well as genes encoding heat shock family and ubiquitin-related proteins.[40] Another study using DNA microarrays provided the first evidence that transcriptional programs associated with the immunoglobulin switch could be maintained through PC differentiation. These findings have been achieved by comparative analysis of IgA versus IgG-MM. Moreover, the authors found several genes differentially expressed by κ and λ MM. Interestingly, Mip-1α was overexpressed in the κ subgroup and these high levels of Mip-1α were correlated to active MM bone disease.[41] By global hierarchical clustering analysis of microarrays data, Croonquist and coworkers found that specific molecular signatures distinguish an intrinsic genetic transformation event (N-Ras activation) and patterns derived from both IL-6 and stromal cells cell contacts in the BM microenvironment.[42]

The multistep progression of PC disorders has been analyzed by supervised analysis of microarray data comparing normal PC *vs* MGUS *vs* MM cells. This approach led to the finding that differences between MGUS and MM are smaller than those between normal PCs and MM cells, or normal PCs and PCs from MGUS, providing an important clue for the understanding of the molecular development of the disease.[43] Moreover, by oligonucleotide microarrays, the anti-apoptotic activity of IL-6 on MM has been evaluated using an IL-6 dependent MM cell line (INA-6). The authors found that an anti-apoptotic system is operative in these cells,

which is STAT-3 dependent.[44] Gene expression profiling of INA-6 cells has also been used to evaluate molecular events triggered by agents that affect the IL-6 signalling pathway. This approach led to the evidence that the combination of the IL6-receptor superantagonist Sant7 and Dex, which results in synergistic *in vitro* and *in vivo* anti-MM activity, down-regulates proliferation/maintenance and cell cycle control genes, while it upregulates pro-apoptotic genes.[45] Munshi and coworkers developed a novel approach for comparative analysis of normal and malignant PCs. These authors performed comparative microarray analysis of $CD138^+$ normal PCs and $CD138^+$ MM cells derived from the BM of genetically identical twins. Genes involved in cell survival pathway (*mcl-1, dad-1, caspase 8*, and FADD-like apoptosis regulator (*FLIP*)), oncogenes/transcriptional factors (*Jun-D, Xbp-1, calmodulin, Calnexin*, and *FGFR-3*), as well as genes related to stress response and ubiquitin/proteasome pathway and ribosomal genes, were all highly expressed in MM cells from the affected twin. On the other hand, the expression of *RAD51*, killer cell immunoglobulin-like receptor protein, and apoptotic protease activating factor were downregulated in the same cells.[46] In a different study, gene expression analysis of MM by DNA microarrays showed overexpression of Amphiregulin (Ar), a growth factor of the erb family. Follow-up studies demonstrated that Ar is indeed a growth factor for MM cells and that targeting of erb family receptor is a potential novel and unpredicted therapeutical approach.[47] Bergsagel and coworkers identified Cyclin D gene dysregulation as an early event in the pathogenesis of MM. This evidences provided the idea that eight TC (Translocation/cyclin D) groups arise from a very early oncogenic step that results in the dysregulation of 1 of the 3 cyclin D genes. These authors demonstrated that the final expression profile, the biology and clinical course of MM are correlated with these molecular phenotypic groups.[48] In a recent report, Agnelli and coworkers provided a further effort to the molecular classification of MM with additional clues on the transcriptional profile linked to CCND1 amplification.[49]

All together, these studies provided important insights and new achievements in the understanding of the biological bases of MM progression and on molecular mechanisms of anti-MM agents.

4. Proteomics

In the last 10 years, many technological advances for the qualitative and quantitative characterization of protein mixtures have been reported. With respect to gene expression profiling, proteomic analysis can give important complementary information, such as the direct readout of protein expression levels and the mapping of post-translational modifications and protein-protein interactions. The progressive introduction of proteomic approaches in cancer research is expected to help improving the understanding of the molecular pathogenesis of cancer and to allow the discovery of novel diagnostic and prognostic markers of disease.[50] Proteomic applications to MM research are reviewed here, preceded by a brief introduction on the main currently available proteomic technologies.

4.1. *Proteomic technologies*

As shown in Fig. 1, proteomic research can rely on a panel of several different technologies. To date, none of these technologies has demonstrated clear superiority over others with respect to parameters such as throughput, reproducibility and sensitivity. Most importantly, none of them has demonstrated the ability of achieving full, comprehensive proteome coverage of complex protein samples such as cell lysates, tissues or body fluids. The proteomic tools described below can be thus considered to yield complementary information.

Two-dimensional gel electrophoresis (2-DE) has been the classical technique of choice for the separation of complex protein mixtures. Its strengths are the unparalleled resolution at the protein level, the efficacy in separating protein isoforms, and the capability of generating a proteome "snapshot" of immediate visual impact.[51] Recent advances based on the use of special fluorescence dyes, namely differential gel electrophoresis (DIGE), has greatly improved its quantitative accuracy,[52] which has been historically one of the weak points of the technique. One drawback of 2-DE is that the Two-dimensional snapshot under-represents proteins which are either highly hydrophobic or highly basic, or at the extremes of the molecular weight range. A second important drawback is throughput: the technology is inherently not suitable to full automation and lacks sufficient speed to screen proteomes in high numbers. A third weak point addressed to 2-DE is that the identity of the protein spots detected requires additional post-analysis steps, very challenging to perform in high throughput, especially for the low abundance analytes.

Matrix-assisted laser desorption ionization coupled to time-of-flight mass spectrometry (MALDI-TOF MS) is a type of mass spectrometric analysis which uses laser energy to ionize biomolecules and bring them into the gas phase, where the analyzer TOF can achieve their mass analysis and detection.[53] MALDI-TOF is a fast and sensitive technique able to analyze both small and large polypeptides. An important drawback is its low dynamic range of detection (about two orders of magnitude). Furthermore, the technique cannot easily provide accurate quantification, even though the peak pattern generated by MALDI-TOF can suggest quantitative changes in protein/peptide abundance levels, provided that samples of very similar composition are screened in a reproducible way.[54] An interesting clinical application of MALDI-TOF is MS imaging, which can be used to profile proteins directly in tissue sections.[55]

A variant of MALDI-TOF, which has been widely used in biofluid clinical proteomics, is surface-enhanced laser desorption ionization (SELDI-TOF).[56] In this approach, clinical samples are applied on chip surfaces tailored to capture specific analyte classes *via* chromatographic interactions (hydrophobic, ionic, affinity). After chip washing, bound substances are analyzed by MALDI-TOF mass spectrometry. This approach has been extensively used to profile low molecular weight proteins (<20 kDa) in serum samples, in the search for diagnostic patterns of disease. SELDI-TOF has generated a lot of expectations from the clinical research world, especially because of its high-throughput capability. Nevertheless, several issues on the technology are still open.[57] Main con-

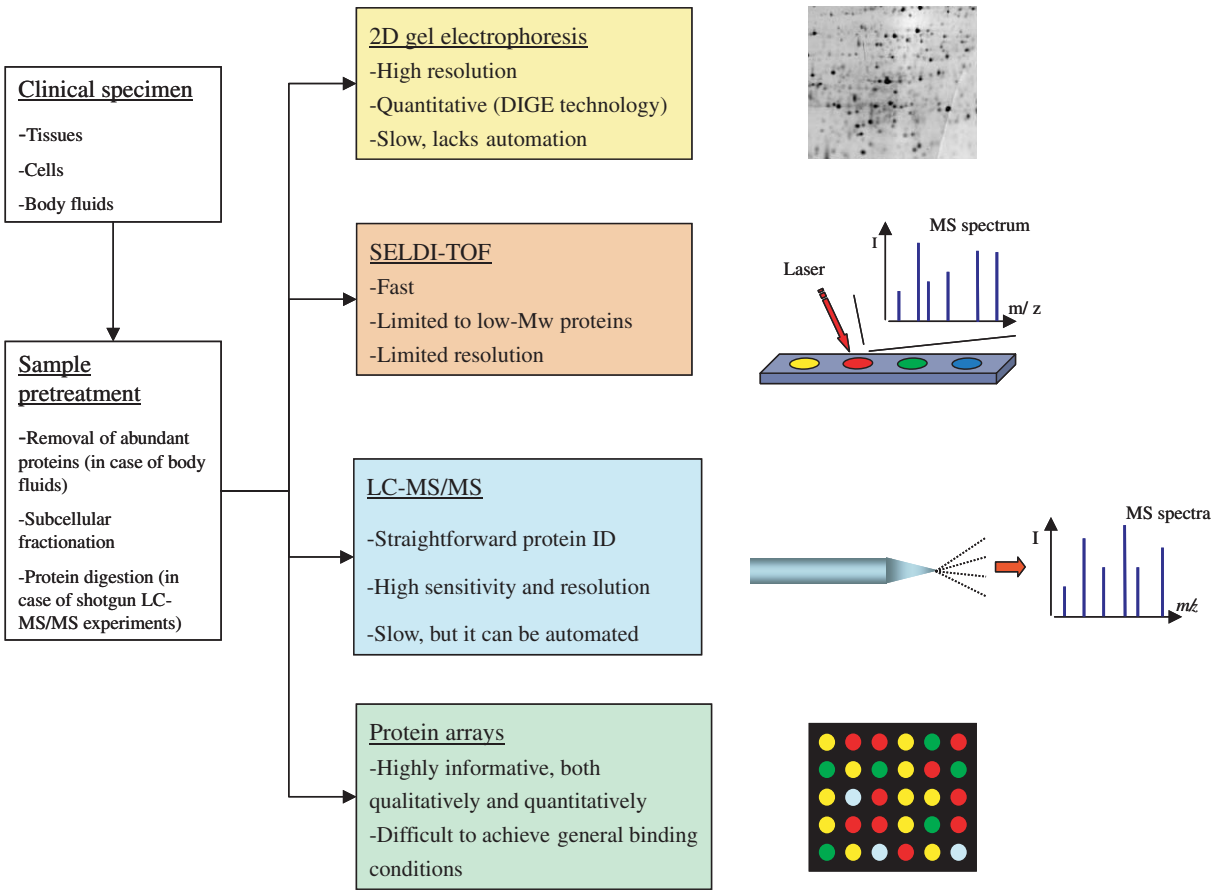

Fig. 1 – Overview of available technologies for clinical proteomics Proteomic analysis. Analysis of proteome can be performed by different techniques. According to the source of the sample and the designed experimental approach, samples undergo a pretreatment process (i.e, removing excess proteins if body fluids are analyzed, collecting only proteins from a specific cellular compartment and/or digesting them), then they are analyzed by different methods: 2D gel electrophoresis. Proteins are separated in a two dimensional gel and quantified by a fluorescent dye (DIfferential Gel Electrophoresis, DIGE); Surface- Enhanced Laser Desorption Ionization (SELDI) coupled to Time-Of-Flight (TOF) Mass Spectrometry (MS). Samples are bound to a chip by chromatographic interactions. Protein are then ionized by a laser and brought to a gas phase, where they are separated and identified through mass spectrometry analysis; Single or multidimensional Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS). Peptides, derived from enzyme digested proteins, are separated by liquid chromatography and then analyzed by Mass Spectrometry; and Protein arrays that provide potentially high proteome coverage and high throughput.

cerns are on its sensitivity of detection, accuracy of quantification, and about its effective capability of generating reproducible diagnostic patterns in different laboratories. In spite of this general concern, attempts have been made to achieve a good interplatform reproducibility in cooperative research programs.[58]

An established gel-free, MS-based approach to qualitatively and quantitatively characterize complex protein mixtures is to perform direct enzymatic digestion, typically using trypsin, and then to analyze the resulting peptide mixture by single- or multi-dimensional liquid chromatography coupled to tandem mass spectrometry analysis (LC-MS/MS).[59] The move from proteins to peptides has several advantages: i) peptides can be more efficiently separated by liquid chromatography; ii) tandem mass spectrometric analysis of peptides can provide full sequence information and, many times, characterize post-translational modifications (while the same is usually not possible at the protein level); iii) analyte solubility is greatly enhanced by breaking down the intact proteins, allowing the use of buffers more "friendly" to both MS and chromatography respect to the ones required for protein analysis. Another advantage of this approach is that it can give straightforward identification of detected species. With the help of isotopic dilution, LC-MS/MS can also provide accurate quantification of the analytes being measured. The greatest drawback of this approach is its low throughput. In fact, the great complexity generated by protein digestion needs extensive chromatographic separations and thus long analysis times. The technique is thus used at its best for the thorough characterization of just a limited number of samples.

Arrays of proteins or antibodies are indicated as emerging proteomic technologies. Their potential for high-proteome coverage and high-throughput analyses might represent a

key advantage over competing approaches. Important drawbacks of protein/antibody arrays are the difficulty of establishing general binding conditions for the proteins under study, and the possible interference from macromolecular complexes, not disrupted in the non-denaturing conditions employed.

4.2. *Proteomic applications to myeloma research*

To date, still very few reports on the application of proteomic technologies to the study of multiple myeloma have been published. Concerning biofluid proteomics, for example, a single work describing the use of SELDI-TOF in profiling sera from MM patients has been recently reported.[60] This work was in fact a study on systematic variability of SELDI-TOF experiments, and described a method to detect and discard low quality spectra generated by SELDI-TOF based on a correlation matrix. Three data sets were used to validate this bioinformatics study, one of which was a set of SELDI-TOF spectra acquired from 64 newly diagnosed MM patients, 36 of which showed evidence of lytic bone lesions, while the remaining 28 patients carried no lytic bone disease. Because the focus of the paper was on the data analysis side, no discussion on potential biomarkers for lytic bone disease originating from the study was reported. Insights on potential clinical findings of this study might be the object of a separate paper.

SELDI-TOF technology was also applied in a recent investigation on the interaction between MM cells and osteoclasts.[61] Secreted proteins from: i) osteoclasts, ii) MM plasma cells and iii) co-cultured osteoclasts/MM plasma cells were profiled by SELDI-TOF mass spectrometry. Protein profiling revealed a remarkable increase of the signal from a 66 kDa protein in the soluble proteins secreted from co-cultured osteoclasts/MM plasma cells. The protein was subsequently identified by running an enriched preparation on SDS-PAGE and then by analyzing the corresponding gel band by mass spectrometry after in-gel digestion. Protein identification as chondroitin synthase 1 (CHSY1) led to the formulation of a hypothesis concerning its role in MM cells/osteoclasts interaction. It has been proposed that CHSY1 directly modulates Notch signalling, known to be involved in interactions between MM cells and their bone marrow microenvironment, by either binding or post-translationally modifying Notch. The hypothesis was supported by biochemical assays on Notch2 and Notch1 activity in myeloma cells co-cultured with osteoclasts.

In the field of cellular proteomics, Fenselau[62] reported the use of MM cell lines in a study focused on the isolation and characterization of the plasma membrane proteome. Their method, based on the use of cationic colloidal silica, allowed for an efficient enrichment of plasma membrane proteins from cell lysates as demonstrated on the two model systems used: breast cancer cell lines MCF-7 and MM cell lines RPMI 8226. After plasma membrane isolation, proteins were separated on SDS-PAGE and protein bands were cut and in-gel digested by trypsin. Protein identification was achieved by MS analysis of the resulting peptide mixtures using either direct infusion in the mass spectrometer by nanoelectrospray ionization or by performing nanoscale liquid chromatography in order to separate the peptide mixture before tandem mass spectrometric analysis (nanoLC-MS/MS). From the MM cell lines, the direct MS approach yielded the identification of 47 proteins, 20 of which were previously reported as being located in the plasma membrane. No analysis using the more powerful nanoLC-MS/MS approach was performed on protein digests from MM cell lines. On the contrary, nanoLC-MS/MS analysis was employed for the characterization of plasma proteins from MCF-7 cell lines, yielding a substantial increase in protein identification (366 proteins in total, 155 of which were reported to be located in the plasma membrane). The method described by Fenselau could be used for comparative proteomics studies aimed at quantitatively profiling the composition of this cellular component.

Antibody-based strategies have been used by Anderson to perform proteomic analysis of myeloma cells. Their approach relied on multiplexed immunoblotting as originally reported by Zhang[63] and it was focused on measuring the levels of abundance of several proteins known to be involved in fundamental cellular mechanisms. In a first study from same group,[64] the signalling state of MM cells was compared to the one of Waldenstrom's macroglobulinemia (WM) cells. Significant overlap between the proteomic profiles of the two cell types was assessed. For example, the absence of expression of the germinal centre kinase (GCK) protein was considered indicative of the post-germinal centre ontogeny of both MM and WM cells. Proteomic profiles were also acquired after cell treatment with anti-tumour agents. Proteasome inhibitor PS-341 (bortezomib), for example, was shown to influence the expression of very specific proteins involved in regulation of proliferation and apoptosis like DNA-PK. Interestingly, the demonstration of the influence of PS-341 on defined biological pathways was not in accordance with the assumption that PS-341 simply generates the accumulation of undegraded proteins in treated cells. Furthermore, as reported in a separate paper from the same group,[65] PS-341 down-regulated the expression of several proteins involved in DNA repair, confirming the observation that PS-341 sensitizes MM cells to the action of DNA-damaging chemotherapeutic agents. These authors reported the use of multiplexed immunoblotting in another study focused on the inhibition of insulin-like growth factor receptor (IGF-1R).[66] In this work, the profile of the signalling state of human myeloma cells (MM-1S) in the absence or presence of IGF-1R inhibitors was analysed. An increase in the molecular events linked to apoptosis in cells treated with the inhibitors was demonstrated. For example, these cells exhibited decreased levels of phosphorylation of kinases and kinase targets in the PI-3K/Akt pathway, and decreased intracellular protein levels of Akt-1, p70S6K, Raf, Src, Bmx, IKK-α and PDK1.

An important advantage of proteomic technologies with respect to gene expression profiling, lies in the possibility of studying protein-protein interactions. This can ultimately give insights into protein function. An interesting example of functional proteomic work in MM research has been reported by Chauhan and coworkers,[67] who investigated resistance of MM cells to Dex. Their investigation started from comparative gene expression analysis of Dex-sensitive (MM.1S) and Dex-resistant (MM.1R) MM cell lines. Among transcripts overexpressed in Dex-resistant cells, they focused their attention on Heat Shock Protein-27 (Hsp-27). Hsp-27 showed a significant up-regulation in Dex-resistant multiple myeloma cells (MM.1R), confirmed by immunoblotting (6-7 fold

overexpression at the protein level). In order to elucidate its functional partners in Dex-resistant cells, the protein was immunoprecipitated and the protein mixture resolved by SDS-PAGE. A strong band of 41 kDa size was detected in the immunoprecipitate from MM.1R cells, and identified as Actin by in-gel digestion and MALDI-TOF peptide mass fingerprinting. The importance of the anti-apoptotic effects of the Hsp-27-Actin complex in MM.1R cells was probed by demonstrating the correlation between disruption of the Hsp-27-actin interaction and apoptosis in MM.1R cells treated with 2ME2 and PS-341.

5. Perspectives

All the reported findings clearly underline the increasing potential of cytogenetic techniques and molecular profiling technology to improve the efficacy of anti-MM treatment. The identification of critical steps in the development of MM[41,46,48,49] clearly defines new opportunities for the development of molecularly designed anti-MM drugs. At the same time the discovery of an anti-apoptotic role of HSP27-actin complex in mediating resistance of MM cells to Dex suggests novel combinational therapeutic approaches.[39,40] The prognostic relevance of specific IgH translocations such as the t(4;14), t(14;16), chromosome 13 deletion and loss of 17p13 might be of major interest for patient stratification in prospective trials.

At present, a major point of discussion is whether cytogenetic techniques and molecular profiling technologies are ready to be translated in the clinical patient management. The translation of novel biomarkers from bench to bedside outside of the research setting proved to be more difficult than expected.[68] This is most likely due to the still early investigational phase of the molecular profiling technologies as demonstrated by the increasing need and efforts for inter-platform and inter-laboratory cross validation. Bioinformatic tools for data management also need to be further cross-validated. Moreover, gene expression data should ideally lead to follow-up studies at the protein level in terms of intracellular content, compartmentalization and functional analysis. Finally, any biomarker, which might modify patient management, needs validation in well-designed prospective clinical studies. It is our opinion that more research is needed for the translation of novel cytogenetic techniques and molecular profiling technologies in the clinical setting. This research is however ready to be performed and will be an important framework for major and individualized advances in the treatment of MM.

Conflict of interest statement

None declared.

REFERENCES

1. Anderson KC. Moving disease biology from the lab to the clinic. *Cancer* 2003;**97**(3 Suppl):796–801.
2. Hideshima T, Anderson KC. Molecular mechanisms of novel therapeutic approaches for multiple myeloma. *Nat Rev Cancer* 2002;**2**(12):927–37.
3. Rajkumar SV, Fonseca R, Dewald GW, Therneau TM, Lacy MQ, Kyle RA, et al. Cytogenetic abnormalities correlate with the plasma cell labelling index and extent of bone marrow involvement in myeloma. *Cancer Genet Cytogenet* 1999;**113**(1):73–7.
4. Dewald GW, Therneau T, Larson D, Lee YK, Fink S, Smoley S, et al. Relationship of patient survival and chromosome anomalies detected in metaphase and/or interphase cells at diagnosis of myeloma. *Blood* 2005;**106**(10):3553–8.
5. Zandecki M, Lai JL, Facon T. Multiple myeloma: almost all patients are cytogenetically abnormal. *Br J Haematol* 1996;**94**(2):217–27.
6. Tabernero D, San Miguel JF, Garcia-Sanz M, Najera L, Garcia-Isidoro M, Perez-Simon JA, et al. Incidence of chromosome numerical changes in multiple myeloma: fluorescence in situ hybridization analysis using 15 chromosome-specific probes. *Am J Pathol* 1996;**149**(1):153–61.
7. Smadja NV, Bastard C, Brigaudeau C, Leroux D, Fruchart C. Hypodiploidy is a major prognostic factor in multiple myeloma. *Blood* 2001;**98**(7):2229–38.
8. Smadja NV, Fruchart C, Isnard F, Louvet C, Dutel JL, Cheron N, et al. Chromosomal analysis in multiple myeloma: cytogenetic evidence of two different diseases. *Leukaemia* 1998;**12**(6):960–9.
9. Greipp PR, Trendle MC, Leong T, Oken MM, Kay NE, Van Ness B, et al. Is flow cytometric DNA content hypodiploidy prognostic in multiple myeloma? *Leuk Lymphoma* 1999;**35**(1–2):83–9.
10. Orfao A, Garcia-Sanz R, Lopez-Berges MC, Belen Vidriales M, Gonzalez M, Caballero MD, et al. A new method for the analysis of plasma cell DNA content in multiple myeloma samples using a CD38/propidium iodide double staining technique. *Cytometry* 1994;**17**(4):332–9.
11. Latreille J, Barlogie B, Dosik G, Johnston DA, Drewinko B, Alexanian R. Cellular DNA content as a marker of human multiple myeloma. *Blood* 1980;**55**(3):403–8.
12. Seong C, Delasalle K, Hayes K, Weber D, Dimopoulos M, Swantkowski J, et al. Prognostic value of cytogenetics in multiple myeloma. *Br J Haematol* 1998;**101**(1):189–94.
13. Fonseca R, Blood E, Rue M, Harrington D, Oken MM, Kyle RA, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood* 2003;**101**(11):4569–75.
14. Nishida K, Tamura A, Nakazawa N, Ueda Y, Abe T, Matsuda F, et al. The Ig heavy chain gene is frequently involved in chromosomal translocations in multiple myeloma and plasma cell leukaemia as detected by in situ hybridization. *Blood* 1997;**90**(2):526–34.
15. Fonseca R, Bailey RJ, Ahmann GJ, Rajkumar SV, Hoyer JD, Lust JA, et al. Genomic abnormalities in monoclonal gammopathy of undetermined significance. *Blood* 2002;**100**(4):1417–24.
16. Fonseca R, Debes-Marun CS, Picken EB, Dewald GW, Bryant JM, Winkler JM, et al. The recurrent IgH translocations are highly associated with non-hyperdiploid variant multiple myeloma. *Blood* 2003;**102**(7):2562–7.
17. Chesi M, Bergsagel PL, Brents LA, Smith CM, Gerhard DS, Kuehl WM. Dysregulation of cyclin D1 by translocation into an IgH gamma switch region in two multiple myeloma cell lines. *Blood* 1996;**88**(2):674–81.
18. Moreau P, Facon T, Leleu X, Morineau N, Huyghe P, Harousseau JL, et al. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. *Blood* 2002;**100**(5):1579–83.
19. Hayman SR, Bailey RJ, Jalal SM, Ahmann GJ, Dispenzieri A, Gertz MA, et al. Translocations involving the immunoglobulin heavy-chain locus are possible early genetic events in patients with primary systemic amyloidosis. *Blood* 2001;**98**(7):2266–8.

20. Fonseca R, Blood EA, Oken MM, Kyle RA, Dewald GW, Bailey RJ, et al. Myeloma and the t(11;14)(q13;q32); evidence for a biologically defined unique subset of patients. *Blood* 2002;**99**(10):3735–41.
21. Chesi M, Nardini E, Lim RS, Smith KD, Kuehl WM, Bergsagel PL. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. *Blood* 1998;**92**(9):3025–34.
22. Trudel S, Li ZH, Wei E, Wiesmann M, Chang H, Chen C, et al. CHIR-258, a novel, multitargeted tyrosine kinase inhibitor for the potential treatment of t(4;14) multiple myeloma. *Blood* 2005;**105**(7):2941–8.
23. Stewart AK, Fonseca R. Prognostic and therapeutic significance of myeloma genetics and gene expression profiling. *J Clin Oncol* 2005;**23**(26):6339–44.
24. Chang H, Sloan S, Li D, Zhuang L, Yi QL, Chen CI, et al. The t(4;14) is associated with poor prognosis in myeloma patients undergoing autologous stem cell transplant. *Br J Haematol* 2004;**125**(1):64–8.
25. Perfetti V, Coluccia AM, Intini D, Malgeri U, Vignarelli MC, Casarini S, et al. Translocation T(4;14)(p16.3;q32) is a recurrent genetic lesion in primary amyloidosis. *Am J Pathol* 2001;**158**(5):1599–603.
26. Chesi M, Bergsagel PL, Shonukan OO, Martelli ML, Brents LA, Chen T, et al. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. *Blood* 1998;**91**(12):4457–63.
27. Sawyer JR, Lukacs JL, Thomas EL, Swanson CM, Goosen LS, Sammartino G, et al. Multicolour spectral karyotyping identifies new translocations and a recurring pathway for chromosome loss in multiple myeloma. *Br J Haematol* 2001;**112**(1):167–74.
28. Shaughnessy Jr J, Gabrea A, Qi Y, Brents L, Zhan F, Tian E, et al. Cyclin D3 at 6p21 is dysregulated by recurrent chromosomal translocations to immunoglobulin loci in multiple myeloma. *Blood* 2001;**98**(1):217–23.
29. Avet-Loiseau H, Daviet A, Brigaudeau C, Callet-Bauchu E, Terre C, Lafage-Pochitaloff M, et al. Cytogenetic, interphase, and multicolor fluorescence in situ hybridization analyses in primary plasma cell leukaemia: a study of 40 patients at diagnosis, on behalf of the Intergroupe Francophone du Myelome and the Groupe Francais de Cytogenetique Hematologique. *Blood* 2001;**97**(3):822–5.
30. Liebisch P, Dohner, H. Cytogenetics and molecular cytogenetics in multiple myeloma. *Eur J Cancer* 2006, in press.
31. Tricot G, Barlogie B, Jagannath S, Bracy D, Mattox S, Vesole DH, et al. Poor prognosis in multiple myeloma is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. *Blood* 1995;**86**(11):4250–6.
32. Facon T, Avet-Loiseau H, Guillerm G, Moreau P, Genevieve F, Zandecki M, et al. Chromosome 13 abnormalities identified by FISH analysis and serum beta2-microglobulin produce a powerful myeloma staging system for patients receiving high-dose therapy. *Blood* 2001;**97**(6):1566–71.
33. Shaughnessy J, Tian E, Sawyer J, Bumm K, Landes R, Badros A, et al. High incidence of chromosome 13 deletion in multiple myeloma detected by multiprobe interphase FISH. *Blood* 2000;**96**(4):1505–11.
34. Liu P, Leong T, Quam L, Billadeau D, Kay NE, Greipp P, et al. Activating mutations of N- and K-ras in multiple myeloma show different clinical associations: analysis of the Eastern Cooperative Oncology Group Phase III Trial. *Blood* 1996;**88**(7):2699–706.
35. Staudt LM, Dave S. The biology of human lymphoid malignancies revealed by gene expression profiling. *Adv Immunol* 2005;**87**:163–208.
36. Zhan F, Hardin J, Kordsmeier B, Bumm K, Zheng M, Tian E, et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. *Blood* 2002;**99**(5):1745–57.
37. De Vos J, Couderc G, Tarte K, Jourdan M, Requirand G, Delteil MC, et al. Identifying intercellular signalling genes expressed in malignant plasma cells by using complementary DNA arrays. *Blood* 2001;**98**(3):771–80.
38. Watts GS, Futscher BW, Isett R, Gleason-Guzman M, Kunkel SE, Salmon SE. cDNA microarray analysis of multidrug resistance: doxorubicin selection produces multiple defects in apoptosis signalling pathways. *J Pharmacol Exp Ther* 2001;**299**(2):434–41.
39. Chauhan D, Auclair D, Robinson EK, Hideshima T, Li G, Podar K, et al. Identification of genes regulated by dexamethasone in multiple myeloma cells using oligonucleotide arrays. *Oncogene* 2002;**21**(9):1346–58.
40. Chauhan D, Li G, Auclair D, Hideshima T, Richardson P, Podar K, et al. Identification of genes regulated by 2-methoxyestradiol (2ME2) in multiple myeloma cells using oligonucleotide arrays. *Blood* 2003;**101**(9):3606–14.
41. Magrangeas F, Nasser V, Avet-Loiseau H, Loriod B, Decaux O, Granjeaud S, et al. Gene expression profiling of multiple myeloma reveals molecular portraits in relation to the pathogenesis of the disease. *Blood* 2003;**101**(12):4998–5006.
42. Croonquist PA, Linden MA, Zhao F, Van Ness BG. Gene profiling of a myeloma cell line reveals similarities and unique signatures among IL-6 response, N-ras-activating mutations, and coculture with bone marrow stromal cells. *Blood* 2003;**102**(7):2581–92.
43. Davies FE, Dring AM, Li C, Rawstron AC, Shammas MA, O'Connor SM, et al. Insights into the multistep transformation of MGUS to myeloma using microarray expression analysis. *Blood* 2003;**102**(13):4504–11.
44. Brocke-Heidrich K, Kretzschmar AK, Pfeifer G, Henze C, Loffler D, Koczan D, et al. Interleukin-6-dependent gene expression profiles in multiple myeloma INA-6 cells reveal a Bcl-2 family-independent survival pathway closely associated with Stat3 activation. *Blood* 2004;**103**(1):242–51.
45. Tassone P, Neri P, Burger R, Savino R, Shammas M, Catley L, et al. Combination therapy with interleukin-6 receptor superantagonist Sant7 and dexamethasone induces antitumour effects in a novel SCID-hu In vivo model of human multiple myeloma. *Clin Cancer Res* 2005;**11**(11):4251–8.
46. Munshi NC, Hideshima T, Carrasco D, Shammas M, Auclair D, Davies F, et al. Identification of genes modulated in multiple myeloma using genetically identical twin samples. *Blood* 2004;**103**(5):1799–806.
47. Mahtouk K, Hose D, Reme T, De Vos J, Jourdan M, Moreaux J, et al. Expression of EGF-family receptors and amphiregulin in multiple myeloma. Amphiregulin is a growth factor for myeloma cells. *Oncogene* 2005;**24**(21):3512–24.
48. Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy Jr J. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. *Blood* 2005;**106**(1):296–303.
49. Agnelli L, Bicciato S, Mattioli M, Fabris S, Intini D, Verdelli D, et al. Molecular classification of multiple myeloma: a distinct transcriptional profile characterizes patients expressing CCND1 and negative for 14q32 translocations. *J Clin Oncol* 2005;**23**(29):7296–306.
50. Srivastava S, Srivastava RG. Proteomics in the forefront of cancer biomarker discovery. *J Proteome Res* 2005;**4**(4):1098–103.
51. Rabilloud T. Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains. *Proteomics* 2002;**2**(1):3–10.

52. Unlu M, Morgan ME, Minden JS. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* 1997;**18**(11):2071–7.
53. Steen H, Mann M. The ABC's (and XYZ's) of peptide sequencing. *Nat Rev Mol Cell Biol* 2004;**5**(9):699–711.
54. Villanueva J, Philip J, Chaparro CA, Li Y, Toledo-Crow R, DeNoyer L, et al. Correcting common errors in identifying cancer-specific serum peptide signatures. *J Proteome Res* 2005;**4**(4):1060–72.
55. Chaurand P, Schwartz SA, Reyzer ML, Caprioli RM. Imaging mass spectrometry: principles and potentials. *Toxicol Pathol* 2005;**33**(1):92–101.
56. Issaq HJ, Conrads TP, Prieto DA, Tirumalai R, Veenstra TD. SELDI-TOF MS for diagnostic proteomics. *Anal Chem* 2003;**75**(7):148A–55A.
57. Diamandis EP. Mass spectrometry as a diagnostic and a cancer biomarker discovery tool: opportunities and potential limitations. *Mol Cell Proteomics* 2004;**3**(4):367–78.
58. Semmes OJ, Feng Z, Adam BL, Banez LL, Bigbee WL, Campos D, et al. Evaluation of serum protein profiling by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry for the detection of prostate cancer: I. Assessment of platform reproducibility. *Clin Chem* 2005;**51**(1):102–12.
59. Swanson SK, Washburn MP. The continuing evolution of shotgun proteomics. *Drug Discov Today* 2005;**10**(10):719–25.
60. Hong H, Dragan Y, Epstein J, Teitel C, Chen B, Xie Q, et al. Quality control and quality assessment of data from surface-enhanced laser desorption/ionization (SELDI) time-of flight (TOF) mass spectrometry (MS). *BMC Bioinformatics* 2005;**6**(Suppl 2):S5.
61. Yin L. Chondroitin synthase 1 is a key molecule in myeloma cell-osteoclast interactions. *J Biol Chem* 2005;**280**(16):15666–72.
62. Rahbar AM, Fenselau C. Integration of Jacobson's pellicle method into proteomic strategies for plasma membrane proteins. *J Proteome Res* 2004;**3**(6):1267–77.
63. Zhang H, Shi X, Zhang QJ, Hampong M, Paddon H, Wahyuningsih D, et al. Nocodazole-induced p53-dependent c-Jun N-terminal kinase activation reduces apoptosis in human colon carcinoma HCT116 cells. *J Biol Chem* 2002;**277**(46):43648–58.
64. Mitsiades CS, Mitsiades N, Treon SP, Anderson KC. Proteomic analyses in Waldenstrom's macroglobulinemia and other plasma cell dyscrasias. *Semin Oncol* 2003;**30**(2):156–60.
65. Mitsiades N, Mitsiades CS, Richardson PG, Poulaki V, Tai YT, Chauhan D, et al. The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. *Blood* 2003;**101**(6):2377–80.
66. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M, et al. Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumours. *Cancer Cell* 2004;**5**(3):221–30.
67. Chauhan D, Li G, Auclair D, Hideshima T, Podar K, Mitsiades N, et al. 2-Methoxyestradiol and bortezomib/proteasome-inhibitor overcome dexamethasone-resistance in multiple myeloma cells by modulating Heat Shock Protein-27. *Apoptosis* 2004;**9**(2):149–55.
68. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. *Nat Rev Cancer* 2005;**5**(11):845–56.